POLYUNSATURED FAT INTAKE AND PROSTATE CANCER

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Epidemiology.

Chapel Hill

2008

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ABSTRACT

DANIEL O. KORALEK: A Prospective Study of Polyunsaturated Fat Intake and Prostate Cancer

(Under the direction of Dr. Jane C. Schroeder)

While the burden of prostate cancer is high, few well-established risk factors exist. Recent efforts have focused on elucidating the role of diet in prostate carcinogenesis, including the roles of individual fatty acids. Evidence has been inconsistent for the relations between specific fatty acids and prostate cancer risk. However, recent evidence suggests that high intakes of alpha-linolenic acid may increase prostate cancer risk while high intakes of longer chain omega-3 fatty acids may reduce prostate cancer risk. We investigated the relations between incident prostate cancer and intakes of specific polyunsaturated fatty acids and their ratios within the screening arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial and in the National Institutes of Health-AARP Diet and Health Study, two large prospective cohort studies of diet and cancer. Cox proportional hazards models were used to estimate hazard ratios and their 95% confidence intervals. In the PLCO population, we found that intake of linoleic acid, the most common ω-6 fatty acid, was inversely associated with total prostate cancer (multivariable-adjusted hazard ratio (HR) for a 4g increment of intake = 0.94; 95%

confidence interval (CI)= 0.89 – 1.00). Dietary intakes of ω-3 fatty acids were positively associated with low-grade prostate cancer (HR for a 0.1g increment of intake = 1.04; 95% CI = 0.99 – 1.09) and trans fatty acid intakes were positively associated with high-grade disease (HR for a 2g increment of intake of total trans fatty acids = 1.07; 95% CI = 0.96 – 1.19). In the NIH-AARP population, we found that intakes of long-chain ω-3 fatty acids were positively associated with total prostate cancer (MV-adjusted HR comparing C5 to C1 = 1.07; 95% CI = 1.02 – 1.12) and inversely associated with fatal tumors (MV-adjusted HR for a 0.1g increment of intake = 0.87; 95% CI = 0.78 – 0.98). Total TFA intake was inversely associated with high-stage disease (MV-adjusted HR for a 2g increment of intake total TFA = 0.95; 95% CI = 0.89 – 1.02) and TFA 16:1 intake was positively associated with fatal disease (MV-adjusted HR for a 0.04g increment of intake = 1.07; 95% CI = 0.97 – 1.18). More research, involving additional prospective studies using instruments with better estimation of PUFA intakes may be used to clarify the role that dietary intakes of these highly interrelated fatty acids may play in prostate carcinogenesis and suggest avenues for primary prevention through the identification of modifiable risk factors for this high burden disease in men.

ACKNOWLLEDGEMENTS

I would like to thank my dissertation advisor, Jane Schroeder, for her patience, guidance, and support throughout the process of my doctoral dissertation. I would like to thank Michael Leitzmann, Richard Hayes, and Arthur Schatzkin for once again giving me the privilege to work with them. In addition, I would like to thank the members of my dissertation committee, Charlie Poole, Jessie Satia, and Joseph Su, for their helpful comments and suggestions during the dissertation process.

Grattitude should also be extended to Leslie Caroll, Matt Moore, and Michael Spriggs of Information Managament Systems for contributions to the data management aspects of this project. In addition, I am indebted to my supervisor at GlaxoSmithKline, Deanna Hill, for her support and understanding during times when deadlines for my dissertation approached. I would like to also acknowledge the financial support afforded by both Andy Olshan through the cancer epidemiology training grant and Michael O'Malley through the Cancer Control Education Program.

I would like to thank the volunteers in the PLCO and NIH-AARP studies, for without their generous commitments to these studies, there would have been no data to analyze.

I owe great thanks to the support of my family and friends, particularly my loving parents, for their support during the past four years.

DEDICATION

"We are at the very beginning of time for the human race. It is not unreasonable that we grapple with problems. But there are tens of thousands of years in the future. Our responsibility is to do what we can, learn what we can, improve the solutions, and pass them on."

- Richard Feynman

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LIST OF ABBREVIATIONS

AA	Arachidonic Acid
ALA	Alpha-linolenic Acid
ATBC	Alpha-Tocopherol and Beta-Carotene Trial
CARET	Carotene and Retinol Efficacy Trial
CI	Confidence Interval
COX	Cyclooxygenase
CSFII	Continuing Survey of Food Intake by Individuals
DGLA	Dihomo-gamma-linolenic Acid
DHA	Docosahexaenoic Acid
DHQ	Diet History Questionnaire
DPA	Docosapentaenoic Acid
DQX	Diet Questionnaire
EPA	Eicosapentaenoic Acid
GLA	Gamma-linolenic Acid
HEPE	Hydroxyeicosapentaenoic Acid
HETE	Hydroxyeicosatetraenoic Acid
HODE	Hydroxyoctadecadienoic Acid
HPEPE	Hydroperoxyeicosapentaenoic Acid
HPETE	Hydroperoxyeicosatetraenoic Acid
ICR	Interaction Contrast Ratio
IMS	Information Management Systems, Inc.
LA	Linoleic Acid

LOX	Lipoxygenase
LT	Leukotriene
MEC	Multiethnic Cohort Study
NCI	the National Cancer Institute
NCOA	National Change of Address
NIH	the National Institutes of Health
NSAID	Non-steroidal Anti-inflammatory Drug
PG	Prostaglandin
PHS	Physician's Health Study
PIN	Prostatic Intraepithelial Neoplasia
PLA_2	Phosopholipase A_2
PLCO	Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial
PSA	Prostate Specific Antigen
PUFA	Polyunsaturated Fatty Acid
RR	Relative Risk
SD	Standard Deviation
SEER	Surveillance, Epidemiology, and End Results
TFA	Trans-Fatty Acid
TX	Thromboxane
ω-3	Omega-3
ω-6	Omega-6

CHAPTER I

REVIEW OF THE LITERATURE

A. Introduction and Historical Background

Prostate cancer is the most common cancer and second leading cause of cancer-related death among men in the United States, accounting for 218,890 new cases and 27,050 deaths in 2007(1). In 2002, the age-standardized incidence rate of prostate cancer in the United States was 124.8 cases/100,000 men and the age-standardized morality rate was 15.8 deaths/100,000 men (both rates standardized to the 2002 World population)(2). Age-standardized prostate cancer incidence has trended upwards over the past three decades, with a sharp peak after the widespread implementation of Prostate Specific Antigen (PSA) testing (Figure 1)(3). Age-standardized prostate cancer mortality continued to increase with a similar trend when PSA testing was introduced, but has shown a small, but steady, decline during the past decade (Figure 2)(4), suggesting that the introduction of PSA screening mainly diagnosed latent tumors that were unlikely to impact mortality. While prostate cancer incidence and mortality vary by race, trends over the past few decades do not differ substantially by race.

While the burden of prostate cancer is high, there are few established risk factors(5). The most widely accepted risk factors for prostate cancer are non-modifiable, including age, race, and family history of prostate cancer(5). A study of

more than 44,000 pairs of twins in Denmark, Finland, and Sweden suggested that 42% (95% confidence interval, CI, = 29 – 50) of prostate cancer risk can be attributed to inherited risk factors(6), but only a small number of genes have been associated with prostate cancer, including the androgen receptor gene and Cytochrome P17(7). Increasing prostate cancer incidence and mortality among Japanese men that have migrated to the United States(8, 9) and increasing rates of prostate cancer within Asian countries (that traditionally have low rates of prostate cancer) suggest that lifestyle factors such as diet and physical activity may contribute substantially to the risk of prostate cancer(10). Studies of lifestyle factors including diet (total energy intake, fat intake, and the intake of micronutrient intakes including lycopene and other antioxidants), physical activity, comorbid conditions (e.g. diabetes), and body size have generated conflicting findings(7).

Autopsy studies have suggested that nearly 30% of men aged 30 – 40 and 65% of men aged 60 – 70 have small cancers of the prostate(11). However, while the lifetime risk of prostate cancer is high, many tumors may be slow-growing cancers with little potential to cause clinically relevant disease or death. On the other hand, the risk of prostate cancer mortality increases with tumor grade and stage at diagnosis(12). Therefore, aggressive prostate cancer may be a greater public health concern than total prostate cancer, and many epidemiologic studies have estimated associations separately for case-subtypes characterized by stage, grade, and/or mortality(7).

B. Review of the Literature

Because studies of total fat intake have been inconclusive, recent efforts have focused on the role of specific types of fat in prostate carcinogenesis(13). Of

particular interest are the roles that polyunsaturated fatty acids (PUFAs) may play. Due to a combination of changes in livestock feeding routines, emphasizing grains, and the perceived health benefit of using cooking oils high in PUFAs, PUFA consumption has increased substantially in the United States(14). Coupled with the perceived health benefits of PUFAs and negative health effects of saturated fat consumption, processed food producers have increased the use of artificially produced trans fats (TFAs), which are now thought to confer potential negative effects on a variety of health outcomes(15). A number of epidemiologic studies have attempted to elucidate relations between specific polyunsaturated and trans fatty acids and prostate cancer, although the evidence has been relatively inconsistent.

B.1. Polyunsaturated Fatty Acids

Fatty acids are a primary building block of phospholipids and glycolipids, two important classes of biological molecules, that modify proteins (to form glycoproteins), generate energy within cells, and are metabolized to form hormones and other intracellular messengers(16). All fatty acids consist of a long hydrocarbon chain bound to a carboxyl (-C(=O))OH) group(16, 17). Fatty acids are essential to normal biologic function and are divided into three broad classes, according to the degree of saturation of the hydrocarbon chain(16, 17); saturated, monounsaturated, and polyunsaturated fatty acids (PUFAs). Naturally occurring monounsaturated fatty acids include a single double cis carbon-carbon bond in the hydrocarbon chain, and naturally occurring PUFAs include at least two double cis carbon-carbon bonds in the hydrocarbon chain(16, 17), although a limited amount of conversion of some double bonds to the trans conformation may occur in ruminants(18). Fatty acids required for normal cellular function and which cannot be synthesized by the body

are classified as essential fatty acids(19) and must be supplied through diet. Furthermore, unsaturated fatty acids may be chemically modified through chemical means, such as the partial hydrogenation of vegetable oils, or in limited quantities through natural processes in the cow gut, to include carbon-carbon double bonds in the trans-fatty acids (TFA)(20). Fatty acids can be identified by two numbers indicating the length of the hydrocarbon chain and the number of double bonds found within the chain (Table 1). For example, a fatty acid labeled "18:0" has a hydrocarbon chain with 18 carbon atoms and no carbon-carbon double bonds, while a fatty acid labeled "18:2" also has 18 carbon atoms, but would include two carbon-carbon double bonds.

PUFA intake has been a recent focus of research because of the potential benefits that this class of fatty acids, or specific types of PUFAs, may have on health outcomes, including cardiovascular disease and cancer(14, 21, 22). PUFAs account for approximately 7% of total energy intake and 20% of energy intake from fats among adults in the United States(14). PUFAs are most generally sub-classified by the location of the first double bond, counting from the methyl group end (the ω-end). The majority of naturally occurring polyunsaturated fatty acids have double bonds in the third or sixth position (ω-3 and ω-6, respectively)(14).

Major ω-3 fatty acids include α-linolenic acid (ALA; 18:3), eicosapentaenoic acid (EPA; 20:5), docosahexaenoic acid (DHA; 22:6), and docosapentaenoic acid (DPA; 22:5)(). Major ω-6 fatty acids include linoleic acid (LA; 18:2) and arachidonic acid (AA; 20:4). The ω-6 fatty acid LA is the most common PUFA in the US diet, accounting for approximately 87% of energy from PUFAs, while ALA is the most common ω-3 fatty acid and second most common PUFA, accounting for

approximately 10% of energy from PUFAs(14). DHA and EPA, two of the so-called "fish fats," get a great deal of publicity even though they account for less than 2% of energy from PUFAs(14). Most ω-3 fatty acids consumed in the US are from terrestrial sources, including vegetable seeds and oils, however the long-chain ω-3 fatty acids, DHA and EPA are most commonly found in fatty fish that obtain these fatty acids by consuming algae and other microscopic organisms(14). The most common dietary sources of DHA and EPA are fatty fish such as salmon and tuna(14). It is important to note that although intake of the ω-3 fatty acid ALA has increased substantially in recent decades in the US(23) there also has been an increase in the ratio of ω-6 to ω-3 fatty acids(14). This shift in the ratio of dietary ω-6 to ω-3 fatty acids consumed in the US has been influenced by increased consumption of modern vegetable oils and the increased use of grains for livestock production, which favors oils rich in ω-6 fatty acids (such as safflower and sunflower oils) and oils rich in ALA (such as canola oil)(14).

Metabolism of the major PUFAs is highly interrelated (Figure 3)(19). Because humans lack the sufficient enzymes to introduce necessary double bonds into hydrocarbon chains, both ALA and LA are essential fatty acids(14). ALA can be converted into EPA and DHA in limited quantities(24). However, because ALA and LA metabolic pathways compete for the same enzymes, this interconversion is limited by both the amounts of ω-3 fatty acids consumed and by the ratio of ω-6 to ω-3 fatty acids(24). DPA can be formed as a metabolic intermediary between EPA and DHA(14).

B.2. Potential Mechanisms for Prostate Carcinogenesis

A number of mechanisms have been proposed to explain potential relations between polyunsaturated fat intake and prostate cancer risk(19). For example, fatty acids may modulate prostate carcinogenesis through the modification of membrane phospholipid composition(25), by increasing lipid peroxidation(26), by modulating cell signaling activity(27-29) or cytokine production(30), and by interfering with androgen activity(31). Some of these mechanisms support a greater role of ω-3 fatty acids and their metabolic byproducts on carcinogenesis, while others support a greater role for ω-6 fatty acids; therefore evidence of both positive and negative effects of each class of fatty acids may be consistent with roles through different carcinogenic mechanisms.

Perhaps, the strongest hypothesis for the role of dietary intakes of PUFAs in prostate carcinogenesis concerns the effects of intakes on the production of metabolic intermediates relevant to pathogenesis. As previously noted, ALA and LA, the primary ω-3 and ω-6 PUFAs in the diet, compete for the same metabolic enzymes that are used produce EPA and AA, respectively (Figure 3)(19). Therefore, increased concentrations of LA relative to ALA will limit conversion of ALA to EPA and DHA and increase conversion of LA of AA. EPA and AA subsequently compete for the same metabolic enzymes (Cyclooxygenases; COXs and Lipoxygenases; LOXs) that are used to form prostaglandins (PGs) and other eicosanoids(19). In addition, high dietary intakes of ω-3 fatty acids increase their incorporation into cell membrane phospholipids, partially displacing AA, which decreases AA-derived eicosanoid production(32).

Hypotheses regarding fatty acids and inflammation suggest a greater carcinogenic potential of ω-6 fatty acids and their intermediates. Specifically, AA-derived eicosanoids, including prostaglandin E_2, have been shown to stimulate prostate tumor growth in both prostate tumor cell lines(33) and animal models(34, 35) and long-chain ω-6 fatty acids (LA) have been shown to enhance growth of human prostate tumor cell lines(36). In addition, AA has been shown to up-regulate COX-2 and COX-1(37), which may increase inflammation-mediated carcinogenesis, while ω-3 fatty acids have been shown to suppress COX-2(38-40).

Potential carcinogenic mechanisms related to oxidative stress suggest a negative effect of the ω-3 fatty acid ALA that may be indirectly related to LA-mediated inhibition of ALA metabolism to DHA and EPA. In particular, ALA has been shown to enhance β-oxidation(41), a process which generates hydrogen peroxide and which may explain why ALA has greater potential to create oxidative damage, and therefore increased tumorgensis, than either DHA or EPA. Animal and cell culture studies suggest that individual ω-3 fatty acids, particularly EPA and DHA, may inhibit prostate carcinogenesis(19). Both DHA and EPA have been shown to inhibit tumor cell growth in both animal models and cell lines derived from human prostate tumors(42). A study of mice fed linseed oil (containing about 50% ALA) showed decreases in prostate tumor DHA levels, but increases in EPA levels, compared with mice fed corn oil(36), suggesting that DHA biosynthesis may be down regulated by high concentrations of ALA(43). In the study of mice fed linseed oil or corn oil rich in LA there was no evidence that prostate tumor growth was prevented with the high ALA diet(36). In a separate study, rats fed perilla oil

(another oil rich in ALA) did not show a reduction in chemically induced prostate tumorgensis as compared with rats fed corn oil(44).

One *in vitro* study showed that ALA increased growth of the TSU, LNCaP, and PC-3 prostate tumor cell lines(45). However, two studies using DU-145 cells found that ALA increased apoptosis at physiological ALA concentrations(46) and suppressed cell proliferation(47). This latter study also showed that urokinase-type plasimongen activator, an important protease enzyme thought to enhance carcinogenesis, production was inhibited by ALA(47). A fourth study found that ALA decreased androgen receptor capacity and increased estrogen receptor capacity in the DU-145 cell line(48), suggesting that ALA may modulate steroid hormone receptor binding. These apparent inconsistencies may be due to variations in cell growth conditions and/or differences in the concentrations of ALA and types of serum used in the cell culture medium(49).

Mechanisms for associations between trans fatty acid (TFA) intake and prostate cancer are less clear. One study found that cis-9, trans-11 conjugated linoleic acid, a naturally occurring trans-fat, inhibited the progression of the cell cycle from the G1 to S phase of DU-145 cell lines, a potential beneficial effect on tumor progression(50). Similarly, tumor necrosis factor (TNF)-α inhibited apoptosis was increased in LNCaP cell lines exposed to this same isomer(51). Hydrogenated fats, the source of the majority of trans-fatty acids in the diet, have been shown to increase production of inflammatory cytokines(15) and other markers of inflammation(52) in humans. Additionally, a recent study found that serum concentrations of trans-fatty acids were associated with increased systemic inflammation(53). Therefore, TFAs, particularly from artificially hydrogenated fats

and oils, may increase prostate cancer risk through a systemic inflammatory response.

B.3. Total Polyunsaturated Fatty Acid, Total ω-3 Fatty Acid, and Total ω-6 Fatty Acid Intakes and Prostate Cancer

Overall, the evidence has been fairly inconclusive for an association between total PUFA intake and prostate cancer risk (Table 2). One prospective cohort(54) and two case-control(55, 56) studies found an increased risk of prostate cancer with increased dietary intake of PUFAs, while two case-control studies found an inverse association(57, 58), and two cohort(59, 60) and six case-control (61-66) studies found no association. Newcomer, et al. reported that ω-3 fatty acid intake was not associated with prostate cancer risk, but high ω-6 fatty acid intake increased risk (OR comparing the 4^{th} quintile of ω-6 fatty acid intake to the 1^{st} quintile = 2.3; 95% CI = 1.0 – 5.4)(67). Harvei, et al. conducted a nested-case control study of serum phospholipids concentrations of blood bank donors and found no association between prostate cancer and concentrations of total PUFAs or ω-3 fatty acids, but a small inverse association with ω-6 fatty acid concentrations(68). In an analysis of the Multiethnic Cohort (MEC), Park, et al. found reported that total PUFA intake and total ω-6 FA intake were not associated with either total or advanced prostate cancer(60). However, they found a slightly reduced risk of advanced prostate cancer with increased intake of total ω-3 FA (RR comparing the 5^{th} to 1^{st} quintiles = 0.90; 95% CI 0.76 – 1.08)(60). In an analysis of the Carotene and Retinol Efficacy Trial (CARET), Neuhouser, et al. found substantially stronger positive associations between total PUFA and total ω-6 FA intakes and prostate cancer among men with a

family history of prostate cancer than among men with no family history of prostate cancer(59)

Because studies of general fatty acid consumption, or classes of fatty acids, and prostate cancer have yielded inconclusive findings, and the fact that evidence suggests that specific fatty acids may act through different mechanisms, recent efforts have focused on studying the relations between specific fatty acids and prostate cancer.

B.4. Linoleic Acid and Arachidonic Acid and Prostate Cancer

As with studies of total PUFAs and prostate cancer, studies of the ω-6 fatty acid LA have been inconclusive (Table 3). Two case-control studies found an increase in prostate cancer risk with increased dietary intake of LA(63, 65), while one case-cohort(58) and one case-control(69) study found an inverse association, and two case-control(57, 66) and one cohort(70) study found no associations. A case-control study in Australia found no association between AA intake and prostate cancer risk(66). Furthermore, in an analysis of the Health Professionals Follow-up Study, Giovannucci, et al. reported a stronger inverse association between dietary intake of LA and advanced prostate cancer than with total prostate cancer (RRs comparing the 5^{th} quintile with the 1^{st} quintile = 0.64 and 0.88; 95% CIs = 0.32 – 1.32 and 0.55 – 1.43, respectively)(71). A later follow-up of the same population found that the early inverse association was attenuated to null for total prostate cancer, and to a RR of 0.80 (0.52 – 1.24) for advanced prostate cancer (72). There are limited data on AA intake and prostate cancer risk as AA is only a minor contributor to dietary fatty acid intakes. However, the case-cohort study by Schuurman, et al. also found a slight positive association between AA intake and prostate cancer risk(58). In

addition, Leitzmann, et al. also found no association between dietary intake of AA and total or advanced prostate cancer(72).

A case-control study nested within the Alpha-Tocopherol and Beta Carotene Trial (ATBC) found an inverse association between prostate cancer and serum concentrations of LA and positive associations between prostate cancer and both serum concentrations and dietary intakes of AA(70). One case-control study nested within the Physician's Health Study (PHS) (73) found an inverse association between plasma cholesterol ester concentrations of LA, but not AA, and prostate cancer risk, while two other case-control studies found positive associations between erythrocyte membrane(67, 74) and adipose tissue(74) concentrations of LA. In a case-control nested within the PHS, Chavarro, et al. reported that whole blood concentrations of LA were associated with a reduced risk of prostate cancer, regardless of aggressiveness (as measured by stage, grade, and a combination of the two), but concentrations of AA were associated with increased risk of aggressive prostate cancer(75).

B.5. Alpha-linolenic Acid and Prostate Cancer

Studies of intake of the ω-3 fatty acid ALA and prostate cancer generally support a positive association (Table 4). Three case-control(57, 69, 76) studies found a positive association between ALA intake and prostate cancer risk. The Giovannucci, et al. analysis(71) of the Health Professionals Follow-up Study found a positive association (RR comparing the 5^{th} quintile to the 1^{st} quintile of ALA intake = 3.43; 95% CI = 1.67 – 7.04) with advanced prostate cancer, but not total prostate cancer. An analysis including greater follow-up time had similar, but slightly attenuated findings(72). These results are compatible with the hypothesis that ALA

may have weaker effects for localized prostate tumors than advanced tumors. One case-cohort study(58) found an inverse association between prostate cancer risk and ALA intake (RR comparing the 5th quintile to the 1st quintile = 0.76, 95% CI = 0.66 – 1.04) and one case-control study(66) one cohort study(60) also found a modest inverse association between ALA intake and prostate cancer risk. Leitzmann, et al. found divergent effects for ALA intake and organ-confined and advanced prostate cancers, suggesting that ALA may act to promote tumor progression(72).

We conducted an analysis of prostate cancer and ALA intake from specific food sources in the screening arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial(49). ALA was not associated with total (RR comparing 5th to 1st quintile of ALA intake = 0.94; 95% CI = 0.81 – 1.09) or advanced prostate cancer risk (either by stage or grade). We also did not find any associations between prostate cancer risk and ALA intake from any specific food source.

Six case control studies(67, 68, 70, 73-75) evaluated ALA biomarkers, rather than dietary intake of ALA, and prostate cancer risk. A case-control study nested within the PHS found that increased plasma cholesterol concentrations of ALA were associated with increased prostate cancer risk(73). Of the four case-control studies(67, 68, 70, 74) that investigated serum concentrations of ALA and prostate cancer, three found positive associations(67, 68, 77). Godley, et al. also investigated the association between ALA concentrations in adipose tissue and prostate cancer; they found a slightly stronger positive association with prostate cancer risk than their analysis of erythrocyte membrane concentrations of ALA(74). Erythrocyte membrane concentrations of fatty acids may be more reflective of short-term dietary intakes of fatty acids and may not reflect long-term intakes measured by dietary assessments. In their analysis of ATBC data, Mannisto, et al. found no association

between prostate cancer risk and either serum concentrations of ALA or dietary intake of ALA(70). In their analysis of PHS data, Chavarro, et al. reported stronger positive associations between high blood concentrations of ALA and non-aggressive prostate cancers (either by stage or a combination of stage and grade), but similar positive associations for low and high grade tumors(75).

B.6. Docosahexaenoic Acid and Eicosapentaenoic Acid and Prostate Cancer

Studies dietary intakes of the long chain ω-3 fatty acids DHA and EPA and prostate cancer generally suggest no associations (Table 5). One case-control(62) and one cohort study(60) found no associations between dietary intakes of DHA and EPA and either local or distant prostate cancer while another(66) found an inverse association between EPA intake and prostate cancer (OR = 0.8; 95% CI = 0.6 – 1.11) and no association between DHA intake and prostate cancer. One case-cohort study(58) found no association between dietary intakes of either EPA or DHA and prostate cancer risk, while another(76) found that DHA and EPA consumption was inversely associated with prostate cancer risk (OR comparing the 4^{th} quartile of consumption to the 1^{st} = 0.70; 95% CI = 0.51 – 0.97). The initial analysis of the Health Professionals Follow-up Study(71) found no association between prostate cancer risk and DHA and EPA intake. However, the follow-up analysis by Leitzmann, et al. (72) suggests that DHA and EPA intakes may be inversely associated with prostate cancer risk, particularly with advanced cases. The analysis of DHA and EPA dietary intakes in ATBC found possible positive associations between dietary intakes of both EPA and DHA and prostate cancer risk.

Studies of biomarkers of DHA and EPA have more consistently been supportive of an inverse association between DHA and EPA and prostate cancer risk

than studies of dietary intakes (Table 5). This may partially be the result of interconversion of ALA to these long-chain ω-3 fatty acids since serum concentrations of DHA and EPA are determined by both dietary consumption of these fatty acids and their formation via ALA metabolism, which may, in turn, be influenced by consumption and metabolism of other PUFAs. One case-control study nested within the PHS(73) found that serum cholesterol ester concentrations of EPA had a modest inverse association with prostate cancer risk (OR comparing the 4^{th} quartile with the 1^{st} = 0.87; 95% CI = 0.41 – 1.82). Godley, et al.(74) found that erythrocyte membrane concentrations of both EPA and DHA and adipose tissue concentrations of EPA were inversely associated with prostate cancer risk while adipose tissue concentrations of DHA were not associated with prostate cancer risk. A nested case-control study by Harvei, et al.(68) found that serum concentrations of DPA were inversely associated with prostate cancer risk while serum concentrations of EPA and DHA were not. A case-control study by Newcomer, et al.(67) found no associations between erythrocyte membrane concentrations of EPA and DHA and prostate cancer risk while another case-control study(78) found inverse associations between erythrocyte membrane concentrations of both EPA and DHA and total and advanced prostate cancer. The case-control study nested within the ATBC study found an inverse association between serum concentrations of DHA, but not EPA, and prostate cancer risk(70). The more recent analysis of PHS data(75) generally found inverse associations between EPA, DHA, and DPA concentrations and prostate cancer regardless of grade, but found no associations with high stage tumors. However, it is important to note that because the metabolism of fatty acids are dependent on one another (Figure 3), serum and tissue concentrations of fatty

acids may not be indicative of dietary intakes. They may also not be as representative of long-term intakes of these fatty acids as dietary assessment methods.

B.7. Trans-fatty Acids and Prostate Cancer

Few studies have investigated relations between TFA intake and prostate cancer risk (Table 6). One case-cohort study(58) found no association between total TFA intake and prostate cancer risk while a case-control study in Australia (66) found a modest positive association between dietary intake of the fatty acid 16:1 TFAs and prostate cancer (RR = 1.2; 95% CI = 0.9 – 1.6) and no associations between 18:1 and 18:2 TFAs. Liu, et al. published a case-control study(79) and found positive associations between advanced cancer and intakes of 16:1, 18:1, 18:2 and total TFAs among Caucasians, but the suggestion of inverse associations among African Americans. Intriguingly, they found that a functional mutant of the RNASEL gene modified this association among Caucasians while the mutation alone was not associated with prostate cancer risk. In their analysis of CARET data(59), Neuhouser, et al, found that total TFA intake was positively associated with prostate cancer risk among men with a family history of prostate cancer, but not among men without a family history.

A single nested-case control study(80) investigated the associations between serum concentrations of specific TFAs and prostate cancer risk and found modest positive associations between prostate cancer and serum concentrations of most of the 18:1 and 18:2 TFAs and no associations with the 16:1 TFAs.

B.8. Ratios of Polyunsaturated Fatty Acids and Prostate Cancer

As discussed previously, the mechanisms for which PUFA intake may modulate prostate carcinogenesis are highly interrelated(19). For example, it is known that conversion of ALA to DHA and EPA is inefficient(81) and is modulated by dietary intakes of ALA as well as concentrations of DHA and EPA(19). Furthermore, it is hypothesized that competition for metabolic enzymes that synthesize AA from LA and DHA and EPA from ALA may influence the concentrations of these long-chain metabolites and their influence on prostate cancer risk(19). Furthermore, because the long-chain ω-3 and ω-6 fatty acids compete for the same enzymes (and act in opposite directions on prostate cancer risk), the relative amounts of these classes of fatty acids may be more influential on prostate cancer risk than the absolute intakes(19).

Few studies have reported associations between ratios of PUFAs and prostate cancer (Table 7). A nested case-control study of serum concentrations of fatty acids and prostate cancer by Harvei, et al.(68) generally found inverse associations between increasing ratios of ω-6 fatty acids to ω-3 fatty acids and prostate cancer risk. In their analysis of PHS data(75), Chavarro, et al. found a modest inverse association between the ratio of serum concentrations of ω-6 fatty acids to ω-3 fatty acids and a modest positive association between the ratio of serum concentrations of AA to EPA. In their analysis of the MEC study, Park, et al.(60) found no associations between high ratios of dietary intakes of ω-6 fatty acids to ω-3 fatty acids and total or advanced prostate cancer. Hedelin, et al.(76) found positive associations between the ratios of dietary intakes of ω-6 fatty acids to ω-3 fatty acids and ω-6 fatty acids to DHA + EPA (results presented are inverse associations for the reciprocal ratios). In

their analysis of the Health Professionals Follow-up Study, Leitzmann et al.(72) found no association between the ratios of dietary intakes of LA to ALA and a positive association between the ratio of LA to EPA and DHA (RR comparing the 5th quintile to the 1st quintile = 1.14; 95% CI = 0.98 – 1.33). They also found an inverse association between the ratio of LA to ALA and advanced prostate cancer and a positive association between the ratio of LA to EPA and DHA and advanced prostate cancer. Most likely, these results are driven by the associations between prostate cancer and ALA, EPA, and DHA, detailed previously.

B.9. Summary of the Prior Literature

A number of studies have investigated associations between prostate cancer and either dietary intakes or biological levels of PUFAs and results are somewhat conflicting. In general, studies support positive associations between ALA and prostate cancer risk and inverse associations with DHA and EPA. However, it is important to note that the majority of studies are retrospective in nature and may be limited by differential recall of diet based on case status, changes in diet as a result of their cancer diagnosis, or in the case of biomarker studies, changes in biological concentrations of fatty acids as a result of the cancer, although biomarker measures of PUFAs have been shown to correlate fairly well with dietary intakes(77).

One difficulty that arises in comparing studies of diet is that dietary assessments vary in their ability to measure complete dietary intakes(82) which makes it difficult to compare categories of intake across studies that use different assessment methods. Therefore, most studies compare ranked intakes (e.g. comparing quintiles of intake). For example, in a validation substudy among male participants in the NIH-AARP Diet and Health Study, the correlation between

PUFA intakes from food frequency questionnaire and diet record was 0.47 and 0.53 after energy intake was adjusted for(83). However, neither assessment method is perfect. Not only are there differences in assessment by method, but also different instruments within the same approach can provide different estimates. Subar, et al. found in a comparison of the Block, Willet, and NIH Diet History Questionnaire (DHQ) that adjustment for energy intake increased the comparability of the three food frequency questionnaires(84). We know, for example, that the dietary assessment used in the screening arm of the PLCO Screening Trial did not include items on the types of cooking oils used. Because vegetable oils are large sources of ALA in the diet, our intake data is expected to be lower than that generated by a food frequency questionnaire (FFQ) that did inquire about cooking oils. A further discussion of the comparability of the two food frequency questionnaires used in the proposed dissertation research can be found in the methods chapter.

Furthermore categories of nutrient intakes by quantile may not be directly comparable across studies as absolute intakes of nutrients in studies may vary across different populations. For example, if the distributions of intakes in two populations differ substantially, individuals who would have been classified in the 5^{th} quintile of intake in one population might be equivalent to individuals in the fourth quintile of another study (based on absolute intakes).

Data from prostate tumor cell lines which has demonstrated that PUFAs can modulate tumor cell replication and growth, supporting the hypothesis that PUFAs act later in carcinogenesis(19). As such, studies that have investigated relations between PUFAs or TFAs and prostate cancer and had sufficient case numbers to stratify by prostate cancer aggressiveness (or mortality) have tended to find stronger associations with more aggressive disease.

Given that PUFA intakes may act to promote tumor growth(19) and that screening is believed to have increased the detection of slow growing tumors(3, 4) recent studies have focused on elucidating relations between PUFA intake and more aggressive or fatal prostate cancers(49, 72). Furthermore, studies with sufficient power (49, 72, 79) have stratified analyses by both potential biological modifiers (e.g. the COX inhibiting non-steroidal anti-inflammatory drugs, NSAIDs) and other purported risk factors for prostate cancer (e.g. race).

While biological levels of PUFAs may be more related to biologic mechanisms, dietary intakes are more relevant in terms of primary prevention as biological concentrations are modifiable through dietary intakes. Godley, et al. found correlations between erythrocyte membrane concentrations and FFQ estimates of EPA and DHA consumption of 0.44 and 0.41, respectively and between adipose tissue concentrations and FFQ estimates of 0.38 and 0.32, respectively(77). These correlations are similar to estimates of the correlations between erythrocyte membrane and adipose tissue concentrations of these same PUFAs(77). Biological concentrations are only modifiable as a function of dietary intakes.

Few large prospective studies have comprehensively examined relations between dietary intakes of specific PUFAs and their ratios, and prostate cancer risk. The majority of studies, including our prior analysis of ALA intake in the PLCO study, has focused on one subset of PUFAs (e.g. long-chain ω-3 fatty acids) and have not thoroughly investigated the entire set of interrelated nutrients and prostate cancer risk. Few studies have investigated dietary intakes of TFAs.

We extended our original analysis of ALA intake in PLCO and used these two large, well-designed prospective studies of diet and prostate cancer to investigate these relations. Results of this study may be used to help understand

how not only individual polyunsaturated fatty acids may influence prostate cancer risk, but also the role that ratios of these fatty acids may influence risk. Furthermore, this study provides additional data on potential negative health effects of trans fatty acids.

CHAPTER II

STATEMENT OF SPECIFIC AIMS

A. Specific Aims

Although prostate cancer is the most common cancer and second leading cause of cancer-related death among men in the United States(1), few established risk factors exist(85). The most widely accepted risk factors for prostate cancer are non-modifiable, including race and family history of prostate cancer(85). However, wide geographic variation in prostate cancer incidence and mortality rates suggests an etiologic role for lifestyle factors(85). Evidence has supported a role for environmental factors associated with a western lifestyle in prostate cancer etiology(85). Studies of dietary consumption of fats and prostate cancer have been inconclusive(86). Recent interest has focused on consumption of specific fatty acids in association with prostate cancer risk(13, 21, 87). Increases in polyunsaturated fat intakes in the western diet, particularly in α-linolenic acid (ALA) and linoleic acid (LA), the most common omega-3 (ω-3) and omega-6 (ω-6) fatty acids(14), respectively, have correlated with increases in prostate cancer incidence, even prior to the widespread introduction of prostate specific antigen (PSA) testing. A number of epidemiologic studies have investigated the relations between total polyunsaturated fat intake(54-57, 61-65, 88), and intakes or biological concentrations of linoleic acid (LA, 18:2, ω-6) (57, 63, 65, 67-74, 88, 89),

α-linolenic acid (ALA, 18:3, ω-3), and docosahexaenoic acid (DHA, 22:6, ω-3) and eicosapentaenoic acid (EPA, 22:5, ω-3) (62, 67, 68, 70-74, 88-90). Although results of these studies have been inconclusive, they generally suggest a modest inverse association between long-chain ω-3 fatty acids (DHA and EPA) and prostate cancer, and modest positive associations between the short chain ω-6 and ω-3 fatty acids (LA and ALA, respectively) and prostate cancer. These associations are biologically plausible as LA is metabolized to arachidonic acid (AA, 20:4, ω-6) by the same enzymes that synthesize EPA and DHA from ALA(19). Eicosanoids formed as metabolites of AA, including prostaglandins and thromboxanes, have been implicated in prostate growth and inflammation(19). Furthermore, ALA has been shown to have the ability to increase oxidative damage, potentially promoting carcinogenesis(19). Because of this enzymatic competition, it is thought that the ratio of ω-6 to ω-3 fatty acid consumption may be more important than the absolute intakes of specific PUFAs(19). An additional strong hypothesis suggests that EPA and DHA may inhibit prostate carcinogenesis by competing for a second set of enzymes, such as COX-2, that are used to form eicosanoids from AA(19). Few studies have reported on relations between trans-fatty acids (TFAs) and prostate cancer(58, 66, 79, 80), but data suggest that there may be a positive association. A positive association is plausible as TFAs from partially hydrogenated have been hypothesized to increase an inflammatory response(15, 52, 53).

We proposed to estimate the associations between prostate cancer incidence and 1) specific polyunsaturated fat intakes, 2) ratios of polyunsaturated fatty acid intakes, and 3) trans fatty acid intakes by conducting separate cohort(91) analyses of men enrolled in the screening arm of the PLCO Cancer Screening Trial(92, 93) and of

in the National Institutes of Health (NIH) – AARP (formerly the American Association of Retired Persons) Diet and Health Study(94).

Specific aims of this study were to:

1. Estimate associations between prostate cancer incidence and dietary intakes of ω-3 and ω-6 fatty acids (ALA, DHA, DPA, LA, and AA) and their ratios and dietary intakes of trans-fatty acids (TFAs) among men in the screening arm of the PLCO trial.
2. Assess the degree to which these associations are modified by race, body mass index (BMI), which may affect fatty acid metabolism, total energy intake, and non-steroidal anti-inflammatory drug (NSAID) use.
3. Evaluate these associations separately for prostate cancer cases classified according to stage and grade.
4. Estimate the associations between prostate cancer incidence and dietary intakes of ω-3 and ω-6 fatty acids (ALA, DHA, DPA, LA, and AA) and their ratios and dietary intakes of TFAs among men in the NIH-AARP Diet and Health Study.
5. Assess the degree to which these associations are modified by race, BMI, and NSAID use.
6. Evaluate these associations separately for advanced and fatal prostate cancer cases.

Study aims one, two, and three were addressed through analyses of data collected through the baseline PLCO questionnaires and screening visit, expanding upon our previous analysis of ALA intake and prostate cancer(49). The PLCO Cancer Screening Trial is a multi-site clinical trial, that enrolled participants at 10

sites throughout the United States (Birmingham, AL, Denver, CO, Detroit, MI, Honolulu, HI, Marshfield, WI, Minneapolis, MN, Pittsburgh, PA, Salt Lake City, UT, St. Louis, MO, and Washington, DC)(93). Participants were recruited from the general populations by advertisements, direct mailings, and other means(93). The PLCO screening arm enrolled 38,350 men between the ages of 55 and 74 years from 1993 through 2001 and will follow-up men for a variety of endpoints, including prostate cancer, for at least 13 years(93). All men were free of cancer at baseline. To date, nearly 1,900 prostate cancer cases have been ascertained, including 285 tumor classified as "advanced" (clinical stage greater than or equal to T3b, N1, or M1)(49). Because we have precise data on time-to-event, associations were estimated using Cox proportional hazards models(95) using age as the underlying time metric(96). Furthermore, the proportional hazards model is free of distributional assumptions and only relies on the proportional hazards assumption being held. To estimate effects between nutrients and tumor stage and/or grade, we fit separate models for each outcome of interest.

Aims four, five, and six were addressed through analyses of data collected from the baseline questionnaires of the NIH-AARP Diet and Health Study(94). The NIH-AARP Diet and Health Study enrolled 340,148 men aged 50-71 years between 1995 and 1996 in six states (CA, FL, PA, NJ, NC, and LA) and two metropolitan areas (Atlanta, GA and Detroit, MI)(94). Participants were recruited through direct mailings to AARP members and will be followed-up for at least 10 years for a variety of endpoints, including prostate cancer(94). More than 10,000 prostate cancer cases have been ascertained through the first five years of follow-up (2000)(50). Associations will be estimated using Cox proportional hazards models(95) using age as the underlying time metric(96). To estimate effects between

nutrients and tumor stage and/or fatality, we fit separate models for each outcome of interest. Study results may be used to help clarify the role that dietary intakes of specific polyunsaturated fats in prostate carcinogenesis and suggest avenues for primary prevention through the identification of modifiable risk factors for this high burden disease in men.

B. Hypotheses and Rationale

Hypotheses and rationales for each aim of the proposed study are:

Aim 1: ALA intake will not be related to either total or advanced prostate cancer as our prior analysis of ALA and prostate cancer in PLCO yielded null findings and only approximately 20 additional cases have been ascertained through additional case-ascertainment during the same follow-up period. However, modest changes in the effect sizes may occur after additional control for intakes of other PUFAs. DHA and EPA intake will be associated with a reduced risk of prostate cancer, particularly among advanced cases, as the majority of prior studies have found an inverse association with increased DHA and EPA and one may expect a stronger effect with advanced cases given the hypothesis that DHA and EPA may have antiproliferative effects. LA intake, and to a lesser extent AA intake, will be associated with a small increase in prostate cancer risk, consistent with the prior literature. Because of the null findings for ALA intake in PLCO, we expect that the ratio of LA/ALA intake will most likely have a modest positive association given the similarities in the PLCO population with the study of these ratios in the Physician's Health Study. A high ratio of LA to DHA + EPA intake will be associated with an increased risk of prostate cancer given that we expect LA, and its proximate metabolite AA, to competitively inhibit the beneficial eicosanoid and

prostaglandin synthesis from DHA and EPA. Due to the interrelated metabolism of these PUFAs, a more thorough investigation of the associations of ALA along with other PUFAs may shed greater insight into the true relations with prostate cancer.

Aim 2: Because NSAID use may be of too small a dose relative to intake, and self-reported use may not accurately reflect true use over the etiologically relevant time period, and NSAID use (and NSAIDs COX inhibitory properties) did not previously modify our relations between ALA intake and prostate cancer, we do not expect that it will modify our associations. However, it is possible that these associations will differ with the other individual fatty acids. Similarly, neither BMI nor race strongly modified risk in our previous analysis, and will be unlikely to modify the current associations. In general, prior studies have either been underpowered to conduct stratified analyses or have not reported substantial effect measure modification by other known risk factors for prostate cancer. In order to remain consistent with the literature, we will evaluate modification by other purported risk factors for prostate cancer.

Aim 3: If associations are present in PLCO, we may expect that they would be stronger for advanced prostate cancer cases (either high stage or grade or a combination of the two). We did not see any associations with ALA intake and advanced prostate cancer in PLCO. We did not, however, comprehensively analyze the role that other PUFAs, and their ratios, had on prostate cancer incidence. However, associations in studies that investigated advanced prostate cancer separately tended to be stronger than those with total cases. Furthermore, these associations are plausible considering some of the proposed mechanisms for relations between PUFAs and prostate cancer risk, such as lycopene intake, smoking history, and NSAID use.

Aim 4: Considering the body of literature, we would expect that ALA intake will be associated with prostate cancer risk in the AARP cohort, particularly with advanced and fatal cases of prostate cancer. The AARP study used an enhanced dietary questionnaire that may better distinguish variability in dietary PUFA intakes, in similar populations. Similarly, we expect that DHA and EPA intake will be associated with a reduced risk of prostate cancer. Consistent with the body of literature, we would expect that the ratio of LA to DHA + EPA will be positively associated with prostate cancer risk and the ratio of LA to ALA intake will not be associated with prostate cancer risk.

Aim 5: As with the PLCO population, we do not expect substantial modification between PUFA intakes and prostate cancer by NSAID use, race, or BMI. In order to remain consistent with the literature, we will evaluate modification by other purported risk factors for prostate cancer.

Aim 6: As with PLCO, we expect that associations will be stronger with advanced (defined by stage) prostate cancers and, in the case of AARP, with fatal cases of prostate cancer.

CHAPTER III

METHODS

A. Overview of Methods

We prospectively investigated relations between prostate cancer and dietary intakes of PUFAs within two large cohort studies, the screening arm of the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial(97) and the NIH-AARP Diet and Health Study (AARP)(94). Diet was assessed at baseline in both cohorts using semi-quantitative food frequency questionnaires (FFQs). Additional data on potential confounders and effect measure modifiers were collected at baseline through extensive background questionnaires. We used a second risk factor questionnaire to collect additional data on potential covariates from AARP participants approximately six months after enrollment. In addition, it was possible to treat screening behavior as a time-varying covariate during follow-up of participants in PLCO, allowing for differential adherence to the PLCO screening protocol (i.e. as our analyses of the PLCO data is not a comparison with the trial control group, we will not use "intention to treat" analyses). We estimated hazard ratios and 95% confidence intervals for associations using Cox Proportional Hazards models(95) and presented both age-adjusted and multivariable-adjusted results.

B. Study Design

B.1. The Screening Arm of the PLCO Cancer Screening Trial

B.1.a. Study Population

The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial is a multi-site (Birmingham, AL, Denver, CO, Detroit, MI, Honolulu, HI, Marshfield, WI, Minneapolis, MN, Pittsburgh, PA, Salt Lake city, UT, St. Louis, MO, and Washington, DC; Figure 4) clinical trial sponsored by the National Cancer Institute (NCI) that was designed to test the effectiveness of screening for these four cancers and to identify early markers and etiologic determinants of cancer(93). Participants were recruited from the general population through direct mailings, advertisements, and other means and were enrolled between November 1993 and June 2001(98). A total of 38,350 men (Table 8) between the ages of 55 and 74 years were enrolled into the screening arm of the trial. Men in the screening arm of the PLCO trial were screened annually, beginning at baseline and continuing through year 5, by prostate specific antigen (PSA) test and were also screened annually through year 3, by digital rectal exam (DRE). Study participants provided written informed consent. The institutional review boards of the NCI and the ten participating screening centers approved the study. Current analyses were exempted from institutional review board review at the University of North Carolina as the research was deemed "non-human subject research."

Men were excluded from our analyses if they had a prior history of cancer other than non-melanoma skin cancer (n= 791), did not undergo an initial screen (n

= 2,471), underwent an initial screen but did not have subsequent contact (n = 1,458), did not complete the baseline or dietary questionnaire (n = 7,493), reported an energy intake in the top or bottom 1% of the reported energy intake distribution (n = 634), or completed their baseline after 30 September, 2002 (n = 71). After exclusions, 29,592 men were eligible for our proposed analyses. The men in the final analytic cohort do not differ substantially from those men excluded from analysis with respect to age, level of education, smoking status, and family history of prostate cancer(49). For these analyses, men were followed-up from completion of the baseline questionnaire until the date of diagnosis, death, date of last questionnaire return, or the end of the study period (30 September, 2002), whichever date came first.

B.1.b. Exposure Assessment

At baseline, participants completed a self-administered semi-quantitative 137-item food frequency questionnaire (FFQ) (DQX; http://www.cancer.gov/prevention/plco/DQX.pdf), which inquired about usual diet over the year prior to completing the questionnaire. The DQX is a grid based FFQ which asked for frequency of consumption for all 137 food items and the usual portion size for 77 items. Gram weights per portion size (small, medium, large) were estimated using data from two 24-hour diet recalls administered in the 1994-1996 Continuing Survey of Food Intake by Individuals (CSFII)(99). For TFA intakes, gram weights were estimated using data from the Nutrition Data System for Research (NDSR; http://www.ncc.umn.edu/products/databasenutrients.html), also estimated from dietary intakes during the same period as completion of the baseline FFQs(99, 100). Food items, assumptions for estimating nutrients and food

groups, and the wording used for the DQX incorporated elements of both database(99) and cognitive(101, 102) research. The DQX was not validated in this population, however both the Block and Willett FFQs from which the DQX was based have been validated in similar populations(84). No data is available for PUFA intakes from supplements such as flax seed or fish-oil capsules.

Food items relevant to PUFA intake that were specifically queried for included fried fish, tuna (including tuna salad and tuna casserole), shellfish (including shrimp, crab, and lobster), and other fish (either broiled or baked). However, the questionnaire did not inquire about specific fats typically used for frying, sautéing, or baking. Responses to the individual food items were converted to average daily intakes of specific fatty acids, including ALA, LA, DHA, DPA, EPA, and AA. Average daily intakes of each fatty acid were then combined in order to estimate the total daily intake of each of these fatty acids.

B.1.c. Outcome Assessment

As part of the PLCO trial, men will be followed-up for cancer mortality for a minimum of 13 years from randomization(92). However, for the purposes of these etiologic analyses, men were followed-up through September 30, 2002. Men found to have either a PSA value greater than 4 ng/ml or an abnormal DRE during a PLCO screening visit were referred to their usual medical care providers for further diagnostic evaluation. Furthermore, participants were asked to report any diagnosis of prostate cancer during the prior year on each annual questionnaire. Medical records were obtained and abstracted for men who had a suspect prostate cancer screen or who reported prostate cancer on their annual questionnaire to confirm the diagnosis and obtain clinical tumor stage(103) and grade data. Death certificates,

autopsy data, and supporting medical/pathologic records were used to confirm the diagnosis and stage and grade information for participants who were reported as deceased by next of kin or the study center directly. Additionally, the National Death Index (NDI) was used to increase completeness of the data. Only confirmed prostate cancer cases will be included in our analysis(97). Due to the ongoing nature of the PLCO Cancer Screening Trial, prostate cancer mortality data is unavailable for these analyses. Through September of 2002, nearly 1,900 incident prostate cancer cases have been ascertained(49). Case ascertainment is believed to be virtually complete. Men were undergoing a standardized screening regimen and death certificate, autopsy data, and supporting medical/pathologic records were used to confirm diagnosis, including tumor stage and grade. Gleason grades are based on biopsy or prostatectomy Gleason scores, whichever value was greater.

B.1.d. Covariate Assessment

In addition to the DQX, PLCO screening arm participants completed an extensive background questionnaire, including questions on body size (current and age 25), physical activity (past year), smoking history (lifetime), medical history (lifetime), NSAID use (past year), and family history of prostate cancer(93). As part of the screening trial, updated data is available on screening behavior throughout the active screening portion of follow-up.

B.2. The NIH-AARP Diet and Health Study

B.2.a. Study Population

The NIH-AARP Diet and Health Study is a prospective cohort study designed to investigate relations between diet and a variety of health outcomes(94). Between 1995 and 1996, 3.5 million current members of the AARP, aged 50 – 71 who resided in one of six US states (CA, FL, PA, NC, NJ, and LA) or one of two metropolitan areas (Atlanta, GA and Detroit, MI) were invited to join the study (Figure 5). Over 500,000 individuals returned the initial mailed questionnaire, including 340,148 men (Table 9). A supplementary risk factor questionnaire was subsequently mailed to respondents during the latter half of 1996. The institutional review board of the NCI has approved the NIH-AARP Diet and Health Study. Current analyses were exempted from institutional review board review at the University of North Carolina as the research was deemed "non-human subject research."

Men were excluded if they had submitted a second baseline questionnaire (n = 103), died or moved out of the study area prior to baseline (n = 373), chose to withdraw from the study (n = 1), had a questionnaire completed by a proxy (n = 14,495), had been previously diagnosed with a cancer other than non-melanoma skin cancer (n = 27,269), or had extreme values (greater than twice the interquartile range for the box-cox transformed energy intake; < 415 and > 6,144 kcal/day) for total energy consumption (n = 2,509), height (n = 1,456), weight (n = 402), or BMI (n = 174). After exclusions, 288,956 men remained in the final analytic cohort, including 178,705 (62%) men who had data available from the supplementary questionnaire. Due to the passive nature of follow-up in AARP, men were followed-up from

completion of the baseline questionnaire until the date of diagnosis, death, or end of the study period. Because detailed data is unavailable for men who have moved out of the study area, there was no censoring for this event.

B.2.b. Exposure Assessment

At baseline, participants completed a grid-based version of the Diet History Questionnaire (DHQ; http://riskfactor.cancer.gov/DHQ/)(94). The DHQ queried participants on the frequency of consumption and typical portion size of 124 food items during the past year prior to completion of the survey. The DHQ included an additional 21 questions on consumption of low-fat foods and food preparation practices, including types of oils and fats used(94). Like the DQX, the DHQ nutrient database is based on national dietary data from the CSFII(99) and NDSR(99, 100).

A separate calibration sub-study was conducted within the AARP cohort(104). Of the 2,053 cohort participants enrolled into the calibration sub-study, 1,986 provided two separate 24-hour dietary recalls (approximately 1-month apart), while the rest provided a single 24-hour recall. All 2,053 sub-study participants received a second DHQ in October of 1996, of which 1,415 were returned. PUFA intakes were fairly well correlated with 24-hour dietary recalls, particularly after nutrients were adjusted for energy intake ($r = 0.53$)(83). No data is available on PUFA intakes from supplements, such as flax seed or fish-oil capsules.

As with dietary assessment in PLCO, average daily nutrient intakes for specific food items were estimated by multiplying the average daily consumption of each food item by the nutrient content of the item. The average daily intake of each nutrient was then estimated by summing the average daily intakes for each of the food items.

Like the DQX, the DHQ inquired about fish consumption, including consumption of tuna (including tuna salad and casserole), fried fish, and other types of seafood. However, the DHQ also queried participants on the types of fats and oils used in cooking, which are common sources of PUFAs in the diet.

B.2.c. Outcome Assessment

Identification of incident prostate cancer cases in the AARP cohort has been conducted through passive follow-up(105, 106) via linkage of the NIH-AARP cohort database with state cancer registries in the eight participating states(107). The AARP cohort database has been matched to the National Change of Address (NCOA) database maintained by the US Postal Service(105) and additional information on address changes has been obtained through direct reporting by study participants in follow-up questionnaires and through data received through US Postal Service processing of undeliverable mail(105). Within three years of follow-up, 98% of AARP cohort members either remained at the same address or relocated within one of the eight AARP states(107) and it has been estimated that over 95% of the cohort met these same criteria over the first five years of follow-up. Therefore, the overwhelming majority of participants remained in follow-up for ascertainment of prostate cancer.

The registries in the eight AARP states are estimated to be 95% complete within two years of cancer diagnosis for all cancer outcomes and have been certified by the North American Association of Central Cancer Registries for meeting the highest standard data quality. Furthermore, the cohort was linked to the NDI to ascertain date and specific cause of death. A validation sub-study was conducted among 12,000 cohort members(105). Using medical record confirmation of self-

reported cancer incidence, approximately 90% of all incident caners were validly identified using the registry-based approach(105), potentially the result of incomplete registry linkage and delayed reporting of cases to the registries. Clinical or pathological tumor stage data(103) was obtained from registry data. However, Gleason grade is unavailable for incident cases of prostate cancer in the AARP cohort. Furthermore, data released in time for the analysis to be completed will have further linkage to cancer registries in Arizona, Texas, and Nevada, the three states in which the largest number of AARP participants have relocated to outside of the eight AARP states and follow-up was extended for incidence through 2003 and for cause-specific mortality through 2005.

B.2.d. Covariate Assessment

The baseline AARP questionnaire included questions on body size (current), physical activity (past year), family history of cancer (ever), and smoking history (lifetime). The supplementary risk factor questionnaire included questions on dietary intakes of selected foods ten-years earlier and during adolescence, as well as questions on cancer screening (including both DRE and PSA testing, past five years) and family medical history (lifetime).

C. Methods

C.1. Classification of Nutrient Intakes and Methods for Energy Adjustment

We considered both continuous and categorical (quintiles, based on the distribution of nutrient intakes among the non-cases) classifications of nutrient exposures, including dietary intakes of ALA, LA, AA, EPA, DHA, and the sum of EPA and DHA. Furthermore, we considered the intakes of major classes of trans-fatty acids (TFAs; trans-16:1, trans-18:1, and trans-18:2), separately and in combination, when correlations were sufficiently weak as to preclude colinearity. Additionally we used splines to explore potential dose-response curves(106).

We used three different approaches to control for energy intake in our analyses(82): a standard model including unadjusted nutrient intakes and terms for energy intake, the residual method, and the nutrient energy-density method.

Briefly, standard models included term(s) for the nutrient whose effect is being modeled (categorical or transformed continuous), energy intake (categorical or transformed continuous), and any covariates used.

For the residual method(82), we first log-transformed the nutrient intake for each individual. We then regressed the log-transformed nutrient on total energy intake or log-transformed energy intake, whichever one had the most linear relationship (simple linear regression), and calculated the residual between each individual's actual nutrient intake and the predicted nutrient intake given his energy intake. The residual nutrient intake was then added to the estimated nutrient intake at the mean energy intake of the study population in order to place the log-transformed, adjusted nutrient intake into a more readily interpretable scale. These values were then exponentiated to generate the energy-adjusted nutrient

intakes. When energy intake is also strongly correlated with the outcome, but not a substantial intermediate, it is said to be appropriate to include terms for energy intake into the final models(82).

For nutrient energy-density models(82) we calculated the percentage of total energy consumed that is accounted for by each nutrient (Density variable = kcal from macronutrient / total kcal). Models incorporating these nutrient density variables also included terms for total energy intake.

In addition to estimating effects of individual fatty acid intakes, we separately estimated effects of ratios of ω-6 to ω-3 fatty acid intakes, including the ratios of AA+LA:ALA+DHA+EPA, LA:ALA, and LA:EPA+DHA+DPA as categorical and continuous exposures.

C.2. Classification of Covariates

C.2.a. PLCO

As discussed previously, at baseline, PLCO screening arm participants completed an extensive background questionnaire at baseline(93). As with nutrient intakes, we evaluated the use of both categorical and continuous variables for use as covariates in our multivariable models. Potential covariates we considered included age (in days; time-varying beginning at baseline), self-reported current BMI, family history of prostate cancer (any blood relative, yes/no), history of diabetes (yes/no), smoking (current/former/ever pipe or cigars/never; categorizations that were selected from a variety of combinations evaluated during our previous analyses of PLCO data), total energy intake (kcal/day), daily red meat consumption (g/day), lycopene intake (μg/day), supplemental vitamin E use (IU/day), regular aspirin

and/or ibuprofen use (never/<2 tablets/week/2+ tablets/week), physical activity (hours of "vigorous exercise"/week), and race (white/African American/Asian or Pacific Islander/Other). Furthermore, detailed screening behavior is available for participants during the first five years of enrollment in the PLCO trial, including the date of DRE and PSA blood draw. Because follow-up data is available for screening history, total numbers of screens were treated as a time-varying covariate in our models. We evaluated patterns of missing data to determine the best method of accounting for this. Missing values for these covariates were estimated using multiple imputation methods based on known values of covariates used in our analysis(108, 109). In the case of PLCO data, the only covariate that was missing in a substantial number of individuals was BMI.

C.2.b. AARP

As discussed previously, AARP participants completed a baseline questionnaire along with a subsequent risk-factor questionnaire(94). Potential covariates included age, BMI, physical activity (number of times per week participating in vigorous physical activity), race (white/black/other), smoking history (never/former/current), education (<12 years/high school graduate/some college/at least a college degree), family history of prostate cancer (yes/no), history of diabetes (yes/no), at least one PSA test in past three years (yes/no), at least one DRE in past three years (yes/no), total energy intake (kcal/day), red meat intake (g/day), lycopene intake (μg/day), vitamin E intake (mg/day), regular NSAID (aspirin and/or ibuprofen) use (yes/no), and calcium intake (mg/day). If necessary and appropriate, missing values for these covariates were estimated using multiple imputation methods based on known values of covariates used in our analysis(108,

109). Subjects who completed the supplemental risk factor questionnaire do not differ substantially from subjects who did not with respect to many of the covariates under investigation. However, we compared estimates from the entire cohort with those in each group as well as evaluated confounding and effect measure modification by factors queried on the supplemental risk factor questionnaire among men for whom that data is available.

C.3. Statistical Analysis

C.3.a. PLCO and AARP

We first presented descriptive statistics for the study populations, including distribution of covariates by major exposure categories (means for continuous covariates, proportions for categorical covariates). Additionally, we described patterns of missing data for covariates.

Because we had individual level data on time to event, we used Cox proportional hazards models(95, 110) to estimate age-adjusted and multivariable-adjusted hazard ratios (HRs) and 95% confidence intervals (95% CIs) using STATA 10.0 (STATACorp, College Station, TX). A strength of the proportional hazards model is that it is free of distributional assumptions and only relies on the assumption that the hazards for each of the exposure levels are proportional over the entire observation period(110). Because age is a stronger risk factor for prostate cancer than time in study, we used age as the underlying time metric in our models(96). Additionally, we generated correlation tables of the non-energy-adjusted and energy-adjusted fatty acid intakes to determine the degree to which colinearity existed when including multiple fatty acid intakes in the same model.

We constructed causal diagrams, including features of directed acyclic graphs (DAGs; Figure 6) to help identify potential confounding relationships between these covariates and the dietary polyunsaturated fat-prostate cancer relationship and determine potential adjustment sets(111). Potential confounders were included in multivariable models as appropriate to each main exposure being examined and we assessed the degree to which covariates acted as confounders by estimating the change in the ratio of precision and bias(112-114). Briefly, after adding a covariate to the model, we calculated the ratio of the change in variance of the point estimate for the main exposure divided by the change in the parameter estimate for the point estimate (on the log-scale). A large value for this ratio indicates relatively decreased precision with little change in the point estimate, and indicates that the covariate should not remain included in the model as a potential confounder. Due to the substantial statistical power afforded by the large sample sizes, we considered keeping covariates that did not appear to change the parameter estimates if they also did not reduce the precision of our estimates using the same diagnostic criteria as above. Furthermore, to maintain consistency with study guidelines, we include standard covariates for studies of diet and prostate cancer within PLCO and AARP, as appropriate, so long as substantial precision was not lost (using the method described previously). Both age-adjusted and multivariable-adjusted effect estimates (HRs and 95%CIs) were presented.

While we modeled intakes of each fatty acid separately, there may be some concern that a given fatty acid intake may have acted as a proxy for another. Given that correlations between specific fatty acid intakes were reasonably weak, we modeled all fatty acid intakes simultaneously, to generate the fatty acid-specific associations.

We tested whether the proportional hazards assumption had been violated in each model by visual inspection(110), by adding a time interaction with each covariate(110), and by the method of Grambsch and Therneau (a test of the linearity of the Schoenfeld residuals)(115). In cases where the proportional hazards assumption was violated, measures were be taken to relax the assumption, such as adding an interaction between the exposure and time into the model(110).

We assessed multiplicative interactions (hazard ratio modification) by including multiplicative interaction terms in our proportional hazards models and comparing the stratum-specific HRs and 95% CIs and additive interactions (risk difference modification) by estimating the interaction contrast ratio (ICR)(116, 117) as both simulations by Li, et al.(118) and our own simulation studies (data not published) suggest that the ICR is a more robust assessment of additive interactions within proportional hazards models than either the attributable proportion due to additive interaction (AP) or the synergy index (S). We examined additive and multiplicative interaction by calculating the difference between the actual and expected interactions (based on a common-reference category)(91). Covariates considered as effect measure modifiers included age at baseline (categorized being below or above the population median), BMI (overweight+/not overweight), race (white/black/other), state of residence/PLCO study center, and family history of prostate cancer (yes/no). Note, that while BMI is technically on the pathway between individual polyunsaturated fatty acids (Figure 6), individual fatty acids do not provide for a substantial amount of total energy intake (e.g. LA, which comprises approximately 88% of PUFA intake, only accounts for approximately 5% of total energy intake in these populations (Table 10) and those individuals with lower intakes of certain types of PUFAs would likely be replacing this energy from

energy from other sources. Thus, any bias induced, should have been fairly minimal and offset by the potential advantages gained by assessing BMI as a confounder and/or effect measure modifier.

Certain covariates, particularly total energy intake, physical activity, and body size, may have been misclassified. To assess the degree to which exposure misclassification may have biased our results, we conducted sensitivity analyses(91). Briefly, the potentially misclassified exposures were reclassified given hypothetical misclassification rates (sensitivity and specificity) and hazard ratios were estimated using the reclassified covariates. These new estimates were then used to evaluate the degree to which effect estimates may have been biased due to misclassification of these covariates by providing bounds to how far the point estimates may have changed given reasonable estimates for how misclassified the data could have been. To investigate the degree to which subclinical manifestations of undiagnosed prostate cancer may have affected our estimates by altering dietary behaviors, we conducted a sub-analysis whereby we removed all subjects who were lost to follow-up or diagnosed with incident prostate cancer within one year of entry into the study. While this period is short relative to tumor progression, it removed latent tumors that were diagnosed as the result of a first screen in the PLCO population.

C.3.b. PLCO-specific statistical analysis

Person-time of follow-up for each participant was calculated from the time of randomization into the screening arm until the date of last questionnaire return, date of diagnosis, date of death, or the end of the study period (30 September 2002), whichever occurred first. Due to active study participation and linkage of the PLCO population to local cancer registries and the NDI, it is unlikely that a substantial

number of men were lost to follow-up with regards to prostate cancer diagnosis. To control for adherence to the prostate cancer screening regimen, we used a time-varying covariate equal to the number of prostate cancer screens that an individual had completed at a given time point in the proportional hazards model.

Due to the ongoing nature of the PLCO trial, information was not available on prostate cancer mortality in this cohort. However, confirmed clinical or pathological stage and grade information (whichever grade was higher if both are available will be used) was available for virtually all prostate cancer cases. We considered as separate end points all prostate cancer cases, regionally invasive or metastatic cases (≥ T3b, N1, or M1), organ-confined cases or cases with minimal extraprostatic extension (T1b – T3a and N0M0), cases with high Gleason sum (≥ 7), and cases with low Gleason sum (<7). Additionally, we considered advanced (meeting either the high stage or high Gleason criteria) and non-advanced (meeting neither criteria) cases. All additional assessed outcomes were evaluated in separate models.

C.3.c. AARP-specific statistical analysis

Person-time of follow-up accrued from the date of return of the baseline questionnaire until the date of prostate cancer diagnosis, death, or the end of the study period (31 December, 2003). As discussed previously, the passive ascertainment methods used in AARP resulted in incomplete case ascertainment. However, it is unlikely that loss to follow-up was associated with dietary fatty acid intake. Approximately 60% of eligible AARP participants completed the supplementary risk factor questionnaire, which is the source of PSA and DRE screening information. We investigated the degree to which participants who

completed both questionnaires differ from participants who completed only the baseline questionnaire and we compared the complete cohort estimates with estimates confined to participants who completed both questionnaires.

We considered separate endpoints of total prostate cancer cases, fatal cases (including cases identified through registry data that subsequently died from prostate cancer after the study period, between 2003 and 2005, localized or organ-confined cases (Stage T1a – T2b and N0 M0), and men with extraprostatic disease (Stage T3 or T4, N1 or M1). A separate set of sensitivity analyses were conducted by excluding T1a cancers from the localized disease group and T3a cancers from the extraprostatic disease group. All additional assessed outcomes were evaluated in separate models. Because it has been estimated that approximately 11% of incident cancer cases will be missed through the cancer registry ascertainment procedure(107), we conducted sensitivity analyses to determine the degree to which misclassification of this nature may have biased our estimates (in a similar manner to the methods used for misclassification of exposure).

D. Power Estimation

We estimated statistical power for Cox proportional hazards models using Power Analysis and Sample Size (PASS) 2005 (NCSS, Kaysville, UT) which uses the methods developed by Schoenfeld(119) and Hsieh and Lavori(120).

We estimated power to detect main effects, at an alpha of 0.05, for the PLCO population, assuming a total sample size of 29,000 men, with a known overall case rate of approximately 6.5%, under two different assumptions regarding correlations between our main exposure and covariates (Table 12). We had excellent power to detect modest (HR = 1.2) associations comparing any given quintile of exposure.

These estimates correlate well with empirical evidence from our prior study of ALA intake and prostate cancer risk in the PLCO population(49).

We estimated power to detect main effects, comparing men classified into one quintile of exposure with those classified into another, at an alpha of 0.05, for the AARP population, assuming a total sample size of 287,760 men, with a known overall case rate of 3.5%, and the same categorization and correlation parameters as with our estimates for the PLCO population (Table 13). We had good power to detect modest associations (HRs = 1.2) within the AARP population.

CHAPTER IV

RESULTS

A. Paper 1: Analysis among males participants in the screening arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial

A.1. Abstract

Epidemiologic research on dietary fats and prostate cancer has increasingly focused on the role of specific types of fat in prostate carcinogenesis, including polyunsaturated (PUFAs) and trans fatty acids that may act through distinct mechanisms. We extended our previous analysis of α-linolenic acid intake and prostate cancer among men in the screening arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, a cohort of 29,594 men enrolled between 1993 and 2001 in ten study centers across the United States, to evaluate associations between prostate cancer and baseline dietary intakes of polyunsaturated fatty acids and their ratios, and dietary intakes of trans fatty acids. Over an average of 5.1 years of follow-up we ascertained 1,914 incident cases of prostate cancer, of which 698 were high-grade (Gleason sum ≥ 7). Intake of linoleic acid, the most common ω-6 fatty acid, was inversely associated with total prostate cancer (multivariable-adjusted hazard ratio (HR) for a 4g increment of intake = 0.94; 95% confidence interval (CI)= 0.89 – 1.00). Dietary intakes of ω-3 fatty acids were positively associated with low-grade prostate cancer (HR for a 0.1g increment of intake = 1.04;

95% CI = 0.99 – 1.09) and trans fatty acid intakes were positively associated with high-grade disease (HR for a 2g increment of intake of total trans fatty acids = 1.07; 95% CI = 0.96 – 1.19). In this study, estimated associations with specific fatty acids appeared to vary by stage and grade. Because the metabolism of the major polyunsaturated fatty acids is highly interrelated, more research is warranted to understand the roles that these nutrients play in prostate carcinogenesis, potentially elucidating further avenues for primary prevention and molecular targets for pharmacological intervention.

A.2. Introduction

The role of specific types of fatty acids in prostate carcinogenesis, particularly the polyunsaturated fatty acids (PUFAs), has been an active area of epidemiologic research. PUFA consumption has increased substantially in the United States over the past few decades due to a combination of increased PUFA content of animal products (resulting from changes in animal feeding practices) and increased use of cooking oils high in PUFAs (e.g. canola oil)(14). In addition, the use artificially hydrogenated trans fats (TFAs) in processed foods had increased until recent concerns about potential negative effects on a variety of health outcomes prompted reductions(15).

PUFAs include ω-3 fatty acids such as α-linolenic acid (ALA; 18:3), eicosapentaenoic acid (EPA; 20:5), docosahexaenoic acid (DHA; 22:6), and docosapentaenoic acid (DPA; 22:5) and ω-6 fatty acids such as linoleic acid (LA; 18:2) and arachidonic acid (AA; 20:4). LA is the most common PUFA in the US diet, accounting for approximately 87% of energy from PUFAs, while ALA is the most

common ω-3 fatty acid and second most common PUFA, accounting for approximately 10% of energy from PUFAs(14).

Major PUFAs act through competing pathways to modulate prostaglandin synthesis, potentially affecting inflammation-mediated carcinogenesis(19). Metabolism of the major PUFAs is highly interrelated (Figure 3). The short-chain ω-3 fatty acid ALA can be converted into the long-chain ω-3 fatty acids EPA and DHA in limited quantities(24), particularly when concentrations of EPA and DHA are low, and DPA can be formed as a metabolic intermediate between EPA and DHA(14). Metabolic enzymes involved in the ALA to EPA conversion are also involved in the conversion of the ω-6 fatty acid LA to AA. Consequently, ALA metabolism is limited both by the amounts of ω-3 fatty acids consumed and by the ratio of ω-6 to ω-3 fatty acids in the diet(24). Prostaglandins and eicosanoids formed from EPA by cycoloxygenase (COX) and lipoxygenase (LOX) enzymes have been associated with decreased growth rates in prostate cancer cell lines and animal models, while prostaglandins and eicosanoids derived from AA via the same COX and LOX enzymes have been associated with increased cell line and tumor growth rates(19). ALA has also been shown to increase prostate tumor growth in animal models through other mechanisms(41); therefore, associations between prostate cancer and ALA might differ from associations with the ALA-derived long-chain ω-3 fatty acids EPA, DPA, and DHA.

Results of studies that have investigated associations between prostate cancer and either dietary intakes or biological concentrations of PUFAs are somewhat inconsistent(49, 54-78, 121). Many(57, 65, 67-71, 73-76), but not all(49, 58, 60, 63, 66), have reported positive associations between prostate cancer and ALA and most(58,

60, 62, 66-68, 71-76, 78), but not all(70), reported null to inverse associations with DHA and EPA. However, many previous studies have important potential limitations. For example, most studies have been retrospective with regards to case status and may have been subject to differential recall of diet, post-diagnostic changes in diet among cases. In studies in which dietary intake is based on biomarker levels, changes in biological concentrations of fatty acids that may have been a consequence of disease or treatment. Furthermore, all studies of that include self-reported dietary instruments suffer from some degree of measurement error.

TFAs have been shown to increase production of cytokines(15) and increase systemic inflammation(52, 53) in clinical crossover studies, and have therefore been hypothesized to increase prostate cancer risk through inflammatory mechanisms, similar to mechanisms proposed to explain associations between TFAs and cardiovascular disease (19, 122). Limited epidemiologic data exist on relations between TFAs and prostate cancer(58, 59, 66, 79, 80) and results are somewhat conflicting, but the majority of studies have reported positive associations between prostate cancer incidence and high intakes of TFAs.

Previously, we evaluated relations between dietary intakes of ALA and prostate cancer among male participants in the screening arm of the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, and found no association between ALA intake and total prostate cancer(49). The current analysis extends this work by comprehensively examining longitudinal associations between prostate cancer, all of the major PUFAs, and TFAs. In addition, because dietary fats may differentially influence aggressive and indolent tumors(62, 71, 123-126), we analyzed high-grade tumors and combined high stage or high-grade tumors as separate endpoints, in addition to estimating associations with all incident cases combined.

A.3. Materials and Methods

A.3.a. Study Population

The PLCO Cancer Screening Trial is a multi-site (Birmingham, AL, Denver, CO, Detroit, MI, Honolulu, HI, Marshfield, WI, Minneapolis, MN, Pittsburgh, PA, Salt Lake city, UT, St. Louis, MO, and Washington, DC) clinical trial sponsored by the National Cancer Institute (NCI) that was designed to test the effectiveness of screening for prostate, lung, colorectal, and ovarian cancers, and to identify early markers and etiologic determinants of major cancers(93). Details on the study design have been published previously(92, 93). Briefly, participants were recruited from the general population through direct mailings, advertisements and other methods(93) and enrolled between November 1993 and June 2001. The screening arm of the PLCO trial included 38,350 men between the ages of 55 and 74 years who received a digital rectal exam (DRE) and prostate specific antigen (PSA) test at baseline followed by annual DREs (through year 3) and PSA tests (through year 5). Study participants provided written informed consent, and institutional review boards of the NCI and the ten participating screening centers approved the study.

At randomization, study participants completed a self-administered baseline questionnaire that asked about medical history, socio-demographic factors, and health-related behaviors (such as smoking history, personal and familial history of cancer). Usual dietary intake was assessed using a 137-item food frequency questionnaire (FFQ) with a referent period of "the past year"(127). In addition, participants were asked to complete annual update questionnaires that inquired about any cancer diagnosed by a health care provider during the prior year.

Men were excluded from the current analysis if they had a prior history of cancer other than non-melanoma skin cancer (n= 791), did not undergo an initial PSA and DRE screen (n = 2,471), underwent an initial screen but did not have subsequent contact (n = 1,458), did not complete the baseline or dietary questionnaire (n = 7,493), reported an energy intake in the top or bottom 1% of the reported energy intake distribution (roughly 5,500 and 750 kcal/day, respectively, which were values considered to be implausible) (n = 634), or completed their baseline screen after 30 September, 2002 (n = 71). After exclusions, 29,594 men remained in our final analytic cohort. These men were similar to those excluded with respect to age, level of education, smoking status, and family history of prostate cancer.

A.3.b. Dietary Assessment

At baseline, participants completed the Diet Health Questionnaire (DQX), a grid-based self-administered semi-quantitative FFQ similar to the Block and Willett FFQs. The DQX inquired about usual diet over the prior year, including frequency of consumption of 137 food items and the usual portion size for 77 items(127). Gram weights of PUFA intakes per portion size (small, medium, large) were estimated using data from two 24-hour diet recalls administered in the 1994-1996 Continuing Survey of Food Intake by Individuals (CSFII),(99) and TFA intakes were estimated using data from the Nutrition Data System for Research (NDSR; http://www.ncc.umn.edu/products/databasenutrients.html, for dietary intakes during the same period as completion of the baseline FFQs)(99, 100). The DQX was not validated in the PLCO population, but is comparable to other frequently used FFQs which have been validated in similar populations(84).

Food items relevant to PUFA intake that were specifically queried for included fried fish, tuna (including tuna salad and tuna casserole), shellfish (including shrimp, crab, and lobster), and other fish (either broiled or baked). However, the questionnaire did not inquire about specific fats used for frying, sautéing, or baking. Responses to the individual food items were converted to average daily intakes of specific fatty acids, including AA, LA, EPA, DPA, DHA, and major TFAs (trans-16:1, 18:1, and 18:2). Average daily intakes of individual fatty acids were combined to estimate the total daily intakes of ω-6 fatty acids (AA and LA), long-chain ω-3 fatty acids (EPA, DPA and DHA, referred to as 'fish fats'), total ω-3 fatty acids (ALA plus fish fats), and total TFAs (sum of trans-16:1, 18:1, and 18:2).

A.3.c. Covariate Assessment

At baseline, PLCO screening arm participants completed an extensive background questionnaire, including questions on body size (current and age 25), physical activity (past year), smoking history (lifetime), medical history (lifetime), aspirin use (past year), and family history of prostate cancer(93). Data on screening behavior throughout the active screening portion of follow-up were also available.

A.3.d. Case Ascertainment

This analysis includes follow-up data through September 30, 2002. Men with a serum PSA greater than 4 ng/ml or an abnormal DRE at a PLCO screening visit were referred to their usual medical care provider for further evaluation, and participants were asked to report prostate cancer diagnoses during the prior year on each annual update questionnaire. Medical records were obtained and abstracted for

men who had an abnormal PLCO screening exam or who reported prostate cancer on an annual update questionnaire to establish or confirm diagnoses and obtain clinical tumor stage(103) and grade data. Death certificates, autopsy data, pathology reports and supporting medical records were used to obtain diagnosis, stage and grade information for participants who were reported as deceased by next of kin or by the study center directly. The National Death Index (NDI) was used to increase completeness of case ascertainment and tumor pathology data. Only prostate cancer cases confirmed by medical record review were included in our analyses(97). In addition to estimating associations with all prostate cancers, we estimated associations separately with high- and low-grade tumors (Gleason sum ≥ 7 and <7, respectively) and combined high-stage or high-grade (Stage ≥ T3b, N1, or M1 or Gleason sum ≥ 7) tumors as separate endpoints. Due to the ongoing nature of the screening trial, data are not currently available on prostate cancer mortality.

A.3.e. Statistical Analysis

Person-time for each participant accrued from the time of randomization into the screening arm until the date of his last questionnaire return, date of prostate cancer diagnosis, date of death, or the end date of the study period (30 September 2002), whichever occurred first. Age-adjusted and multivariable-adjusted relative risks (hazard ratios, HRs) and 95% confidence intervals (95% CIs) were estimated using Cox proportional hazards regression (Stata/MP 10.0, StataCorp, College Station, TX) with age as the underlying time metric(95, 96). We adjusted nutrient values for total energy intake using the residual method(82) and categorized adjusted nutrient intakes into five levels that were comparably spaced along the range of intakes for each nutrient. Additionally, we adjusted for total energy intake by modeling raw

nutrient intakes and energy intakes simultaneously, and by modeling the ratio of the specific nutrient to total energy intake (i.e. using the nutrient density method). We used directed acyclic graphs to identify confounders(111), including covariates associated with prostate cancer and fatty acid intakes in our study population, and confounders identified from previous reports. Standard multivariable-adjusted (MV) models included study center (nine indicator variables), race (white, African American, Asian/Pacific Islander, other), and baseline data on family history of prostate cancer (yes, no), diabetes (yes, no), smoking history (never, current, former, pipe/cigar), body mass index (BMI; categorized as < 20, 20 - < 25, 25 - < 30, ≥ 30 kg/m^2), hours of vigorous physical activity (0, 1, 2, 3, ≥ 4 h/week), aspirin use (never, < 2 tablets/week, ≥ 2 tablets/week), vitamin E supplement use (0, 1 – 30, 31 – 400, > 400 IU/day), total energy intake (5 categories), lycopene intake (5 categories) and the total number of screens since baseline (as a time varying categorical variable). Education (as a proxy for socioeconomic status) and alcohol intake (potentially related to inflammation) were also confounders based on our analysis of directed acyclic graphs, but were excluded from final models because they had a negligible impact on effect estimates. Missing data for BMI (~1% of subjects) were imputed using the multiple imputation by chained equations method(128-130) implemented by the 'ICE' procedure in Stata(131-133). Additionally, we simultaneously modeled the major PUFAs (AA, LA, ALA, and Fish fats) in a separate multivariable-adjusted model. Ratios of PUFA intakes were estimated by dividing residually adjusted nutrients by each other (e.g. LA:ALA ratio = energy-adjusted LA intake/energy-adjusted ALA intake) and categorized into five levels that were comparably spaced along the range of observed values for each ratio. We modeled the main exposure as a continuous variable and estimated the hazard ratio

for an increment of change of exposure equal to the inter-quartile range (rounded to one significant figure) for that covariate to estimate the linear trend. Our overall interpretation was based on both the categorical and continuous effect estimates, but less weight was given to continuous estimates when categorical estimates were inconsistent with a linear trend.

We evaluated potential effect measure modification by race, BMI, total energy intake, aspirin use, alcohol use, education, and family history of prostate cancer, covariates which may either modulate inflammatory pathways (e.g. alcohol intake) or metabolism of fatty acids (e.g. modulation of prostaglandin synthesis by inhibition of the COX pathways by aspirin intake). Multiplicative interaction (HR modification) was assessed by evaluating the magnitude of the ratios of HRs across strata of the modifier (via exponentiation of interaction terms)(134) and departures from additive hazards were assessed using the interaction contrast ratio (ICR)(116, 118).

A.4. Results

During the follow-up period we ascertained 1,914 incident cases of prostate cancer in our population of 29,594 men, including 698 cases that were Gleason sum 7 or higher (37% of all cases). Polyunsaturated fatty acids contributed approximately 6.5% of total energy intake and 20% of energy intake from fat in the PLCO population (**Table 15**), consistent with estimates of PUFA intake in the US(14). Ratios of ω-6 to ω-3 fatty acids, LA to ALA, and LA to fish fats (EPA, DPA and DHA combined) were also consistent with expectations. Energy-adjusted intakes of the long-chain ω-3 fatty acids (EPA, DPA, and DHA) were correlated (Pearson correlation coefficients ranging from 0.92 – 0.99) as were intakes of EPA and LA, but

correlations between other PUFAs were relatively weak. Pearson correlation coefficients for individual TFAs ranged from 0.34 – 0.93 (data not shown).

Background characteristics of the study cohort are described in **Table 16**. PLCO participants were 63 years old on average at baseline (range 55 – 70 years) and were overwhelmingly white (~91%). Data were nearly complete for all covariates. Baseline BMI, the covariate most likely to be missing, was available for more than 99% of observations. Estimates from complete case models (data not shown) were similar to those from models with imputed BMI.

There was an inverse association between total prostate cancer and LA intake for all categories of intake above the reference (MV-adjusted HR for a 4g increment of intake = 0.94; 95% CI = 0.89 – 1.00) (Table 17), but no clear associations between total prostate cancer and intakes of other individual PUFAs or ratios of PUFAs. Trans fatty acid intakes also were not associated with total prostate cancer.

Low grade prostate cancer (Gleason sum < 7, **Table 18**) was inversely associated with LA intake (MV-adjusted HR for a 4g increment of intake = 0.89; 95%CI = 0.82 – 0.96), ALA intake (MV-adjusted HR for a 0.3g increment of consumption = 0.97; 95% CI = 0.91 – 1.04), ω-6:ω-3 and LA:ALA ratios (MV-adjusted HR for a 5 unit increment of the ratio of LA:ALA = 0.83; 95% CI = 0.73 – 0.94), and intakes of individual TFA (MV-adjusted HR for a 2g increment of consumption = 0.93; 95% CI 0.86 – 1.01). Low-grade prostate cancer was positively associated with long-chain ω-3 fatty acid intake ("fish fats") across all categories of consumption above the reference (MV-adjusted HR for a 0.1g increment of consumption = 1.04; 95% CI = 0.99 – 1.09).

High-grade prostate cancer (Gleason sum ≥ 7, **Table 19**) was not clearly associated with intakes of individual PUFAs but was positively associated with the LA:ALA ratio for all categories above the reference (e.g., MV-adjusted HR comparing C5 to C1 = 1.20; 95% CI = 0.75 – 1.90); however, confidence limits were wide for all high-grade HR estimates relative to estimates for total and low grade prostate cancer. High-grade prostate cancer was positively associated with total and individual TFA intakes (MV-adjusted HR for a 0.04g increment of consumption of TFA 16:1 = 1.10; 95%CI = 1.01 – 1.20).

We confirmed that the proportional hazards assumption was met for all models using the method of Grambsch and Therneau(115), which tests departure from linearity of Schoenfeld residuals. The magnitude and precision of our multivariable-adjusted associations did not differ substantially (changes in estimates of HRs and CIs ≈ ± 0.02) from age-adjusted estimates, and effect estimates shown for individual PUFAs were comparable to estimates from models that included the major PUFAs simultaneously. Results for intakes that were energy adjusted using the residual method were comparable to estimates that were directly adjusted for total energy intake, or adjusted using the nutrient density method (data not shown). Results were also comparable to those shown when we restricted the analysis to cases diagnosed after one year of follow-up. Sub-group analyses based on tumor stage or tumor stage and grade together did not differ appreciably from those for case-subtypes defined by grade only. Observed estimates from models that included interaction terms were comparable to those expected for both additive and multiplicative risks for joint exposures to PUFAs and race, family history of prostate cancer, BMI, total energy intake, alcohol intake, education, and aspirin use as potential effect measure modifiers.

A.5. Discussion

The results of our prospective study suggest that consumption of LA may be inversely associated with prostate cancer, particularly low-grade disease. Ratios of ω-6: ω-3 fatty acids were also inversely associated with low-grade disease, and to a lesser extent with total prostate cancer. Intakes of ω-3 fatty acids did not appear to be associated with total or high-grade prostate cancer, but had small positive associations with low-grade prostate cancer. Of the PUFAs examined, only TFAs appeared to be associated with high-grade disease.

We did not find an association between AA and total prostate cancer, consistent with the Health Professionals Follow-up Study(HPFS)(72) and a case-control study in Australia(66), but not with the findings of the Netherlands Cohort Study(58) or a nested case-control study of blood bank donors(68) that reported slight positive associations. We also found no association between AA and high-grade prostate cancer, in contrast with a case-control study nested within the Physicians Health Study (PHS)(75) which reported that whole blood AA concentrations were associated with aggressive prostate cancer but not non-aggressive cases.

Our finding of a possible inverse association between LA and total and low-grade prostate cancer but not high-grade prostate cancer is consistent with some published studies, but not others. For example, Giovannucci, et al.(71) found a stronger inverse association between dietary intake of LA and advanced prostate cancer than with total prostate cancer in an analysis of the HPFS, whereas Leitzmann, et al.(72) reported no association between LA intake and total prostate

cancer but an inverse association with advanced disease in a subsequent analysis of the HPFS with longer follow-up(72).

Our findings for ALA intake, which suggest no association with total or high-grade prostate cancer but an inverse association with low-grade disease, are comparable to one prospective study (70) and four case-control studies (135-138) among Caucasian men that observed no relation between dietary or tissue ALA and total or advanced prostate cancer, and are consistent with our earlier analysis of ALA intake in PLCO(49). However, other case-control and cohort studies have reported inverse (58, 60, 66) or positive(57, 69, 76) associations between ALA intake and prostate cancer. In addition, three(67, 68, 77) of four case-control studies(67, 68, 70, 74) that investigated serum concentrations of ALA and prostate cancer found positive associations, while Mannisto, et al.(70) reported no association. In their analysis of PHS data, Chavarro, et al. reported strong positive associations between blood concentrations of ALA and non-aggressive prostate cancers (low stage or low stage and grade) and positive associations with low and high-grade tumors(75).

Our findings for long-chain ω-3 fatty acid intakes (i.e. the "fish fats" EPA, DPA, and DHA), which suggest no associations with total or high-grade prostate cancer but a weak positive association with low-grade disease, are largely compatible with the prior literature which has suggests that EPA and DHA are not associated with prostate cancer(58, 60, 62, 71). A more recent analysis of HPFS data(71), suggested that DHA and EPA intake may be inversely associated with prostate cancer, particularly with advanced cases (high stage or fatal), while an analysis of data from the Alpha-Tocopherol, Beta-Carotene Trial(70) suggested that EPA and DHA intakes may be associated with elevated risk of prostate cancer. Studies of serum and adipose tissue concentrations of long-chain ω-3 fatty acids

generally support inverse associations with prostate cancer(67, 68, 70, 73-75, 78). This apparent discrepancy between studies of dietary intakes and biological concentrations of fish fats may partially be the result of metabolic interconversion of ALA to long-chain ω-3 fatty acids since serum concentrations of DHA and EPA are determined both by dietary consumption and their formation via ALA metabolism, which may, in turn, be influenced by consumption and metabolism of other PUFAs(19).

Our results for ratios of ω-6 to ω-3 PUFAs, which suggest inverse associations with total and low-grade prostate cancer but no associations with high-grade disease, are similar to results from two nested-case control studies(68, 75) that found modest inverse associations between prostate cancer and ratios of serum concentrations of major PUFAs, and with an analysis of the Multiethnic Cohort (MEC)(60), which, like the current analysis, found no association between ratios of PUFAs and advanced prostate cancer. However the HPFS and the MEC found no association between these ratios and total prostate cancer, while a Swedish case-control study(76) reported positive associations with total prostate cancer. Our finding of inverse associations between the LA:ALA ratio specifically and total or low-grade but not high-grade prostate cancer also differ from for an analysis of the HPFS(72) which found no association with total prostate cancer but an inverse association with advanced prostate cancer.

Intakes of total or individual TFAs were not associated with total prostate cancer overall, but were positively associated with high-grade disease. A previous case-control study(58) also found no association between TFA intakes and total prostate cancer, while another case-control study(79) reported positive associations

with advanced (high stage, high-grade, or high total PSA) prostate cancer. Another case-control study(66) found a modest positive association between total prostate cancer and intakes of trans-16:1, but not trans-18:1 and trans-18:2. Neuhouser, et al.(59) found that total TFA intake was positively associated with prostate cancer among men with a family history of prostate cancer, but not men without a family history.

Potential causal mechanisms for associations between trans fatty acid (TFA) intake and prostate cancer are not clear, but TFAs are hypothesized to increase inflammation-mediated carcinogenesis(59, 66, 79). A number of studies, including some randomized trials, have found that high TFA consumption is associated with elevated levels of markers of inflammation such as C-reactive protein(15, 52). In a randomized crossover study, Baer, et al. found that increased consumption of TFAs relative to unsaturated fatty acids increased inflammatory markers, particularly C-reactive protein(52). A recent study of heart patients found that serum concentrations of trans-fatty acids were associated with increased systemic inflammation(53). Therefore, TFAs may increase advanced prostate cancer risk through a systemic inflammatory response.

Our study had a number of strengths. We used data from a large, well-designed prospective study to comprehensively estimate associations between prostate cancer and intakes of major PUFAs and TFAs. PUFA intakes (as a fraction of total energy intake and energy from fat) among men in our study were similar to estimated intakes for men in the United States as a whole. The entire PLCO study benefits from the fact that the study population has undergone standardized screening examinations for prostate cancer, which will reduce the effect of detection bias on our analyses relative to observational population-based studies.

Furthermore, adjudicated data were available on both prostate cancer stage and grade. The study population enrolled men from sites across the United States.

There are some limitations to our analysis of the PLCO population. There is published evidence of a "healthy volunteer effect" in the PLCO population(139), but this limitation is shared by most epidemiologic studies that require active participation. In addition, the proportion of minority participants was low, potentially limiting our ability to generalize our findings to the US as a whole. We also know that in this study population, family history of prostate cancer was reported less often than expected (0.52 times the expected rate based on SEER data), and that African Americans reported family history of cancer less frequently than whites(140), potentially limiting our ability to control for this potentially important covariate as either a confounder or effect measure modifier. Diet was only assessed for the year prior to enrollment, which was fairly late in life; although adult diets are fairly stable, it is possible that estimated nutrient intakes did not represent those during the etiologically relevant time period for this slow-developing disease(82). Estimated PUFA intakes do not account for supplement use, and data on fish consumption and cooking oil use were limited, which may have affected our estimates of long-chain ω-3 fatty acid intakes. We do know, however, that the databases(99, 100) used to estimate nutrient intakes were generated at a comparable time period to enrollment in the PLCO Cancer Screening Trial. Therefore our estimates of nutrient intakes for specific food items fairly accurately represented the nutrient contents at baseline. Because of the relatively late age at entry into the study (~63 years at baseline), we may have missed early onset forms of prostate cancer that may be more aggressive or clinically relevant. In theory, controlling for prostate cancer screening behavior, which may be a proxy measure of information

quality, may induce information bias; however, the magnitude of any such bias would have been negligible given the minimal effect of adjustment on our effect measure estimates. Finally, we did not have data on prostate cancer mortality, but did estimate associations with high-grade prostate cancer, which is strongly associated with disease progression, recurrence, and mortality(141).

It may clarify the public health relevance of our results to place them in the context of the absolute risk of prostate cancer. Recent SEER data indicate that the five-year probability of developing prostate cancer for 60 year-old men is approximately 3%(3, 4). Therefore, a hazard ratio of 1.10 for prostate cancer in association with high versus low consumption of fish fats suggests an increase in the absolute risk of prostate cancer from 3% to 3.3%, a risk difference of only 0.3%. This risk difference suggests that it would be necessary to switch 333 men from the highest category of intake of fish fats to the lowest category to reduce the number of cases of prostate cancer per 100,000 men by one.

In summary, we found evidence of a weak inverse association between total prostate cancer and dietary intake of LA among men enrolled in the screening arm of the PLCO Cancer Screening Trial, and positive associations between dietary intakes of ω-3 fatty acids and low-grade prostate cancer. Positive associations between TFAs and high-grade prostate cancer may have greater public health significance than associations between PUFAs and total or low-grade prostate cancer since grade is strongly associated with mortality. Because of the highly interrelated nature of the major PUFAs, more research exploring associations between the total set of major PUFAs and aggressive or fatal prostate cancer are warranted.

A.6. Acknowledgements

The authors would like to thank Amy Subar of the NCI for her comments and suggestions on this manuscript.

B. Paper 2: Analysis among male participants in the National Institutes of Health-AARP Diet and Health Study

B.1. Abstract

We recently reported on associations between intakes of polyunsaturated (PUFAs) and trans-fatty acids (TFAs) and prostate cancer among male participants in the screening arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Due to limitations in dietary assessment in that population along with the lack of data on prostate cancer mortality, an outcome of potentially greater public health significance, we analyzed similar relations among male participants in the National Institutes of Health-AARP Diet and Health Study, a cohort of 288,956 men residing in one of six states or two metropolitan areas in the United States in 1996 and followed-up for prostate cancer incidence through the end of 2003. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (95% CIs). During the follow-up period, we identified 17,095 cases of prostate cancer, 1,891 of which were advanced tumors and through the end of 2005 we identified 427 fatal cases of prostate cancer. We found that intakes of long-chain ω-3 fatty acids were positively associated with total prostate cancer (MV-adjusted HR comparing C5 to C1 = 1.07; 95% CI = 1.02 – 1.12) and inversely associated with fatal tumors (MV-adjusted HR for a 0.1g increment of intake = 0.87; 95% CI = 0.78 – 0.98). Total TFA intake was inversely associated with high-stage

disease (MV-adjusted HR for a 2g increment of intake total TFA = 0.95; 95% CI = 0.89 – 1.02) and TFA 16:1 intake was positively associated with fatal disease (MV-adjusted HR for a 0.04g increment of intake = 1.07; 95% CI = 0.97 – 1.18). Because of limitations in our study, more research may be warranted to understand associations between these highly interrelated nutrients and prostate cancer, potentially identifying further targets for pharmacological intervention and targets for primary prevention.

B.2. Introduction

We recently reported our findings on associations between dietary intakes of polyunsaturated fatty acids (PUFAs) and trans-fatty acids (TFAs) and prostate cancer among male participants in the screening arm of the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial(142). Briefly, we found an inverse association between total and low-grade prostate cancer and dietary intake of linoleic acid (LA), the most common ω-6 fatty acid in the U.S. diet(14), and positive associations between high-grade (Gleason sum ≥ 7) prostate cancer and dietary intakes of TFAs. Here we report the results of a similar study we conducted among participants in the NIH-AARP Diet and Health Study(94).

The major classes of PUFAs are ω-3 fatty acids, including α-linolenic acid (ALA; 18:3), eicosapentaenoic acid (EPA; 20:5), docosahexaenoic acid (DHA; 22:6), and docosapentaenoic acid (DPA; 22:5), and ω-6 fatty acids, including linoleic acid (LA; 18:2) and arachidonic acid (AA; 20:4). LA accounts for approximately 87% of energy from PUFAs, while ALA accounts for approximately 10% of energy from PUFAs(14).

Metabolism of the major PUFAs is highly interrelated. For example, the short-chain ω-3 fatty acid ALA can be converted into the long-chain ω-3 fatty acids EPA, DPA, and DHA in limited quantities(24), particularly when dietary intakes of EPA and DHA are low(14). Metabolic enzymes that help convert ALA to EPA are also involved in the conversion from the ω-6 fatty acid LA to AA. Consequently, ALA metabolism is limited both by the absolute dietary intakes of ω-3 fatty acids and by the relative dietary intakes of ω-6 to ω-3 fatty acids (i.e. the ratio of ω-6 to ω-3 fatty acids)(24). PUFAs, whether consumed in the diet or formed through metabolic conversion, act through competing pathways to modulate prostaglandin synthesis, which may be relevant to inflammation-mediated carcinogenesis(19). Prostaglandins and eicosanoids formed from EPA by the enzymatic action of cycoloxygenases (COX) and lipoxygenases (LOX) have been associated with decreased prostate tumor growth in human prostate cancer cell lines and animal models, while prostaglandins and eicosanoids derived from AA via the same COX and LOX enzymes have been associated with increased prostate tumor growth(19). In addition, ALA has been shown to increase prostate tumor growth in animal models through mechanisms that are independent of the metabolism of ALA to long chain ω-3 fatty acids, and the subsequent production of EPA-derived eicosanoids(41). Therefore, associations between prostate cancer and dietary ALA may be independent of associations with dietary intakes of the long-chain ω-3 fatty acids EPA, DPA, and DHA.

Results of studies that have investigated associations between prostate cancer and either dietary intakes or biological concentrations of PUFAs have yielded inconsistent findings(54-78, 121, 142), but many studies have found positive

associations between prostate cancer and ALA(57, 67-69, 76, 77) and null to inverse associations with DHA and EPA(67, 68, 70, 71, 73-75, 78).

Trans-fatty acids, which are artificially formed through the partial hydrogenation of vegetable oils, have been hypothesized to increase prostate cancer risk through inflammatory mechanisms, similar to those hypothesized to explain relations with cardiovascular disease(19, 122). TFAs have been shown in clinical crossover studies to increase production of cytokines(15) and to increase systemic inflammation(52, 53). Limited epidemiologic data exist on associations between TFAs and prostate cancer(58, 59, 66, 79, 80, 142) and although the results are somewhat conflicting, the majority of studies have reported that high TFA consumption is positively associated with prostate cancer. We noted a positive association between TFA intakes and high-grade prostate cancer, but not with incident prostate cancer, in our previous analysis of data from the screening arm of the PLCO Cancer Screening Trial(142).

The goal of the current study was to investigate relations between dietary intakes of PUFAs and TFAs and incident prostate cancer, prostate cancer with extraprostatic extension at diagnosis, and fatal prostate cancer in the NIH-AARP Diet and Health Study cohort (94). The NIH-AARP Diet and Health Study offered some additional advantages; namely the ability to investigate relations with fatal prostate cancer and potentially greater ability to ascertain PUFA intake due to the fact that cooking oil (a substantial source of PUFAs in the diet) use, was inquired about. Outcome subtypes and data collection instruments differ somewhat from those used in our study of dietary PUFAs and TFAs in association with total and high-grade prostate cancer in PLCO Cancer Screening Trial participants, but

comparable analytic approaches were used to facilitate comparisons between the two study cohorts.

B.3. Materials and Methods

B.3.a. Study Population

The NIH-AARP Diet and Health Study is a prospective cohort study designed to investigate relations between diet and a variety of health outcomes(94). Between 1995 and 1996, 3.5 million current AARP members, aged 50 – 71 who resided in one of six US states (CA, FL, PA, NC, NJ, and LA) or one of two metropolitan areas (Atlanta, GA and Detroit, MI) were invited to participate in the study. Over 500,000 individuals returned the initial mailed questionnaire, including 340,148 men. A supplementary risk factor questionnaire (RFQ) was mailed to these respondents during the latter half of 1996.

Men were excluded from the current analysis if they submitted more than one baseline questionnaire (n = 103, died or moved out of the study area prior to baseline (n = 373), chose to withdraw from the study (n = 1), had a questionnaire completed by a proxy (n = 14,495), or had been previously diagnosed with a cancer other than non-melanoma skin cancer (n = 27,269). Men were also excluded if they reported extreme values for total energy consumption (greater than twice the interquartile range for the box-cox transformed energy intake (< 415 and > 6,144 kcal/day), n = 2,509), height (< 1.22 m and > 2.41 m) (n = 1,456), or weight (< 0.90 kg and > 450 kg)(n= 402) or if their derived body mass index (BMI; kg/m^2 based on self-reported weight and height) was implausible (< 0.28 and > 151) (n = 174). After exclusions, 288,956 men remained in the final analytic cohort, including 178,705 (62%) men for whom data were available from the RFQ. Men were followed-up

from completion of the baseline questionnaire until the date of a prostate or other malignant cancer diagnosis (other than a non-melanoma skin cancer), death from any cause, or the end of the study period.

The Special Studies Institutional Review Board of the U.S. National Cancer Institute approved the NIH-AARP Diet and Health Study. Current analyses were exempted from institutional review board review at the University of North Carolina.

B.3.b. Dietary Assessment

At baseline, participants completed a grid-based version of the Diet History Questionnaire (DHQ; http://riskfactor.cancer.gov/DHQ/)(94). The DHQ queried participants on the frequency of consumption and typical portion size of 124 food items during the prior 12 months. The DHQ also included an additional 21 questions on consumption of low-fat foods and food preparation practices, including types of oils and fats used in cooking, which are common sources of PUFAs in the diet(94). The DHQ inquired about fish consumption, including consumption of tuna (including tuna salad and casserole), fried fish, and other types of seafood that are important sources of PUFAs. Data were not available on PUFA supplement use, including flax seed or fish-oil supplements.

A separate calibration sub-study based on 24-hour recalls was conducted among 2,053 participants in the NIH-AARP cohort(104) including 1,986 who completed two separate 24-hour dietary recalls (approximately one-month apart) and 1,415 who completed and returned a second DHQ in late 1996. PUFA intakes were correlated with 24-hour dietary recalls, particularly after nutrients were

adjusted for energy intake (r = 0.47 when not adjusted for energy intake and 0.53 when adjusted) (143).

Gram weights per portion size (small, medium, large) for a given food item were estimated using data from two 24-hour diet recalls administered in the 1994-1996 Continuing Survey of Food Intake by Individuals (CSFII)(99). For TFA intakes, gram weights were estimated using data from the Nutrition Data System for Research (NDSR; http://www.ncc.umn.edu/products/databasenutrients.html) for the same time period, which overlapped with baseline data collection for the cohort(99, 100). Average daily nutrient intakes of specific fatty acids (including ALA, LA, DHA, DPA, EPA, and AA) and specific TFAs (trans- 16:1, 18:1, and 18:2) were estimated by multiplying the average daily consumption of each food item by its estimated nutrient content and summing the average nutrient intakes from each food item.

B.3.c. Covariate Assessment

The baseline NIH-AARP study questionnaire included questions on body size (current), physical activity (past year), family history of cancer (ever), and smoking history (lifetime). The supplementary RFQ included questions on cancer screening (including both digital rectal exam, DRE, and prostate specific antigen, PSA, testing, past five years) and family medical history (lifetime).

B.3.d. Case Ascertainment

Identification of incident prostate cancer cases in the NIH-AARP cohort has been conducted through passive follow-up(105, 106) via linkage of the NIH-AARP cohort database with state cancer registries in the participating states(107). Cancers other

than prostate cancer were also ascertained since men were censored at the time of any malignant cancer diagnosis (other than non-melanoma skin cancer). Furthermore, the cohort was linked to the NDI to ascertain date and specific cause of death, identify fatal prostate cancer cases and determine a censoring date for men who died of other causes. Clinical or pathological tumor stage(103) were obtained from cancer registry data.

Cancer registries in the eight NIH-AARP states have been certified by the North American Association of Central Cancer Registries for meeting the highest standard data quality and are estimated to ascertain 95% of cancer cases within two years of cancer diagnosis. Based on medical record confirmation of self-reported cancer incidence in a validation sub-study of 12,000 cohort members, approximately 90% of all incident cancers were correctly identified using the registry-based approach(105). Failure to ascertain all cancers may be the result of incomplete registry linkage, delayed reporting of cases to the registries, and movement of participants outside of the areas covered by the study area cancer registries. Changes in residence were monitored by linkage of the NIH-AARP cohort database to the US Postal Service's National Change of Address (NCOA) database(105), and additional information was obtained through direct reporting by study participants in follow-up questionnaires, and through data received through US Postal Service processing of undeliverable mail(105). Within three years of follow-up, 98% of NIH-AARP cohort members either remained at the same address or relocated within one of the eight NIH-AARP study states(107), and it has been estimated that over 95% of the cohort met these same criteria over the first five years of follow-up. Therefore, the overwhelming majority of participants remained in follow-up for ascertainment of prostate cancer. Furthermore, linkage was recently extended to include cancer

registries in Arizona, Texas, and Nevada, where NIH-AARP study participants were most likely to have relocated to when moving out of the study area, increasing the completeness of case ascertainment.

B.3.e. Statistical Analysis

We used Cox proportional hazards models(95, 110) to estimate age-adjusted and multivariable-adjusted hazard ratios (HRs) and 95% confidence intervals (95% CIs) using STATA 10.0 (STATACorp, College Station, TX). Person-time of follow-up for incident prostate cancer analyses accrued from the date of return of the baseline questionnaire until the date of prostate cancer diagnosis, date of any other malignant cancer diagnosis, date of death from any cause, or the end of the study period (31 December, 2003). Person-time of follow-up for fatal prostate cancer analyses accrued from the date of return of the baseline questionnaire until the date of death or the end of the study period (31 December, 2005). For subanalyses among participants who completed the supplementary RFQ, person-time accrued from the date of return of the RFQ.

We adjusted for total energy intake, using the residual method(82). We log-transformed the nutrient intake for each individual. We then regressed the log-transformed nutrient on log-transformed energy intake (simple linear regression), and calculated the residual between each individual's actual nutrient intake and the predicted nutrient intake given his energy intake. In order to place the log-transformed adjusted nutrient intake into a more readily interpretable scale, we added the residual nutrient intake to the estimated nutrient intake at the mean energy intake of the study population and then exponentiated the resulting values to generate the energy-adjusted nutrient intakes.

We considered both continuous and categorical classifications of nutrient exposures, including dietary intakes of ALA, LA, AA, EPA, DHA, and the sum of EPA and DHA. In addition to estimating effects of individual fatty acid intakes, we separately estimated effects of ratios of -6 to -3 fatty acid intakes, including the ratios of AA+LA:ALA+DHA+EPA, LA:ALA, and LA:EPA+DHA+DPA, as categorical and continuous exposures. Ratios of intakes of PUFAs were estimated by dividing the residually adjusted nutrients by each other (e.g. ratio LA:ALA = energy-adjusted LA intake/energy-adjusted ALA intake) and ratios were categorized based on distributions in the total study population. Furthermore, we considered the intakes of major classes of trans-fatty acids (TFAs; trans-16:1, trans-18:1, and trans-18:2) separately, as well as total TFA intake.

To maintain consistency with our prior analysis in the PLCO cohort, we used the same cutpoints for categorizing energy-adjusted nutrient intakes(142). Briefly, we generated five separate evenly spaced categories (C1 – C5) by creating cutpoints near the bottom and top 10-15% of the PLCO population and divided the range of exposures between those two points into three groups that were evenly spaced along the range of intakes.

We estimated linear trends by modeling the main exposure as a continuous variable and estimating the hazard ratio for an increment of intake equal to the inter-quartile range (rounded to one significant figure) for that nutrient (or nutrient ratio) within the PLCO population. We interpreted categorical and continuous effect estimates together, when categorical estimates were consistent with a linear trend. Where the two results appeared to be contradictory, we further explored the associations by using both flexible spline models and by subsetting the data into smaller categories to determine whether the continuous linear trend estimates

appeared to be a useful summary of the underlying dose-response relation, or should be discounted relative to the categorical estimates when interpreting the overall results.

Confounders were identified based on analysis of directed acyclic graphs (DAGs)(111), which included covariates reported or hypothesized to be associated with prostate cancer in the prior literature. Specifically, we evaluated age (implicit in the model), BMI, physical activity (number of times per week participating in vigorous physical activity), race (white/black/other), smoking history (never/former/current), education (<12 years/high school graduate/some college/at least a college degree), family history of prostate cancer (yes/no), history of diabetes (yes/no), total energy intake (kcal/day), red meat intake (g/day), lycopene intake (μg/day), vitamin E intake (mg/day),calcium intake (mg/day), and state of residence. Missing data for BMI and race were imputed using the multiple imputation by chained equations method(128-130) implemented by the 'ICE' procedure in Stata(131-133). Confounders identified in or analysis of DAGs that did not alter the effect estimates substantially (e.g. differences in HRs of ± 0.02) and were not controlled for in our prior analysis of the PLCO cohort, such as education, were not included in our final multivariable-adjusted models. Furthermore, we conducted subanalyses among participants who completed the supplementary RFQ to additionally control for a history of at least one DRE in the past three years (yes/no) and regular NSAID (aspirin and/or ibuprofen) use (yes/no).

We considered separate endpoints of incident prostate cancer cases, fatal cases (deaths with prostate cancer listed as the underlying cause, including cases identified through registry data that subsequently died from prostate cancer after

the incident cancer study period, between 2003 and 2005, localized or organ-confined cases (Stage T1a – T2b and N0M0), and men with advanced or extraprostatic disease (Stage T3 or T4, N1 or M0). Fatal cases of prostate cancer that occurred during the incident prostate cancer analysis period, were included as incident cases and as advanced tumors.

We evaluated potential effect measure modification by race, body mass index, total energy intake, aspirin use, prostate cancer screening (DRE and PSA) history, and family history of prostate cancer. Multiplicative interaction (HR modification) was assessed by evaluating interaction terms and departures from additive hazards were assessed using the interaction contrast ratio (ICR)(116, 118).

We tested whether the proportional hazards assumption was met for all models using the method of Grambsch and Therneau(115), which tests departure from linearity of Schoenfeld residuals. Sensitivity analyses were conducted to place bounds on the potential effect of bias on our estimates.

B.4. Results

There were 17,095 incident cases of prostate cancer over approximately 2,000,000 person-years of follow-up, of which 1,891 were advanced tumors (T3 or T4, N1 or M1) and 427 were fatal cases of prostate cancer over approximately 2,600,000 person-years of follow-up. Polyunsaturated fatty acids contributed 22.6% of energy from fats and 7.5% of total energy intake in the NIH-AARP study population (**Table 20**), consistent with estimated intakes in the PLCO population and with national estimates for the United States(14, 142). As expected, energy-adjusted intakes of individual long-chain ω-3 fatty acids (EPA, DPA, and DHA; "marine fatty acids")

were positively correlated with each other (Pearson correlation coefficients ranging from 0.81 – 0.94)(**Table 20**).

Baseline characteristics of male participants in the NIH-AARP cohort were fairly similar to those of male participants in the screening arm of the PLCO Cancer Screening Trial (**Table 21**). Participants were overwhelmingly white (~94%) with a mean age of 62 years at baseline. Participants in this cohort reported lower average total energy intakes, and correspondingly lower intakes of specific nutrients compared with the PLCO study cohort. This is not unexpected, as the NIH-AARP DHQ included fewer food items than the PLCO FFQ. NIH-AARP study participants were also more likely than PLCO participants to report a positive family history of prostate cancer (13.5 vs. 8.0%) and were less likely to report a history of diabetes (7.3 vs. 8.5%).

Subjects who completed the supplemental RFQ did not differ substantially from subjects who did not with respect to many of the covariates under investigation. Adjusting for screening history and NSAID use, which were only ascertained for participants who had completed the RFQ, had little impact on effect estimates within this subset of cohort participants; therefore, final models did not include adjustment for these factors.

We found no clear associations between dietary intakes of AA, LA, and ALA and incident prostate cancer (**Table 22**). We noted evidence of positive associations between dietary intakes of individual and combined long-chain ω-3 fatty acids ("marine fatty acids") and incident prostate cancer for intake categories above the reference level. However, continuous (linear) trend estimates indicated a flat dose-response with increasing intakes, and further categorical analyses with marine fatty acid consumption divided into smaller categories and modeling exposures using

flexible splines confirmed that linear trend estimates were driven by a small number of non-case observations at extreme levels of intake. We found no associations between ratios of PUFAs and incident prostate cancer. However, we found inverse linear trends for the ratios of ω-6: ω-3 and LA:ALA. Flexible spline analysis further confirmed that an inverse continuous trend estimate for the ratio of LA:ALA was driven by high leverage data points at extreme values of the ratios and by the large increments used for the estimates based on the interquartile range for this ratio in the PLCO population. Furthermore, we found no associations between intake of total TFA, or individual TFAs, and incident prostate cancer.

AA intake was positively associated with advanced prostate cancer (MV-adjusted HR for a 0.05g increment of intake = 1.04; 95% CI = 0.99 – 1.10) (**Table 23**). LA was inversely associated with advanced cancer for all categories of intake (MV-adjusted HR comparing C5 to C1 = 0.91; 95% CI = 0.77 – 1.08); however, there was a flat linear dose-response between LA intake and advanced disease. Further analysis, in which LA intake was divided into smaller categories and using flexible splines suggests that linear trend estimates were biased by values at extreme levels of LA intake. EPA intake was inversely associated with advanced cancer (MV-adjusted HR for 0.03g of intake = 0.97; 95% CI = 0.94 – 1.02) and that DHA intake was positively associated at all levels of intake relative to the lowest level of intake with advanced cancer. Again our finding of no linear-dose response for this exposure was apparently driven by estimates at extremely high levels of DHA intake. Similarly, we found that total marine fatty acid consumption was positively associated with advanced prostate cancer. Ratios of ω-6:ω-3 fatty acids and LA:ALA were positively associated with advanced prostate cancer at most levels of intake

relative to the lowest level of these ratios, although we found inverse linear dose-responses (MV-adjusted HR for a 2-unit change in the ratio of ω-6:ω-3 fatty acids = 0.95; 95% CI = 0.91 – 1.00); MV-adjusted HR for a 5-unit change in the ratio of LA:ALA = 0.95; 95% CI = 0.88 – 1.02). Further exploration of these associations using flexible splines and further subsetting of the categories suggest that these inverse trends are driven by the combination of strong associations in the second category of the ratios and inverse associations at very high levels of these ratios. We found no association between the ratio of LA:marine fatty acids and advanced prostate cancer. Total TFA intake was inversely associated with advanced prostate cancer (MV-adjusted HR for a 2g increment of intake = 0.95; 95% CI = 0.89 – 1.02), an association that appeared to be driven by intakes of TFA 18:1 and 18:2; in contrast, TFA 16:1 appeared to be positively associated with advanced prostate cancer.

Fatal prostate cancer was inversely associated with LA intake, although the slope of the continuous was relatively flat (**Table 24**). Similar to our analysis of advanced disease, high leverage data points contributed to apparently discrepant estimates for linear trends. Total and individual intakes of marine fatty acids were inversely associated with fatal prostate cancer (MV-adjusted HR for a 0.1g increment of intake of total marine fatty acids = 0.87; 95% CI = 0.78 – 0.98). The ratio of LA:ALA was inversely associated with fatal prostate cancer, but the ratio of LA:marine fatty acids was positively associated with fatal cancer. Fatal prostate cancer was also positively associated with TFA 16:1 (MV-adjusted HR for a 0.04g increment of intake = 1.07; 95% CI = 0.97 – 1.18), but not with other specific TFAs or total TFAs.

Multivariable-adjusted estimates were comparable to our age-adjusted estimates(data not shown), as were models that controlled for additional

confounders such as education and red meat intake. Simultaneous modeling of individual PUFAs did not result in substantially different estimates. We did not find evidence of hazard ratio modification either on the additive or multiplicative scale. All models met the proportional hazards assumption.

B.5. Discussion

In this large cohort study, dietary intakes of most PUFAs and TFAs were not associated with incident prostate cancer, although intakes of long-chain ω-3 fatty acids ("marine fatty acids") is positively associated with incident cancer. However, consumption of large amounts of AA was positively associated with advanced disease while total TFA intake was inversely associated with advanced disease. Furthermore, the ratio of LA:ALA was inversely associated with advanced prostate cancer. Conversely, we found that consumption of large amounts of long-chain ω-3 fatty acids ("marine fatty acids") and LA was inversely associated with fatal prostate cancer and intake of TFA 16:1 was associated with an increased risk of fatal prostate cancer, although estimates for categorical associations with fatal prostate cancer were imprecise.

Our results for dietary intakes of fatty acids and incident disease are largely comparable to our prior analysis of fat consumption and prostate cancer among male participants in the screening arm of the PLCO Cancer Screening Trial(142). However, in the PLCO analysis, LA intake was inversely associated with incident disease (MV-adjusted HR for a 4g increment of LA intake = 0.89; 95% CI = 0.82 – 0.96), but not in the present study.

We did not have sufficient data on tumor grade to evaluate associations with tumors defined by grade, but we were able to estimate associations with advanced

stage and fatal prostate cancers. In our prior analyses, we found that only TFA intakes were positively associated with high-grade disease among PLCO study participants. In the NIH-AARP study population, TFA intakes were inversely associated with high stage disease, but were positively associated with fatal disease, particularly for TFA 16:1. Unlike our PLCO analyses, we found evidence of an inverse association between consumption of marine fatty acids and LA and fatal disease and a positive association between AA intake and advanced (high stage) disease.

Our findings for AA intake are largely compatible with our analysis of ω-6 fatty acids and prostate cancer in PLCO(142), a case-control study conducted in Australia(66) and an analysis of the Health Professionals Follow-up Study (HPFS)(72) which found no associations between AA intake and incident prostate cancer. Our results for LA intake and incident prostate cancer were also similar to our earlier analysis (MV-adjusted HR for a 4g increment of LA intake = 0.94; 95% CI = 0.89 – 1.00)(142). Like two analyses of the HPFS(71, 72), we found that LA intake may be inversely associated with aggressive disease.

Consistent with our prior analyses of ALA in PLCO(49, 142) and a number of other studies (70, 135-138), we did not find associations between ALA intake and incident or advanced prostate cancer However, they were inconsistent with several other studies that reported either positive(57, 69, 76) or inverse(58, 60, 66) associations between intakes of ALA and prostate cancer.

Our findings of inverse associations between long-chain ω-3 fatty acids and fatal prostate cancer are largely compatible with a recent study in the HPFS, which found that DHA and EPA intake may be inversely associated with advanced

(defined as high stage or fatal) prostate cancer(72). Animal and cell culture studies suggest that individual ω-3 fatty acids, particularly EPA and DHA, may inhibit prostate carcinogenesis(19).

Both DHA and EPA have been shown to inhibit tumor cell growth in both animal models and cell lines derived from human prostate tumors(42). Relative to DHA or EPA, ALA may have greater potential to create oxidative damage which could contribute to prostate cancer tumorgenesis(41). Also, while animal and cell cultures suggest that long-chain ω-3 fatty acids may inhibit carcinogenesis(19), other studies have suggested that ALA does not prevent prostate tumor growth(36, 42-44), supporting the notion that ALA may modulate prostate cancer growth through mechanisms independent of its metabolism to EPA and DHA.

As with our analysis of men in the screening arm of the PLCO study, we found no evidence of an association between TFA intakes and incident prostate cancer. However, we found conflicting evidence when we stratified cases by aggressiveness. Specifically, intakes of TFAs were inversely associated with high stage disease, but some TFAs were positively associated with fatal disease, a result consistent with our finding of a positive association between high-grade disease in the PLCO cohort. However, our estimates are fairly imprecise, and it is important to note that we used the same cutpoints for categorization as we did in PLCO. Because these estimates were based on a different questionnaires, estimated intakes for the same individual would be expected to vary. One case-control study(58) reported no association between TFA intake and incident prostate cancer, while another(79) found a positive association with aggressive (high stage, high grade, or high total PSA) disease. Intriguingly, another case-control study(66) found no association

between incident prostate cancer and intakes of trans-18:1 and trans-18:2, but a positive association with intake of trans-16:1, similar to our findings for fatal prostate cancer.

It has been hypothesized that TFAs may increase prostate cancer risk through inflammation-mediated carcinogenesis(15, 52). A number of clinical crossover studies of TFA supplementation have found that TFA intake is associated with increased markers of systemic inflammation, including C-reactive protein(15, 52, 53).

This study has a number of strengths. It is one of the few studies to comprehensively examine relations between prostate cancer and intakes of the most common PUFAs and TFAs and it was conducted within a well-designed, large prospective cohort study. As with men in the screening arm of the PLCO trial, men in the NIH-AARP study consumed amounts of the major PUFAs as a fraction of energy from fat and total energy intake that were similar to estimated intakes for men in the United States. Intakes of TFAs as a fraction of total energy intake were slightly lower in the NIH-AARP study population than in the PLCO population, but it is unclear whether this reflects true differences in consumption or measurement error due to differences in specific food items queried for by the two FFQs or differences in assumptions used to calculate nutrient contents of similar food items. In addition, the FFQ used in the NIH-AARP study included a question on usual cooking oil used, potentially providing a better estimate of dietary intakes of PUFAs. This FFQ was developed using cognitive research to help identify and correct deficiencies in prior dietary assessments including comprehension of food items, poor ordering of food items, and difficulties in averaging intakes of seasonal foods and multiple foods included in a single line item. (84, 101)Furthermore, unlike our

prior analyses in PLCO, we had the ability to investigate associations with fatal prostate cancer, potentially the most clinically relevant form of the disease.

We know that there are some limitations to our current analysis. Case ascertainment is entirely passive. A validation sub-study found that approximately 89% of cancer cases were ascertained(105), although ascertainment could potentially be lower for prostate cancer. Because there is no reason to suspect that diet is associated with the likelihood of a diagnosed case being reported to the cancer registry, there is no reason to suspect that this misclassification should be differential with respect to exposure status, resulting most likely in a bias towards the null. Furthermore, it is highly unlikely that we falsely identified men without prostate cancer as cases through this method. A sensitivity analysis or our estimates, randomly assigning positive case status to 2,000, assuming that ascertainment is not associated with fatty acid intakes, of the non-cases (~10% of the number of cases ascertained during follow-up) and rerunning the analyses, suggests that the amount of bias that this would introduce in our analysis is limited, but would bias estimates towards the null. This type of case ascertainment also limited our ability to obtain tumor grade data and information. Like the PLCO population, the NIH-AARP study recruited participants in a broad range of U.S. states; however, generalizability may be limited to individuals with similar sociodemographic characteristics to the study population, which was predominantly white and of higher socio-economic status than the United States population as a whole. Similarly, we assessed diet at a single point, which was relatively late in life, which may not have captured diet at the etiologically relevant time period. Limited data were available on specific types of fish consumed and no data were available on fish oil or flaxseed supplements; consequently, we may have underestimated ω-3 fatty

acid intakes. Furthermore, we noted a wide variation in intakes of some nutrients (in some instances, the standard deviation equaling in the mean), which may have contributed to additional imprecision in our effect estimates.

While we have estimated relative risks of prostate cancer in relation to dietary intakes of PUFAs and TFAs, it may be more relevant to consider the absolute impact that consumption of these nutrients may have. According to SEER data(3, 4), the 5-year probability of developing prostate cancer beginning at age 60 (the closest 5-year increment to the mean age of entry into the NIH-AARP Diet and Health Study) is approximately 3%. Given, an HR for prostate cancer of 1.18 for consuming an amount of AA that would place one in the highest category of AA intake compared with the lowest, one would increase the absolute 5-year risk of prostate cancer from 3 to 3.54%. This risk difference is equivalent to the requirement that185 men switch from the highest to lowest category of AA consumption in order to reduce the number of cases of prostate cancer per 100,000 men by one.

In summary, we found no evidence of associations between intakes of most PUFAs or TFAs and incident prostate cancer. We found that long-chain ω-3 fatty acids ("marine fatty acids") were positively associated with incident cancer. We found that long-chain ω-3 fatty acids were inversely associated with fatal prostate cancer and that TFA intakes were inversely associated with high-stage disease and positively associated with fatal prostate cancer.

Other large prospective studies that include younger men and that also capture a more complete estimation of fish consumption and supplement use are warranted to further elucidate the roles that these highly interrelated nutrients may play in prostate carcinogenesis.

B.6. Acknowledgements

This research was supported in part by the Intramural Research Program of the NIH, National Cancer Institute. Cancer incidence data from the Atlanta metropolitan area were collected by the Georgia Center for Cancer Statistics, Department of Epidemiology, Rollins School of Public Health, Emory University. Cancer incidence data from California were collected by the California Department of Health Services, Cancer Surveillance Section. Cancer incidence data from the Detroit metropolitan area were collected by the Michigan Cancer Surveillance Program, Community Health Administration, State of Michigan. The Florida cancer incidence data used in this report were collected by the Florida Cancer Data System under contract to the Department of Health (DOH). The views expressed herein are solely those of the authors and do not necessarily reflect those of the contractor or DOH. Cancer incidence data from Louisiana were collected by the Louisiana Tumor Registry, Louisiana State University Medical Center in New Orleans. Cancer incidence data from New Jersey were collected by the New Jersey State Cancer Registry, Cancer Epidemiology Services, New Jersey State Department of Health and Senior Services. Cancer incidence data from North Carolina were collected by the North Carolina Central Cancer Registry. Cancer incidence data from Pennsylvania were supplied by the Division of Health Statistics and Research, Pennsylvania Department of Health, Harrisburg, Pennsylvania. The Pennsylvania Department of Health specifically disclaims responsibility for any analyses, interpretations or conclusions. We are indebted to the participants in the NIH-AARP Diet and Health Study for their outstanding cooperation.

CHAPTER V

DISCUSSION

A. Summary of Findings

We investigated relations between intakes of major PUFAs, their ratios, and TFAs and prostate cancer in two large prospective cohort studies, the screening arm of the PLCO Cancer Screening Trial and the NIH-AARP Diet and Health Study.

Among men in the PLCO study, we found that intake of LA, the most common ω-6 fatty acid in the diet, was inversely associated with total prostate cancer (MV-adjusted HR for a 4g increment of intake = 0.94; 95% CI = 0.89 – 1.00). Intakes of the long-chain ω -3 fatty acids (EPA, DPA, and DHA) were positively associated with low-grade (Gleason sum < 7) prostate cancer (MV-adjusted HR for a 0.1g increment of intake = 1.04; 95% CI = 0.99 – 1.09), while TFA intakes were positively associated with high-grade disease (MV-adjusted HR for a 2g increment of total TFA intake = 1.07; 95% CI = 0.96 – 1.19). We found no other associations between PUFAs or their ratios and TFAs and total, low-grade, or high-grade prostate cancer.

Among men in the NIH-AARP Diet and Health Study, we found that intakes of long-chain ω-3 fatty acids were positively associated with total prostate cancer (MV-adjusted HR comparing C5 to C1 = 1.07; 95% CI = 1.02 – 1.12) and inversely associated with fatal disease (MV-adjusted HR for a 0.1g increment of intake = 0.87;

95% CI = 0.78 – 0.98). We also found that total TFA intake was inversely associated with high-stage disease (MV-adjusted HR for a 2g increment of intake total TFA = 0.95; 95% CI = 0.89 – 1.02) and TFA 16:1 intake was positively associated with fatal disease (MV-adjusted HR for a 0.04g increment of intake = 1.07; 95% CI = 0.97 – 1.18).

B. Synthesis of Results of the Two Studies

In general, we found no associations between intakes of individual PUFAs, their ratios, or individual TFAs and total or advanced (based on tumor stage, grade, or fatality) prostate cancer. However, in the PLCO population we found an inverse association between intake of LA and total prostate cancer, an association we did not see in the NIH-AARP study population. We found apparent divergent associations between intakes of long-chain ω-3 fatty acids and prostate cancer; a positive association with low-grade disease in the PLCO population, positive association with total prostate cancer in the NIH-AARP study population, and an inverse association with fatal cancer in the NIH-AARP study population. We also found apparent differences between associations with TFAs and prostate cancer; a positive association between TFA intake and high-grade prostate cancer in the PLCO population and inverse association with advanced prostate cancer in the NIH-AARP Diet and Health Study (although intake of TFA 16:1 was positively associated with fatal disease in the NIH-AARP study population).

A number of explanations may account for these apparent discrepancies between the two study populations and from our initial hypotheses. First, it is possible that the true associations differ due to differences in the study populations. Furthermore, due to the fact that true associations between dietary risk factors for

prostate cancer are of low magnitude and that there is a high likelihood of non-differential misclassification of exposure in prospective, with respect to disease status, studies of diet, it is not surprising that results may be attenuated towards a null value of 1.0. While both study populations are large, and power substantial (an indicator of relative precision; Table 12 and Table 13), it becomes difficult to determine whether small estimates are true representations of the actual association or simply given added weight due to increased precision. That said, while the two study populations were fairly similar, differences in both assessment of diet and ascertainment of case status may help account for differences in our findings for the two study populations.

As discussed briefly previously, there are a number of differences between the dietary assessments used in the PLCO study and the NIH-AARP study. In the PLCO population, we used the DQX to assess average dietary intake of food items during the year prior to enrollment in the study. The DQX was based on both the Willett and Block FFQs, two frequently used and validated dietary instruments(127). However, Subar, et al. found, through cognitive interviewing, that there are substantial limitations to the traditional approaches used in these FFQs(84, 101). These include the fact that participants had difficulty averaging intakes of multiple food items that were listed for a single line-item (e.g. a single line item on the DQX included "Meatloaf, burritos, tacos (beef only)"), difficulty averaging intakes of seasonal foods (such as fruits and vegetables), difficulty understanding what foods would be included in a single line-item, and difficulties arising from the poor ordering of food items (e.g. not realizing until the end of the FFQ that a specific food belonged in one of the last line-items rather than one that occurred near the beginning of the FFQ)(84, 101). The DHQ was developed to attempt to correct these

deficiencies and produce a more accurate estimate of nutrient intakes than older FFQs. Subar, et al. did note that the DHQ performed better than either the Block or Willett FFQs, although energy-adjustment of nutrients helped attenuate some of these differences(84).

Additionally, the DHQ specifically inquired about usual fats and oils used in cooking, which could help differentiate intakes of PUFAs as fats and oils provide a substantial amount of fatty acids in the diet(14). Along with questions on whether butter and margarine is used on bread (and corresponding frequency) and whether mayonnaise is consumed, the DHQ included a set of questions on fats and oils used in cooking. First, the DHQ queries on the use of butter or margarine (and type of margarine used such as diet tub-based margarine) and the frequency of use in cooking. For frying and sautéing, the DHQ inquired about whether margarine, butter, lard, vegetable shortening (e.g. "Crisco"), spray oil, or oil was used (allowing the respondent to select as many choices as used). For those respondents who selected oil, the DHQ queried on the specific type of oil used (allowing for multiple responses ("don't know," corn, olive, safflower, sunflower, canola, and other). Because different types of fats and shortening contain different amounts of trans- and polyunsaturated fatty acids, the additional questions used in the DHQ may help differentiate specific fatty acid consumption more readily than the DQX and, therefore, the DHQ may provide for a better assessment of nutrient intakes than the DQX used in the PLCO study.

Both studies used similar methods for estimating nutrient intakes for given food items and both studies were limited in their assessment of types of fish consumed, potentially limiting our ability to measure intakes of long-chain ω-3 fatty

acids. Neither the DHQ nor the DQX are able to distinguish intakes of fatty fish (e.g. salmon, sword fish) from less fatty-fish (e.g. catfish, cod). Therefore, consumers of large amounts of fatty fish will have reported intakes of long-chain ω-3 fatty acids similar to those for consumers of less fatty fish within strata of total fish consumption. And consumers of large amounts of non-fatty fish will have elevated estimates of long-chain ω-3 fatty acids. Most likely this would have biased our estimates towards null. Neither FFQ included questions on fish oil supplementation. Kris-Etherton, et al. reported that in 1998 it was estimated that yearly per capita EPA and DHA intake from fish oil supplements was approximately 0.6 – 0.9 mg/person(14), suggesting that during the time period of these two studies, fish oil supplementation would contribute negligibly to total intakes of long-chain ω-3 fatty acid consumption, although we might expect that members of these two cohorts may be more likely to have taken supplements than the average man. No data on correlations for specific fatty acids estimated by the DQX or DHQ and those estimated by other methods (such as 24-hour diet recall) were not available, although correlations for total PUFA intake were fairly strong for the DHQ in a validation substudy conducted within the NIH-AARP study population(143). Some have claimed that 24-hour diet recalls or diet diaries administered over a range of days may serve as appropriate alloyed gold standards to correct for systematic and random error in nutrient estimates from FFQ data by providing for unbiased correction of the FFQ estimates(144-147). It is assumed that there is no correlated systematic error between both measurements(146) and that the major sources of within and between person variation are averaged out through the use of large population samples, variation in seasonal coverage, and a large span of

coverage for each individual(147). However, it may be reasonable to assume that some amount of correlated systematic error may also exist in diet recall or diet diaries(144). For example, an individual might underreport intakes of foods considered unhealthy on both an FFQ and a diet recall. Adjustment for total energy intake(82, 84) was used to help correct for systematic measurement error. It should also be noted, that even if absolute intakes were not measured accurately, variation in intakes may have been, and caution must be used in interpreting associations for given differences in reported intake as reflective of the association for an equal difference in true intake of that nutrient. We would, however, hypothesize that the DHQ provides a more accurate estimate of average daily intake of nutrients than the DQX. In order to facilitate comparison of associations between the two studies, we used the same cutpoints for the categorization of exposures, but as reported total energy intake varied across the two studies, this may not provide for a completely accurate comparison.

There were substantial differences in case ascertainment between the two study populations. The PLCO population was fairly uniformly screened for prostate caner using regular PSA tests and DREs, whereas the NIH-AARP study population did not undergo a standardized screening regimen, although approximately 90% of men who responded to the supplementary RFQ had undergone at least one DRE or PSA during the five years prior to completion of the RFQ. More importantly, self-reported diagnoses of prostate cancer were confirmed through standardized procedures, including medical record review for both tumor stage and grade data. We did not, however, have data on prostate cancer mortality in this cohort due to the ongoing nature of the screening trial and associations with fatal prostate cancer may have the greatest public health significance as localized prostate cancer has

nearly 100% 5-year survival(3, 4). In contrast, case ascertainment in the NIH-AARP Diet and Health Study was entirely passive. Linkage to cancer registries and the NDI was used to identify prostate cancer incidence, mortality, and tumor stage data. It was estimated that approximately 89% of cases were successfully ascertained using these methods(105). It may be more difficult to compare results on prostate cancer aggressiveness between the two studies due to the differences in data collected for tumor aggressiveness. Additionally, some differences between associations for advanced and fatal disease within the NIH-AARP study population may be accounted for by the additional two years of follow-up time available for the mortality analysis. These limitations in outcome data may, in part, mitigate some of the strengths of a nearly 10-fold larger study population and more accurate estimates of dietary intakes of fatty acids.

C. Strengths and Limitations

The completed study had a number of strengths. It included analyses of two of the few large, well-designed prospective studies of PUFA intake and prostate cancer risk. We have the additional benefit of having been able to leverage two different studies to investigate these relations.

The PLCO study benefited from the fact that the entire population has undergone standardized screening practices, reducing the effect of detection bias on our analyses. Furthermore, prostate cancer ascertainment was of high quality and data was available on both prostate cancer stage and grade. The study population included sites across the United States and efforts had been made to recruit minorities(148). While minority participation was not as great as had been hoped, the incidence of prostate cancer during follow-up among African American participants was substantially higher than among whites (~10% vs. ~6%). In

addition, we had extensive data on potential covariates, aiding in our ability to control for potential confounding factors and effect measure modifiers.

There are some limitations to our analysis of the PLCO population. There is published evidence of a "healthy volunteer effect" in the PLCO population(139), potentially limiting our ability to generalize our findings to the US or World population. However, this limitation is not unique to our population and is a limitation for most other epidemiologic studies. We also know that in this study population, family history of cancer was underreported more frequently among males and African Americans(140), potentially limiting the utility of this potentially important confounder. Diet was only assessed once in this population, and it was assessed fairly late in life. It is possible that the assessed diet does not represent diet during the etiologically relevant time period for this slow-developing disease. Because of the relatively late age at entry into the study (~63 years at baseline), we may have missed some of the more aggressive, early onset forms of prostate cancer. Finally, due to the ongoing nature of the PLCO Cancer Screening Trial, we did not have data on prostate cancer mortality, which limited our ability to investigate all aspects of prostate cancer aggressiveness. Additionally, there may be some concern that controlling for screening behavior may be more of a control of information quality, rather than confounding by screening behavior and may have induced bias into our estimates.

As with the PLCO population, the NIH-AARP population provided some strengths in investigating relations between diet and prostate cancer. The NIH-AARP population is the largest prospective study of diet and health and as such we had increased power to detect modest associations over other studies. However, this increased power may have been mitigated somewhat by the fact that case

ascertainment was largely passive and likely missed a substantial number of prostate cancer cases(105). While there is no published data on participants in AARP being healthier than average, it is reasonable to assume that AARP suffers from a similar "healthy participant effects" as PLCO. The FFQ used in the AARP cohort (DHQ) may have provided a better assessment of dietary intakes of some of the PUFAs than that used in PLCO (DQX). Unlike the PLCO population, we had data on prostate cancer mortality in the AARP cohort. However, data was not available on prostate cancer grade, further mitigating the potential benefits of having had increased power. The age at entry into the study was somewhat younger, potentially increasing the incidence of aggressive tumors while decreasing the incidence of asymptomatic cases.

Like PLCO, there are some additional limitations to the AARP cohort. Extensive screening data was only available on approximately 60% of the study population. Because the entire population will not have undergone routine prostate cancer screening, detection of asymptomatic prostate tumors will likely have been reduced, decreasing the incidence of prostate cancer in this population relative to PLCO. Like PLCO, the AARP cohort was limited by a single dietary assessment. However, the DHQ may have provided a better assessment of PUFA intake than the DQX used in PLCO. Like the PLCO population, while the AARP cohort recruited participants in a broad range of U.S. states, generalizability may be limited to individuals with similar sociodemographic characteristics to the study population.

As discussed previously, different instruments for the same type of dietary assessment method can produce different absolute estimates of nutrient intakes. For example, the DQX used in PLCO generally had higher estimates for most of the exposures of interest in the proposed study(Table 10). These differences may be

accounted for by a combination of the fact that the DQX estimates a higher total energy intake on average (2,397.7 kcal/day) than the DHQ does (2,012.2 kcal/day) as well as differences in certain specific food items (e.g. in AARP respondents were queried on specific types of cooking oils used).

Treatment of variables for dietary exposure in statistical models can be a difficult process. First and foremost, dietary assessment is an error-prone endeavor. This mismeasurement, combined with a lack of *a priori* knowledge of the shape of the dose-response curve for the associations between a nutrient and given outcome make it difficult to model associations with continuous covariates. Categorization based on evenly spaced quantiles can potentially induce bias(91). We included additional categorization schemes for our exposures based on the results of spline analyses of the data(106). Additional concern existed for the potential to introduce bias through the large number of men excluded from our analyses based on study protocols. We compared known characteristics of excluded men with those in the included analyses to qualitatively evaluate the degree to which the two populations (excluded/included) differed.

D. Public Health Significance of Findings

We did not find substantial associations between PUFAs, their ratios, or TFAs and prostate cancer in either the PLCO or NIH-AARP study populations. Typical associations found would require the movement of more than 300 men from low to high intake of a specific fatty acid in order to decrease or increase (depending on the direction of the association) 1 case per 100,000 men. However, we consistently found that intake of ALA was not associated with prostate cancer regardless of aggressiveness. This may be due to the fact that the potential increased risk of

prostate cancer due to oxidative stress may be balanced by the potential benefits afforded by the conversion of ALA to the longer-chain ω-3 fatty acid metabolite(19). Our evidence does suggest that high intakes of at least some TFAs is positively associated with more advanced prostate cancer. This provides additional evidence that TFA intake may have negative health effects.

E. Conclusions

Both the PLCO and AARP populations provided a unique opportunity to investigate relations between PUFA intake and prostate cancer risk. Each study has unique strengths that may help provide insights into these associations. By conducting both sets of analyses simultaneously, we had a unique opportunity to compare the findings from these two large cohorts. Overall, we did not report many strong associations between dietary intakes of unsaturated fatty acids and prostate cancer and those associations that we did report were not consistent across both studies.

Limitations in our ability to assess fish consumption and the lack of data on fish oil and flaxseed supplementation may account for some deficiencies in our ability to estimate fatty acid consumption. One of the most important improvements in understanding these complex relations is to generate better estimates of PUFA and TFA intakes. As mentioned previously, the food items included in the DQX and DHQ did not separate fatty and non-fatty fish species. Future studies should include broad categories of these types of fish separately (giving common examples for the fatty fish). Furthermore, neither questionnaire included items on fish or flaxseed supplementation. While fish oil supplementation was relatively rare during the mid 1990s(14), it is likely more common now and can

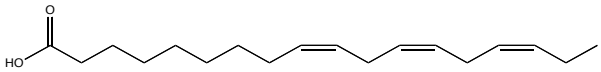
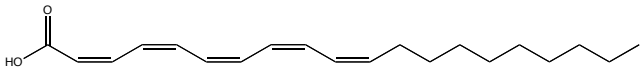
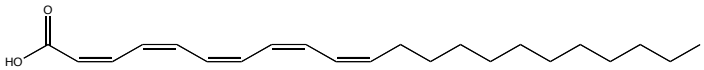
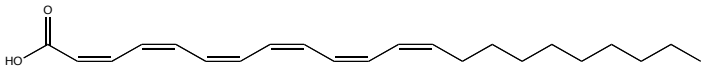
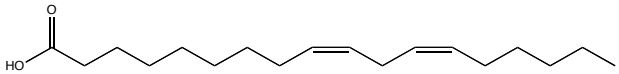
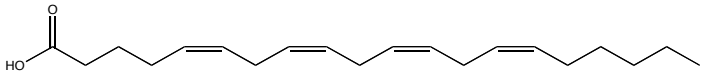
shift an individual's consumption of these fatty acids substantially. In order to further elucidate the associations involving these highly interrelated fatty acids, it may be worthwhile to collect serum or adipose tissue and conduct a nested-case control or case-cohort study within a large prospective study which has collected the proposed dietary and supplement data(77).

A large, well-designed prospective study including more detailed questions on fish consumption and supplement use may be warranted to further assess relations between PUFA and TFA intakes and prostate cancer.

Appendix 1

Tables

Table 1. Major polyunsaturated fatty acids, their abbreviations, numerical notations, and structures.

Fatty Acid Name	Abbr.	Num*	Structure
ω-3			
Alpha-linolenic	ALA	18:3	
Eicosapentaenoic	EPA	20:5	
Docosapentaenoic	DPA	22:5	
Docosahexaenoic	DHA	22:6	
ω-6			
Linoleic	LA	18:2	
Arachidonic	AA	20:4	

*The first number corresponds to the length of the hydrocarbon chain while the second number corresponds to the number of double bonds in the hydrocarbon chain.

Table 2. Selected results from prior studies of total polyunsaturated fatty acids and prostate cancer.

Author, Year	Country	Type of Study	Population	Comparison	Results
Andersson, et al., 1996	Sweden	Case-Control	522 cases/536 population-based controls	Q4 vs. Q1	For total prostate cancer: age-adjusted OR = 1.27 (0.92 – 1.82) and age- and energy adjusted OR = 0.98 (0.70 – 1.38) For advanced prostate cancer: ORs = 1.26 (0.83 – 1.93) and 0.96 (0.65 – 1.43)
Ghadirian, et al. , 1996	Canada	Case-Control	232 cases/231 population-based controls	Q4 vs. Q1	Multivariate OR = 1.46 (0.74 – 2.87)
Key, et al., 1997	UK	Case-Control	328 cases/328 practice-based controls	T3 vs. T1	OR = 0.94 (0.64 – 1.38
Kristal, et al., 2002	US	Case-Control	605 cases/592 population-based controls	Q5 vs. Q1 (% energy from PUFA)	OR (Local) = 0.91 (0.58 – 1.43), OR (Regional/Distant) = 1.17 (0.64 – 2.12)
Meyer, et al., 1997	Canada	Case-Control	215 cases/593 population-based controls	Q4 vs. Q1	OR = 1.10 (0.60 – 1.99)
Ramon, et al., 2000	Spain	Case-Control	217 cases/217 hospital-based controls/217 population-based controls	Q4 vs. Q1	OR = 0.85 (0.54 – 1.3)
Rohan, et al., 1995	Canada	Case-Control	207 cases/207 populations-based controls	Q4 vs. Q1	OR = 1.17 (0.66 – 2.08)
Schuurman, et al., 1999	The Netherlands	Case-Cohort (from a prospective cohort)	Subcohort of 1,688 men/642 incident cases (full cohort of 582,279 men)	Q5 vs. Q1	RR = 0.78 (0.56 – 1.10)
Tzonou, et al., 1999	Greece	Case-Control	320 cases/246 hospital-based controls	1SD of intake among controls	OR = 1.79 (1.13 – 2.84)
Veierod, et al., 1997	Norway	Prospective Cohort	25,708 men/72 incident cases	Q5 vs. Q1	IRR = 1.4 (0.6 – 3.0)
Harvei, et al., 1997	Norway	Nested Case-Control (blood bank donors)	141 cases/141 population-based controls	Q4 vs. Q1 (serum phospholipids)	OR = 1.1 (0.6 2.1) OR (ω-6) = 0.7 (0.3 – 1.3) OR (ω-3) = 1.1 (0.6 – 2.1)
Newcomer, et al., 2001	US	Case-Control	67 cases/156 population-based controls	Q4 vs. Q1 (erythrocyte membrane phospholipids)	OR (ω-3) = 1.1 (0.5 – 2.5) OR (ω-6) = 2.3 (1.0 – 5.4)
Hodge, et al., 2004	Australia	Case-Control	964 cases/911 frequency matched controls	Q5 vs. Q1	OR = 1.0 (0.7 – 1.3)

Park, et al., 2007	US (MEC)	Cohort	82,483 men/4,404 incident cases	Q5 vs. Q1	For total prostate cancer: RR (PUFA) = 1.01 (0.91 – 1.11) RR (ω-3) = 0.95 (0.86 – 1.05) RR (ω-6) = 1.03 (0.93 – 1.14) For advanced prostate cancer: RR (PUFA) = 1.01 (0.84 – 1.23 RR (ω-3) = 0.90 (0.76 – 1.08) RR (ω-6) = 1.04 (0.86 – 1.27)
Hedelin, et al., 2006	Sweden	Case-Control	1,499 cases/1,130 frequency matched controls	Q4 vs. Q1	OR (ω-3) = 1.25 (0.88 – 1.78) OR (ω-6) = 1.36 (1.01 – 1.84)
Neuhouser, et al., 2007	US (CARET)	Prospective Cohort	12,000 men/890 incident cases	Q4 vs. Q1	All: RR (PUFA) = 1.17 (0.88 – 1.32) RR (ω-6) = 1.19 (0.90 – 1.58) Positive family history: RR (PUFA) = 2.47 (0.96 – 6.37) RR (ω-6) = 2.61 (1.01 – 6.72) Negative family history: RR (PUFA) = 1.13 (0.84 – 1.51) RR (ω-6) = 1.14 (0.86 – 1.52)

Table 3. Selected results from prior studies of linoleic acid and arachidonic acid and prostate cancer.

Author, Year	Country	Type of Study	Population	Comparison	Results
Andersson, et al., 1996	Sweden	Case-Control	522 cases/536 population-based controls	Q4 vs. Q1	For total prostate cancer: Age-adjusted OR = 1.38 (0.96 – 1.97) and age- and energy-adjusted OR = 1.19 (0.84 – 1.68). For advanced prostate cancer: ORs = 1.8 (0.97 – 2.25) and 1.19 (0.79 – 1.77)
Meyer, et al., 1997	Canada	Case-Control	215 cases/593 population-based controls	Q4 vs. Q1Pr	OR = 1.57 (0.85 – 2.93)
Ramon, et al., 2000	Spain	Case-Control	217 cases/217 hospital-based controls/217 population-based controls	Q4 vs. Q1	OR = 0.92 (0.64 – 1.3)
Schuurman, et al., 1999	The Netherlands	Case-Cohort (from a prospective cohort)	Subcohort of 1,688 men/642 incident cases (full cohort of 582,279 men)	Q5 vs. Q1	RR = 0.78 (0.56 – 1.09) (for AA, RR = 1.20 (0.87 – 1.66))
De Stefani, et al., 2000	Uruguay	Case-Control	217 cases/431hospital-based controls	Q4 vs. Q1	OR = 0.69 (0.39 – 1.19)
Gann, et al., 1994	US (PHS)	Nested Case-Control (from a prospective cohort)	120 cases/120 population-based controls	Q4 vs. Q1 (plasma cholesterol ester)	OR = 0.62 (0.30 – 1.30) (for AA, OR = 1.36 (0.63 – 2.90)
Giovannucci, et al., 1993	US (HPFS)	Prospective Cohort	47,885 men/279 incident cases (126 advanced cases)	Q5 vs. Q1	RR = 0.88 (0.55 – 1.43) RR (advanced) = 0.64 (0.32 – 1.32)
Godley, et al., 1996	US	Case-Control	89 cases/38 clinic-based controls	Q4 vs. Q1 (erythrocyte membran and adipose tissue)	OR (eryth) = 3.54 (1.0 – 12.53) OR (adip) = 2.47 (0.66 – 9.26)
Harvei, et al., 1997	Norway	Nested Case-Control (blood bank donors)	141 cases/141 population-based controls	Q4 vs. Q1 (serum phospholipids)	OR = 2.0 (0.4 – 1.2) OR (AA) = 0.8 (0.4 – 1.5)
Mannisto, et al., 2003	Finalnd (ATBC)	Nested Case-Control (from prospective cohort)	198 cases/198 population-based controls (29,133 men in cohort)	Q4 vs. Q1 (serum fatty acid and FFQ)	OR (serum) = 0.77 (0.43 – 1.39) OR (serum, AA) = 1.39 (0.79 – 2.44) OR (FFQ) = 0.92 (0.54 – 1.59) OR (FFQ, AA) = 1.31 (0.77 – 2.21)
Newcomer, et al., 2001	US	Case-Control	67 cases/156 population-based controls	Q4 vs. Q1 (erythrocyte membrane phospholipids)	OR = 2.1 (0.9 – 4.8) OR (AA) = 0.9 (0.4 – 2.3)
Leitzmann, et al., 2004	US	Prospective Cohort (NPFS)	47,866 men/2,965 incident cases (448 advanced cases)	Q5 vs. Q1	RR = 1.06 (0.89 – 1.26) RR (advanced) = 0.80 (0.52 – 1.24) RR (AA) = 1.08 (0.94 – 1.25) RR (AA, adv.) = 1.11 (0.78 – 1.59)
Hodge, et al., 2004	Australia	Case-Control	964 cases/911 frequency matched controls	Q5 vs. Q1	OR = 1.0 (0.7 – 1.3) OR (AA) = 1.0 (0.7 – 1.4)

Chavarro, et al., 2007	US (PHS)	Nested Case-Control (from a prospective cohort)	476 cases/476 matched controls	Q5 vs. Q1 (whole blood)	For total: OR (LA) = 0.62 (0.41 – 0.95) OR (AA) = 1.09 (0.72 – 1.64) For localized: OR (LA) = 0.55 (0.32 – 0.94) OR (AA) = 0.68 (0.40 – 1.15) For advanced: OR (LA) = 0.67 (0.28 – 1.58) OR (AA) = 2.45 (1.02 – 5.09) For Gleason < 7: OR (LA) = 0.79 (0.44 – 1.43) OR (AA) = 0.98 (0.55 – 1.74) For Gleason ≥ 7: OR (LA) = 0.38 (0.17 – 086) OR (AA) = 1.43 (0.61 – 3.32) For non-aggressive: OR (LA) = 0.61 (0.33 – 1.16) OR (AA) = 0.83 (0.45 – 1.54) For aggressive: OR (LA) = 0.52 (0.28 – 0.95) OR (AA) = 1.25 (0.69 – 2.28)

Table 4. Selected results from prior studies of alpha-linolenic acid and prostate cancer.

Author, Year	Country	Type of Study	Population	Comparison	Results
Andersson, et al., 1996	Sweden	Case-Control	522 cases/536 population-based controls	Q4 vs. Q1	For total prostate cancer: Age-adjusted OR = 1.23 (0.86 – 1.74) and age- and energy-adjusted OR = 0.93 (0.65 – 1.32) For advanced prostate cancer: ORs = 1.21 (0.80 – 1.82) and 0.82 (0.54 – 1.23)
Meyer, et al., 1997	Canada	Case-Control	215 cases/593 population-based controls	Q4 vs. Q1	OR = 0.98 (0.54 – 1.78)
Ramon, et al., 2000	Spain	Case-Control	217 cases/217 hospital-based controls/217 population-based controls	Q4 vs. Q1	OR = 3.1 (2.2 – 4.7)
Schuurman, et al., 1999	The Netherlands	Case-Cohort (from a prospective cohort)	Subcohort of 1,688 men/642 incident cases (full cohort of 582,279 men)	Q5 vs. Q1	RR = 0.76 (0.66 – 1.04)
De Stefani, et al., 2000	Uruguay	Case-Control	217 cases/431hospital-based controls	Q4 vs. Q1	OR = 3.91 (1.50 – 10.1)
Gann, et al., 1994	US (PHS)	Nested Case-Control (from a prospective cohort)	120 cases/120 population-based controls	Q4 vs. Q1 (plasma cholesterol ester)	OR = 2.14 (0.93 – 4.93)
Giovannucci, et al., 1993	US (HPFS)	Prospective Cohort	47,885 men/279 incident cases (126 advanced cases)	Q5 vs. Q1	RR = 1.25 (0.82 – 1.92) RR (advanced) = 3.43 (1.67 – 7.04)
Godley, et al., 1996	US	Case-Control	89 cases/38 clinic-based controls	Q4 vs. Q1 (erythrocyte membrane and adipose tissue)	OR (eryth) = 1.69 (0.54 – 5.26) OR (adip) = 2.73 (0.70 – 10.61)
Harvei, et al., 1997	Norway	Nested Case-Control (blood bank donors)	141 cases/141 population-based controls	Q4 vs. Q1 (serum phospholipids)	OR = 2.0 (1.1 – 3.6)
Mannisto, et al., 2003	Finalnd (ATBC)	Nested Case-Control (from prospective cohort)	198 cases/198 population-based controls (29,133 men in cohort)	Q4 vs. Q1 (serum fatty acid and FFQ)	OR (serum) = 0.97 (0.54 – 1.75) OR (FFQ) = 1.31 (0.77 – 2.21)
Newcomer, et al., 2001	US	Case-Control	67 cases/156 population-based controls	Q4 vs. Q1 (erythrocyte membrane phospholipids)	OR = 2.6 (1.1 – 5.8)
Leitzmann, et al., 2004	US	Prospective Cohort (NPFS)	47,866 men/2,965 incident cases (448 advanced cases)	Q5 vs. Q1	RR = 1.09 (0.93 – 1.26) RR (advanced) = 1.98 (1.34 – 2.93)
Koralek, et al., 2006	US	Prospective Cohort (PLCO)	29,592 men/1,898 incident cases (285 advanced)	Q5 vs. Q1 (T3 vs. T1)	RR = 0.94 (0.81 – 1.09)
Hodge, et al., 2004	Australia	Case-Control	964 cases/911 frequency matched controls	Q5 vs. Q1	OR = 0.8 (0.6 – 1.0)

Park, et al., 2007	US (MEC)	Cohort	82,483 men/4,404 incident cases	Q5 vs. Q1	RR (total) = 0.92 (0.84 – 1.02) RR (advanced) = 0.89 (0.74 – 1.06)
Hedelin, et al., 2006	Sweden	Case-Control	1,499 cases/1,130 frequency matched controls	Q4 vs. Q1	OR = 1.35 (0.99 – 1.84)
Chavarro, et al., 2007	US (PHS)	Nested Case-Control (from a prospective cohort)	476 cases/476 matched controls	Q5 vs. Q1 (whole blood)	For total: OR = 1.31 (0.89 – 1.95) For localized: OR = 1.66 (1.02 – 2.71) For advanced: OR = 1.04 (0.45 – 2.38) For Gleason < 7: OR = 1.56 (0.90 – 2.71) For Gleason ≥ 7: OR = 1.49 (0.67 – 3.27) For non-aggressive: OR = 1.73 (0.98 – 3.07) For aggressive: OR = 1.14 (0.64 – 2.03)

Table 5. Selected results from prior studies of docosahexaenoic acid and eicosapentaenoic acid and prostate cancer.

Author, Year	Country	Type of Study	Population	Comparison	Results
Kristal, et al., 2002	US	Case-Control	605 cases/592 population-based controls	Q5 vs. Q1	OR (local) = 1.05 (0.68 – 1.63), OR (Regional/distant) = 0.84 (0.44 – 1.58)
Schuurman, et al., 1999	The Netherlands	Case-Cohort (from a prospective cohort)	Subcohort of 1,688 men/642 incident cases (full cohort of 582,279 men)	Q5 vs. Q1	RR for EPA = 1.00 (0.73 – 1.35) RR for DHA = 1.03 (0.75 – 1.40)
Gann, et al., 1994	US (PHS)	Nested Case-Control (from a prospective cohort)	120 cases/120 population-based controls	Q4 vs. Q1 (plasma cholesterol ester)	OR (EPA only) = 0.87 (0.41 – 1.82)
Giovannucci, et al., 1993	US (HPFS)	Prospective Cohort	47,885 men/279 incident cases (126 advanced cases)	Q5 vs. Q1	RR (advanced) = 0.90 (0.51 – 1.61)
Godley, et al., 1996	US	Case-Control	89 cases/38 clinic-based controls	Q4 vs. Q1 (erythrocyte membrane and adipose tissue)	OR (eryth, EPA) = 0.74 (0.23 – 2.33) OR (adip, EPA) = 0.54 (0.18 – 1.62) OR (eryth, DHA) = 0.36 (0.10 – 1.27) OR (adip, DHA) = 1.11 (0.30 – 4.14)
Harvei, et al., 1997	Norway	Nested Case-Control (blood bank donors)	141 cases/141 population-based controls	Q4 vs. Q1 (serum phospholipids)	OR (EPA) = 1.2 (0.6 – 2.1) OR (DPA = 0.7 (0.3 – 1.3) OR (DHA) = 1.0 (0.5 – 1.8)
Mannisto, et al., 2003	Finalnd (ATBC)	Nested Case-Control (from prospective cohort)	198 cases/198 population-based controls (29,133 men in cohort)	Q4 vs. Q1 (serum fatty acid and FFQ)	OR (Serum, EPA) = 1.12 (0.61 – 2.04) OR (Serum, DHA) = 0.71 (0.40 – 1.26) OR (FFQ, EPA) = 1.22 (0.68 – 2.20) OR (FFQ, DHA) = 1.31 (0.74 – 2.32)
Newcomer, et al., 2001	US	Case-Control	67 cases/156 population-based controls	Q4 vs. Q1 (erythrocyte membrane phospholipids)	OR (EPA) = 1.3 (0.6 – 3.0) OR (DHA) = 10. (0.4 – 2.3)
Leitzmann, et al., 2004	US	Prospective Cohort (NPFS)	47,866 men/2,965 incident cases (448 advanced cases)	Q5 vs. Q1	OR (EPA) = 0.88 (0.76 – 1.01) OR (DHA) = 0.89 (0.78 – 1.04) OR (EPA + DHA) = 0.89 (0.77 – 1.04) OR (EPA, advanced) = 0.82 (0.58 – 1.17) OR (DHA, advanced) = 0.71 (0.49 – 1.08) OR (EPA + DHA, advanced) = 0.74 (0.49 – 1.08)
Norrish, et al., 1999	New Zealand	Case-Control	317 cases/480 population-based controls	Q4 vs. Q1 (erythrocyte phospholipids)	OR (EPA) = 0.59 (0.37 – 0.95) OR (EPA, advanced) = 0.54 (0.31 – 0.96) OR (DHA) = 0.73 (0.45 – 1.18) OR (DHA, advanced) = 0.66 (0.39 – 1.13)
Hodge, et al., 2004	Australia	Case-Control	964 cases/911 frequency matched controls	Q5 vs. Q1	OR (EPA) = 0.8 (0.6 – 1.1) OR (DHA) = 1.0 (0.7 – 1.4)

Park, et al., 2007	US (MEC)	Cohort	82,483 men/4,404 incident cases	Q5 vs. Q1	Total prostate cancer: RR (EPA) = 1.01 (0.91 – 1.13) RR (DHA) = 0.99 (0.89 – 1.09) Advanced prostate cancer: RR (EPA) = 1.05 (0.86 – 1.28) RR (DHA) = 1.07 (0.88 – 1.30)
Hedelin, et al., 2006	Sweden	Case-Control	1,499 cases/1,130 frequency matched controls	Q4 vs. Q1	OR (EPA + DHA) = 0.70 (0.51 – 0.97)
Chavarro, et al., 2007	US (PHS)	Nested Case-Control (from a prospective cohort)	476 cases/476 matched controls	Q5 vs. Q1 (whole blood)	For total: OR (EPA) = 0.57 (0.36 – 0.92) OR (DPA) = 0.60 (0.38 – 0.93) OR (DHA) = 0.60 (0.39 – 0.93) OR (EPA+DPA+DHA) = 0.59 (0.38 – 0.93) For localized: OR (EPA) = 0.46 (0.24 – 0.86) OR (DPA) = 0.46 (0.26 – 0.83) OR (DHA) = 0.53 (0.30 – 0.94) OR (EPA+DPA+DHA) = 0.52 (0.28 – 0.94) For advanced: OR (EPA) = 1.27 (0.49 – 3.29) OR (DPA) = 0.72 (0.30 – 1.73) OR (DHA) = 0.98 (0.39 – 2.50) OR (EPA+DPA+DHA) = 1.03 (0.41 – 2.63) For Gleason < 7: OR (EPA) = 0.57 (0.28 – 1.11) OR (DPA) = 0.72 (0.39 – 1.32) OR (DHA) = 0.64 (0.35 – 1.17 OR (EPA+DPA+DHA) = 0.58 (0.31 – 1.10) For Gleason ≥ 7: OR (EPA) = 0.42 (0.15 – 1.14) OR (DPA) = 0.30 (0.12 – 0.80) OR (DHA) = 0.53 (0.21 – 1.31) OR (EPA+DPA+DHA) = 0.63 (0.26 – 1.55) For non-aggressive: OR (EPA) = 0.58 (0.28 – 1.17) OR (DPA) = 0.62 (0.32 – 1.21) OR (DHA) = 0.64 (0.33 – 1.24) OR (EPA+DPA+DHA) = 0.61 (0.31 – 1.20) For aggressive: OR (EPA) = 0.61 (0.30 – 1.25) OR (DPA) = 0.42 (0.21 – 0.83) OR (DHA) = 0.53 (0.26 – 1.05) OR (EPA+DPA+DHA) = 0.45 (0.27 – 1.13)

Table 6. Selected results from prior studies of trans-fatty acids and prostate cancer

Author, Year	Country	Type of Study	Population	Comparison	Results
Schuurman, et al., 1999	The Netherlands	Case-Cohort (from a prospective cohort)	Subcohort of 1,688 men/642 incident cases (full cohort of 582,279 men)	Q5 vs. Q1	RR (total TFA)= 0.99 (0.70 – 1.40)
Hodge, et al., 2004	Australia	Case-Control	964 cases/911 frequency matched controls	Q5 vs. Q1	OR (16:1 trans (t)) = 1.2 (0.9 – 1.6) OR (18:1 t) = 0.9 (0.6 – 1.2) OR (18:2 t) = 1.0 (0.7 – 1.4)
Liu, et al., 2007	US	Case-Control	506 cases (advanced)/506 matched controls	Q4 vs. Q1	Caucasians OR (16:1 t) = 2.05 (1.23 – 3.42) OR (18:1 t) = 2.95 (1.71 – 5.09) OR (18:2 t) = 2.84 (1.57 – 5.14) OR (total TFA) = 2.77 (1.60 – 4.79) African Americans OR (16:1 t) = 0.60 (0.18 – 2.03) OR (18:1 t) = 0.64 (0.16 – 2.50) OR (18:2 t) = 0.31 (0.07 – 1.37) OR (total TFA) = 0.43 (0.10 – 1.78)
King, et al., 2005	US	Nested Case-Control (CARET)	272 cases/426 matched controls	Q4 vs. Q1 (serum concentrations)	OR (16:1/Δ9t) = 0.71 (0.44 – 1.15) OR (16:1/Δ7t) = 0.98 (0.59 – 1.62) OR (18:1/Δ8t) = 1.38 (0.86 – 2.22) OR (18:1/Δ9t) = 1.39 (0.87 – 2.23) OR (18:1/Δ10t) = 1.41 (0.87 – 2.28) OR (18:1/Δ11t) = 1.69 (1.03 – 2.77) OR (18:1/Δ12t) = 1.53 (0.94 – 2.50) OR (18:2/Δ9c,12t) = 1.79 (1.02 – 3.15) OR (18:2/Δ9t,12c) = 1.31 (0.80 – 2.12) OR(18:2/Δ9t,Δ12t) = 1.19 (0.76 – 1.87)
Neuhouser, et al., 2007	US (CARET)	Prospective Cohort	12,000 men/890 incident cases	Q4 vs. Q1	All: OR (total TFA) = 1.51 (0.48 – 4.69) Positive family history: OR (total TFA) = 1.46 (0.61 – 3.47) Negative family history: OR (total TFA) = 1.06 (0.81 – 1.39)

Table 7. Selected results from prior studies of ratios of polyunsaturated fatty acids and prostate cancer.

Author, Year	Country	Type of Study	Population	Comparison	Results
Harvei, et al., 1997	Norway	Nested Case-Control (blood bank donors)	141 cases/141 population-based controls	Q4 vs. Q1 (serum phospholipids)	OR (PUFA/saturated) = 1.8 (0.9 – 3.6) OR (ω-6/ω-3) = 0.8 (0.4 – 1.6) OR (LA/EPA) = 0.8 (0.4 – 1.6) OR (LA/ALA) = 0.3 (0.2 – 0.8) OR (AA/EPA) = 0.8 (0.4 – 1.3)
Leitzmann, et al., 2004	US	Prospective Cohort (NPFS)	47,866 men/2,965 incident cases (448 advanced cases)	Q5 vs. Q1	OR (LA/ALA) = 1.00 (0.89 – 1.14) OR (LA/EPA + DHA) = 1.14 (0.98 – 1.33) OR (LA/ALA, advanced) = 0.62 (0.45 – 0.86) OR (LA/EPA + DHA, advanced) = 1.38 (0.94 – 2.04)
Park, et al., 2007	US (MEC)	Cohort	82,483 men/4,404 incident cases	Q5 vs. Q1	RR (ω-6/ω-3, total) = 1.04 (0.95 – 1.15) RR (ω-6/ω-3, adv) = 1.10 (0.92 – 1.13)
Hedelin, et al., 2006	Sweden	Case-Control	1,499 cases/1,130 frequency matched controls	Q4 vs. Q1	OR (ω-3/ω-6) = 0.71 (0.55 – 0.92) OR (DHA+EPA/ω-6) =0.66 (0.51 – 0.84)
Chavarro, et al., 2007	US (PHS)	Nested Case-Control (from a prospective cohort)	476 cases/476 matched controls	Q5 vs. Q1 (whole blood)	OR (ω-6/ω-3) = 0.80 (0.54 – 1.19) OR (AA/EPA) = 1.30 (0.80 – 2.11)

Table 8. Selected baseline characteristics (SD) of men in the screening arm of the PLCO Trial.

Fatty Acid	Covariate	Quintile 1	2	3	4	5
ALA						
	Age at baseline	62.7 (5.3)	62.8 (5.3)	62.8 (5.3)	62.7 (5.2)	62.6 (5.3)
	Current BMI	27.0 (3.9)	27.5 (4.0)	27.5 (4.1)	27.8 (4.2)	28.0 (4.6)
	Total energy intake (kcal/day)	2,316.9 (860.5)	2,303.3 (821.7)	2,341.3 (844.7)	2,383.3 (865.5)	2,353,6 (856.5)
	Family history of prostate cancer (%)	7.8	8.6	8.3	8.3	8.3
	History of diabetes (%)	5.9	7.5	8.3	8.8	11.8
LA						
	Age at baseline	63.2 (5.3)	62.9 (5.3)	62.6 (5.3)	62.4 (5.2)	62.3 (5.2)
	Current BMI	27.1 (4.0)	27.4 (4.0)	27.6 (4.1)	27.8 (4.2)	28.0 (4.4)
	Total energy intake (kcal/day)	2,333.7 (878.6)	2,318.0 (817.1)	2,315.6 (831.4)	2,353.7 (840.2)	2,377.6 (881.0)
	Family history of prostate cancer (%)	8.0	8.0	8.4	8.1	8.7
	History of diabetes (%)	6.1	7.8	7.4	9.1	11.8
TFA						
	Age at baseline	62.8 (5.4)	62.8 (5.3)	62.7 (5.3)	62.7 (5.2)	62.5 (5.2)
	Current BMI	26.8 (4.0)	27.3 (4.1)	27.7 (4.1)	27.9 (4.2)	28.1 (4.3)
	Total energy intake (kcal/day)	2,316.6 (850.4)	2,320.8 (830.2)	2,3477.8 (840.7)	2,364.2 (850.1)	2,349.1 (878.9)
	Family history of prostate cancer (%)	7.9	8.0	8.6	8.5	8.3
	History of diabetes (%)	7.0	7.9	8.2	9.1	10.2

Table 9. Selected baseline characteristics (SD) of male participants in the NIH-AARP Diet and Health Study.

Fatty Acid	Covariate	Quintile 1	2	3	4	5
ALA						
	Age at baseline	62.1 (5.4)	62.0 (5.4)	62.1 (5.4)	62.2 (5.3)	62.3 (5.3)
	Current BMI	26.6 (4.1)	27.1 (4.1)	27.4 (4.3)	27.5 (4.3)	27.6 (4.5)
	Total energy intake (kcal/day)	1,999.5 (942.7)	1,951.5 (790.3)	2,019.6 (806.6)	2,061.4 (825.2)	2,029.2 (836.4)
	Family history of prostate cancer (%)	9.8	10.3	10.5	10.8	10.6
	History of diabetes (%)	7.2	8.6	9.8	10.8	12.0
LA						
	Age at baseline	62.2 (5.4)	62.1 (5.4)	62.1 (5.4)	62.1 (5.3)	62.2 (5.3)
	Current BMI	26.7 (4.1)	27.1 (4.1)	27.4 (4.3)	27.5 (4.3)	27.7 (4.4)
	Total energy intake (kcal/day)	2,023.7 (952.5)	1,960.1 (798.1)	1,998.4 (798.3)	2,032.7 (807.5)	2,046.3 (844.2)
	Family history of prostate cancer (%)	9.6	10.5	10.4	10.7	10.6
	History of diabetes (%)	6.8	8.4	9.4	10.5	13.4
TFA						
	Age at baseline	62.1 (5.3)	62.1 (5.4)	62.0 (5.4)	62.1 (5.4)	62.4 (5.3)
	Current BMI	26.5 (4.0)	27.1 (4.1)	27.4 (4.3)	27.6 (4.4)	27.7 (4.4)
	Total energy intake (kcal/day)	2,005.5 (933.9)	1,978.4 (804.6)	2,025.2 (824.5)	2,047.9 (831.4)	2,004.2 (811.0)
	Family history of prostate cancer (%)	10.0	10.4	10.4	10.5	10.7
	History of diabetes (%)	7.1	8.8	9.8	10.7	12.1

Table 10. Comparison of intakes of selected nutrients (SD) in the PLCO and NIH-AARP studies.

Nutrient	PLCO	AARP
Total Energy (kcal/day)	2,339.7 (850.3)	2,012.2 (842.7)
ALA (g/day)	1.44 (0.60)	1.40 (0.73)
EPA* (g/day)	0.044 (0.046)	NA
DPA (g/day)	0.016 (0.016)	0.015 (0.014)
DHA (g/day)	0.087 (0.078)	0.076 (0.068)
EPA + DPA + DHA* (g/day)	0.15 (0.14)	NA
LA (g/day)	14.1 (6.5)	13.5 (7.3)
AA (g/day)	0.12 (0.07)	0.11 (0.07)
Total TFA (g/day)	6.7 (3.1)	4.8 (2.9)
TFA 16:1 (g/day)	0.075 (0.053)	0.060 (0.053)
TFA 18:1 (g/day)	5.9 (2.7)	4.2 (2.9)
TFA 18:2 (g/day)	0.71 (0.32)	0.52 (0.30)
Lycopene (μg/day)	11,709.0 (8,514.5)	8,064.9 (8,144.6)
Calcium (mg/day)	1,165.6 (578.4)	813.1 (472.5)
Alcohol (g/day)	16.0 (29.2)	16.8 (38.5)

* Note, that EPA data was not available for AARP at the date of the proposal defense

Table 11. Comparison of the PLCO and NIH-AARP Studies.

	PLCO	AARP
Sample Size	29,592 men	287,760 men (172,961 with second questionnaire)
Time Period	1993-2001 through September 2003	1996 through 2003
Setting	Subjects residing around major medical centers	Subjects residing in one of six states or two metropolitan regions
Age	55 – 74 years at baseline	50 – 71 years at baseline
Dietary Assessment	137-item FFQ (DQX) no information on cooking oils	124-item FFQ (DHQ) includes data on cooking oils
Outcome Assessment	Men with positive screens referred for follow-up, self-report, all confirmed with medical chart review, linkage to NDI	Linkage to state cancer registries and the NDI
Outcomes Available	Case status, stage, and grade	Case status, stage, prostate cancer mortality
Screening Data	Dates of PLCO trial screening behavior available	Self-reported history available only for men who provided the second questionnaire

Table 12. Power estimates for main effect analyses in the PLCO population.

Hazard Ratio	Sample Size	R-squared between exposure and covariates	Power
1.1	11,600	0	0.56
1.2	11,600	0	0.98
1.3	11,600	0	1.00
1.4	11,600	0	1.00
1.5	11,600	0	1.00
1.1	11,600	0.2	0.47
1.2	11,600	0.2	0.95
1.3	11,600	0.2	1.00
1.4	11,600	0.2	1.00
1.5	11,600	0.2	1.00

Table 13. Power estimates for main effect analyses in the AARP population.

Hazard Ratio	Sample Size	R-squared between exposure and covariates	Power
1.1	115,104	0	0.84
1.2	115,104	0	1.00
1.3	115,104	0	1.00
1.4	115,104	0	1.00
1.5	115,104	0	1.00
1.1	115,104	0.2	0.77
1.2	115,104	0.2	1.00
1.3	115,104	0.2	1.00
1.4	115,104	0.2	1.00
1.5	115,104	0.2	1.00

Table 14. Timeline for Completion of the Dissertation.

Event	2007					2008	
	September	October	November	December	January	February	March
Defense of the Proposal							
PLCO Analysis							
PLCO Write-up							
AARP Analysis							
AARP Write-up							
Interim Meeting							
Dissertation Write-up							
Dissertation Defense							

Table 15. Contributions and correlations of energy-adjusted intakes of major polyunsaturated fatty acids among 29,594 male participants in the screening arm of the PLCO Cancer Screening Trial in the year prior to randomization.

	ALA	EPA	DPA	DHA	Fish Fats*	LA	AA
% Energy	0.60	0.02	0.00	0.04	0.06	5.86	0.05
% Energy from Fat	1.87	0.06	0.02	0.12	0.20	17.88	0.16
Correlation†	Pearson Correlation Coefficients						
ALA	1.						
EPA	0.16	1.					
DPA	0.18	0.92	1.				
DHA	0.19	0.98	0.93	1.			
Fish Fats*	0.18	0.99	0.94	1.00	1.		
LA	0.65	0.94	0.14	0.13	0.11	1.	
AA	0.29	0.36	0.47	0.43	0.42	0.24	1.

*Fish Fats are the sum of intakes of EPA, DPA, and DHA.
†Correlations are between residually-adjusted nutrient intakes

Table 16. Selected baseline characteristics of 29,594 male participants in the screening arm of the PLCO Cancer Screening Trial.

Characteristic	**Cohort**
N	29,594
Mean Age at Baseline (yrs) (SD)	62.7 (5.3)
Body Mass Index (kg/m^2) (%)*	
BMI < 20	1.2
BMI 20 - < 25	24.9
BMI 25 - < 30	50.5
BMI ≥ 30	23.5
Mean Daily Intake†, g/day (SD)	
AA	0.12 (0.04)
LA	13.11 (3.08)
ALA	1.35 (0.27)
EPA	0.04 (0.04)
DPA	0.02 (0.01)
DHA	0.08 (0.07)
Total TFA	6.28 (1.50)
TFA 16:1	0.07 (0.04)
TFA 18:1	5.51 (1.34)
TFA 18:2	0.66 (0.15)
Mean Energy Intake, kcal/day (SD)	2,339.7 (850.3)
Mean Lycopene Intake†, μg/day (SD)	11,057.9 (6,639.2)
Mean Supplemental Vit. E, IU/day (SD)	64.8 (108.1)
Vigorous Exercise (h/week) (SD)	2.3 (1.9)
Regular Aspirin Use (%)	
< twice weekly	23.2
≥ twice weekly	30.7
Race (%)	
White	90.7
African American	3.3
Asian/Pacific Islander	4.0
Other (Hispanic/Native American)	1.9
Family History of Prostate Cancer (%)	8.0
History of Diabetes (%)	8.5
Smoking history (%)	
Never	29.5
Current Cigarettes	10.6
Former Cigarettes	52.0
Ever Pipe/Cigars	7.9

*BMI was missing in 0.9% of the sample
†Nutrient values adjusted for total energy intake

Table 17. Hazard ratios and 95% confidence intervals for total prostate cancer in relation to intakes of major polyunsaturated fatty acids and trans fatty acids (TFAs) in the screening arm of the PLCO Cancer Screening Trial.

	C1	C2	C3	C4	C5	Trend
AA						
Range of ntakes(g/day)	0.01 – 0.08	0.08 – 0.11	0.11 – 0.14	0.14 – 0.17	0.17 – 0.34	Per 0.05g
Number of Cases	394	599	515	233	173	
MV-adjusted HR*	1.	0.97 (0.85 – 1.11)	1.05 (0.92 – 1.21)	0.92 (0.78 – 1.09)	0.99 (0.82 – 1.20)	1.01 (0.95 – 1.07)
LA						
Range of Intakes(g/day)	2.06 – 9.60	9.60 – 12.10	12.10 – 14.60	14.60 – 17.10	17.10 – 38.73	Per 4g
Number of Cases	223	584	641	331	135	
MV-adjusted HR*	1.	0.90 (0.77 – 1.06)	0.89 (0.76 – 1.04)	0.91 (0.76 – 1.08)	0.77 (0.61 – 0.95)	0.94 (0.89 – 1.00)
ALA						
Range of Intakes(g/day)	0.21 – 1.10	1.10 – 1.30	1.30 – 1.50	1.50 – 1.70	1.70 – 4.37	Per 0.3g
Number of Cases	310	536	595	317	156	
MV-adjusted HR*	1.	0.90 (0.78 – 1.04)	0.93 (0.81 – 1.08)	0.94 (0.79 – 1.10)	0.92 (0.75 – 1.12)	1.00 (0.95 – 1.06)
EPA						
Range of Intakes(g/day)	0.000 – 0.015	0.015 – 0.031	0.031 – 0.048	0.048 – 0.064	0.64 – 0.770	Per 0.03g
Number of Cases	337	630	399	226	322	
MV-adjusted HR*	1.	1.17 (1.02 – 1.34)	1.13 (0.97 – 1.31)	1.13 (0.95 – 1.34)	1.10 (0.94 – 1.29)	1.02 (0.98 – 1.05)
DPA						
Range of Intakes(g/day)	0.000 – 0.006	0.006 – 0.012	0.012 – 0.019	0.019 – 0.024	0.024 – 0.218	Per 0.01g
Number of Cases	304	681	437	199	293	
MV-adjusted HR*	1.	1.02 (0.88 – 1.17)	1.00 (0.86 – 1.17)	1.08 (0.89 – 1.29)	0.98 (0.83 – 1.16)	1.02 (0.98 – 1.05)
DHA						
Range of Intakes(g/day)	0.00 – 0.03	0.03 – 0.06	0.06 – 0.09	0.09 – 0.12	0.12 – 1.29	Per 0.06g
Number of Cases	225	620	481	258	330	
MV-adjusted HR*	1.	1.07 (0.92 – 1.25)	1.12 (0.95 – 1.32)	1.08 (0.89 – 1.30)	1.06 (0.89 – 1.26)	1.02 (0.98 – 1.06)
Fish Fats†						
Range of Intakes(g/day)	0.000 – 0.050	0.050 – 0.088	0.088 – 0.125	0.125 – 0.162	0.163 – 2.257	Per 0.1g
Number of Cases	227	467	395	297	528	
MV-adjusted HR*	1.	1.11 (0.94 – 1.31)	1.11 (0.94 – 1.31)	1.23 (1.03 – 1.47)	1.10 (0.93 – 1.29)	1.02 (0.98 – 1.06)
ω6:ω3						
Range of Intakes	3.66 – 7.20	7.20 – 8.20	8.20 – 9.20	9.20 – 10.20	10.20 – 51.57	Per 2
Number of Cases	215	512	574	344	269	
MV-adjusted HR*	1.	1.07 (0.91 – 1.26)	1.06 (0.90 – 1.24)	1.05 (0.88 – 1.25)	0.86 (0.72 – 1.04)	0.94 (0.89 – 0.99)
LA:ALA						
Range of Intakes	2.20 – 6.20	6.20 – 8.70	8.70 – 11.20	11.20 – 12.70	12.70 – 65.85	Per 5

	C1	C2	C3	C4	C5	Trend
Number of Cases	243	562	499	203	407	
MV-adjusted HR*	1.	1.08 (0.89 – 1.30)	1.03 (0.83 – 1.29)	0.98 (0.75 – 1.28)	0.84 (0.64 – 1.12)	0.91 (0.83 – 1.00)
LA: Fish Fats†						
Range of Intakes	7.8 – 50.0	50.0 – 100.0	100.0 – 150.0	150.0 – 200.0	200.0 – 62,630.6	Per 100
Number of Cases	222	583	447	257	405	
MV-adjusted HR*	1.	0.95 (0.81 – 1.12)	1.00 (0.85 – 1.19)	0.92 (0.76 – 1.11)	0.92 (0.77 – 1.09)	0.96 (0.93 – 1.00)
Total TFA‡						
Range of Intakes(g/day)	1.08 – 5.00	5.00 – 6.00	6.00 – 7.00	7.00 – 8.00	8.00 – 15.20	Per 2g
Number of Cases	368	521	484	325	216	
MV-adjusted HR*	1.	1.09 (0.95 – 1.25)	0.95 (0.83 – 1.09)	0.97 (0.83 – 1.13)	1.02 (0.85 – 1.21)	0.98 (0.92 – 1.04)
TFA 16:1						
Range of Intakes(g/day)	0.00 – 0.04	0.04 – 0.06	0.06 – 0.08	0.08 – 0.10	0.10 – 0.43	Per 0.04g
Number of Cases	410	565	434	236	269	
MV-adjusted HR*	1.	0.87 (0.76 – 1.00)	0.90 (0.78 – 1.04)	0.94 (0.80 – 1.12)	0.99 (0.83 – 1.16)	1.02 (0.97 – 1.08)
TFA 18:1						
Range of Intakes(g/day)	0.94 – 4.50	4.50 – 5.50	5.50 – 6.50	6.50 – 7.50	7.50 – 13.29	Per 2g
Number of Cases	430	596	501	263	124	
MV-adjusted HR*	1.	1.05 (0.92 – 1.19)	0.97 (0.85 – 1.10)	1.00 (0.85 – 1.18)	1.00 (0.81 – 1.23)	0.98 (0.91 – 1.05)
TFA 18:2						
Range of Intakes(g/day)	0.10 – 0.52	0.52 – 0.65	0.65 – 0.77	0.78 – 0.90	0.90 – 1.50	Per 0.2g
Number of Cases	360	556	599	294	105	
MV-adjusted HR*	1.	0.98 (0.84 – 1.12)	0.95 (0.83 – 1.10)	0.92 (0.78 – 1.09)	0.99 (0.78 – 1.24)	0.98 (0.92 – 1.04)

*MV-adjusted HRs adjusted for: age at baseline, current body mass index, family history of prostate cancer, history of diabetes, smoking history, intakes of total energy, lycopene, and supplemental vitamin E, aspirin use, physical activity, study center, and race plus the number of prostate cancer screening exams since baseline (time varying covariate)

† Fish Fats is the sum of intakes from EPA, DPA, and DHA

‡ Total TFA is the sum of intakes from TFA 16:1, TFA 18:1, and TFA 18:2

§ Nutrient intakes adjusted for total energy intake using the residual method

** All HRs and 95% CIs estimated using Cox proportional hazards models

Table 18. Hazard ratios and 95% confidence intervals for low-grade prostate cancer (Gleason sum < 7) in relation to intakes of major polyunsaturated fatty acids and trans fatty acids (TFAs) in the screening arm of the PLCO Cancer Screening Trial.

	C1	C2	C3	C4	C5	Trend
AA						
Range of ntakes(g/day)	0.01 – 0.08	0.08 – 0.11	0.11 – 0.14	0.14 – 0.17	0.17 – 0.61	Per 0.05g
Number of Cases	245	390	302	155	90	
MV-adjusted HR*	1.	1.04 (0.88 – 1.23)	1.00 (0.84 – 1.20)	1.00 (0.81 – 1.24)	0.86 (0.66 – 1.11)	0.96 (0.89 – 1.04)
LA						
Range of Intakes(g/day)	2.06 – 9.60	9.60 – 12.10	12.10 – 14.60	14.60 – 17.10	17.10 – 38.73	Per 4g
Number of Cases	149	374	399	186	74	
MV-adjusted HR*	1.	0.87 (0.72 – 1.06)	0.84 (0.69 – 1.02)	0.80 (0.64 – 1.00)	0.62 (0.47 – 0.83)	0.89 (0.82 – 0.96)
ALA						
Range of Intakes(g/day)	0.21 – 1.10	1.10 – 1.30	1.30 – 1.50	1.50 – 1.70	1.70 – 4.37	Per 0.3g
Number of Cases	209	328	376	172	97	
MV-adjusted HR*	1.	0.82 (0.69 – 0.98)	0.89 (0.75 – 1.06)	0.78 (0.63 – 0.96)	0.87 (0.68 – 1.11)	0.97 (0.91 – 1.04)
EPA						
Range of Intakes(g/day)	0.000 – 0.015	0.015 – 0.031	0.031 – 0.047	0.047 – 0.064	0.064 – 0.770	Per 0.03g
Number of Cases	207	374	258	145	198	
MV-adjusted HR*	1.	1.14 (0.96 – 1.36)	1.19 (0.98 – 1.44)	1.20 (0.97 – 1.50)	1.14 (0.93 – 1.40)	1.04 (0.99 – 1.08)
DPA						
Range of Intakes(g/day)	0.000 – 0.006	0.006 – 0.012	0.012 – 0.018	0.019 – 0.024	0.024 – 0.218	Per 0.01g
Number of Cases	182	423	265	126	186	
MV-adjusted HR*	1.	1.05 (0.88 – 1.26)	1.03 (0.84 – 1.24)	1.17 (0.93 – 1.48)	1.07 (0.87 – 1.33)	1.03 (0.98 – 1.07)
DHA						
Range of Intakes(g/day)	0.00 – 0.03	0.03 – 0.06	0.06 – 0.09	0.09 – 0.12	0.12 – 1.29	Per 0.06g
Number of Cases	134	377	304	156	211	
MV-adjusted HR*	1.	1.10 (0.90 – 1.35)	1.19 (0.97 – 1.48)	1.12 (0.88 – 1.42)	1.19 (0.95 – 1.49)	1.04 (0.98 – 1.10)
Fish Fats†						
Range of Intakes(g/day)	0.000 – 0.050	0.50 – 0.087	0.088 – 0.125	0.125 – 0.162	0.163 – 2.257	Per 0.1g
Number of Cases	134	290	241	185	332	
MV-adjusted HR*	1.	1.17 (0.95 – 1.45)	1.15 (0.92 – 1.43)	1.30 (1.04 – 1.64)	1.21 (0.98 – 1.49)	1.04 (0.99 – 1.09)
ω6:ω3						
Range of Intakes	3.92 – 7.20	7.20 – 8.20	8.20 – 9.20	9.21 – 10.20	10.20 – 51.57	Per 2
Number of Cases	144	320	362	194	162	
MV-adjusted HR*	1.	0.99 (0.81 – 1.21)	0.97 (0.80 – 1.19)	0.87 (0.70 – 1.09)	0.75 (0.60 – 0.95)	0.89 (0.83 – 0.95)
LA:ALA						
Range of Intakes	2.19 – 6.20	6.20 – 8.70	8.70 – 11.20	11.20 – 12.70	12.70 – 65.85	Per 5

	C1	C2	C3	C4	C5	Trend
Number of Cases	158	354	305	128	237	
MV-adjusted HR*	1.	0.98 (0.78 – 1.24)	0.88 (0.67 – 1.17)	0.85 (0.60 – 1.19)	0.68 (0.48 – 0.97)	0.83 (0.73 – 0.94)
LA: Fish Fats†						
Range of Intakes	7.83 – 50.00	50.00 – 99.99	100.00 – 149.99	150.01 – 200.00	200.00 – 62,631	Per 100
Number of Cases	145	367	272	152	246	
MV-adjusted HR*	1.	0.89 (0.74 – 1.09)	0.90 (0.73 – 1.11)	0.80 (0.63 – 1.01)	0.82 (0.66 – 1.02)	0.94 (0.90 – 0.99)
Total TFA‡						
Range of Intakes(g/day)	1.08 – 5.00	5.00 – 6.00	6.00 – 7.00	7.00 – 8.00	8.00 – 15.20	Per 2g
Number of Cases	240	332	297	185	128	
MV-adjusted HR*	1.	1.05 (0.88 – 1.24)	0.88 (0.74 – 1.05)	0.84 (0.69 – 1.02)	0.92 (0.73 – 1.15)	0.93 (0.86 – 1.01)
TFA 16:1						
Range of Intakes(g/day)	0.00 – 0.04	0.04 – 0.06	0.06 – 0.08	0.08 – 0.10	0.10 – 0.43	Per 0.04g
Number of Cases	263	354	261	141	163	
MV-adjusted HR*	1.	0.81 (0.69 – 0.96)	0.81 (0.68 – 0.97)	0.84 (0.68 – 1.04)	0.87 (0.71 – 1.08)	0.98 (0.91 – 1.05)
TFA 18:1						
Range of Intakes(g/day)	0.94 – 4.50	4.50 – 5.50	5.50 – 6.50	6.50 – 7.50	7.50 – 13.29	Per 2g
Number of Cases	278	379	302	150	73	
MV-adjusted HR*	1.	1.02 (0.87 – 1.20)	0.89 (0.75 – 1.05)	0.89 (0.73 – 1.10)	0.90 (0.69 – 1.17)	0.92 (0.84 – 1.01)
TFA 18:2						
Range of Intakes(g/day)	0.10 – 0.52	0.53 – 0.65	0.65 – 0.77	0.78 – 0.90	0.90 – 1.50	Per 0.2g
Number of Cases	237	349	364	170	62	
MV-adjusted HR*	1.	0.91 (0.77 – 1.08)	0.85 (0.71 – 1.01)	0.80 (0.65 – 0.98)	0.88 (0.65 – 1.17)	0.94 (0.87 – 1.02)

* MV-adjusted HRs adjusted for: age at baseline, current body mass index, family history of prostate cancer, history of diabetes, smoking history, intakes of total energy, lycopene, and supplemental vitamin E, aspirin use, physical activity, study center, and race plus the number of prostate cancer screening exams since baseline (time varying covariate)

† Fish Fats is the sum of intakes from EPA, DPA, and DHA

‡ Total TFA is the sum of intakes from TFA 16:1, TFA 18:1, and TFA 18:2

§ Nutrient intakes adjusted for total energy intake using the residual method

** All HRs and 95% CIs estimated using Cox proportional hazards models

Table 19. Hazard ratios and 95% confidence intervals for high-grade prostate cancer (Gleason sum ≥ 7) in relation to intakes of major polyunsaturated fatty acids and trans fatty acids (TFAs) in the screening arm of the PLCO Cancer Screening Trial

	C1	C2	C3	C4	C5	Trend
AA						
Range of ntakes(g/day)	0.01 – 0.08	0.08 – 0.11	0.11 – 0.14	0.14 – 0.17	0.17 – 0.61	Per 0.05g
Number of Cases	145	197	202	75	80	
MV-adjusted HR*	1.	0.84 (0.67 – 1.05)	1.11 (0.88 – 1.38)	0.77 (0.58 – 1.04)	1.20 (0.90 – 1.60)	1.06 (0.97 – 1.16)
LA						
Range of Intakes(g/day)	2.06 – 9.60	9.60 – 12.10	12.10 – 14.60	14.60 – 17.10	17.10 – 38.73	Per 4g
Number of Cases	72	200	229	139	59	
MV-adjusted HR*	1.	0.94 (0.71 – 1.24)	0.95 (0.72 – 1.25)	1.09 (0.81 – 1.47)	1.01 (0.71 – 1.44)	1.03 (0.93 – 1.14)
ALA						
Range of Intakes(g/day)	0.21 – 1.10	1.10 – 1.30	1.30 – 1.50	1.50 – 1.70	1.70 – 4.37	Per 0.3g
Number of Cases	97	197	210	139	56	
MV-adjusted HR*	1.	1.04 (0.81 – 1.34)	1.02 (0.79 – 1.31)	1.24 (0.94 – 1.62)	1.00 (0.71 – 1.40)	1.05 (0.96 – 1.14)
EPA						
Range of Intakes(g/day)	0.000 – 0.015	0.015 – 0.031	0.031 – 0.047	0.048 – 0.064	0.064 – 0.770	Per 0.03g
Number of Cases	126	241	135	79	118	
MV-adjusted HR*	1.	1.20 (0.96 – 1.50)	1.04 (0.81 – 1.34)	1.03 (0.77 – 1.38)	1.03 (0.79 – 1.34)	0.99 (0.93 – 1.05)
DPA						
Range of Intakes(g/day)	0.000 – 0.006	0.006 – 0.012	0.012 – 0.018	0.019 – 0.024	0.024 – 0.218	Per 0.01g
Number of Cases	116	247	164	69	103	
MV-adjusted HR*	1.	0.96 (0.76 – 1.21)	0.98 (0.76 – 1.26)	0.94 (0.69 – 1.28)	0.86 (0.65 – 1.14)	1.00 (0.94 – 1.06)
DHA						
Range of Intakes(g/day)	0.00 – 0.03	0.03 – 0.06	0.06 – 0.09	0.09 – 0.12	0.12 – 1.29	Per 0.06g
Number of Cases	89	230	168	99	113	
MV-adjusted HR*	1.	1.01 (0.78 – 1.30)	1.00 (0.77 – 1.31)	1.02 (0.76 – 1.38)	0.86 (0.64 – 1.16)	0.98 (0.92 – 1.06)
Fish Fats†						
Range of Intakes(g/day)	0.000 – 0.050	0.050 – 0.088	0.088 – 0.125	0.125 – 0.162	0.163 – 2.257	Per 0.1g
Number of Cases	90	168	146	108	187	
MV-adjusted HR*	1.	1.01 (0.77 – 1.32)	1.04 (0.79 – 1.37)	1.14 (0.85 – 1.52)	0.94 (0.72 – 1.22)	0.99 (0.92 – 1.06)
ω6:ω3						
Range of Intakes	3.66 – 7.20	7.20 – 8.20	8.20 – 9.20	9.20 – 10.20	10.20 – 51.57	Per 2
Number of Cases	67	182	203	147	100	
MV-adjusted HR*	1.	1.25 (0.94 – 1.67)	1.25 (0.94 – 1.66)	1.47 (1.09 – 1.98)	1.06 (0.77 – 1.47)	1.00 (0.92 – 1.08)
LA:ALA						
Range of Intakes	2.20 – 6.20	6.20 – 8.70	8.70 – 11.20	11.20 – 12.70	12.70 – 65.85	Per 5
Number of Cases	80	201	185	71	162	

	C1	C2	C3	C4	C5	Trend
MV-adjusted HR*	1.	1.30 (0.94 – 1.78)	1.34 (0.92 – 1.96)	1.22 (0.78 – 1.91)	1.20 (0.75 – 1.90)	1.03 (0.89 – 1.18)
LA: Fish Fats†						
Range of Intakes	7.8 – 50.0	50.0 – 100.0	100.0 – 150.0	150.0 – 200.	200.0 – 62,630.6	Per 100
Number of Cases	73	206	168	98	154	
MV-adjusted HR*	1.	1.07 (0.81 – 1.41)	1.23 (0.92 – 1.63)	1.15 (0.83 – 1.57)	1.12 (0.83 – 1.50)	0.99 (0.93 – 1.04)
Total TFA‡						
Range of Intakes(g/day)	1.08 – 5.00	5.00 – 5.99	6.00 – 7.00	7.00 – 8.00	8.00 – 15.20	Per 2g
Number of Cases	123	179	175	137	85	
MV-adjusted HR*	1.	1.16 (0.92 – 1.47)	1.06 (0.84 – 1.35)	1.24 (0.96 – 1.60)	1.21 (0.90 – 1.62)	1.07 (0.96 – 1.19)
TFA 16:1						
Range of Intakes(g/day)	0.00 – 0.04	0.04 – 0.06	0.06 – 0.08	0.08 – 0.10	0.10 – 0.43	Per 0.04g
Number of Cases	141	200	164	94	100	
MV-adjusted HR*	1.	0.96 (0.76 – 1.21)	1.05 (0.82 – 1.33)	1.18 (0.89 – 1.56)	1.19 (0.89 – 1.56)	1.10 (1.01 – 1.20)
TFA 18:1						
Range of Intakes(g/day)	0.95 – 4.50	4.50 – 5.50	5.50 – 6.50	6.50 – 7.50	7.50 – 13.29	Per 2g
Number of Cases	145	207	187	110	50	
MV-adjusted HR*	1.	1.09 (0.88 – 1.36)	1.10 (0.88 – 1.38)	1.23 (0.95 – 1.60)	1.21 (0.87 – 1.69)	1.07 (0.96 – 1.21)
TFA 18:2						
Range of Intakes(g/day)	0.10 – 0.52	0.53 – 0.65	0.65 – 0.77	0.78 – 0.90	0.90 – 1.50	Per 0.2g
Number of Cases	118	195	225	119	42	
MV-adjusted HR*	1.	1.09 (0.86 – 1.38)	1.17 (0.93 – 1.48)	1.18 (0.90 – 1.55)	1.22 (0.84 – 1.79)	1.06 (0.95 – 1.18)

*MV-adjusted HRs adjusted for: age at baseline, current body mass index, family history of prostate cancer, history of diabetes, smoking history, intakes of total energy, lycopene, and supplemental vitamin E, aspirin use, physical activity, study center, and race plus the number of prostate cancer screening exams since baseline (time varying covariate)

† Fish Fats is the sum of intakes from EPA, DPA, and DHA

‡ Total TFA is the sum of intakes from TFA 16:1, TFA 18:1, and TFA 18:2

§ Nutrient intakes adjusted for total energy intake using the residual method

** All HRs and 95% CIs estimated using Cox proportional hazards models

Table 20. Contributions and correlations of energy-adjusted intakes of major polyunsaturated fatty acids among 287,468 male participants in the NIH-AARP Diet and Health Study.

	ALA	EPA	DPA	DHA	Marine fatty acids*	LA	AA
% Energy	0.68	0.02	0.00	0.04	0.06	6.60	0.06
% Energy from Fat	2.10	0.06	0.02	0.13	0.21	20.07	0.17
Correlation†	Pearson Correlation Coefficients						
ALA	1.						
EPA	0.03	1.					
DPA	0.03	0.83	1.				
DHA	0.07	0.95	0.81	1.			
Marine fatty acids*	0.05	0.98	0.84	0.99	1.		
LA	0.78	0.00	0.03	0.04	0.03	1.	
AA	0.17	0.35	0.50	0.47	0.45	0.20	1.

*Marine fatty acids are the sum of intakes of EPA, DPA, and DHA.
†Correlations are between residually-adjusted nutrient intakes

Table 21. Selected baseline characteristics of 29,594 male participants in the screening arm of the PLCO Cancer Screening Trial and 287,468 male participants in the NIH-AARP Diet and Health Study

Characteristic	PLCO	NIH-AARP
N	29,594	287,468
Mean Age at Baseline (yrs) (SD)	62.7 (5.3)	62.0 (5.4)
Body Mass Index (kg/m^2) (%)*		
BMI < 20	1.2	1.5
BMI 20 - < 25	24.9	28.0
BMI 25 - < 30	50.5	49.5
BMI ≥ 30	23.5	21.0
Mean Daily Intake†, g/day (SD)		
AA	0.12 (0.04)	0.11 (0.05)
LA	13.11 (3.08)	12.49 (3.97)
ALA	1.35 (0.27)	1.30 (0.41)
EPA	0.04 (0.04)	0.03 (0.04)
DPA	0.02 (0.01)	0.01 (0.01)
DHA	0.08 (0.07)	0.07 (0.06)
Total TFA	6.28 (1.50)	4.36 (1.70)
TFA 16:1	0.07 (0.04)	0.05 (0.04)
TFA 18:1	5.51 (1.34)	3.80 (1.53)
TFA 18:2	0.66 (0.15)	0.47 (0.17)
Mean Energy Intake, kcal/day (SD)	2,339.7 (850.3)	2,013.6 (843.8)
Mean Lycopene Intake†, μg/day (SD)	11,057.9 (6,639.2)	7,588.5 (6,773.1)
Mean Supplemental Vit. E, IU/day (SD)	64.8 (108.1)	-
Vigorous Exercise (h/week) (SD)	2.3 (1.9)	2.6 (1.6)
Regular Aspirin Use (%)		
< twice weekly	23.2	‡
≥ twice weekly	30.7	‡
Race (%)§		
White	90.7	93.6
African American	3.3	2.7
Asian/Pacific Islander	4.0	1.9
Other (Hispanic/Native American)	1.9	1.7
Family History of Prostate Cancer (%)	8.0	13.5
History of Diabetes (%)	8.5	7.3
Smoking history (%)		
Never	29.5	30.7
Current Cigarettes	10.6	10.7
Former Cigarettes	52.0	58.7

*BMI was missing in 0.9% of the PLCO population, 1.9% of the AARP population
†Nutrient values adjusted for total energy intake
‡Only available in the supplementary risk factor questionnaire
§Race missing among 1.2% of the AARP population

Table 22. Hazard ratios and 95% confidence intervals for incident prostate cancer in relation to intakes of major polyunsaturated fatty acids and trans fatty acids (TFAs) in the NIH-AARP Diet and Health Study.

	C1	C2	C3	C4	C5	Trend
AA						
Person Years	621,312	564,122	416,655	221,945	182,537	
Range of ntakes(g/day)	0.00 – < 0.08	0.08 – < 0.11	0.11 – < 0.14	0.14 – < 0.17	0.17 – < 0.66	Per 0.05g
Number of Cases	5,505	4,889	3,535	1,768	1,398	
MV-adjusted HR*	1.	1.01 (0.97 – 1.06)	1.04 (0.99 – 1.09)	1.00 (0.94– 1.06)	0.99 (0.93 – 1.06)	1.00 (0.98 – 1.02)
LA						
Person Years	466,301	531,315	484,338	290,073	234,542	
Range of Intakes(g/day)	0.33 – < 9.60	9.60 – < 12.10	12.10 – < 14.60	14.60 – < 17.10	17.10 – < 54.87	Per 4g
Number of Cases	4,077	4,470	4,146	2,457	1,945	
MV-adjusted HR*	1.	0.97 (0.93 – 1.02)	0.99 (0.95 – 1.04)	1.00 (0.94 – 1.05)	0.97 (0.92 – 1.03)	1.00 (0.98 – 1.01)
ALA						
Person Years	677,554	457,742	360,680	227,783	282,811	
Range of Intakes(g/day)	0.03 – < 1.10	1.10 – < 1.30	1.30 – < 1.50	1.50 – < 1.70	1.70 – < 7.19	Per 0.3g
Number of Cases	5,755	3,940	3,097	1,912	2,391	
MV-adjusted HR*	1.	1.03 (0.98 – 1.07)	1.03 (0.99 – 1.08)	0.99 (0.94 – 1.05)	1.02 (0.97 – 1.08)	1.00 (0.99 – 1.02)
EPA						
Person Years	622,188	624,985	308,850	175,142	275,405	
Range of Intakes(g/day)	0.00 – < 0.015	0.015 – < 0.031	0.031 – < 0.048	0.048 – < 0.064	0.064 – < 1.16	Per 0.03g
Number of Cases	5,071	5,375	2,707	1,497	2,445	
MV-adjusted HR*	1.	1.06 (1.02 – 1.11)	1.09 (1.04 – 1.15)	1.04 (0.97 – 1.10)	1.07 (1.02 – 1.13)	1.01 (1.00 – 1.02)
DPA						
Person Years	505,509	534,595	401,448	202,098	362,919	
Range of Intakes(g/day)	0.000 – < 0.006	0.006 – < 0.012	0.012 – < 0.018	0.019 – < 0.024	0.024 – < 0.404	Per 0.01g
Number of Cases	4,176	4,581	3,450	1,745	3,143	
MV-adjusted HR*	1.	1.05 (1.01 – 1.11)	1.05 (1.00 – 1.11)	1.06 (1.00 – 1.13)	1.05 (1.00 – 1.11)	1.01 (0.99 – 1.02)
DHA						
Person Years	394,487	689,483	412,746	227,008	282,846	
Range of Intakes(g/day)	0.00 – < 0.03	0.03 – < 0.06	0.06 – < 0.09	0.09 – < 0.12	0.12 – < 1.47	Per 0.06g
Number of Cases	3,202	5,949	3,517	2,007	2,420	
MV-adjusted HR*	1.	1.08 (1.03 – 1.13)	1.09 (1.03 – 1.16)	1.09 (1.03 – 1.16)	1.07 (1.01 – 1.13)	1.01 (0.99 – 1.03)
Marine fatty acids‡						
Person Years	415,533	522,297	383,048	227,483	458,209	
Range of Intakes(g/day)	0.000 – < 0.050	0.050 – < 0.087	0.088 – < 0.125	0.125 – < 0.162	0.162 – < 2.78	Per 0.1g
Number of Cases	3,427	4,415	3,358	1,897	3,998	
MV-adjusted HR*	1.	1.04 (1.00 – 1.10)	1.09 (1.03 – 1.15)	1.04 (0.98 – 1.10)	1.07 (1.02 – 1.12)	1.01 (0.99 – 1.02)
ω6:ω3						
Person Years	274,029	415,369	511,716	379,800	425,656	

	C1	C2	C3	C4	C5	Trend
Range of Intakes	1.86 – < 7.20	7.20 – < 8.20	8.20 – < 9.20	9.20 – < 10.20	10.20 – < 81.82	Per 2
Number of Cases	2,390	3,584	4,410	3,252	3,459	
MV-adjusted HR*	1.	1.02 (0.96 – 1.08)	1.01 (0.96 – 1.07)	1.02 (0.96 – 1.07)	0.96 (0.91 – 1.01)	0.98 (0.96 – 0.99)
LA:ALA						
Person Years	304,791	518,072	478,623	202,112	502,972	
Range of Intakes	1.11 – < 6.20	6.20 – < 8.70	8.70 – < 11.20	11.20 – < 12.70	12.70 – < 115.31	Per 5
Number of Cases	2,488	4,374	4,094	1,794	4,345	
MV-adjusted HR*	1.	0.99 (0.93 – 1.06)	0.98 (0.91 – 1.06)	1.00 (0.92 – 1.09)	0.96 (0.88 – 1.05)	0.97 (0.94 – 0.99)
LA: Marine fatty acids‡						
Person Years	230,260	510,237	413,104	273,297	579,672	
Range of Intakes	2.36 – < 50.00	50.00 – < 100.00	100.00 – < 150.00	150.00 – < 200.00	200.00 – <529,633	Per 100
Number of Cases	2,033	4,448	3,542	2,342	4,730	
MV-adjusted HR*	1.	1.01 (0.95 – 1.07)	1.00 (0.95 – 1.06)	1.01 (0.94 – 1.07)	0.94 (0.89 – 0.99)	1.00 (1.00 – 1.00)
Total TFA§						
Person Years	1,391,765	313,153	163,865	76,547	61,241	
Range of Intakes(g/day)	0.03 – < 5.00	5.00 – < 6.00	6.00 – < 7.00	7.00 – < 8.00	8.00 – < 27.43	Per 2g
Number of Cases	11,834	2,700	1,401	627	533	
MV-adjusted HR*	1.	1.01 (0.97 – 1.06)	1.01 (0.95 – 1.07)	0.94 (0.86 – 1.02)	0.99 (0.90 – 1.08)	0.99 (0.97 – 1.01)
TFA 16:1						
Person Years	899,055	482,564	268,095	153,718	203,138	
Range of Intakes(g/day)	0.00 – < 0.04	0.04 – < 0.06	0.06 – < 0.08	0.08 – < 0.10	0.10 – < 0.72	Per 0.04g
Number of Cases	7,794	4,046	2,245	1,341	1,669	
MV-adjusted HR*	1.	1.01 (0.97 – 1.06)	1.02 (0.97 – 1.07)	1.06 (1.00 – 1.13)	0.99 (0.94 – 1.05)	1.01 (0.99 – 1.02)
TFA 18:1						
Person Years	1,452,688	304,481	143,889	61,249	44,263	
Range of Intakes(g/day)	0.03 – < 4.50	4.50 – < 5.50	5.50 – < 6.50	6.50 – < 7.50	7.50 – < 25.00	Per 2g
Number of Cases	12,364	2,606	1,233	504	388	
MV-adjusted HR*	1.	1.01 (0.96 – 1.06)	1.00 (0.94 – 1.06)	0.95 (0.87 – 1.05)	0.97 (0.87 – 1.08)	0.99 (0.97 – 1.01)
TFA 18:2						
Person Years	1,310,714	424,370	185,895	61,562	24,028	
Range of Intakes(g/day)	0.00 – < 0.52	0.52 – < 0.65	0.65 – < 0.77	0.78 – < 0.90	0.90 – < 2.34	Per 0.2g
Number of Cases	11,208	3,546	1,601	526	214	
MV-adjusted HR*	1.	0.98 (0.94 – 1.02)	1.00 (0.94 – 1.06)	0.95 (0.87 – 1.05)	0.98 (0.85 – 1.13)	0.99 (0.97 – 1.01)

*MV-adjusted HRs adjusted for: total energy intake, body mass index, family history of prostate cancer, history of diabetes, smoking history, race, physical activity, lycopene intake, and state of residence at baseline

‡ Marine fatty acids is the sum of intakes from EPA, DPA, and DHA

‡ Total TFA is the sum of intakes from TFA 16:1, TFA 18:1, and TFA 18:2

§ Nutrient intakes adjusted for total energy intake using the residual method

** All HRs and 95% CIs estimated using Cox proportional hazards models

Table 23. Hazard ratios and 95% confidence intervals for advanced prostate cancer in relation to intakes of major polyunsaturated fatty acids and trans fatty acids (TFAs) in the NIH-AARP Diet and Health Study.

	C1	C2	C3	C4	C5	Trend
AA						
Person Years	602,103	547,099	404,428	215,766	177,900	
Range of ntakes(g/day)	0.00 – < 0.08	0.08 – < 0.11	0.11 – < 0.14	0.14 – < 0.17	0.17 – < 0.66	Per 0.05g
Number of Cases	568	530	407	202	184	
MV-adjusted HR*	1.	1.03 (0.91 – 1.17)	1.08 (0.94 – 1.24)	1.04 (0.88 – 1.23)	1.18 (0.99 – 1.41)	1.04 (0.99 – 1.10)
LA						
Person Years	452,057	515,518	470,150	281,659	227,911	
Range of Intakes(g/day)	0.33 – < 9.60	9.60 – < 12.10	12.10 – < 14.60	14.60 – < 17.10	17.10 – < 54.87	Per 4g
Number of Cases	448	503	444	276	220	
MV-adjusted HR*	1.	0.95 (0.83 – 1.08)	0.89 (0.78 – 1.03)	0.96 (0.82 – 1.12)	0.91 (0.77 – 1.08)	0.99 (0.94 – 1.04)
ALA						
Person Years	657,281	444,091	349,907	221,230	274,787	
Range of Intakes(g/day)	0.03 – < 1.10	1.10 – < 1.30	1.30 – < 1.50	1.50 – < 1.70	1.70 – < 7.20	Per 0.3g
Number of Cases	605	442	358	205	281	
MV-adjusted HR*	1.	1.07 (0.94 – 1.22)	1.07 (0.93 – 1.23)	0.97 (0.82 – 1.15)	1.06 (0.91 – 1.23)	1.01 (0.98 – 1.05)
EPA						
Person Years	604,708	606,401	299,661	169,861	266,666	
Range of Intakes(g/day)	0.00 – < 0.015	0.015 – < 0.031	0.031 – < 0.047	0.048 – < 0.064	0.064 – < 1.16	Per 0.03g
Number of Cases	584	586	345	155	221	
MV-adjusted HR*	1.	1.03 (0.91 – 1.17)	1.24 (1.08 – 1.43)	0.98 (0.81 – 1.18)	0.92 (0.78 – 1.08)	0.97 (0.94 - 1.02)
DPA						
Person Years	491,213	518,832	389,406	195,880	351,966	
Range of Intakes(g/day)	0.000 – < 0.006	0.006 – < 0.012	0.012 – < 0.018	0.019 – < 0.024	0.024 – < 0.404	Per 0.01g
Number of Cases	468	525	392	180	326	
MV-adjusted HR*	1.	1.10 (0.95 – 1.27)	1.06 (0.91 – 1.23)	1.01 (0.84 – 1.22)	1.02 (0.87 – 1.19)	0.99 (0.96 – 1.03)
DHA						
Person Years	383,316	668,947	400,678	220,014	274,342	
Range of Intakes(g/day)	0.00 – < 0.03	0.03 – < 0.06	0.06 – < 0.09	0.09 – < 0.12	0.12 – < 1.47	Per 0.06g
Number of Cases	340	679	412	228	232	
MV-adjusted HR*	1.	1.19 (1.04 – 1.37)	1.25 (1.07 – 1.46)	1.23 (1.03 – 1.48)	1.02 (0.85 – 1.22)	0.98 (0.93 – 1.03)
Marine fatty acids‡						
Person Years	403,691	506,998	371,598	220,851	444,158	
Range of Intakes(g/day)	0.000 – < 0.050	0.050 – < 0.087	0.088 – < 0.125	0.125 – < 0.162	0.163 – < 2.782	Per 0.1g
Number of Cases	370	511	384	216	410	
MV-adjusted HR*	1.	1.15 (1.00 – 1.33)	1.21 (1.04 – 1.41)	1.13 (0.95 – 1.36)	1.08 (0.93 – 1.25)	0.98 (0.93 – 1.03)
ω6:ω3						

	C1	C2	C3	C4	C5	Trend
Person Years	265,768	402,890	496,621	368,315	413,702	
Range of Intakes	1.86 – < 7.20	7.20 – < 8.20	8.20 – < 9.20	9.20 – < 10.20	10.20 – < 81,82	Per 2
Number of Cases	273	378	529	337	374	
MV-adjusted HR*	1.	0.90 (0.76 – 1.06)	1.02 (0.87 – 1.19)	0.85 (0.72 – 1.01)	0.87 (0.74 – 1.02)	0.95 (0.91 – 1.00)
LA:ALA						
Person Years	296,157	502,970	464,691	195,951	487,527	
Range of Intakes	1.11 – < 6.20	6.20 – < 8.70	8.70 – < 11.20	11.20 – < 12.70	12.70 – < 115.31	Per 5
Number of Cases	268	507	469	193	454	
MV-adjusted HR*	1.	1.10 (0.91 – 1.33)	1.07 (0.86 – 1.34)	0.99 (0.76 – 1.30)	0.95 (0.73 – 1.24)	0.95 (0.88 – 1.02)
LA: Marine fatty acids‡						
Person Years	222,988	494,857	400,953	265,077	563,421	
Range of Intakes	2.36 – < 50.00	50.00 – < 100.00	100.00 – < 150.00	150.00 – < 200.00	200.00 – < 529,633	Per 100
Number of Cases	184	511	399	271	526	
MV-adjusted HR*	1.	1.17 (0.98 – 1.39)	1.16 (0.96 – 1.38)	1.17 (0.96 – 1.42)	1.01 (0.85 – 1.20)	1.00 (1.00 – 1.00)
Total TFA§						
Person Years	1,350,785	303,920	158,874	74,343	59,374	
Range of Intakes(g/day)	0.03 – < 5.00	5.00 – < 6.00	6.00 – < 7.00	7.00 – < 8.00	8.00 – < 27.43	Per 2g
Number of Cases	1,347	292	140	60	52	
MV-adjusted HR*	1.	0.96 (0.84 – 1.09)	0.84 (0.70 – 1.01)	0.82 (0.63 – 1.07)	0.79 (0.59 – 1.07)	0.95 (0.89 – 1.02)
TFA 16:1						
Person Years	872,013	468,451	260,490	148,917	197,424	
Range of Intakes(g/day)	0.00 – < 0.04	0.04 – < 0.06	0.06 – < 0.08	0.08 – < 0.10	0.10 – < 0.72	Per 0.04g
Number of Cases	849	438	267	146	191	
MV-adjusted HR*	1.	1.02 (0.90 – 1.15)	1.11 (0.96 – 1.29)	1.07 (0.88 – 1.29)	1.09 (0.92 – 1.29)	1.05 (1.00 – 1.10)
TFA 18:1						
Person Years	1,409,945	295,480	139,451	59,542	42,877	
Range of Intakes(g/day)	0.03 – < 4.50	4.50 – < 5.50	5.50 – < 6.50	6.50 – < 7.50	7.50 – < 25.00	Per 2g
Number of Cases	1,408	271	118	57	37	
MV-adjusted HR*	1.	0.89 (0.78 – 1.03)	0.84 (0.69 – 1.02)	0.94 (0.71 – 1.24)	0.75 (0.53 – 1.08)	0.94 (0.88 – 1.00)
TFA 18:2						
Person Years	1,272,013	412,000	180,250	59,723	23,309	
Range of Intakes(g/day)	0.00 – < 0.52	0.53 – < 0.65	0.65 – < 0.77	0.78 – < 0.90	0.90 – < 2.34	Per 0.2g
Number of Cases	1,258	402	152	53	26	
MV-adjusted HR*	1.	0.97 (0.86 – 1.09)	0.85 (0.71 – 1.01)	0.88 (0.66 – 1.18)	0.93 (0.60 – 1.43)	0.96 (0.91 – 1.02)

*MV-adjusted HRs adjusted for: total energy intake, body mass index, family history of prostate cancer, history of diabetes, smoking history, race, physical activity, lycopene intake, and state of residence at baseline

‡ Marine fatty acids is the sum of intakes from EPA, DPA, and DHA

‡ Total TFA is the sum of intakes from TFA 16:1, TFA 18:1, and TFA 18:2

§ Nutrient intakes adjusted for total energy intake using the residual method

** All HRs and 95% CIs estimated using Cox proportional hazards models

Table 24. Hazard ratios and 95% confidence intervals for fatal prostate cancer in relation to intakes of major polyunsaturated fatty acids and trans fatty acids (TFAs) in the NIH-AARP Diet and Health Study.

	C1	C2	C3	C4	C5	Trend
AA						
Person Years	816,263	741,372	547,486	290,663	238,504	
Range of ntakes(g/day)	0.00 – < 0.08	0.08 – < 0.11	0.11 – < 0.14	0.14 – < 0.17	0.17 – < 0.66	Per 0.05g
Number of Cases	128	127	93	38	41	
MV-adjusted HR*	1.	1.04 (0.80 – 1.35)	1.09 (0.82 – 1.44)	0.81 (0.55 – 1.20)	1.16 (0.80 – 1.70)	0.99 (0.89 – 1.11)
LA						
Person Years	611,767	697,244	636,868	380,975	307,436	
Range of Intakes(g/day)	0.33 – < 9.60	9.60 – < 12.10	12.10 – < 14.60	14.60 – < 17.10	17.10 – < 54.88	Per 4g
Number of Cases	114	99	100	59	55	
MV-adjusted HR*	1.	0.71 (0.54 – 0.95)	0.80 (0.60 – 1.06)	0.75 (0.54 – 1.05)	0.83 (0.59 – 1.18)	0.97 (0.88 – 1.07)
ALA						
Person Years	888,989	601,376	473,872	299,065	370,986	
Range of Intakes(g/day)	0.03 – < 1.10	1.10 – < 1.30	1.30 – < 1.50	1.50 – < 1.70	1.70 – < 7.19	Per 0.3g
Number of Cases	144	90	83	41	69	
MV-adjusted HR*	1.	0.89 (0.67 – 1.17)	0.97 (0.73 – 1.30)	0.72 (0.49 – 1.04)	1.02 (0.75 – 1.38)	0.99 (0.92 – 1.07)
EPA						
Person Years	813,253	820,951	406,527	230,385	363,173	
Range of Intakes(g/day)	0.000 – < 0.015	0.015 – < 0.031	0.031 – < 0.047	0.048 – < 0.064	0.064 – < 1.16	Per 0.03g
Number of Cases	170	120	60	35	42	
MV-adjusted HR*	1.	0.70 (0.55 – 0.91)	0.72 (0.53 – 0.99)	0.86 (0.59 – 1.25)	0.65 (0.45 – 0.92)	0.89 (0.80 – 0.98)
DPA						
Person Years	660,379	702,301	527,965	266,047	477,596	
Range of Intakes(g/day)	0.000 – < 0.006	0.006 – < 0.012	0.012 – < 0.019	0.019 – < 0.024	0.024 – < 0.404	Per 0.01g
Number of Cases	138	121	68	27	73	
MV-adjusted HR*	1.	0.89 (0.67 – 1.19)	0.65 (0.50 – 0.90)	0.53 (0.33 – 0.83)	0.83 (0.60 – 1.13)	0.92 (0.84 – 1.00)
DHA						
Person Years	515,676	905,048	542,097	299,051	372,417	
Range of Intakes(g/day)	0.00 – < 0.03	0.03 – < 0.06	0.06 – < 0.09	0.09 – < 0.12	0.12 – < 1.47	Per 0.06g
Number of Cases	89	167	76	50	45	
AA-adjusted HR	1.	1.16 (0.88 – 1.53)	0.89 (0.64 – 1.24)	1.10 (0.76 – 1.60)	0.86 (0.58 – 1.25)	0.87 (0.77 – 0.99)
Marine fatty acids‡						
Person Years	543,029	685,169	503,853	298,486	603,751	
Range of Intakes(g/day)	0.000 – < 0.050	0.050 – < 0.088	0.088 – < 0.125	0.125 – < 0.162	0.163 – < 2.782	Per 0.1g
Number of Cases	102	137	62	47	79	
MV-adjusted HR*	1.	1.21 (0.92 – 1.58)	0.72 (0.51 – 1.01)	0.86 (0.59 – 1.26)	0.86 (0.62 – 1.17)	0.87 (0.78 – 0.98)
ω6:ω3						
Person Years	359,957	545,734	671,774	498,742	558,083	

	C1	C2	C3	C4	C5	Trend
Range of Intakes	1.86 – < 7.20	7.20 – < 8.20	8.20 – < 9.20	9.20 – < 10.20	10.20 – < 81,83	Per 2
Number of Cases	59	91	109	74	94	
MV-adjusted HR*	1.	1.08 (0.76 – 1.52)	1.01 (0.72 – 1.41)	0.79 (0.54 – 1.15)	1.09 (0.77 – 1.54)	0.99 (0.90 – 1.09)
LA:ALA						
Person Years	398,116	680,391	629,530	266,177	660,075	
Range of Intakes	1.11 – < 6.20	6.20 – < 8.70	8.70 – < 11.20	11.20 – < 12.70	12.70 – < 115.32	Per 5
Number of Cases	79	108	100	40	100	
MV-adjusted HR*	1.	0.78 (0.54 – 1.14)	0.70 (0.44 – 1.09)	0.64 (0.37 – 1.11)	0.59 (0.34 – 1.02)	1.01 (0.86 – 1.18)
LA: Marine fatty acids‡						
Person Years	303,549	671,266	542,894	358,376	758,204	
Range of Intakes	2.36 – < 50.00	50.00 – < 100.00	100.00 – < 150.00	150.00 – < 200.00	200.00 – < 529,587	Per 100
Number of Cases	34	104	81	59	149	
MV-adjusted HR*	1.	1.23 (0.82 – 1.84)	1.17 (0.77 – 1.78)	1.33 (0.86 – 2.05)	1.43 (0.97 – 2.11)	1.00 (1.00 – 1.00)
Total TFA§						
Person Years	1,828,881	411,170	214,454	99,929	79,854	
Range of Intakes(g/day)	0.03 – < 5.00	5.00 – < 6.00	6.00 – < 7.00	7.00 – < 8.00	8.00 – < 27.43	Per 2g
Number of Cases	290	66	37	16	18	
MV-adjusted HR*	1.	0.92 (0.69 – 1.23)	0.93 (0.64 – 1.35)	0.97 (0.58 – 1.61)	1.10 (0.65 – 1.85)	0.97 (0.86 – 1.09)
TFA 16:1						
Person Years	1,182,603	633,242	351,392	201,360	265,691	
Range of Intakes(g/day)	0.00 – < 0.04	0.04 – < 0.06	0.06 – < 0.08	0.08 – < 0.10	0.10 – < 0.72	Per 0.04g
Number of Cases	173	104	61	40	49	
MV-adjusted HR*	1.	1.12 (0.86 – 1.44)	1.12 (0.82 – 1.53)	1.23 (0.85 – 1.79)	1.19 (0.85 – 1.67)	1.07 (0.97 – 1.18)
TFA 18:1						
Person Years	1,908,712	399,809	188,137	79,992	57,638	
Range of Intakes(g/day)	0.03 – < 4.50	4.50 – < 5.50	5.50 – < 6.50	6.50 – < 7.50	7.50 – < 25.00	Per 2g
Number of Cases	304	63	31	18	11	
MV-adjusted HR*	1.	0.89 (0.66 – 1.19)	0.96 (0.66 – 1.42)	1.28 (0.78 – 2.10)	0.90 (0.46 – 1.76)	0.96 (0.84 – 1.10)
TFA 18:2						
Person Years	1,723,622	555,660	243,385	80,357	31,265	
Range of Intakes(g/day)	0.00 – < 0.52	0.53 – < 0.65	0.5 – < 0.77	0.78 – < 0.90	0.90 – < 2.34	Per 0.2g
Number of Cases	267	90	44	21	5	
MV-adjusted HR*	1.	0.93 (0.72 – 1.20)	1.06 (0.76 – 1.48)	1.40 (0.87 – 2.25)	0.71 (0.26 – 1.92)	0.99 (0.88 – 1.12)

* MV-adjusted HRs adjusted for: total energy intake, body mass index, family history of prostate cancer, history of diabetes, smoking history, race, physical activity, lycopene intake, and state of residence at baseline

† Marine fatty acids is the sum of intakes from EPA, DPA, and DHA

‡ Total TFA is the sum of intakes from TFA 16:1, TFA 18:1, and TFA 18:2

§ Nutrient intakes adjusted for total energy intake using the residual method

** All HRs and 95% CIs estimated using Cox proportional hazards models

Appendix 2

Figures

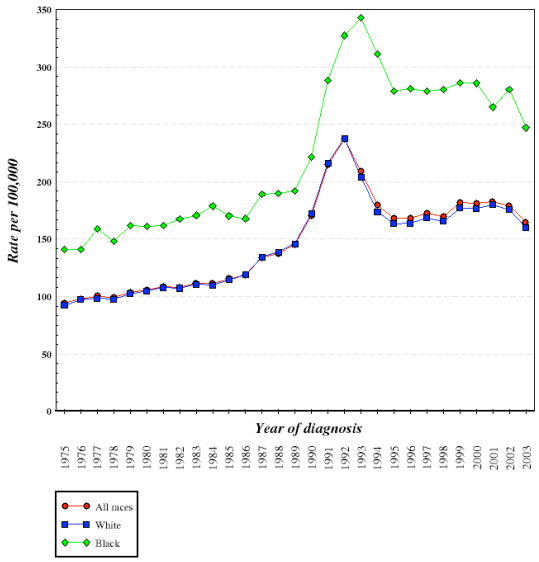


Figure 1. Prostate cancer incidence in the United States from 1975-2003 (from (3)).

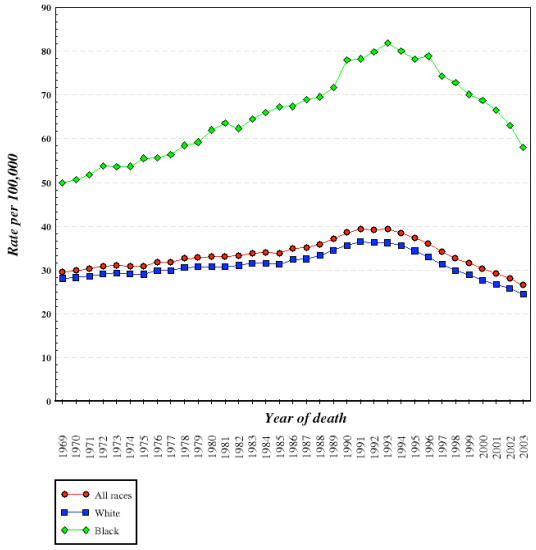


Figure 2. Prostate cancer mortality in the United States from 1975 – 2003 (from(4))

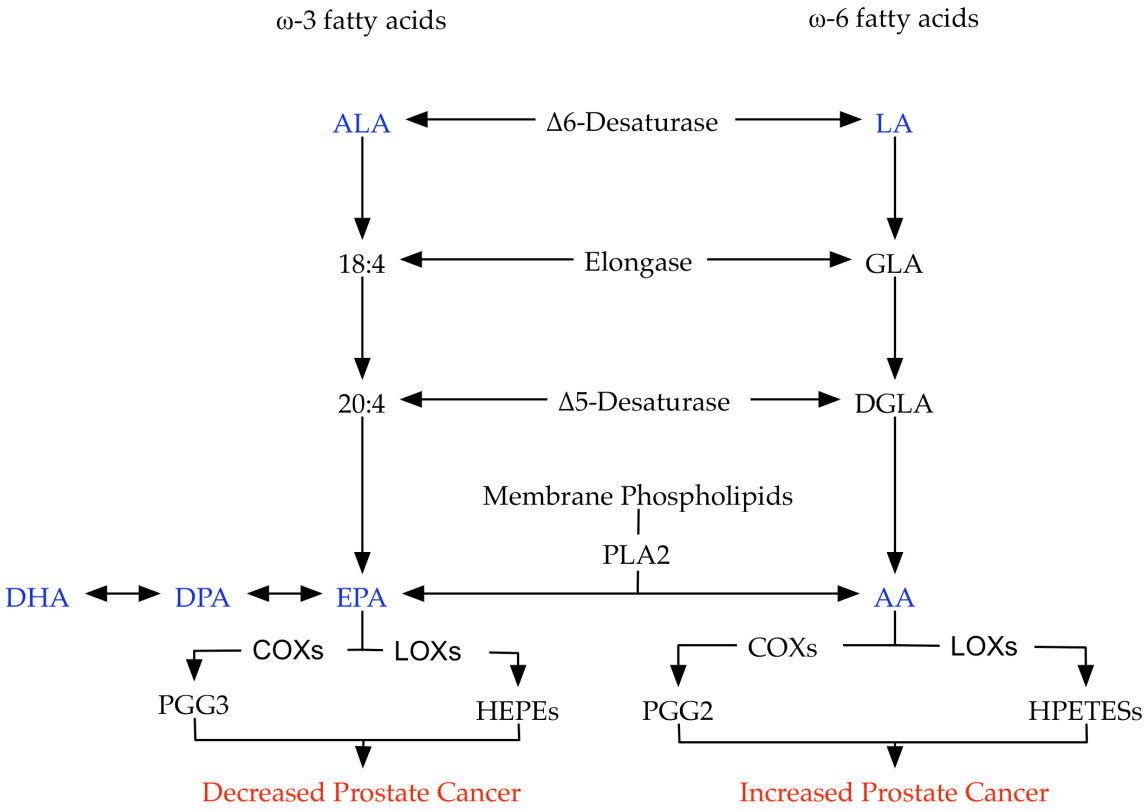


Figure 3. Mechanistic model for relations between polyunsaturated fatty acids and prostate carcinogenesis (adapted from (19)).

Abbreviations: AA – Arachidonic Acid, ALA - α-Linolenic Acid, COX – Cyclooxygenase, DGLA – Dihomo-γ-Linolenic Acid, DHA – Docosahexaenoic Acid, DPA – Docosapentaenoic Acid, GLA – γ-Linolenic Acid, HEPE – Hydroxyeicosapentaenoic Acid, HPETE – Hydroperoxyeicsoatetraenoic Acid, LOX – Lipoxygenase, PGG – Prostaglandin G, PLA_2 – Phospholipase A_2.

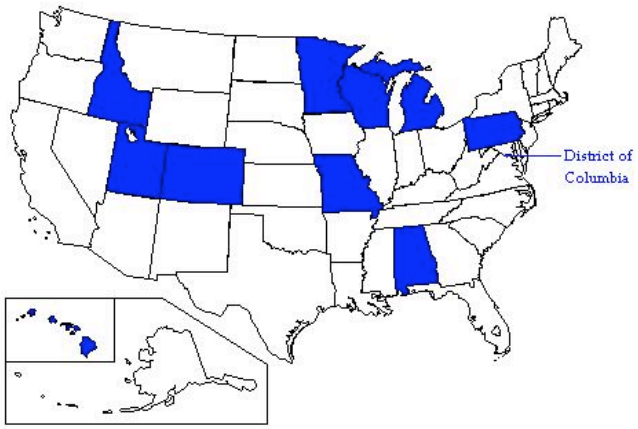


Figure 4. States represented in the PLCO Cancer Screening Trial.

Figure 5. States represented in the NIH-AARP Diet and Health Study.

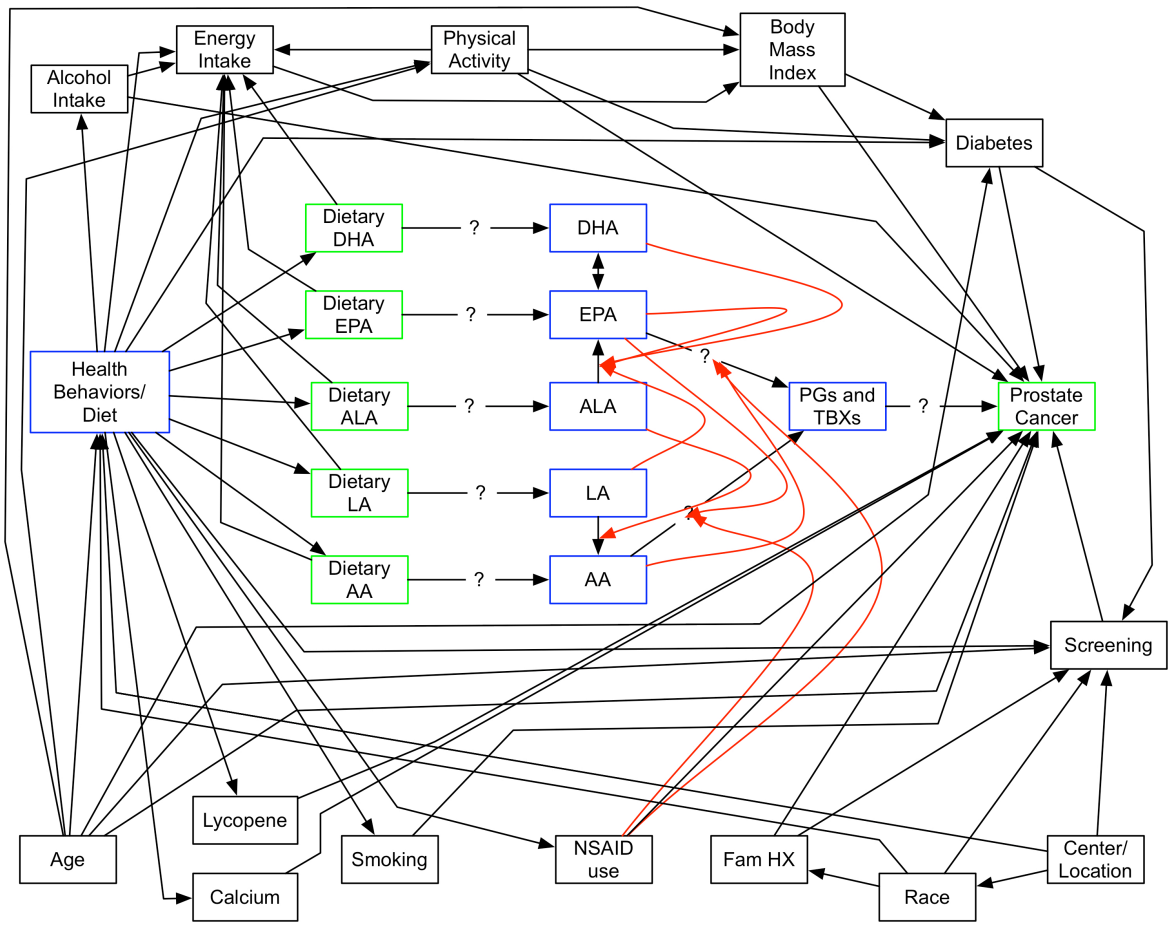


Figure 6. Conceptual diagram, sharing features of a Directed Acyclic Graph, for the associations between dietary intakes of major polyunsaturated fatty acids and prostate cancer.

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