Prostate Cancer and Alpha-Linolenic Acid

by

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Abstract

The objectives were to 1) conduct a meta-analysis to assess the association between alpha-linolenic acid (ALA) and prostate cancer; 2) analyze a trial of ALA on coronary heart disease with PSA as a post hoc outcome; 3) assess the effect of trial serum and also ALA directly on LNCaP cell growth. 1) The ALA meta-analysis of prospective and case-control studies showed no overall effect on prostate cancer. However, removal of one study from the analysis of prospective studies changed the result to a significant protective effect (RR=0.91; 95%CI:0.83,0.99). 2) No significant treatment difference was seen in the change in PSA in the randomized controlled trial. 3) The ALA treatment serum from the clinical trial did not affect LNCaP cell growth. However, ALA decreased LNCaP cell growth in a dose dependent manner when added to cell culture. The results provide no positive evidence for an effect of ALA on prostate cancer.

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13-HODE – 13-hydroxy-octadecadienoic acid
AF – Atrial Fibrillation
ALA – Alpha-Linolenic Acid
ATBC – Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study
BMI – Body Mass Index
CHD – Coronary Heart Disease
CI – Confidence Interval
CV – Coefficient of Variation
DHA – Docosahexaenoic Acid
DHT – Dihydrotestosterone
DMAB – 3,2’-dimethyl-4-aminobiphenyl
DRI – Dietary Reference Intakes
EPA – Eicosapentaenoic Acid
FBS – Fetal Bovine Serum
FFQ – Food Frequency Questionnaire
ITT – Intent-To-Treat
LA – Linoleic Acid
mRNA – Messenger Ribonucleic Acid
MTS - [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt]
OR – Odds Ratio
PLCO – Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial
PSA – Prostate-Specific Antigen
PUFA – Polyunsaturated Fatty Acid
RR – Relative Risk
SD – Standard Deviation
SE – Standard Error
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1. Introduction
1 Introduction

There are divergent health views on alpha-linolenic acid (ALA). Numerous epidemiological [1-6] and clinical studies [7-9] have shown that ALA is associated with a reduction in coronary heart disease (CHD) incidence and heart disease mortality. However, ALA has also been associated with an increased risk of prostate cancer [10-17]. The meta-analysis by Brouwer et al. [10] demonstrates this dichotomy, reporting that increasing intake of ALA by 1.2 g/d decreases the risk of fatal CHD by at least 20%, but also that high ALA intake increases the risk of prostate cancer by 70%. This alarming result demonstrated the need to weigh any favourable effects of ALA on CHD against its possible adverse effects on prostate cancer.

Several studies have investigated the relationship between ALA and prostate cancer risk, however, the results from these studies are both controversial and conflicting and range from a positive [10-18] to a not significant [19, 20] to a negative association [21, 22]. A meta-analysis by Simon et al. [23] illustrates this inconsistency when they considered 16 epidemiological studies taken together and showed that when compared with men in the lowest ALA quantile, men in the highest ALA quantile had approximately 20% increased risk of prostate cancer, but with evidence of significant heterogeneity. After performing subgroup analyses on the basis of differences in study design, studies that used dietary intake estimates, showed no evidence linking ALA with increased prostate cancer risk, whereas those that used blood or tissue markers suggested a 54% increased risk. Further, results from case-control studies are more heterogeneous compared to results from prospective cohort studies. For example, comparing the highest to lowest category of ALA intake, Spanish [14] and Uruguayan [15] case-control studies both reported an over 300% risk of prostate cancer, whereas Australian [24] and Swedish [19] case-control studies found non-significant protective effects. However, results from prospective cohort studies are more homogenous with hazard ratios close to one [18, 20, 22, 25].

Despite the question of ALA’s effect on the risk of prostate cancer, no randomized controlled trials have been undertaken to specifically assess the effect of ALA on prostate cancer development or markers of risk. All the data incriminating ALA have come from cross sectional, cohort, or case-control studies [10, 26], where red meat, margarine, and creamy salad dressings have been some of the chief sources of ALA. As a result, the question remains of whether ALA is responsible for the increased prostate cancer risk, or whether an unidentified aspect of lifestyle associated with ALA consumption is to blame, and that dietary or serum ALA are simply
markers. Attar-Bashi et al. [26] indicate in their meta-analysis the need for future research to include dietary intervention studies of patients with prostate cancer to investigate the effects of a diet rich in ALA on prostate cancer biomarkers. An ALA-enriched diet, such as the Mediterranean heart-healthy diet used in the Lyon-Diet-Heart Study [7], would be a appropriate intervention for such a trial. The Lyon-Diet-Heart Study [27] demonstrated that vegetable ω-3 fatty acid (ALA) derived from canola margarine and oil reduced fatal and non-fatal cardiac events. Testing a similar diet would be advantageous. A biomarker for prostate cancer that would be suitable to analyze in this dietary intervention would be prostate specific antigen (PSA), a protein produced by the prostate gland that is elevated in the presence of prostate cancer. Currently, PSA testing of the blood is the most effective test available for the early detection of prostate cancer and a PSA greater than 4 ng/mL is generally considered abnormal [28]. Like cholesterol level, however, PSA also may have a predictive value to indicate those at risk for a later diagnosis of clinical cancer [29-34].

In addition to no randomized controlled trials, there are a lack of in vitro studies assessing the association of ALA and prostate cancer. In vitro studies provide the potential for assessing the total impact of a diet on cancer promotion or inhibition in short term studies. It is known that diet and lifestyle can significantly alter the constituents of serum and consequently the cellular environment. These inter-individual differences in serum profile have been shown to be reflected in cell growth when sera from different individuals were incubated with various cancer cell lines, conceptually suggesting an in vitro approach to assessing the impact of diet and dietary components [35, 36]. This approach has produced interesting results where in vivo and in vitro data are mutually supportive. Ornish et al. [36] studied the effects of intensive lifestyle changes in prostate cancer patients (diet, exercise, stress management, and support) on PSA and LNCaP (human Caucasian prostate carcinoma) cell growth incubated with participants’ serum over the length of the trial. They found that the healthier lifestyle changes in prostate cancer in the intervention group were associated with a significant decrease both in serum PSA and on LNCaP cell growth. This methodology can be of use in examining the association between ALA and prostate cancer by analyzing the effect of serum from individuals on an ALA-enriched diet on prostate cancer cells.

Since most of the available evidence on the influence of ALA on prostate cancer comes from observational studies and the results are very heterogeneous, a broader approach to ALA’s
involvement in prostate cancer needs to be undertaken in order to produce a more complete picture of the relationship between ALA and prostate cancer. In the present study, the association between ALA and prostate cancer has been investigated in three ways: 1) through a meta-analysis to assess the evidence for an association between dietary ALA intake and prostate cancer risk from observational studies, which has not been assessed specifically in the field as of yet, 2) through analyses of a randomized controlled trial of ALA on CHD with PSA as a post hoc outcome, and 3) through cell culture studies to a) determine whether serum from the ALA trial subjects stimulate LNCaP cell growth more than serum from control subjects, b) compare the effect of C18 fatty acids on LNCaP cell growth, and c) determine if there is an ALA dose response.
2. Literature Review
2.1 Role of Diet in Prostate Cancer

Prostate cancer is the second most common cancer in men in the world [37] and the third leading cause of cancer death amongst Canadian men [38]. Prostate cancer incidence rates vary widely between countries, populations, and races. While prostate cancer has an important genetic component, twin studies have shown that environmental factors contribute 58% to the risk of developing prostate cancer [39]. The lowest rates are found in Asian countries (China, India, Japan) and the highest rates in Northern Europe and North America [40]. North Americans have the highest incidence of prostate cancer, with rates that are almost eighty times higher when compared to the Chinese [37]. The large difference in prostate cancer incidence rates between Asians and Caucasians have led to many migration and ecologic studies, which have provided strong evidence for the role of environmental factors, such as diet, in the etiology of prostate cancer [41-50]. Dunn et al. [44] first reported that the risk of developing prostate cancer in successive generations of early Japanese migrants to the United States gradually approaches that of white men. Further, the work of Sim et al. [41] showed that with an increase in westernization between the periods 1978-1982 and 1993-1997, incidence and mortality rates of prostate cancer have significantly increased in Asian countries. Historically, dietary factors that have been cited as possible contributing factors to the low incidence in Asians included low dietary fat, isoflavonoids in soybeans, polyphenols in green tea, lycopene, selenium, and vitamin E [41], but increasing incidence and mortality rates indicate that many Asian countries may be losing their protective cultural factors and acquiring high-risk ones. With respect to dietary fat intake, the case-control study by Lee et al. [51] illustrates this suggestion, finding that from interviewing 133 cases and 265 controls in 12 cities in China, daily fat intake and percentage energy from fat were 3.6 times higher in cases than controls. Since 1975, when Armstrong and Doll first hypothesized that there was an association between dietary fat and death from prostate cancer [48], many studies have examined this connection. However, the evidence is inconsistent and conflicting [52-55], with some studies suggesting a positive association between fat consumption and prostate cancer [52, 53], and others finding no support for such an association [54, 55], perhaps indicating a need for further investigation into specific types of fat.
2.1.1 Saturated Fat

Epidemiological studies suggest that saturated fat may have a role in the development of advanced prostate cancer [11, 12, 53, 56-59]. In a case-control study, West et al. [53] reported a 2-fold risk of aggressive cancer with increased saturated fat intake. They hypothesized that saturated fat may have a role in the promotion of prostate cancer by stimulating the progression of occult tumour foci whose numbers increase with age. The analysis of Slattery et al. [56] comparing the association between saturated fats consumed during adolescence and adulthood with aggressive prostate cancer seems to corroborate this hypothesis, since only saturated fat consumption in adulthood was associated with an increased risk of aggressive prostate cancer. Bairati et al. [59] found a similar 2-fold increased risk of advanced prostate cancer among those in the highest quartile of saturated fat intake and further determined that the relationship between saturated fat intake and advanced prostate cancer was dose dependent. Meat consumption, (particularly red meat) as an indicator of saturated fat intake, has also been associated with an increased risk of advanced [11, 58] and fatal prostate cancer [60]. However, there are inconsistencies since other epidemiological studies suggest a weaker association between saturated fat and prostate cancer risk [19] and some suggest no association at all [61, 62].

2.1.2 Polyunsaturated Fatty Acids

The influence of omega-6 and omega-3 polyunsaturated fatty acids (PUFA) on cancer has been the subject of much research, both epidemiological and experimental [63-68]. Experimental studies reporting the effects of PUFA on cancer cells and animal models of cancer suggest that omega-3 fatty acids, especially long-chain omega-3 fatty acids, are potentially protective against several cancers, whereas in contrast, omega-6 fatty acids tend to favour cancer development.

2.1.2.1 Omega-6 Fatty Acids

Linoleic acid (LA) is the parent fatty acid of the omega-6 fatty acid series and the precursor for the biosynthesis of arachidonic acid. A cross-national ecological study in 20 countries [69] showed a non-significant positive relation between prostate cancer incidence and omega-6 fatty acid intake. However, another study [70] found no such relation between omega-6 fatty acid adipose tissue content and prostate cancer incidence in 11 centres from eight countries in Europe. In a case-control study in Sweden, Hedelin et al. [71] reported that omega-6 fatty acid
intake was significantly associated with a 36% increased risk of prostate cancer risk in the highest when compared to the lowest quartile of intake. Further, they found in separate analyses of LA and arachidonic acid, only high intake of LA was associated with an increased risk of prostate cancer and contributed the most to the positive association between omega-6 fatty acids and prostate cancer risk. In addition, three other case-control studies (two in the United States and one in Greece) [16, 17, 72] also observed a significant positive association of LA with prostate cancer risk, but 10 other studies [11-15, 19, 22, 73, 74] based either on biomarkers or on a dietary questionnaire, of which all were prospective studies [11-13, 22, 25, 74], did not find any significant association. Four biomarker-based studies, one case-control [17] and three nested case-controls [12, 13, 74], did not report any association of arachidonic acid level with prostate cancer risk.

Investigating the in vitro effects of omega-6 fatty acids on the growth of human prostatic cell lines has also produced contradictory results. At concentrations varying from 0.003 to 25 µM, LA and arachidonic acid have been shown to increase the growth of the androgen-insensitive line PC-3 [75-78], but using the androgen-insensitive DU-145 cell line, cell growth was inhibited by omega-6 fatty acids (LA, 18:3n-6, and arachidonic acid) at concentrations up to 8 µM [75, 79]. Further, LA was shown to have no effect on androgen-sensitive LNCaP cells [76], but arachidonic acid stimulated LNCaP cell growth at concentrations up to 5 µM [77]. There is therefore no clear evidence of an association of omega-6 fatty acids with prostate cancer risk.

### 2.1.2.2 Omega-3 Fatty Acids

Omega-3 fatty acids can be subdivided into ALA and long-chain omega-3 fatty acids, mainly from animal foods (fish, meat, poultry, eggs, and seafood), with the most predominant fatty acids being eicosapentaenoic acid (EPA) and docosahexaenoic (DHA).

#### 2.1.2.2.1 Long-Chain Omega-3 Fatty Acids

Long chain omega-3 fatty acids, such as EPA and DHA can be biosynthesized by ALA, but the bioconversion is much less active than in other species [80], so that the majority of long-chain omega-3 fatty acids in blood and tissues is attributable to the dietary supply, rather than by their biosynthesis from ALA. Fish and seafood, especially fatty fish, are by far the main dietary
sources of long-chain omega-3 fatty acids. In epidemiological studies, dietary omega-3 long-chain fatty acids are highly correlated with the intake of fish, so that fish intake can be a proxy for omega-3 fatty acids intake and vice versa. Further, long-chain omega-3 fatty acids in blood fractions or in adipose tissue are well correlated with fish intake [81, 82].

Ecological studies have investigated the relation between prostate cancer incidence or mortality and fish consumption and found either no relation [83] or a significant protective relation [46]. The latter result is consistent with a study by Kobayashi et al. [84] that showed a negative relation between prostate cancer mortality and serum omega-3 fatty acids (mainly EPA and DHA) in Japanese men from five cities in Japan and Brazil. However, case-control studies also report inconsistent results. In a Japanese study [85], regular fish consumers were found to have a 57% lower risk of prostate cancer than those who ate fish ‘never or only occasionally’ and in studies from Italy [86, 87] and United Kingdom [88], a non-significant decrease for prostate cancer risk was found in fish consumers, with odds ratios (OR) ranging from 0.7 to 0.8. Conversely, one study in Uruguay [89] found no significant association between prostate cancer and fish consumption and similarly, a large study in United States [90] found no association between long-chain omega-3 fatty acids intake and prostate cancer incidence, including advanced prostate cancer incidence.

Prospective studies report an even wider range of results with two earlier studies finding an increased risk of prostate cancer with fish intake, significantly in Seventh-day Adventists [91], and not significantly in men from Japanese ancestry in Hawaii [92]. However, no association was found between fish or long-chain omega-3 fatty acids consumption and prostate cancer risk, including advanced prostate cancer, in the Netherlands Cohort Study [22, 93], and this result is corroborated by three nested case-control studies that did not find any significant association between EPA and/or DHA blood levels and prostate cancer risk [12, 13, 74]. In contrast, two cohort studies [94, 95] reported a negative association of fish consumption with prostate cancer. Terry et al. [94] observed that after more than 20 years of follow-up, a significant reduction of prostate cancer risk (Relative Risk (RR)=0.43, \( P=0.05 \)) and an even stronger reduction of prostate cancer death (\( RR=0.27, \ P=0.01 \)). Further, although Augustsson et al. [95] found no association between fish intake and cases of prostate cancer in the Health Professional’s Follow-Up Study, they found a non-significant decreased risk of advanced prostate cancer and a significant decreased risk of metastatic prostate cancer.
In a meta-analysis by Brouwer [96], 8 observational studies (5 prospective, 3 case-control) on EPA intake or blood concentrations and prostate cancer were analyzed as well as 7 observational studies (4 prospective, 3 case-control) on DHA intake or blood concentrations and prostate cancer. The combined estimates of risk of prostate cancer for men with a high intake or status of EPA and DHA suggest a protective, but not significant association.

The in vitro effects of omega-3 fatty acids on the growth of human prostatic cell lines varies depending on the cell lines used. Using the androgen-insensitive PC-3 cell line, at concentrations varying from 0.003 to 25 µM, EPA and DHA inhibited cell growth [75, 76, 78]. Conversely, using the androgen-sensitive LNCaP cell line, Pandalai et al. [76] found low concentrations of EPA to be slightly stimulatory in 0.5% fetal bovine serum (FBS) medium. However, in the same cell line, Chung et al. [97] found that at high concentrations (100-200 µM), EPA and DHA inhibited androgen-stimulated cell growth and PSA protein expression. Therefore, long-chain omega-3 fatty acids show potential protective effects on prostate cancer risk, however, there are some discrepancies in the literature, indicating the need for further research.

2.1.2.2.2 Alpha-Linolenic Acid

Unlike the long-chain omega-3 fatty acids, EPA and DHA, that have typically been associated with a decreased risk of prostate cancer [98], ALA has been reported, albeit inconsistently, to be associated with an increased risk of prostate cancer [10]. ALA is of particular importance since it is an essential fatty acid and accounts for approximately 85 to 94% of the total omega-3 fatty acid dietary intake [99]. The dietary reference intake (DRI) range for ALA is 1.1 to 1.6 g/d [100]. The concentration of ALA in phospholipids in plasma, cells and tissues is typically less than 0.5% of total fatty acids and is about 0.7% in adipose tissue [101]. ALA is the parent fatty acid of omega-3 fatty acids and can be converted into EPA and DHA through a desaturation and elongation pathway [101]. However, the extent of conversion is low, with approximately 8% of ALA converted to EPA [102, 103] and less than 0.05% converted to DHA [104]. An advantage of ALA over the long-chain omega-3 fatty acids from fish is that it is easier to incorporate into food products because it does not have the pronounced smell and taste of fish oil. Further, replacing long-chain omega-3 fatty acids from fish with ALA-rich vegetable sources, such as flaxseed, perilla, chia seed, canola, green leafy vegetables, beans (soybeans,
navy beans), and nuts (walnuts), would prevent further depletion of the already low stocks of edible fish in the ocean [105]. To shed light on the nature of this possible adverse relationship, many different lines of evidence have been explored to elucidate the effect of ALA on prostate cancer.

### 2.1.2.2.2.1 Meta-Analyses

Since the advent of the hypothesis of an adverse relationship between ALA and prostate cancer, a number of meta-analyses have been undertaken to examine the data on this relationship [10, 23, 96, 106]. The most recent of these analyzes the data from prospective studies concerned with dietary ALA intake and prostate cancer risk [106]. In comparing the highest with the lowest ALA intake, Carayol et al. [106] found a non-significant protective association between ALA intake and prostate cancer risk (pooled RR=0.97; 95%CI: 0.86-1.10), but significant heterogeneity, which was principally due to one study by Giovannucci et al. [18]. Upon exclusion of this study, the protective effect of ALA intake became significant without heterogeneity. Further, choosing a cut-point value of 1.5 g/d ALA, Carayol et al. [106] found the results supported a significant protective association between a consumption of ALA greater than 1.5 g/d and prostate cancer risk. Carayol et al. [106] also explored publication bias considering both case-control and prospective studies and determined there was an absence of prospective studies with small numbers of subjects and that published studies all have hazard ratios close to 1. They also found an absence of case-control studies with both small numbers of subjects and small or null effects, which Carayol et al. [106] suggest may reflect a bias that studies reporting strong and significant associations are easier to publish than non-significant associations in small case-control studies, or that the results are biased toward larger effects due to low methodological quality of smaller case-control studies. These potential publication biases argue for caution in interpretation of the results and moderation of the strength of the conclusions since the results can be considered qualified and the combined estimate may overestimate the RR.

The meta-analysis conducted by Simon et al. [23] delved deeper into subgroup analyses to try and explain the inconsistent findings by examining data from 16 studies (8 prospective studies, 8 retrospective case-control studies) on ALA intake, blood, or adipose tissue concentrations and prostate cancer risk. In the overall analysis, the highest quantiles of ALA intake, blood, or adipose tissue concentrations were significantly associated with approximately
20% increased risk of prostate cancer (RR=1.20, 95%CI:1.01-1.43, P=0.04) [23], but with significant heterogeneity. In their subgroup analyses, Simon et al. [23] found no association between ALA concentrations and prostate cancer among the 7 older studies (published from 1993 through 1999), but found a trend toward increased risk among the 10 newer studies (published 2000 through 2007). When they examined the 8 prospective studies alone, they found no association between dietary or blood ALA concentrations and risk of prostate cancer, but results from the 8 retrospective case-control studies revealed a non-significant 51% increased risk of prostate cancer among men in the highest quantile for ALA [23]. When Simon et al. [23] analyzed the 11 studies that estimated ALA intake by using dietary assessment, they found no association between dietary ALA intake and risk of prostate cancer. However, the 6 studies that measured ALA directly by using serum fatty acids, whole blood, or adipose tissue, of which 2 were case-control studies, revealed that men in the highest ALA quantile were at a significantly increased risk of 54% (RR=1.54, 95%CI:1.16-2.06, P<0.01) for prostate cancer [23]. Further, Simon et al. [23] reported significant heterogeneity across all subgroups except the subgroup analysis of serum, whole blood, and adipose tissue.

Although the overall result of the effect of ALA on prostate cancer risk in these meta-analyses differed, with Carayol et al. [106] reporting a non-significant protective effect, and Simon et al. [23] finding an association, there was significant heterogeneity in both studies. The differences in the overall results may be attributable to two important factors: study design of the included studies, and dietary ALA versus blood and tissue ALA. These factors should be taken into consideration for future meta-analyses. These meta-analyses [23, 106] highlight the inconsistencies in the data relating ALA to prostate cancer risk.

2.1.2.2.2.2.2 Prospective Studies

2.1.2.2.2.2.2.1 Dietary Alpha-Linolenic Acid Intake

The possible adverse association between ALA and prostate cancer was initially observed by Giovannucci et al. [11] in the prospective Health Professionals Follow-Up Study after 3.5 years of follow-up. They found a positive association of ALA with prostate cancer risk (RR=1.32, 95%CI:0.82-1.92, P=0.04) and an even stronger association with advanced prostate cancer (RR=3.06, 95%CI:1.67-7.04, P=0.0005). After 16 years of follow-up, Giovannucci et al. [18] reported that higher ALA intakes were significantly associated with increased risk of
incident, fatal, advanced, and low-grade prostate cancer, with an especially strong relationship with low-grade advanced prostate cancer (RR=2.23, 95%CI:1.11-4.48, P=0.04), suggesting that ALA may stimulate prostate cancer from a latent to an invasive disease. Conversely, Schuurman et al. [22] found a non-significant decrease in prostate cancer risk with the highest intake of ALA in the Netherlands Cohort Study. Compared with men who had the lowest ALA intake, the screening arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO) [20], found that men who had the highest ALA intake were at no increased risk of total prostate cancer, organ-confined prostate cancer, invasive prostate cancer, or high-grade/aggressive prostate cancer. Additionally, when examining specific dietary sources of ALA, there was no difference in prostate cancer risk from animal-derived or plant-derived ALA. No association between dietary ALA intake and prostate cancer risk was also observed in the Multiethnic Cohort study in United States [25] and in a nested case-control study within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) cohort in Finland [74].

2.1.2.2.2.2.2 Blood Alpha-Linolenic Acid Levels

Of the five prospective studies examining the relationship between blood ALA levels and prostate cancer, two have shown an increased risk of prostate cancer with higher ALA levels: prostate cancer risk was doubled for the highest quintile of ALA in serum phospholipids (OR=2.0, P=0.03) [13] or in plasma cholesteryl ester fatty acids (OR=2.14, P=0.03) [12]. Further, in the US Physician’s Health Study, Gann et al. [12] reported prostate cancer risk as doubled already within the second quartile of plasma ALA, with the first quartile only including subjects with no detectable ALA peak, that is, less than 0.02% of plasma cholesteryl ester fatty acids. In terms of intake, Gann et al. [12] found that plasma ALA levels were significantly positively correlated with meat and dairy product intake, and similar to the prospective analysis from the Health Professionals Follow-Up Study, they found that red meat was positively associated with advanced prostate cancer, where as dairy foods were not. This corroboration suggests a correlation between ALA intake and serum ALA levels. Conversely, a nested case-control study from the ATBC cohort in Finland [74] did not find any association between prostate cancer risk and the level of ALA in serum cholesteryl esters at baseline. Two other studies, one nested case-control examining whole blood ALA [107] and one prospective cohort analyzing serum ALA [108], corroborated these results. However, the results reported by Gann
et al. and Mannisto et al. may warrant additional weight due to the biomarker used since the percentage of ALA in plasma cholesteryl esters appears to be a better biomarker of ALA intake than its percentage in plasma phospholipids or in erythrocyte fatty acids [109, 110].

### 2.1.2.2.2.3 Case-Control Studies

#### 2.1.2.2.3.1 Dietary Alpha-Linolenic Acid Intake

Two case-control studies based on a dietary questionnaire, one in Uruguay [15], the other in Spain [14], reported a significant positive association between prostate cancer risk and dietary ALA intake. This association persisted and was strengthened in both studies after adjustment for intake of other fatty acids (saturated, monounsaturated, linoleic) and red meat (OR=4.4 [15] and 3.1 [14]). Further, in the study by De Stéfani et al. [15], the positive association was observed for ALA of both vegetable and animal origin. The Swedish study by Hedelin et al. [71] supports this positive association, finding a significant trend toward higher risk with increasing intake of ALA. However, Bidoli et al. [111] found the converse, reporting a significant protective association ALA intake and prostate cancer risk (OR=0.7; 95% Confidence Interval (CI): 0.6-0.9). Two other case-control studies, one in Canada with only preclinical (localized) prostate cancer cases [73], the other in Sweden on all-stage prostate cancer, including advanced prostate cancer cases [19], did not find any association of prostate cancer risk with ALA intake. Further, comparing advanced prostate cancer cases to local prostate cancer cases, Bairati et al. [59] found no association with the intake of ALA.

#### 2.1.2.2.3.2 Blood and Tissue Alpha-Linolenic Acid Levels

Two case-control studies using ALA biomarkers, found a positive association between prostate cancer risk and the percentage of ALA in adipose tissue or in erythrocyte fatty acids [16, 17]. In the small study (89 cases, 38 controls) by Godley et al. [16], although the risk was strongly increased with the second quartile of ALA both in erythrocytes (OR=3.02) and in adipose tissue (OR=3.73), the OR only reached significance with the third quartile of ALA in adipose tissue (OR=4.31; 95%CI: 1.17-15.8). Newcomer et al. [17] found a significant association of erythrocyte ALA with prostate cancer risk (OR=2.6, P=0.01). However, since the percentage of ALA was low and its measure relatively imprecise in erythrocytes, as well as in plasma phospholipids, adipose tissue ALA may be a better marker of ALA intake [112].
Interestingly, in both these studies [16, 17], the risk of prostate cancer was also positively associated with blood or tissue linoleic acid levels, which may suggest a non-specific association of prostate cancer risk with PUFA intake, rather than a specific association with ALA [113]. Using serum ALA concentrations, Yang et al. [114] found that serum ALA levels were significantly higher (approximately 46%), among the 19 Korean men who had prostate cancer compared with 21 normal control subjects.

Conversely, Freeman et al. [21] found the opposite result in their study that examined prostatic levels of individual fatty acids in relation to histopathological characteristics of cancer in men undergoing radical prostatectomy for localized disease. The results showed that prostatic ALA levels were significantly lower in patients with advanced prostate cancer than in control subjects. Measurement of prostatic levels of fatty acids, as in this study, may offer advantages over self-reported dietary intake and plasma ALA as it provides an estimate of exposure at the target organ level, where the concentrations likely reflect long-term dietary intake [21, 26]. Thus far, only three reports [21, 115, 116] have examined human prostate fatty acid levels, with two studies [21, 115] reporting ALA levels in the prostate tissue from 0.5 to 2.7% of total fatty acids. However, a pilot study by Attar-Bashi et al. [117] did not find any significant relationship between prostatic ALA and serum ALA levels in 28 subjects undergoing exploratory surgery for prostate cancer.

2.1.2.2.2.4 Clinical Trials with Flaxseed Supplementation

To date, no clinical trial has investigated the relation between dietary intake of ALA and prostate cancer risk. However, a randomized controlled trial by Demark-Wahnefriend et al. [118] investigated the effect of dietary supplementation with flaxseed (30 g/day), which contains approximately 56% ALA [26], and fat restriction (20% of kilocalories) on PSA levels, proliferation, and other biomarkers of prostatic neoplasia. After 30 days of treatment in 161 men with prostate cancer, the results demonstrated that flaxseed had no effect on PSA levels between baseline and follow-up, but did have a significant protective effect on tumour proliferation rate as measured through biopsies. However, since flaxseed is also an exceptionally rich source of dietary lignans, possessing over 800-fold the amount in most other foods [119, 120], it is not possible to conclude whether the effect seen was due to ALA, lignans, a synergy between these two factors, or another unidentified factor.
2.1.2.2.2.5 Animal Models of Prostate Cancer

There are few studies investigating the effect of ALA or omega-3 fatty acids on animal models of prostate carcinogenesis or on prostatic tumour cell growth in animals. In studies observing the growth of DU-145 human prostatic tumour cells transplanted in athymic nude mice fed different types of lipids, it was found that tumour growth was reduced in mice fed fish oil (rich in EPA and DHA), as compared to mice fed corn oil (rich in LA) [121, 122], but not in mice fed linseed oil, containing about 50% ALA [123]. In rats, in which prostate cancer was induced by 3,2’-dimethyl-4-aminobiphenyl (DMAB) treatment alone, or by DMAB and testosterone treatment, feeding perilla oil, an ALA-rich oil, did not modify the incidence of prostate carcinoma as compared to rats fed corn oil [124].

2.1.2.2.2.6 Cell Culture Studies

The results with regard to cell culture studies are still controversial, with some studies showing ALA inhibits the growth of prostate cancer cells in vitro and others demonstrating growth promotional effects. A study by Motaung et al. [125] of androgen-insensitive DU-145 prostate cancer cells showed that there was an increase in dead cells at physiological concentrations of ALA (4-40 µM). Liu et al. [126] reported a similar result using androgen-sensitive LNCaP cells, finding that for ALA, inhibition of cell growth occurred initially at 100 µM and increased as the dose was increased. Another study investigating the effects of various PUFA on DU-145 cells and the production of urokinase-type plasminogen activator, an important protease enzyme in carcinogenesis and is involved in invasion and metastasis of cancer, found that ALA both suppressed cell proliferation and inhibited urokinase-type plasminogen activator production [127]. Since it is believed PUFA may change the physical properties of the plasma membrane and thus the permeability of the cell, the binding of steroid hormones to their receptors may be altered. Prinsloo et al. [128] studied this effect in DU-145 cells and found that ALA inhibited androgen receptor capacity to testosterone, a prostate stimulatory hormone. In contrast, Pandalai et al. [76] found that ALA exhibited growth promotional effects on human metastatic PC-3, LNCaP, and TSU prostate cell lines, the rat metastatic May-Ly-Lu cell line, and rat nonmetastatic epithelial EPYP1 cell line. Specifically, this study found that the growth of LNCaP cells was stimulated by 25% by very low concentrations of ALA (0.003-0.33 µM) in 10% FBS [76]. Pandalai et al. [76] further showed
that this proliferative effect of ALA was strikingly enhanced, with up to 800% increase in LNCaP cell growth, when the FBS content of the culture medium was reduced to 0.5%. The apparently inconsistent results of the effect of ALA on prostate cancer cell growth may perhaps be attributable to differences in cell lines and differences in growth conditions of cells in culture, including the concentration of ALA and the percentage of serum in the cell culture medium.

**2.1.2.2.7 Possible Mechanisms for Alpha-Linolenic Acid Promotion of Prostate Cancer**

There has been little work on the mechanism by which ALA promotes prostate cancer, so currently, only hypotheses exist. Studies in humans and in animals show that ALA is subject to extensive β-oxidation [129] and that it is also deposited in the adipose tissue and skin in animals [129]. Hydroxy PUFA derivatives, such as 13-hydroxy-octadecadienoic acid (13-HODE), are produced in proliferating cells in mammals from LA via a lipoxygenase reaction [130]. Perhaps ALA competes with LA, leading to a reduction in 13-HODE production [131], which has been suggested, along with other hydroxy-PUFA, to be related to the metastatic potential of cells through modulation of endothelial cell behavior as well as by inducing cell adhesion molecule expression by itself [132]. It is also possible that ALA is metabolized to hydroxyl fatty acids in mammals, since in plants, ALA is extensively metabolized by 15-lipoxygenase to a variety of compounds [129]. Other proposed mechanisms to explain the possible effect of ALA in prostatic carcinogenesis include interference with 5α-reductase and formation of free radicals from fatty acid oxidation [11, 12]. Although the biological mechanisms for ALA are not yet characterized, it is known that ALA can be converted to EPA and DHA [101-104], long-chain omega-3 fatty acids also postulated to affect prostate cancer risk [113]. Larsson et al. [133] published a review on potential mechanisms whereby omega-3 fatty acids affect carcinogenesis. Suggested mechanisms include: 1) suppression of arachidonic acid derived eicosanoid biosynthesis, which have proinflammatory effects and may promote carcinogenesis, resulting in altered immune response to cancer cells and modulation of inflammation, cell proliferation, apoptosis, metastasis, and angiogenesis; 2) influence on transcription factor activity, gene expression, and signal transduction, which leads to changes in metabolism, cell growth, and differentiation; 3) alteration of estrogen metabolism, which leads to reduced estrogen-stimulated cell growth; 4)
increased or decreased production of free radicals and reactive oxygen species; and 5) mechanisms involving insulin sensitivity and membrane fluidity [133].

2.2 Prostate-Specific Antigen as a Predictor of Prostate Cancer

Current prostate cancer screening is prostate-specific antigen (PSA) based [28]. Its value as a possible tumour marker was first described in 1979 by Wang et al. [134]. The US Food and Drug Administration approved PSA as a marker to monitor patients treated for prostate cancer in 1986 and as a diagnostic marker in 1994 [135]. In 1984, 5.1% of all newly diagnosed prostate cancer cases were detected by PSA testing. By 1990, this percentage had already increased to 60.6% of the prostate cancer diagnosed in the United States [136]. PSA has also been used to monitor prostate cancer treatment outcome. However, PSA levels can be raised not only in prostate cancer, but also in nonmalignant conditions such as benign prostatic hyperplasia, infection, or chronic inflammation [137, 138]. With the increasing use of PSA screening, increasing controversy has been generated, with some believing that PSA screening had led to diagnosis and treatment of prostate cancers that pose no real threat without reducing prostate cancer mortality. Further, there is also disagreement regarding the PSA threshold level that should indicate biopsy [28].

2.2.1 Biology of Prostate-Specific Antigen

PSA transcription is regulated by androgens and is produced almost exclusively by prostate epithelial cells. It is released into seminal fluid with PSA concentrations ranging from approximately 0.3 to 3 ng/mL (10 to 100 µmol/L). The majority of PSA is an active serine protease with chymotrypsin-like activity and it proteolyses the gel proteins of seminal fluid thereby liquefying it [28].

Normally, the prostate keeps PSA tightly confined so that only a minute amount leaks into the blood. In healthy adult males 50 years of age or younger, the concentrations are 106 times higher in seminal fluid than in blood, in which the median PSA level is approximately 0.6 ng/mL [139]. PSA levels in the blood span about a 105-fold range, from less than 0.1 to 104 ng/mL, with levels above 102 ng/mL found almost exclusively in men with advanced prostate cancer. The increased blood PSA levels are caused by an increased release of PSA into the blood, rather than increased PSA expression. It is believed that the increased release is a result of
disruption of prostate structure seen in prostate tumours, such as dis-ordering of the basement membrane and loss of basal cell layer, ductal lumen architecture, and epithelial cell polarity [28].

2.2.2 Effectiveness of Prostate-Specific Antigen Testing in Predicting Prostate Cancer

The introduction of PSA-based screening has led to a sharp increase in the incidence of prostate cancer due to a shift towards diagnosis at earlier stages, with arguably considerable overdiagnosis, that is, men diagnosed with prostate cancer whose cancer would never have affected their lives if they had not had a PSA test. However, a decline of 30% in prostate cancer mortality rate was observed in the United States during the 1990’s [28] and on the basis of mathematical modeling, Etzioni et al. [140] reported that 45-70% of the observed decline in prostate cancer mortality could be attributed to the decreased proportion of men with advanced stage disease at diagnosis, as a result of PSA screening.

Several studies have shown that PSA has predictive value in indicating the risk of prostate cancer years, or even decades, before diagnosis. A prospective study by Gann et al. reported a lead time between PSA levels greater than or equal to 4 ng/mL and subsequent prostate cancer diagnosis of 5.5 years [29]. Similarly, Fang et al. [30] found that after 13 years of follow-up, a baseline PSA above the age-adjusted median conveyed a relative risk of prostate cancer diagnosis of approximately 3.6. Further, two larger studies have extended prediction to lower PSA ranges and longer intervals. Loeb et al. [31] studied 1178 men in their 40’s with risk factors for prostate cancer and found that men with a baseline PSA level between 0.7 and 2.5 ng/mL were at a 14.6-fold higher risk of prostate cancer diagnosis compared to men with PSA less than 0.7 ng/mL. In a large study consisting of 21,277 men, Lilja et al. [33] reported that PSA level at 44 to 55 years of age was associated with prostate diagnosis up to 25 years later and that this association applied even to low levels of PSA; for example, the odds ratio for developing prostate cancer for PSA 0.51 to 1.0 ng/mL compared with less than 0.5 ng/mL was 2.51. In a further analysis, these men were compared to men ages 59 to 61 years and it was reported that the prognostic accuracy of PSA decreased with age, a difference hypothesized to result from greater prevalence of benign prostatic hyperplasia, and therefore of non-cancer related PSA increase, among older men [34]. Ulmert et al. [32] showed that a single PSA test taken before 50 years of age is a strong predictor of advanced prostate cancer diagnosed up to 25 years. All of
these studies indicate that men who will eventually develop prostate cancer have increased PSA levels years before prostate cancer is diagnosed, signifying the predictive capabilities of PSA testing and consequently its importance in identifying men at risk of developing prostate cancer.

2.3 Cell Culture Studies in Assessing the Impact of Diet

A major criticism of cell culture studies is that due to the dissimilarities between in vitro models, which are in isolation from whole body interactions, and human physiology, the applicability of cell culture results to humans is questionable. However, it is known that diet and lifestyle can significantly alter the constituents of serum and consequently the cellular environment. So, while there are many studies that have established in vitro links between diet and lifestyle and cancer cell growth, there is a lack of studies assessing the total impact of diet on cancer promotion or inhibition.

In 2001, using their specific cell culture bioassay of incubating an individual’s serum with cancer cell lines, Dr. R. J. Barnard and his group showed that diet and exercise can affect the risk of prostate cancer acutely and chronically [35]. Thirteen overweight men were placed on an 11 day low fat, high fiber diet and exercise intervention, and incubated the subjects’ sera from before and after the trial with two prostate cancer cell lines (LNCaP and PC3). As controls, eight men who had complied with the regime for a mean of 14.2 years were also included along with seven men not following the intervention. For the cell culture experiments they incubated the androgen sensitive LNCaP cells and androgen independent PC3 cells for the first 24 hours with 10% FBS (to allow the cells to attach to the bottom of the wells) and from there an additional 48 hours in 10% subject sera. The post-intervention serum from each of the 11 day intervention subjects resulted in a 30% reduction in LNCaP cell growth compared with their baseline serum. Further, serum from long-term subjects significantly inhibited LNCaP cell growth by an additional 15% compared to those who had completed the 11 day study. This study demonstrates the capability of the bioassay for effectively assessing the effect of diet on changes in cancer risk by incubating serum from a particular individual on a specific diet with cancer cell lines.

Furthermore, a later study conducted by the Barnard group demonstrated how using this approach can, importantly, produce results where in vivo and in vitro data are mutually supportive [36]. The study consisted of 93 men with early, biopsy proven prostate cancer, but had chose not to undergo any conventional treatment, avoiding confounding effects of
interventions such as radiation, surgery, or androgen deprivation therapy. All the men had a serum PSA level between 4 and 10 ng/mL and cancer Gleason scores less than 7. They were randomly assigned either to an experimental group, consisting of an intensive lifestyle program that included a vegan diet supplemented with soy, fish oil, vitamin E, selenium, and vitamin C, moderate aerobic exercise, stress management techniques, and participation in a one hour support group once a week to enhance adherence to the intervention, or to a usual care control group, consisting of the advice of their physicians regarding lifestyle changes. The effect of changes in diet and lifestyle on the progression of prostate cancer was assessed using PSA measurements and the serum stimulated LNCaP cell growth bioassay experiments. It was reported that after one year of adherence to the interventions, PSA levels decreased 4% from baseline in the experimental group, but increased 6% in the control group. In corroboration, the growth of LNCaP cells was inhibited almost 8 times more by subject serum from the experimental intervention (70% inhibition) than serum from control group subjects (9% inhibition). Further, the extent to which participants made changes in diet and lifestyle was significantly related to decreases in PSA (in vivo) and to LNCaP cell growth (in vitro). Therefore, the inter-individual differences in serum profile as a result of diet and lifestyle are reflected in cell growth when sera from different individuals are incubated with cancer cell lines, which provide in vitro data that is consistent with that of in vivo, indicating the ability of this bioassay to produce a meaningful picture of diet and cancer interaction.
3. Hypothesis, Objectives, and Rationale
3.1 Hypothesis

Our hypothesis is that while ALA may decrease the risk of CHD, it may also increase the risk or stimulate the progression of prostate cancer.

1. Observational studies will show that dietary ALA intake increases the risk of prostate cancer.
2. Participants on an ALA-enriched diet will experience a significant rise in their PSA levels, which are indicators of increased risk of prostate cancer.
3. Serum from subjects on an ALA-enriched diet and ALA added directly to serum will stimulate or promote prostate cancer (LNCaP cell) growth in vitro.

3.2 Objectives

Overall objective: To investigate the association between ALA and prostate cancer.

Specifically:

1. To assess through a meta-analysis the evidence for an association between dietary ALA and prostate cancer risk from observational studies, with a subgroup analysis based on study design.
2. To assess the effect of ALA on PSA change as a post hoc outcome in an ALA supplemented cardiovascular randomized controlled trial.
3. To determine through cell culture studies: a) whether serum from intervention subjects, who consumed ALA, stimulates LNCaP cell growth more than serum from control subjects, b) the effect of ALA on LNCaP cell growth compared to other C18 fatty acids, and c) whether there is an ALA dose response.

3.3 Rationale

There are divergent health views on the effects of ALA. Epidemiological [1-6] and clinical studies [7-9] have shown that ALA is associated with a reduction in coronary heart disease morbidity and heart disease mortality, but also with an increase in the risk of prostate cancer [10-17]. The effect of ALA on prostate cancer needs to be studied more thoroughly. Meta-analyses are needed to clarify the association between ALA and prostate cancer in light of the inconsistent epidemiological data. Currently, no randomized controlled trials have been undertaken to specifically assess the effect of ALA on prostate cancer development or markers of
risk. Clinical trials are required for more definitive answers on ALA intake and prostate cancer and at the same time, studies of PSA can provide a surrogate marker for studying the effect of ALA intake on prostate cancer risk [28-34]. In addition, in vitro studies of ALA and prostate cancer would help corroborate results, particularly using a physiological bioassay that demonstrates minimal intra-individual differences and significant inter-individual differences in terms of cancer cell growth. Since canola oil, a rich source of ALA, is currently being promoted for cardiovascular health, it is important for ALA recommendations to weigh any favourable effects on CHD against possible adverse effects on prostate cancer.
4. Case-Control and Prospective Studies of Dietary
    Alpha-Linolenic Acid Intake and Prostate Cancer
    Risk: a Meta-Analysis
Case-Control and Prospective Studies of Dietary Alpha-Linolenic Acid Intake and Prostate Cancer Risk: a Meta-Analysis

4.1 Abstract

**Background:** There are divergent lines of evidence of the effect of alpha-linolenic acid (ALA) on chronic disease. Although ALA is considered a cardioprotective nutrient, epidemiological studies have suggested that dietary ALA intake increases the risk of prostate cancer. However, this association is currently based on inconsistent and heterogeneous results.

**Objective:** To assess through a meta-analysis the evidence for an association between dietary ALA and prostate cancer risk from case-control and prospective studies.

**Methods:** MEDLINE and EMBASE were searched for relevant prospective and case-control studies that included data on dietary ALA intake and the risk of prostate cancer. Data were pooled using the generic inverse variance method and a random-effects model from studies that compared the highest ALA quantile with the lowest ALA quantile, and were expressed as risk estimates with 95% confidence intervals. Heterogeneity was assessed by $\chi^2$ and quantified by $I^2$.

**Results:** Data from 5 prospective and 6 case-control studies were pooled and the overall estimate showed ALA intake to be positively, but non-significantly associated with prostate cancer risk (relative risk (RR): 1.09; 95%CI: 0.91, 1.31, $P=0.34$), but the association was heterogeneous. When examined by study design, that is, prospective compared with case-control, the weak positive association persisted among the case-control studies alone (RR: 1.39; 95%CI: 0.83, 2.33, $P=0.21$), but the prospective studies revealed a weak protective effect of ALA intake on prostate cancer risk (RR: 0.95; 95%CI: 0.84, 1.09, $P=0.48$). Inter-study heterogeneity was high for both, but a sensitivity analysis with the prospective studies alone showed that removal of one study reduced heterogeneity ($I^2=8\%$, $P=0.35$) and changed the overall result to a significant protective effect (RR=0.91; 95%CI: 0.83,0.99, $P=0.02$).

**Conclusions:** There is no clear evidence of an association between dietary ALA intake and prostate cancer risk, but it is of concern and warrants further study. Additional research, both epidemiological and experimental, is needed to clarify the association.
4.2 Introduction

Prostate cancer is the second most common cancer in men worldwide [37] and the third leading cause of cancer death amongst Canadian men [38]. Prostate cancer incidence rates vary widely between countries, populations, and races. The large differences in prostate cancer incidence rates have led to many migration and ecologic studies, which have provided strong evidence for the role of environmental factors, such as diet, in the etiology of prostate cancer [41-50]. Since 1975, when Armstrong and Doll first hypothesized that there was an association between dietary fat and death from prostate cancer [48], many studies have examined this connection [52-55], but in recent years more attention has been focused on specific fatty acids. Several studies have examined the association between polyunsaturated fatty acids (PUFAs) and risk of prostate cancer [11, 18, 20, 22, 25, 74, 141]. There has been particular interest in alpha-linolenic acid (ALA), the parent fatty acid for the ω-3 PUFAs since increased consumption of ω-3 fatty acids are being advised especially in relation to cardiovascular disease risk reduction [142-145] even though its effect on prostate cancer remains in question due to inconsistent results.

Dietary ALA occurs mainly in plants and vegetable oils with certain seed oils (flaxseed, perilla, chia seed, and canola), beans (soybeans, navy beans), and nuts (walnuts) singled out as examples of healthy foods due to their high ALA content [146]. However, in the United States in particular, the main sources of ALA are from typically unhealthy foods including creamy mayonnaise, creamy salad dressings, margarine, butter, beef, pork, lamb, and oil-and-vinegar-based dressings, rather than from healthy ALA-rich vegetable sources [11]. There is considerable variability in the primary sources of ALA between countries, for example the largest proportion of ALA (53.8%) comes from red meat in Uruguay [15], but comes from margarine (25%) in the Netherlands [147]. Furthermore, food such as bread, eggs, and margarine are now being enriched with sources of ALA to increase their health value. Therefore, it appears timely to determine whether there are associations between healthy ω-3 fatty acid-rich foods and prostate cancer risk.
4.3 Methods

We followed the Cochrane handbook for systematic reviews of interventions version 5.0.2 updated September 2009 for the planning and conduct of this meta-analysis [148]. The reporting followed the QUOROM (Quality of Reporting of Meta-analyses) guidelines [149].

4.3.1 Study Selection

We conducted a search of MEDLINE (1950-April 17, 2009) and EMBASE (1980-April 17, 2009) using the following search terms and Boolean operators: prostate AND (cancer OR adenoma OR adenocarcinoma OR neoplasia OR gleason score) AND (alpha-linolenic acid OR n-3 fatty acids OR omega-3 fatty acids). The search was restricted to human research studies. No limit was placed on language. Manual searches of references cited by the published original studies and review articles supplemented the database search strategy. This search strategy was updated on March 30, 2010. We included all prospective cohort, case-control, nested case-cohort, and nested case-control studies that investigated the effect of dietary ALA intake on the incidence (or diagnosis) of prostate cancer and provided relative risk (RR), hazard ratios (HR), or odds ratios (OR) estimates. No randomized controlled trials were identified. In cases where multiple publications existed for the same study, the article with the most recent information was included.

4.3.2 Data Extraction

Two investigators (AJC, JLS) independently extracted relevant data on study characteristics and outcomes using a standardized proforma. These data included information about study design (prospective cohort, case-control, randomized controlled trial, etc.), sample size and characteristics (nationality, race, study cohort, gender, age, disease status, preexisting medical conditions), country of origin, follow-up, sources of ALA, method of ALA status assessment, endpoints (incidence of prostate cancer, prostate specific antigen (PSA), Gleason score etc.), endpoint assessment (self-reporting, medical records, biopsy, etc.), and number of new incident cases. Bounds of intake categories, quartiles or quintiles, were also recorded. RR, HR, or OR with the greatest degree of control for other environmental and dietary risk factors (adjusted for confounding factors), and their corresponding 95% CIs for incident prostate cancer risk were extracted as the main endpoint. Disagreements were reconciled by consensus and
where necessary by discussion with another investigator (DJAJ). Authors were not contacted to request additional information.

4.3.3 Statistical Analysis

Data were analyzed using Review Manager (RevMan) 5.0.24 (Cochrane Library software, Oxford, UK). We used the reported RR or OR of the highest versus lowest intake category, as the measure of the relation between ALA intake and prostate cancer risk. A pooled analysis of all reports was conducted using the Generic Inverse Variance fixed method where the log RR for cohort studies or log OR for case-control studies were weighted by the inverse of the variance to obtain a pooled RR estimate. Since nested case-cohort and nested case-control studies are temporally prospective, we analyzed data from these studies with the prospective studies. As in other meta-analyses that have examined prostate cancer, ORs were considered as approximations of RRs. The results of the analyses are reported as the more conservative pooled RR estimates generated by random effect models rather than from fixed effect models [150]. Inter-study heterogeneity was tested by Cochrane’s Q (Chi$^2$ at significance level of P<0.10) and quantified by $I^2$, where $I^2 \geq 50\%$ is considered to be evidence of substantial heterogeneity and $\geq 75\%$, considerable heterogeneity [151]. Sources of heterogeneity were investigated by sensitivity analyses, in which the effect of systematically removing each study was assessed. An a priori subgroup analysis by study design, that is, prospective compared to case-control, was also undertaken to investigate sources of heterogeneity.

4.4 Results

4.4.1 Search Results

Figure 1 shows the flow of the literature applying the systematic search and selection strategies to identify eligible reports. One hundred and seventy two reports were identified by the search. Of these, 162 were determined to be irrelevant on review of the titles and abstracts. Four reports were manually included after the database search. The remaining 14 reports were retrieved and reviewed in full, of which 3 were excluded. Results for The Health Professionals’ Follow-up Study were published in three separate publications at different times of follow-up [11, 18, 141]. Only the most recent publication of the results, by Giovannucci et al. in 2007, was
included in the analyses [18]. A total of 11 reports, 5 prospective and 6 case-control studies, were selected for pooled analyses.

4.4.2 Study Characteristics

Table 1 shows the characteristics of the 11 included studies, which are composed of 6 case-control studies and 5 prospective studies that used 3 designs: cohort, nested case-cohort, and nested case-control. Four studies were conducted in North America, 1 in South America, and 6 in Europe. The 11 included studies contained a total of 14,716 cases of prostate cancer and 222,956 comparative subjects. All the studies obtained dietary data using food frequency questionnaires (FFQ). Dietary ALA intake in these studies ranged from about 0.05 to 2.3 g/d and the reported relative risk or odds ratio of the highest versus the lowest intake category ranged from 0.7 to 3.91.

4.4.3 Primary Analysis

The overall analysis of the 11 studies examined prostate cancer, comparing the highest with the lowest ALA intake category. Six studies reported a protective effect of ALA intake on prostate cancer, 2 of which were significant, and the remaining five studies reported a positive association, of which 3 were significant. Overall, we found that the highest quantiles of ALA intake were weakly associated with increased prostate cancer risk (RR: 1.09; 95%CI: 0.91, 1.31, P=0.34) (Figure 2). However, there was evidence of considerable inter-study heterogeneity (I^2=86%, P<0.00001). Systematic removal of each study during sensitivity analyses did not explain the heterogeneity.

4.4.4 Case-Control Subgroup Analysis

An a priori subgroup analysis by study design, that is, case-control compared with prospective studies, was used to explore a possible cause of heterogeneity in the primary analysis. This subgroup analysis of case-control studies alone showed a similar association as the primary analysis, in that, ALA intake was weakly associated with increased prostate cancer risk (RR: 1.39; 95%CI: 0.83, 2.33, P=0.21), but with even further inter-study heterogeneity (I^2=91%, P<0.00001) (Figure 3). Systematic removal of each study during sensitivity analyses did not explain the heterogeneity.
4.4.5 Prospective Subgroup Analysis

Conversely, in the subgroup analysis of prospective studies alone, a weak protective association between ALA intake and prostate cancer risk was revealed (RR: 0.95; 95% CI: 0.84, 1.09, P=0.48) (Figure 4). Although there was also evidence of considerable inter-study heterogeneity ($I^2=69\%, P=0.01$), sensitivity analyses through the systematic removal of each study showed that removal of the study conducted by Giovannucci et al. [18] reduced heterogeneity to non-significant levels ($I^2=8\%, P=0.35$). However, upon removal of this study, the conclusions were altered to a significant protective effect (RR=0.91; 95% CI: 0.83, 0.99, P=0.02) (Figure 5).

4.5 Discussion

The present meta-analysis of 11 case-control and prospective studies (5 case-control and 6 prospective) comparing the highest with the lowest categories of dietary ALA intake demonstrates heterogeneous effects of ALA on prostate cancer risk. Overall, there was a non-significantly positive and heterogeneous association between ALA intake and risk of prostate cancer, which persisted when looking at case-control studies alone. However, when examining prospective studies alone, this association became weakly protective and after removal of the study by Giovannucci et al. [18] became significantly protective with no heterogeneity. These results are similar to that of a previous published finding [106], but our study additionally investigated the association between dietary ALA intake and prostate cancer risk among case-control studies as well.

The difference in results may be explained by a number of factors. First, the differences could be a reflection of variation in ALA consumption, which may be a result of the population’s dietary patterns or the use of different FFQs and food databases. In terms of dietary patterns, the main sources of ALA differ between countries, which may have an impact on the results. In the Netherlands, the chief sources of ALA include margarine (25% of daily intake), meat (11%), bread (10%), and vegetables (8%) [147], whereas in the United States, major sources of ALA come from mayonnaise, creamy salad dressings, margarine, butter, beef, pork, lamb, and oil and vinegar-based dressings [11]. Interestingly, the prospective study from the Netherlands reported a weak protective effect of ALA intake on prostate cancer risk [22], but the most recent study from the United States reported a 25% increase in risk [18]. This difference may be due to the
nature of the foods that contain ALA since in the United States, the sources of ALA are not the “healthy” sources where ALA is naturally found (e.g. flaxseed, walnuts, and canola oil), but rather profiled an unhealthy diet (e.g. canola oil in the form of mayonnaise and creamy salad dressings), which may be indicative of a less healthy lifestyle and this in itself may contribute to an increased risk of prostate cancer independent of ALA intake levels. In addition, in the case-control study from Uruguay that showed over 3 times the risk of prostate cancer at the highest levels of ALA intake compared to the lowest [15], meat and not vegetable was the major source of ALA, indicating that high meat intake instead of high ALA could potentially explain ALA’s apparent adverse effect. However, it is important to note that in this study, ALA from both animal and vegetable sources was associated with an increased risk of prostate cancer [15]. These studies indicate the importance of not only identifying the dietary sources of ALA, but taking into account what the nature of the foods may indicate in terms of diet and lifestyle since these also may affect prostate cancer risk. In terms of utilizing different FFQs and food databases, each study used a different dietary FFQ. ALA content of processed food can vary, which can be of concern when using food databases to translate food intake into fatty acid intake. For example, the ALA content of 12 margarines available in Australia range from 0.2% to 5.9% [152].

Secondly, the dietary ALA intake levels observed in these studies were all within the dietary reference intake (DRI) range of 1.1 to 1.6 g/d [100], suggesting that ALA may not increase the risk of cancer more than any other nutrient which provides a stimulus to cell growth and since ALA is a nutrient in which the Western diet is deficient [153], it may be that a deficiency prevents the growth of cancer rather than an excess causing prostate cancer growth.

Lastly, confounding from other polyunsaturated fatty acids such as omega-6 or other omega-3 fatty acids (eicosapentaenoic and docosahexaenoic fatty acids) might affect ALA metabolism [133] and consequently may introduce bias.

In considering the limitations of the meta-analysis, it should be noted that all data currently available for inclusion come from epidemiological studies since there are no data from randomized controlled trials due to ethical concerns. Interpretation of the analyses is complicated by the evidence of considerable heterogeneity among the studies, therefore a number of potential contributing factors should be considered. First, study design should be taken into account. The association between ALA intake and prostate cancer risk was stronger in the case-control studies
than in the prospective. However, since case-control studies collect dietary intake information after disease development there is the possibility of recall bias, whereas prospective studies collect intake information before disease diagnosis. Secondly, follow-up time could also have an effect on heterogeneity, especially since the study by Giovannucci et al. [18] had the longest follow-up duration (16 years). So, the heterogeneity induced by this study may indicate that follow-up duration is related to the strength of the association between ALA and prostate cancer risk. Another important aspect to consider is the differing exposure levels between the studies. Each study had different cut-offs for each quantile, which makes a true comparison of ALA intake exposure difficult since some studies had higher levels of ALA in their highest intake quantile than others. Further, some studies did not adequately define the absolute upper and lower limits of ALA intake [15, 18, 111] and one study did not report numerical exposure levels [73].

In conclusion, there is no clear evidence of an association between dietary ALA intake and prostate cancer risk since studies that show an association between ALA intake and prostate cancer are observational and causation is difficult to establish. Therefore, additional research from epidemiological, clinical, and in vitro studies are required to elucidate the effect of ALA on prostate cancer risk and development. However, the relation of dietary intake of ALA to prostate cancer risk is likely to continue to be difficult to resolve through randomized controlled trials due to the significant public health implications of reducing/eliminating a dietary fatty acid which is essential and has suggested heart health benefits. Despite the lack of randomized controlled trials using ALA with prostate cancer as the primary outcome, the present study failed to provide evidence for the promotion by ALA of prostate cancer.
### Table 1 - Characteristics of studies included in the meta-analysis of alpha-linolenic acid intake and prostate cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Country of Origin</th>
<th>Study Design</th>
<th>Sample size</th>
<th>Age (years)</th>
<th>Incident Cases</th>
<th>Follow-up (years)</th>
<th>Exposure level (g/d)</th>
<th>Relative Risk or Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andersson et al. 1996 [19]</td>
<td>Sweden</td>
<td>Case-control</td>
<td>526 cases/536 controls</td>
<td>&lt;80</td>
<td>-</td>
<td>-</td>
<td>0.817 - 1.352</td>
<td>0.93</td>
<td>0.65-1.32</td>
</tr>
<tr>
<td>Meyer et al. 1997 [71]</td>
<td>Canada</td>
<td>Case-control</td>
<td>215 cases/593 controls</td>
<td>≥45</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.98</td>
<td>0.54-1.78</td>
</tr>
<tr>
<td>Schuurman et al. 1999 [22]*</td>
<td>Netherlands</td>
<td>Nested case-cohort</td>
<td>58279 (1525 subcohort)</td>
<td>55-69</td>
<td>642</td>
<td>6.3</td>
<td>0.7 - 2.1</td>
<td>0.76</td>
<td>0.66-1.04</td>
</tr>
<tr>
<td>De Stefani et al. 2000 [15]</td>
<td>Uruguay</td>
<td>Case-control</td>
<td>217 cases/513 controls</td>
<td>40-89</td>
<td>-</td>
<td>-</td>
<td>≤0.8 - ≥1.5</td>
<td>3.1</td>
<td>2.2-4.7</td>
</tr>
<tr>
<td>Ramon et al. 2000 [14]</td>
<td>Spain</td>
<td>Case-control</td>
<td>217 cases/431 controls</td>
<td>&lt;60-80</td>
<td>-</td>
<td>-</td>
<td>0.72 - 2.1</td>
<td>3.91</td>
<td>1.50-10.1</td>
</tr>
<tr>
<td>Mannisto et al. 2003* [72]</td>
<td>Finland</td>
<td>Nested case-control</td>
<td>198 cases/198 controls</td>
<td>50-69</td>
<td>246</td>
<td>5-8</td>
<td>1.0 - 2.3</td>
<td>1.16</td>
<td>0.64-2.13</td>
</tr>
<tr>
<td>Bidoli et al. 2005 [104]</td>
<td>Italy</td>
<td>Case-control</td>
<td>1294 cases/1451 controls</td>
<td>45-74</td>
<td>-</td>
<td>-</td>
<td>mean 1.6</td>
<td>0.7</td>
<td>0.6-0.9</td>
</tr>
<tr>
<td>Koralek et al. 2006 [20]*</td>
<td>United States</td>
<td>Prospective cohort</td>
<td>29,592</td>
<td>55-74</td>
<td>1898</td>
<td>5.1</td>
<td>1.09 - 1.75</td>
<td>0.94</td>
<td>0.81-1.09</td>
</tr>
<tr>
<td>Hedelin et al. 2007 [69]</td>
<td>Sweden</td>
<td>Case-control</td>
<td>1499 cases/1130 controls</td>
<td>mean 67.3</td>
<td>-</td>
<td>-</td>
<td>0.05 - 0.60</td>
<td>1.35</td>
<td>0.99-1.84</td>
</tr>
<tr>
<td>Giovannucci et al. 2007 [18]*</td>
<td>United States</td>
<td>Prospective cohort</td>
<td>47,750</td>
<td>40-75</td>
<td>3544</td>
<td>16</td>
<td>&lt;0.79 - ≥1.32</td>
<td>1.25</td>
<td>0.82-1.92</td>
</tr>
<tr>
<td>Park et al. 2007 [25]*</td>
<td>United States</td>
<td>Prospective cohort</td>
<td>82,483</td>
<td>≥45</td>
<td>4404</td>
<td>8</td>
<td>1.1 - 2.14†</td>
<td>0.92</td>
<td>0.84-1.02</td>
</tr>
</tbody>
</table>

* Prospective studies.
† Based on a 2000 kcal diet.
Figure 1 - Flow of the literature.
### Figure 2

Pooled effect of dietary ALA intake on prostate cancer risk in case-control, nested case-control, nested case-cohort, and cohort studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.0.24, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane’s Q (Chi²) at a significance level of P<0.10 and quantified by I², where I² ≥ 50% is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [148].

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Weight</th>
<th>Risk Ratio IV, Random, 95% CI</th>
<th>Year</th>
<th>Risk Ratio IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andersson 1996 [19]</td>
<td>8.8%</td>
<td>0.93 [0.65, 1.33]</td>
<td>1996</td>
<td></td>
</tr>
<tr>
<td>Meyer 1997 [71]</td>
<td>5.4%</td>
<td>0.98 [0.54, 1.78]</td>
<td>1997</td>
<td></td>
</tr>
<tr>
<td>Schuurman 1999 [22]</td>
<td>10.9%</td>
<td>0.76 [0.61, 0.95]</td>
<td>1999</td>
<td></td>
</tr>
<tr>
<td>Mannisto 2003 [72]</td>
<td>5.4%</td>
<td>1.16 [0.64, 2.12]</td>
<td>2003</td>
<td></td>
</tr>
<tr>
<td>Bidoli 2005 [104]</td>
<td>11.3%</td>
<td>0.70 [0.57, 0.86]</td>
<td>2005</td>
<td></td>
</tr>
<tr>
<td>Koralek 2006 [20]</td>
<td>12.1%</td>
<td>0.94 [0.81, 1.09]</td>
<td>2006</td>
<td></td>
</tr>
<tr>
<td>Park 2007 [25]</td>
<td>12.7%</td>
<td>0.92 [0.83, 1.01]</td>
<td>2007</td>
<td></td>
</tr>
<tr>
<td>Hedelin 2007 [69]</td>
<td>9.5%</td>
<td>1.35 [0.99, 1.84]</td>
<td>2007</td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>100.0%</td>
<td>1.09 [0.91, 1.31]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 0.07; Chi² = 71.18, df = 10 (P < 0.00001); I² = 86%
Test for overall effect: Z = 0.95 (P = 0.34)

### Figure 3

Pooled effect of dietary ALA intake on prostate cancer risk in case-control studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.0.24, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane’s Q (Chi²) at a significance level of P<0.10 and quantified by I², where I² ≥ 50% is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [148].

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Weight</th>
<th>Risk Ratio IV, Random, 95% CI</th>
<th>Year</th>
<th>Risk Ratio IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andersson 1996 [19]</td>
<td>17.9%</td>
<td>0.93 [0.65, 1.33]</td>
<td>1996</td>
<td></td>
</tr>
<tr>
<td>Meyer 1997 [71]</td>
<td>15.5%</td>
<td>0.98 [0.54, 1.78]</td>
<td>1997</td>
<td></td>
</tr>
<tr>
<td>Bidoli 2005 [104]</td>
<td>19.0%</td>
<td>0.70 [0.57, 0.86]</td>
<td>2005</td>
<td></td>
</tr>
<tr>
<td>Hedelin 2007 [69]</td>
<td>18.3%</td>
<td>1.35 [0.99, 1.84]</td>
<td>2007</td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>100.0%</td>
<td>1.39 [0.83, 2.33]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 0.35; Chi² = 56.93, df = 5 (P < 0.00001); I² = 91%
Test for overall effect: Z = 1.26 (P = 0.21)
Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.0.24, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane’s Q (Chi²) at a significance level of P<0.10 and quantified by I², where I² ≥ 50 % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [148].

**Figure 4** – Pooled effect of dietary ALA intake on prostate cancer risk in prospective studies after the systematic removal of the study by Giovannucci et al. [18] following a sensitivity analysis. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.0.24, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane’s Q (Chi²) at a significance level of P<0.10 and quantified by I², where I² ≥ 50 % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [148].
5. Effect of Alpha-Linolenic Acid Supplementation on Serum Prostate Specific Antigen: a Randomized Controlled Study
Effect of Alpha-Linolenic Acid Supplementation on Serum Prostate Specific Antigen: a Randomized Controlled Study

5.1 Abstract

**Background:** Cohort studies suggest a possible link between α-linolenic acid (ALA) consumption and prostatic cancer despite apparent benefits for cardiovascular disease.

**Objective:** To assess the effect of ALA on serum prostate specific antigen (PSA) in men treated with ALA to reduce recurrence of atrial fibrillation.

**Methods:** For atrial fibrillation, 79 men were randomized to either a supplemented ALA margarine (1.4 g/d ALA) Mediterranean diet or conventional care and followed for one year or until atrial fibrillation occurred. Fasting bloods were obtained at 0, 2, 6, and 12 months and serum from at least two time points was available for 54 men (ALA, n=31; conventional care, n=23). Seven years after the end of the study participant medical records were assessed for evidence of prostate disease before, during, and after the study.

**Results:** Of the 79 men, 3 on the ALA treatment and one on the control were diagnosed with prostate cancer prior to the study, and 2 on the control were diagnosed with prostate cancer 2 and 3 years after the study completion. Excluding participants with treated and untreated prostate disease, the intention to treat analysis showed no significant difference in change in PSA between study treatments. Using data from those with serum samples who completed 6 or 12 months of the trial also showed no treatment difference between change in PSA on the control -0.02 ± 0.19 ng/mL (n=10) and the ALA phase -0.43 ± 0.45 ng/mL (n=20) (P=0.40). Change in serum PSA did not relate to serum ALA (r=-0.10, n=29, P=0.60).

**Conclusion:** This small randomized controlled trial failed to provide evidence for a potential stimulation of prostatic tissue by ALA, which might favor prostate cancer development in the long-term.
5.2 Introduction

Increased ω-3 fatty acid consumption has attracted attention as a potentially important strategy to reduce cardiovascular disease risk [7, 27, 154-159]. However, data have emerged suggesting that increased consumption of the vegetable ω-3 fatty acid, alpha-linolenic acid (ALA), may be associated with an increased risk of prostate cancer [10-18]. The dilemma therefore is whether to continue to advise increased ALA consumption to reduce the risk of heart disease or whether to deemphasize the use of foods rich in ALA. Many of these foods have previously been promoted as healthy, including flaxseed, walnuts, canola and soybean oils and have not been implicated individually with prostate cancer risk.

Data incriminating ALA have come from cohort or case-control studies [10, 11, 15, 18, 26, 141] where red meat, margarine, and creamy salad dressings have been some of the chief sources of ALA. As a result in the absence of randomized controlled trials, the question remains of whether ALA is the culprit or whether some other aspect of lifestyle associated with ALA consumption is to blame and dietary or serum ALA are simply markers.

In an attempt to address this issue, we have used blood samples from a randomized controlled trial in which ALA was part of the test intervention to reduce recurrence of atrial fibrillation in subjects after electrocardioversion. This study allowed us to measure the potential rise in PSA after ALA consumption as an indication of increased prostatic stimulation which in the long term would translate into increased prostate cancer risk [29-34].

5.3 Methods

5.3.1 Study Subjects and Protocol

A total of 126 subjects were screened and of these, 111 subjects were randomized after successful cardioversion for atrial fibrillation (AF) (Figure 1). Successful cardioversion was defined as no recurrence within the first 24 hours. The inclusion criteria included patients hospitalized in one of the three study centers for electrocardioversion for atrial fibrillation between the ages of 18 and 77 years. Exclusion criteria included patients who were unable to receive electrocardioversion or those who were already enrolled in another trial; patients who were unable or unwilling to comply with the diet recommendations (experimental or control) or follow-up requirements; and patients with cardiac disease, advanced heart failure, cardiac cachexia, hyperthyroidism, clinically significant hepatic or renal disease or a history of
malignant disease or alcohol abuse. A further inclusion criterion for the present study was a sufficient amount of blood for further analyses from at least two time intervals. Subjects were recruited from three centers in the Bordeaux region (Hôpital du Haut-Lévêque, Girac Hospital, and Robert Boulin Hospital). After successful cardioversion, patients were randomly assigned to continue eating their usual diet or to an ALA-rich diet starting on the same day, since AF often recurs early after cardioversion. Fasting bloods were collected at enrollment and 2, 6, and 12 months later or until recurrence of AF after which participants were withdrawn from the study. Twenty-four hour dietary recalls were obtained at the end of months 2, 6 and 12. Subject characteristics and 2 month study measurements were available for the group of 75 subjects who completed the study per protocol and of these, there were 40 ALA and 35 control subjects with a mean age of 65.8 ± 5.9 years and 68.6 ± 6.7 years, and mean body mass index (BMI) 28.7 ± 4.7 kg/m² and 27.4 ± 4.1 kg/m², respectively. It has been assessed that these results are representative of the larger group. In total, serum from 90 subjects were available from at least 2 time points for analysis (60 men, 30 women), 50 of which were on the ALA-supplemented diet and 40 on the control.

Sera from the trial together with diet history were analyzed for ALA at 2 months to assess dietary compliance. Seven years after the completion of the study, the medical records of participants at the 3 centres were assessed by a nurse, who had coordinated one center during the study, to determine whether there had been any evidence of prostatic disease noted prior to, during, or since completion of the study. Those individuals with evidence of prostatic disease were eliminated from the final analysis due to concerns over possible confounding by therapeutic interventions and lack of randomization for prostatic disease at baseline.

5.3.2 Dietary Intervention

Dietary instructions given to the ALA group were similar to those given in the Lyon Heart Study [7]. Patients in the ALA group were instructed to use a 7% ALA canola oil margarine and a 9% ALA canola oil instead of other fats and oils. The margarine was used to replace butter and cream in the patients’ diets. Other suggestions given to the test subjects were to use 2% milk, cheese low in fat, more cereals, and vegetables and fruit. The main objective was to decrease the intake of saturated fat (less than 10% of calories) without increasing the polyunsaturated fats with the ratio of linolenic/linoleic approximately 1/5. Patients in the control
group were advised to continue with a conventional low saturated fat – low cholesterol cardiovascular therapeutic diet.

5.3.3 Analyses

Serum samples were sent by rail and air on frozen CO₂ along with temperature probes from Bordeaux via Paris to Toronto (World Courier, Toronto, Canada). Samples were first analyzed in two batches at the Toronto Medical Laboratories, Mitchener Institute, Toronto for both total and free PSA using the Axzym PSA assay. The coefficient of variation (CV) for control serum samples run in the same assay as the study samples was 17%. The complete set of samples was again analyzed for total PSA at St. Joseph’s Health Center, Toronto using an automated chemiluminescence immunoassay (Bayer Centaur, Toronto, Canada). These samples had undergone one freeze-thaw cycle. The CV for total PSA in the control serum run in this assay was 10%. Sera from at least 2 time points were available from 54 men (ALA, n=31; control, n=23). Serum from time zero and two months (n=62) were also analyzed for fatty acids by gas chromatography in Bordeaux.

Dietary intakes from the one day dietary food recalls at 2, 6 and 12 months were analyzed for macronutrients and fatty acids in Bordeaux with available carbohydrate by difference using CIQUAL French food composition tables (Agence Française de Sécurité Sanitaire des Aliments, France) and BILNUT software (SCDA, Nutrisoft, Cerelles, France).

5.3.4 Statistical Analysis

Results are expressed as means ± standard error (SE). The total PSA values used in the final analysis represented the mean of the Axzym and Bayer Centaur analyses. Since the primary objective of this study was to determine the change in PSA over time, the Bayer Centaur values for each subject were adjusted such that the mean of each individual’s Bayer Centaur PSA values was identical to the mean of that individual’s Axzym PSA value. On average the Bayer Centaur total PSA values were multiplied by a factor of 1.3. Subjects with a history of prostatic disease (cancer or hypertrophy) were included in only one analysis. Completers were defined in two ways: those with month 12 data or those with either 6 or 12 month data. Students two sample t-test was used to determine the difference between treatments. Pearson correlations were used to establish associations between the dietary variables, serum fatty acids, and PSA concentrations.
Outliers beyond ± 2 standard deviations (SD) of the mean were removed from the correlation analyses.

5.4 Results

5.4.1 Dietary and Serum ALA

Compliance with the study margarine appeared good. The difference in ALA intake between the test and control diets was 1.4 g/d, equivalent to approximately 20 g/d of 7% ALA margarine (2.1 ± 0.2 g/d test, 0.7 ± 0.1 g/d control, P<0.0001) (Table 1). Furthermore, ALA margarine consumption was associated with a 0.4 ± 0.3% increase in serum ALA on the test compared with a minor rise in the control 0.08 ± 0.2% (P<0.0001) (Table 2). At two months, change in serum ALA was highly significantly related to the corresponding dietary ALA intake (r=0.52, n=67, P<0.0001) (Figure 2).

5.4.2 Prostate Cancer

Of the 79 men, 3 participants on the ALA treatment and one on the control were diagnosed with prostate cancer prior to the start of the study. Two underwent prostatectomies and one received medication. After the treatment concluded, two further patients on the control were diagnosed with prostate cancer at 2 and 3 years after study completion. Prostate adenoma and hypertrophy were detected in 2 control participants prior to the study, and 3 control and 3 treatment subjects after the study conclusion (Table 3).

5.4.3 Prostate Specific Antigen

No significant treatment differences in PSA change over time were detected. In the intent-to-treat (ITT) analysis of 54 participants, the mean change on ALA was -0.10 ± 0.32 ng/mL and for control it was 0.09 ± 0.20 ng/mL (n=54, P=0.62). After elimination of the participants with prostate disease, the respective ITT PSA changes were -0.24 ± 0.35 ng/mL for ALA versus 0.05 ± 0.26 ng/mL for control (n=45, P=0.50). If only those with a 6 or 12 month visit were assessed then the PSA changes for ALA versus control, respectively, were -0.43 ± 0.45 ng/mL versus -0.02 ± 0.19 ng/mL (n=30, P=0.40).
5.4.4 Relation of PSA to Dietary and Serum ALA

Change in PSA at 2 months did not relate to change in serum ALA at 2 months ($r=-0.10$, $n=29$, $P=0.60$) nor to change in dietary ALA at 2 months ($r=0.025$, $n=25$, $P=0.90$) (Figure 3a and b).

5.5 Discussion

The present data indicate that ALA supplementation at a level which may have favorable effects on the cardiovascular system (2.1 g/d) [7] had no consistent effect on total serum PSA. The implication of these results is that overall ALA did not stimulate prostatic metabolism as reflected by significantly increased PSA production and activity, which in the long term could relate to the promotion of advanced prostatic cancer. Lack of effect was seen despite some prospective studies implicating ALA in increasing the risk of aggressive prostate cancer [11, 18, 141].

The first study to link ALA with prostate cancer was the Health Professionals Follow-up Study [11]. Initially it appeared that meat membrane associated ALA was the culprit and that meat consumption may be responsible for the association. However, further analysis indicated a stronger relative risk for vegetable than animal sources of ALA [141]. In addition, the link between ALA and prostatic cancer was strengthened by evidence from the Physicians Health Study in which serum phospholipid ALA concentrations were shown to be related to increased prostate cancer risk [12]. In the Health Professionals study, the major sources of ALA were creamy salad dressings, mayonnaise, and olive oil as a minor contributor [11, 18, 141]. It is possible that such sources of ALA represent a specific type of North American lifestyle and it is the lifestyle which confers risk and not the increased ALA intake per se. Nevertheless, since the early 1990s, these results have been confirmed by case-control studies from Uruguay and Spain and five studies where ALA measured in serum, red cell membranes or prostatic tissue were shown to relate prostate cancer or tumor invasiveness [12-15, 17, 114, 160].

The argument has been raised that it is the aggressive prostate cancer which is related to serum ALA intake and serum concentrations. This concept would be compatible with the promotion of growth of a preexisting tumor rather than the initiation of carcinogenesis. ALA would therefore be expected to increase PSA concentrations, as a marker of increased prostatic activity. No relation was seen in our data between ALA and change in total PSA nor was there a
significant positive relation between percentage increase in PSA and the baseline PSA concentration in those taking ALA ($r=0.169$, $n=16$, $P=0.53$) (Figure 4). Such a finding would have indicated that the effect of ALA was greatest in those at greatest risk.

Not all the epidemiological data suggest an effect of ALA in increasing prostate cancer risk. A case-control study from Sweden and a Dutch cohort study both showed no effect of dietary ALA [19, 22] nor did five studies assessing serum ALA and prostate cancer [16, 74, 107, 108, 141], while a further study demonstrated that ALA related negatively to the extension of prostatic tumors to the anatomical or surgical margins at the time of prostatectomy [21]. In addition, a more recent Italian case-control study provided evidence that dietary ALA in their population was apparently protective for prostate cancer [111]. The epidemiological data are therefore not consistent in all populations in demonstrating a clear health disadvantage for ALA.

The disadvantages of our study include the relatively small sample size, the length of the follow-up, the lack of cases with clearly defined biopsy proven prostate cancer, the use of ALA in the context of a Mediterranean diet, and the lack of a control margarine. However the mean length of follow-up on the control was 7.0 months and on the test it was 7.8 months. These durations are certainly long enough in dietary studies to see changes in PSA [161]. A significant group of subjects with high PSA levels and biopsy proven prostate cancer would have been helpful. Nevertheless, no relation to percentage change and baseline PSA level was found. The test intervention involved both an increase in ALA and a Mediterranean diet. We are not aware that a Mediterranean diet per se has been shown to influence serum PSA. Furthermore, the type of diet recommended in the Lyon Heart Study, which was the model for this diet, even failed to alter serum lipids significantly suggesting a modest metabolic effect [7, 27]. Similarly, a control margarine would have allowed a clearer comparison between $\omega-3$ and $\omega-6$ fatty acids. Despite this failing, a good relation was seen between the serum and dietary ALA, and the goal of adding 1.4 g/d ALA to the diet appeared to have been achieved.

We conclude that the lack of consistent association between ALA intake and change in PSA argues against significant stimulation of the prostate by ALA. Prospective studies have related ALA intake to prostate cancer, especially advanced, progressive cancer [11, 18, 141]. It is possible in these studies that ALA intake may be a marker for other lifestyle changes. Further clarification of the situation may come from the assessment of the change in prostatic cancer risk.
in countries which have reduced their cardiovascular disease risk though increased consumption of ALA rich vegetable oil.
### Table 1 - Mean (±SD) dietary nutrient intakes* at 2 months in subjects consuming ALA (n=40) or control diets (n=35)†.

<table>
<thead>
<tr>
<th>Dietary Intake</th>
<th>Control diet</th>
<th>ALA diet</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total kcal/d</td>
<td>1770 ± 436</td>
<td>1839 ± 466</td>
<td>0.551</td>
</tr>
<tr>
<td>Proteins (g/d)</td>
<td>79.7 ± 22.9</td>
<td>82.0 ± 19.7</td>
<td>0.665</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>65.7 ± 24.0</td>
<td>67.8 ± 20.8</td>
<td>0.709</td>
</tr>
<tr>
<td>Total Saturated fatty acids (g/d)</td>
<td>21.2 ± 10.3</td>
<td>19.3 ± 30.5</td>
<td>0.011‡</td>
</tr>
<tr>
<td>18:1 (n-9) Oleic acid (g/d)</td>
<td>22.9 ± 9.7</td>
<td>30.3 ± 9.1</td>
<td>0.003‡</td>
</tr>
<tr>
<td>18:2 (n-6) Linoleic acid (g/d)</td>
<td>11.2 ± 6.3</td>
<td>10.4 ± 3.5</td>
<td>0.858</td>
</tr>
<tr>
<td>18:3 (n-3) Alpha-Linolenic Acid (ALA) (g/d)</td>
<td>0.7 ± 0.4</td>
<td>2.1 ± 0.9</td>
<td>0.000‡</td>
</tr>
</tbody>
</table>

* Dietary 24 hour recall at 2 months.
† n = number of patients, SD = standard deviation.
‡ Indicates significant difference between treatments, P<0.05.

### Table 2 - Mean (±SD) plasma fatty acids at baseline and 2 months in subjects consuming ALA (n=29) or control diets (n=33)*†

<table>
<thead>
<tr>
<th>Fatty Acid‡</th>
<th>Baseline</th>
<th>2 Months</th>
<th>Change</th>
<th>Baseline</th>
<th>2 Months</th>
<th>Change</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0 Lauric Acid (%)</td>
<td>0.07 ± 0.07</td>
<td>0.08 ± 0.04</td>
<td>0.008 ± 0.07</td>
<td>0.07 ± 0.07</td>
<td>0.09 ± 0.06</td>
<td>0.02 ± 0.08</td>
<td>0.572</td>
</tr>
<tr>
<td>14:0 Myristic Acid (%)</td>
<td>1.0 ± 0.3</td>
<td>1.0 ± 0.3</td>
<td>0.02 ± 0.3</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.4</td>
<td>0.1 ± 0.4</td>
<td>0.299</td>
</tr>
<tr>
<td>16:0 Palmitic Acid (%)</td>
<td>22.9 ± 1.7</td>
<td>21.9 ± 1.8</td>
<td>-1.1 ± 1.6</td>
<td>22.6 ± 1.6</td>
<td>22.2 ± 1.8</td>
<td>-0.4 ± 2.2</td>
<td>0.156</td>
</tr>
<tr>
<td>18:0 Stearic Acid (%)</td>
<td>6.8 ± 0.9</td>
<td>6.6 ± 0.6</td>
<td>-0.2 ± 0.9</td>
<td>6.6 ± 0.7</td>
<td>6.6 ± 0.7</td>
<td>0.002 ± 0.9</td>
<td>0.294</td>
</tr>
<tr>
<td>18:1 (n-9) Oleic Acid (%)</td>
<td>19.3 ± 3.1</td>
<td>19.0 ± 2.8</td>
<td>-0.2 ± 2.5</td>
<td>19.8 ± 2.8</td>
<td>20.1 ± 2.6</td>
<td>0.3 ± 2.5</td>
<td>0.421</td>
</tr>
<tr>
<td>18:2 (n-6) Linoleic Acid (%)</td>
<td>27.2 ± 4.4</td>
<td>28.8 ± 5.5</td>
<td>1.9 ± 3.4</td>
<td>26.6 ± 4.5</td>
<td>25.3 ± 4.4</td>
<td>-1.3 ± 4.2</td>
<td>0.002§</td>
</tr>
<tr>
<td>18:3 (n-3) Alpha-Linolenic Acid (ALA) (%)</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td>0.08 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.8 ± 0.3</td>
<td>0.4 ± 0.3</td>
<td>0.0001§</td>
</tr>
<tr>
<td>20:5 (n-3) Eicosapentaenoic Acid (EPA) (%)</td>
<td>0.8 ± 0.6</td>
<td>1.1 ± 0.8</td>
<td>0.3 ± 0.5</td>
<td>0.9 ± 0.5</td>
<td>1.7 ± 1.6</td>
<td>0.8 ± 1.6</td>
<td>0.102</td>
</tr>
<tr>
<td>22:6 (n-3) Docosahexaenoic Acid (DHA) (%)</td>
<td>2.3 ± 1.0</td>
<td>2.3 ± 0.8</td>
<td>0.04 ± 0.7</td>
<td>2.2 ± 0.9</td>
<td>2.4 ± 0.8</td>
<td>0.2 ± 0.8</td>
<td>0.453</td>
</tr>
</tbody>
</table>

* n = number of patients. SD = standard deviation.
† Only 62 participants were available out of the larger group of 75 per protocol subjects.
‡ Individual plasma fatty acids as a percent of total plasma fatty acids.
§ Indicates significant difference between treatments, P<0.05.
<table>
<thead>
<tr>
<th>Group</th>
<th>Prostate Diagnosis</th>
<th>Treatment</th>
<th>Time of Event</th>
<th>PSA (ng/mL) at Month</th>
<th>Change in PSA (ng/mL)</th>
<th>Change in PSA (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0 2 6 12</td>
<td>End PSA - Baseline†</td>
<td>Mean PSA - Baseline‡</td>
</tr>
<tr>
<td>ALA</td>
<td>Prostate Cancer</td>
<td>Prostatectomy</td>
<td>13 yrs before study</td>
<td>3.77 4.74 - -</td>
<td>0.97</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Prostate Cancer</td>
<td></td>
<td>3 yrs before study</td>
<td>0.46 - - -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Prostate Cancer</td>
<td>Radiation</td>
<td>1 yr before study</td>
<td>7.78 4.42 1.84 -</td>
<td>-5.94</td>
<td>-4.65</td>
</tr>
<tr>
<td></td>
<td>Prostatic Hypertrophy</td>
<td>Extract of Serenoa repens plant (5-α reductase inhibitor)</td>
<td>11 mths into study</td>
<td>0.56 - - 1.45</td>
<td>0.89</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Doxazosin mesylate (α1 adrenergic blocker) 4mg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prostatic Adenoma</td>
<td>Alfuzosin (α1 adrenergic blocker) 10mg/d</td>
<td>1 yr after study</td>
<td>2.68 2.75 2.99 4.63</td>
<td>1.95</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>Prostatic Hypertrophy</td>
<td>Doxazosin mesylate (α1 adrenergic blocker) 4mg/d</td>
<td>2 yrs after study</td>
<td>- - - -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Prostatic Hypertrophy</td>
<td>Doxazosin mesylate (α1 adrenergic blocker) 1cp/d</td>
<td>2 yrs after study</td>
<td>3.79 4.91 3.56 -</td>
<td>-0.23</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td></td>
<td>3.17 4.20 2.80 3.04</td>
<td>-0.47</td>
<td>-0.31</td>
</tr>
<tr>
<td>Control</td>
<td>Prostatic Adenoma</td>
<td>14 yrs before study</td>
<td></td>
<td>0.51 0.83 0.33 0.53</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Prostatic Adenoma</td>
<td>5 yrs before study</td>
<td></td>
<td>0.09 0.16 0.37 0.41</td>
<td>0.31</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Prostate Cancer</td>
<td>Prostatectomy</td>
<td></td>
<td>0.38 - - 0.41</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Prostate Cancer</td>
<td>Bicalutamide (anti-androgen) 50mg/d</td>
<td>3 yrs before study</td>
<td>- 9.08 10.24 9.91</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leuprolide (GnRH) 11.25mg injection/month§</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prostatic Hypertrophy</td>
<td>Alfuzosin (α1 adrenergic blocker)</td>
<td>2 yrs after study</td>
<td>- - - -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Prostatic Hypertrophy</td>
<td>3 yrs after study</td>
<td></td>
<td>- - - -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Prostate Cancer</td>
<td>Prostatectomy</td>
<td>3 yrs after study</td>
<td>2.99 2.5 3.79 -</td>
<td>0.80</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>Prostatic Hypertrophy</td>
<td>Endoscopic Ressection</td>
<td>4 yrs after study</td>
<td>0.87 0.78 0.78 0.78</td>
<td>-0.09</td>
<td>-0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td></td>
<td>0.97 2.67 3.68 2.41</td>
<td>0.22</td>
<td>0.07</td>
</tr>
</tbody>
</table>

* Empty cells denote no available data.
† End PSA refers to the last recorded PSA value for patient.
‡ Mean PSA refers to the mean of the PSA values from months 2, 6, and 12 where available.
§ GnRH, gonadotropin-releasing hormone.
Figure 1 – Patient consort diagram for the cardiovascular ALA-supplemented randomized controlled trial and post-hoc PSA analysis.
**Figure 2** – Correlation of change in serum ALA and ALA intake after 2 months.

**Figure 3a** – Correlation of change in PSA and change in serum ALA after 2 months.
**Figure 3b** – Correlation of change in PSA and ALA intake after 2 months.

**Figure 4** – Correlation of percentage change in PSA after 2 months and baseline PSA.
6. Effect of Alpha-Linolenic Acid on Human Prostate Cancer Cells
Effect of Alpha-Linolenic Acid on Human Prostate Cancer Cells

6.1 Abstract

**Background:** The effect of alpha-linolenic acid (ALA) on prostate cancer growth remains in question since some in vitro studies have shown that ALA inhibits the growth of prostate cancer cells whereas others demonstrate growth promotional effects.

**Objective:** To determine through cell culture studies: whether serum from intervention subjects, who consumed ALA, stimulates LNCaP cell growth more than serum from control subjects, the effect of ALA on LNCaP cell growth compared to other C18 fatty acids, and whether there is an ALA dose response.

**Methods:** Serum was obtained from 23 subjects before and after undergoing 12 months of either an ALA-supplemented Mediterranean diet or a conventional care cardiovascular diet. LNCaP prostate cancer cells were grown in culture medium containing 10% of subject pre-intervention or post-intervention serum and viable cells were counted using a colorimetric assay after 72 hours. Pure ALA was added to 10% pooled human serum (PHS) and its effect on LNCaP cell growth in culture was compared to stearic, oleic, and linoleic acids, and also its effect was assessed in dose response experiments (1.5626 µM to 400 µM ALA).

**Results:** There was no significant stimulation of LNCaP cells with study serum from the ALA-supplemented diet (P=0.74) or from the control diet (P=0.97), with no significant difference between treatments (P=0.73). The growth of LNCaP cells was inhibited by ALA (P<0.05), but not by other C18 fatty acids and increasing ALA concentrations led to decreased LNCaP cell growth (r=-0.920, P<0.0001).

**Conclusions:** An ALA-supplemented diet did not result in serum changes that increased the growth of LNCaP prostate cancer cells in vitro, as evidence of cancer promotion, and pure ALA added to serum decreased LNCaP cell growth and in a dose-dependent manner.
6.2 Introduction

A number of epidemiological [1-6] and clinical studies [7-9] have reported that alpha-linolenic acid (ALA) was associated with a reduction in coronary heart disease (CHD) incidence and mortality. However, ALA has also been associated with an increased risk of prostate cancer [10-18]. The possible adverse association between ALA and prostate cancer was first observed by Giovannucci et al. [11] in the prospective Health Professionals Follow-Up Study after 3.5 years of follow-up. Since then, further epidemiological studies have been conducted to investigate this relationship, but so far have reported inconsistent results ranging from positive [10-18] to a non-significant [19, 20] to a negative association [21, 22]. However, despite the question of ALA’s effect on the risk of prostate cancer, no randomized controlled trials have been undertaken specifically to assess the effect of ALA on prostate cancer development or markers of risk. The results with regard to cell culture studies are controversial, with some studies showing ALA inhibited the growth of prostate cancer cells in vitro [125-127] and others demonstrated growth promotional effects [76].

In vitro studies provide the potential for assessing the total impact of a diet on cancer promotion or inhibition in short term studies. It is known that diet and lifestyle can significantly alter the constituents of serum and consequently the cellular environment. These inter-individual differences in serum profile have been shown to be reflected in cell growth when serum from different individuals was incubated with various cancer cell lines, conceptually promising a picture of diet and cancer interaction [35, 36]. This approach has produced interesting results where in vivo and in vitro data are mutually supportive. To examine the effects of ALA in subjects’ serum from a dietary intervention and also in its purified form applied directly, a physiological bioassay system was used to stimulate the growth of prostate cancer cells in vitro.

6.3 Methods

6.3.1 Study Subjects and Protocol

Please see the Methods section of Chapter 2 for study subject recruitment, inclusion criteria, and characteristics, and for ALA clinical trial protocol. The primary objective of this clinical trial was to determine the effect of an ALA rich diet in reducing recurrence of atrial fibrillation.
Serum from the trial was analyzed for ALA at 2 months to assess dietary compliance. Seven years after the completion of the study, the medical records of participants at the 3 centres were assessed by a nurse, who had coordinated one center during the study, to determine whether there had been any evidence of prostatic disease noted prior to, during, or since completion of the study. Those individuals with evidence of prostatic disease were eliminated from the analyses due to concerns over possible confounding by therapeutic interventions and lack of randomization at baseline. Only those who completed the 12 month intervention and had serum available were included in the experiments in order to observe the effect of long-term ALA supplementation.

6.3.2 Dietary Intervention

Dietary instructions given to the ALA group were similar to those given in the Lyon Heart Study [7]. Patients in the ALA group were instructed to use a 7% ALA canola oil margarine and a 9% ALA canola oil instead of other fats and oils. The margarine was used to replace butter and cream in the patients’ diets. Other suggestions given to the test subjects were to use 2% milk, cheese low in fat, more cereals, and vegetables and fruit. The main objective was to decrease the intake of saturated fat (less than 10% of calories) without increasing the polyunsaturated fats with the ratio of linolenic/linoleic approximately 1/5. Patients in the control group were advised to continue with a conventional low saturated fat –low cholesterol cardiovascular therapeutic diet.

6.3.3 Cell Culture Assay

LNCaP prostate cancer cell lines (American Type Culture Collection Rockville, Maryland) were raised in RPMI 1640 medium without phenol red and supplemented with 10% pooled human serum (PHS) (Labquip Ltd., Woodbridge, ON, Canada) in humidified incubators maintained at 37°C in 5% CO₂. LNCaP cell concentrations were kept at levels that would allow cell passaging every 7 days (confluence of 85% to 90%) and the medium was changed every 2 days. All cell culture experiments were performed within a specific range of passages, that is, 6 to 13.

LNCaP cells were harvested 7 days after a subculture and seeded in clear 96 well plates at a density of 5000 cells/well. The control medium was a standard medium supplemented with
10% PHS for a total volume of 100µL in each well. The cells were observed under the microscope and placed in a standard incubator (37°C, 5% CO₂). The cells were allowed to stay in the incubator for 24 hours to ensure adherence to bottom of the wells. After 24 hours, the cells were observed under a microscope to ensure that there were no morphological abnormalities and that the cells had adhered to the bottom of the wells. The control medium was then removed using a P200 pipette and replaced with 100µL of treatment medium (refer to sections 6.3.3.1 to 6.3.3.3 for treatment methods). The cells were checked again under the microscope and placed back into the incubator. The cells were then removed from the incubator after 72 hours of incubation and observed under a microscope for abnormalities and confluency. To determine the number of viable cells in proliferation, the colorimetric assay CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI, USA) containing an electron coupling reagent [Phenazine ethosulfate; PES] and the tetrazolium compound, [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt], in short, titled MTS, was used. The treatment medium was removed and replaced with 120µL of MTS mixed with serum free medium (ratio of 1:5). The cells were placed back in the incubator for 2 hours. The 96 well plates were then taken to the heated Fusion plate reader (at 37°C) and read with the 485nm filter. The experiments were repeated in triplicate with 8 wells used per treatment repetition. All results were expressed as a percentage of the 10% PHS control.

### 6.3.3.1 Treatments for Human Serum

Serum from 23 subjects (12 men, 11 women), from the cardiovascular ALA-feeding trial, that had completed 12 months of either the ALA-supplemented Mediterranean diet or a conventional care cardiovascular diet were available for the experiments. Twelve month samples were selected to maximize the chances of detecting a change in the stimulus to cell growth. The treatments consisted of 10% PHS (as a control), 10% pre-intervention subject serum, and 10% post-intervention subject serum.

### 6.3.3.2 Treatments for C18 Fatty Acid Comparison

Purified C18 stearic, oleic, linoleic, and alpha-linolenic acids (Nu-Chek Prep Inc., Minnesota, USA), which increase in unsaturation from 0 to 3 double bonds, were each added at a concentration of 100 µM to 10% PHS. This concentration is approximately four times the
physiological estimated mean free fatty acid ALA level in the serum, but may still be within the physiological range.

6.3.3.3 Treatments for Alpha-Linolenic Acid Dose Response

Purified ALA (Nu-Chek Prep Inc., Minnesota, USA) was added in a range of different concentrations to 10% PHS. The ALA concentrations were 1.5625 µM, 3.125 µM, 6.25 µM, 12.5 µM, 25 µM, 100 µM, 200 µM, 300 µM, and 400 µM. These concentrations are below, include, and are above the physiological range of ALA in the serum.

6.3.4 Statistical Analysis

Statistical analyses were performed with SPSS 17.0 statistical package (SPSS Inc., Chicago, Illinois) and consisted of one-way ANOVA analyses and Tukey’s tests to determine statistically significant differences. The alpha value for significance was set at 0.05. Differences are reported as mean ± standard error (SE). For the correlation analysis, Pearson’s correlation was used.

6.4 Results

6.4.1 Human Serum Experiment

Of the 23 subjects with available serum that had completed 12 months of either the ALA-supplemented Mediterranean diet or a conventional care cardiovascular diet and that had available serum, 13 subjects were randomized to the ALA intervention and 10 subjects to the control. There was no significant stimulation of LNCaP cells with post-intervention study serum compared to baseline serum from the ALA-supplemented group (109.0 ± 4.6% vs. 106.8 ± 4.4% P=0.74) or from the control group (117.4 ± 5.4% vs. 117.8 ± 5.7%, P=0.97) (Figure 2). Comparing the mean percentage change in LNCaP cell growth from baseline of the control group (0.76 ± 4.2%) with that of the ALA group (2.8 ± 4.0%) showed no significant treatment differences (P=0.73) (Figure 3). Looking at men and women separately, there were also no significant treatment differences in LNCaP cell growth for either (P=0.52 and P=0.29, respectively). Further, there was no effect of sex on change in cell growth in either the ALA (P=0.49) or control group (P=0.30).
6.4.2 C18 Fatty Acid Comparison Experiment

The effects of other C18 fatty acids on LNCaP cell growth were compared to ALA to determine whether the effect of ALA on cell growth is specific. At an effective dose of 100 µM, the mean reduction in LNCaP cell growth by ALA was 11%. Only ALA significantly decreased LNCaP cell growth compared with the 10% PHS control (P<0.05), whereas the other C18 fatty acids did not, therefore indicating specificity of ALA (Figure 4). The overall trend was that with increasing unsaturation, there is decreased LNCaP cell growth (r=0.934).

6.4.3 Alpha-Linolenic Acid Dose Response Experiment

After adding a range of ALA concentrations as the free fatty acid from 1.5626 µM to 400 µM to 10% PHS for dose response experiments, ALA concentrations of 100 µM and above were found to result in significantly decreased LNCaP cell growth compared with the 10% PHS control. ALA concentrations of 300 µM and 400 µM resulted in the most LNCaP cell growth inhibition compared to all other concentrations (Figure 5). The overall trend was that with increasing ALA concentrations, there is decreased LNCaP cell growth (r=−0.920, P<0.0001) (Figure 6).

6.5 Discussion

The present study provided no evidence that either diet derived ALA or ALA added to an in vitro system stimulated or promoted the growth of prostate cancer cells. Further, pure ALA appeared to inhibit prostate cancer growth in a dose-response manner.

Serum from subjects who completed a cardiovascular ALA-feeding randomized controlled trial allowed use of this unique in vitro approach to investigate the effect of ALA on prostate cancer since its effect can be evaluated at the same time as its effect on cardiovascular outcomes. The results from the clinical trial showed that adding 1.4 g/d of ALA to a Mediterranean diet led to significantly reduced recurrence of atrial fibrillation (AF) in patients at 2, 6, and 12 months with the Kaplan-Meier survival curve indicating a significant advantage of the ALA-supplemented diet over the control at one year [162]. Using serum from participants in the trial therefore allowed us to test the same ALA dose that was shown to improve AF outcomes in this study [162] and cardiovascular outcomes in the Lyon Diet Heart Study [7, 27] on prostate cancer growth. Since serum from patients on the ALA intervention did not stimulate prostate
cancer growth more than those on the control intervention, this suggests that ALA levels that confer cardioprotective benefits, may not have adverse effects on prostate cancer.

The estimated mean free fatty acid ALA level in the serum is approximately 22.5 ± 5.6 µM (0.0225 ± 0.0056 mmol/L). There are no data on the much lower levels, which are likely to be found in the interstitial fluid to which cells are actually exposed. However, we also conducted an experiment with a range of free fatty acid ALA doses (1.5626 µM to 400 µM) that is below, includes, and is far higher than what is typically found in the serum, with particular interest in the higher dosages since there is concern of a “more is better” effect, that is, people may consume more ALA to prevent cardiovascular disease, but these higher dosages may promote prostate cancer. However, our results corroborate those of Liu et al. [126] and demonstrate the converse in that increasing ALA concentrations led to further inhibition of prostate cancer growth.

The limitations of this study include the small number of serum samples available from study subjects, possible cytotoxicity of fatty acids in general, and its short time period and isolation from whole body metabolic and immune processes, which may limit the applicability of the data. The data and serum from those who completed 2 and 6 months of the clinical trial were not included in the analyses. It is possible that inclusion of these shorter exposures to ALA may have strengthened the data. However, the lack of an obvious effect at 12 months suggests that the shorter-timed studies may not have resulted in a different picture. However, the possible cytotoxicity of all fatty acids used in cell culture is of particular concern and has been investigated in a number of studies [125, 127, 163, 164]. The study by Du Toit et al. [127] on single fatty acids concluded that most of the prostate cancer cells were dead after 2 days in the presence of 200 µM several fatty acids (ALA, eicosapentaenoic acid, oleic acid, linoleic acid, gamma-linolenic acid, and arachidonic acid) indicating a toxicity at this level. However, another study found that ALA exhibited cytotoxic effects between 500 µM to 2 mM [163], concentrations much higher than previously reported and higher than the concentrations included in our study. Therefore the possible cytotoxic properties of fatty acids remain an issue of debate.

The main strength of this study is its novel approach to evaluating the effect of ALA prostate cancer growth. No studies thus far have used serum from subjects on a dietary ALA intervention and applied it to prostate cancer cells. This approach takes into account the
absorption and metabolism of the fatty acid and its effect on serum composition and through this, cancer growth.

In conclusion, an ALA-supplemented diet did not result in prostate cancer promotion and pure ALA added directly to serum inhibited prostate cancer cell growth in a dose-dependent manner.
126 Subjects Assessed for Eligibility

- 15 Excluded
  - 11 Not Meeting Inclusion Criteria
  - 1 Unable to Speak French
  - 3 Involved in another Randomized Trial

111 Randomized

60 Assigned to Receive ALA Intervention

- 50 Serum Samples Available from at least 2 Time Points
  - 36 Men
  - 14 Women

- 28 Completers with both 0 and 12 Month Samples
  - 20 Men
  - 8 Women

- 13 Completer Serum Samples Available for Experiments
  - 6 Men
  - 7 Women

51 Assigned to Control

- 40 Serum Samples Available from at least 2 Time Points
  - 24 Men
  - 16 Women

- 17 Completers with both 0 and 12 Month Samples
  - 10 Men
  - 7 Women

- 10 Completer Serum Samples Available for Experiments
  - 6 Men
  - 4 Women

Figure 1 – Patient consort diagram for the cardiovascular ALA-supplemented randomized controlled trial and subsequent cell culture studies.
Figure 2 – LNCaP cell growth using medium containing pre-intervention subject serum or post-intervention serum from either the alpha-linolenic acid (ALA) (n=13) or control (n=10) treatments. Results are compared with % pooled human serum stimulated control (PHS) and expressed as mean ± SE.

Figure 3 – Mean (SE) percentage change in LNCaP cell growth from baseline for alph-linolenic (ALA) (n=13) and control treatments (n=10) (P=0.73). Results are compared with % pooled human serum stimulated control (PHS).
Figure 4 – Effect of different C18 fatty acids at a concentration of 100 µM, in increasing unsaturation, on LNCaP cell growth. Results are compared with % pooled human serum stimulated control (PHS) and expressed as mean ± SE. Treatment means with different letters are significantly different compared to all other treatments (Tukey's test, P<0.05).

Figure 5 – Effect of different ALA concentrations on LNCaP cell growth. Results are compared with % pooled human serum stimulated control (PHS) and expressed as mean ± SE. Treatment means with different letters are significantly different compared to all other treatments (Tukey's test, P<0.05).
Figure 6 – Correlation of LNCaP cell growth and ALA concentration.
7. **Overall Discussion**
7.1 Overall Discussion

Alpha-linolenic acid has been shown through epidemiological [1-6] and clinical studies [7-9] to reduce the risk of CHD incidence and mortality. However, ALA has also been associated, albeit inconsistently, with an increased risk of prostate cancer [10-22]. The majority of the data implicating ALA comes from observational studies from which causation is difficult to establish. So, a broader approach using three different types of studies – epidemiological, clinical, and cell culture – was undertaken to investigate the effect of ALA on prostate cancer risk in order to elucidate a clearer picture of the nature of the association.

In the meta-analysis of prospective and case-control studies, dietary ALA intake was not significantly associated with increased prostate cancer risk and after a sensitivity analysis among the prospective studies, ALA was even found to have a significant protective effect. In the post-hoc analysis of a cardiovascular randomized controlled trial, we found no prostate cancer diagnoses among participants in the ALA group up to seven years after study completion, no significant difference in change in PSA between ALA and control treatments, and no relation between changes in PSA and changes in serum ALA. In the cell culture studies, there was no significant stimulation of prostate cancer cells with study serum from either the ALA or control treatment and no difference between treatments, and inhibition of prostate cancer cell growth by ALA compared to other fatty acids in a dose-dependent manner. These results all indicate no positive evidence for an effect of ALA on increased prostate cancer risk.

7.2 Limitations and Strengths

7.2.1 Meta-Analysis

In considering the limitations of the meta-analysis, it should be noted that all data currently available for inclusion come from epidemiological studies since there are no data from randomized controlled trials due to ethical concerns. Interpretation of the analyses is complicated by the evidence of considerable heterogeneity among the studies. The significant heterogeneity seen in the overall analysis, and which persisted in the case-control studies alone and prospective studies alone subgroup analyses as well, may be explained by a number of differences between the studies. First, study design should be taken into account. The association between ALA intake and prostate cancer risk was stronger in the case-control studies than in the prospective. However, since case-control studies collect dietary intake information after disease development
there is the possibility of recall bias, whereas prospective studies collect intake information before disease diagnosis. In support of this, the weak positive association found among the case-control studies was highly heterogenous (91%), while the weak protective effect among the prospective studies was less heterogenous (69%) and became non-significant after the removal of one study by Giovannucci et al. [18], indicating that this study was the principle source of heterogeneity within the prospective studies. Conversely, a sensitivity analysis had no effect on the heterogeneity among the case-control studies. Secondly, follow-up time could also have an effect on heterogeneity, especially since the study by Giovannucci et al. [18] had the longest follow-up duration (16 years). So, the heterogeneity induced by this study may indicate that follow-up duration is related to the strength of the association between ALA and prostate cancer risk. Another important aspect to consider is the differing exposure levels between the studies. Each study had different cut-offs for each quantile, which makes a true comparison of ALA intake exposure difficult since some studies had higher levels of ALA in their highest intake quantile than others. Further, some studies did not adequately define the absolute upper and lower limits of ALA intake [15, 18, 111] and one study did not report numerical exposure levels [73]. Inadequate reporting of ALA exposure may be a result of differences in ALA intake assessment and differences in ALA consumption between the populations. In terms of utilizing different FFQs and food databases, each study used a different dietary FFQ. ALA content of processed food can vary, which can be of concern when using food databases to translate food intake into fatty acid intake. For example, the ALA content of 12 margarines available in Australia ranged from 0.2% to 5.9% [152]. The differences in ALA consumption may be a result of the population’s dietary patterns. In terms of dietary patterns, the main sources of ALA differ between countries, which may have an impact on the results. In the Netherlands, the chief sources of ALA include margarine (25% of daily intake), meat (11%), bread (10%), and vegetables (8%) [147], whereas in the United States, major sources of ALA come from mayonnaise, creamy salad dressings, margarine, butter, beef, pork, lamb, and oil and vinegar-based dressings [11]. Interestingly, the prospective study from the Netherlands reported a weak protective effect of ALA intake on prostate cancer risk [22], but the most recent study from the United States reported a 25% increase in risk [18]. This difference may be due to the nature of the foods that contain ALA since in the United States, the sources of ALA are not the “healthy” sources where ALA is naturally found (e.g. flaxseed, walnuts, and canola oil), but rather profiled
an unhealthy diet (e.g. canola oil in the form of mayonnaise and creamy salad dressings), which may be indicative of a less healthy lifestyle and this in itself may contribute to an increased risk of prostate cancer independent of ALA intake levels. In addition, in the case-control study from Uruguay that showed over 3 times the risk of prostate cancer at the highest levels of ALA intake compared to the lowest [15], meat and not vegetable was the major source of ALA, indicating that high meat intake instead of high ALA could potentially explain the ALA’s apparent adverse effect. However, it is important to note that in this study, ALA from both animal and vegetable sources was associated with an increased risk of prostate cancer [15]. These studies indicate the importance of identifying and assessing the effect of specific dietary sources of ALA on prostate cancer risk, and further, what the nature of the foods may indicate in terms of diet and lifestyle since these also may affect prostate cancer risk. Another consideration is possible confounding from the background diet, particularly from other polyunsaturated fatty acids such as omega-6 or other omega-3 fatty acids (EPA and DHA), which may affect ALA metabolism [133] and consequently introduce bias.

7.2.2 Randomized Controlled Trial

There were a number of limitations in the analysis of a cardiovascular randomized controlled trial. First, our analysis was a post-hoc secondary analysis, where PSA was not the primary outcome of the trial. Further, the assessment of patient records for prostatic disease was also done post-hoc rather than prospectively. Despite these shortcomings, this trial provided a unique opportunity to assess the effect of ALA on both heart disease (AF) and prostate cancer at the same time, using PSA as a surrogate marker for prostate cancer, which has been shown capable of predicting prostate cancer risk years and even decades before clinical diagnosis [28-34]. This trial has significant clinical relevance since the levels of ALA given to the patients represent a therapeutic dose that decreased AF and improved cardiovascular outcomes, and at the same time these levels did not adversely affect prostate cancer stimulation and progression. To our knowledge, these data represent the first clinical data on the association between ALA and prostate cancer. Another limitation is that the study had a relatively small sample size since we were limited to only men that had available serum for PSA analyses from at least two time points. And since the subjects were unselected for prostate cancer risk, the study lacked “high risk” patients with high serum PSA levels and cases of clearly defined biopsy proven prostate
cancer. Larger numbers of subjects with urological assessments to evaluate prostatic health before and in the years after the study would be helpful. The current study depended on a nurse who may have missed valuable chart data, for example due to prostate disease diagnosis or treatment outside the Bordeaux region. Validation of her findings was not undertaken even on a subset by an independent observer. However, to ensure accurate data, the serum PSA analyses were conducted twice using two different assays at two different centres. Another limitation was the short duration of the study, with the mean length of follow-up being 7.0 months on the control and 7.8 months on the ALA intervention. While these may seem to be short follow-up periods, these durations have been shown to be long enough in dietary studies to see changes in PSA [161]. In terms of the ALA-supplemented intervention, there may have been confounding effects from the background Mediterranean diet. However, to our knowledge, the Mediterranean diet itself has not shown to influence serum PSA. Further, the type of diet recommended in the Lyon Heart Study, which was the model for this diet, even failed to alter serum lipids significantly suggesting a modest metabolic effect [7, 27]. In addition, a control margarine would have allowed a clearer comparison between \( \omega-3 \) and \( \omega-6 \) fatty acids. Despite this, a good relation was seen between the serum and dietary ALA, and the goal of adding 1.4 g/d ALA to the diet appeared to have been achieved.

### 7.2.3 Cell Culture

Lastly, there were several limitations in the cell culture studies. First, as with most in vitro studies, the relevance and applicability of the findings can be questioned due to their isolation from whole body metabolic and immune processes. However, our approach by using serum from a dietary intervention trial, accounts for the absorption and metabolism of ALA and its effect on serum composition and through this, cancer growth and progression. This approach contrasts with traditional in vitro studies that simply incubate cells with various concentrations of a specific dietary nutrient that have been isolated from physiological processes that may in vivo affect the bioavailability and biotransformation of the nutrient. No studies thus far have used serum from subjects on a dietary ALA intervention and applied it to prostate cancer cells. Therefore, one of the main strengths of this study is its novel approach to evaluating the effect of ALA and prostate cancer growth in that it may be as representative of whole body interactions as is possible for any in vitro system. Another key strength lies in the practicality of the assay since
over a short period of 5 days and with a small volume of serum, the effects of diet and lifestyle on cancer growth can be determined [35, 36]. Barnard et al. [35, 36] demonstrated that this method was capable of producing mutually supportive in vivo and in vitro data, in particular, that in vitro cell growth inhibition was supported by reduction in clinical risk factors, growth hormones, and cancer biomarkers. However, there are some disadvantages associated with this particular cell culture technique. There are differences between serum and interstitial fluid (the fluid to which cells are actually exposed) profiles, namely in protein concentration. Further, LNCaP cells were used as a model for prostate cancer growth, but these cells respond differently than healthy cells to diet and lifestyle changes. As a parallel example, although high folate intake in healthy individuals has been shown to protect against colorectal cancer, folate intake in patients with existing undiagnosed preneoplastic lesions exacerbated the disease [165-167]. Another limitation was the small number of serum samples that were available for analysis. More serum samples would have increased the power of the study and allowed for more effective detection of significant inter-individual differences. Perhaps, serum from subjects who completed 2 and 6 months of the trial could have been included in the experiments, but we wanted to determine the longer term effects of ALA and quantify the largest effect possible within the study, therefore we used the 12 month samples. Due to the lack of effect seen using the 12 month serum, the addition of the 2 and 6 month samples containing shorter exposures to ALA, may not have changed the result. However, the possible cytotoxicity of all fatty acids used in cell culture is of particular concern and has been investigated in a number of studies [125, 127, 163, 164]. The study by Du Toit et al. [127] on single fatty acids concluded that most of the prostate cancer cells were dead after 2 days in the presence of 200 µM several fatty acids (ALA, EPA, oleic acid, LA, gamma-linolenic acid, and arachidonic acid) indicating a toxicity at this level. However, another study found that ALA exhibited cytotoxic effects between 500 µM to 2 mM [163], concentrations much higher than previously reported and higher than the concentrations included in our study. In addition, Motaung et al. [125] found that combining fatty acids, such as ALA and LA or ALA and gamma-linolenic acid, resulted in less dead cells than single fatty acids, indicating an interaction or interference between the fatty acids, thereby further complicating the issue of fatty acid toxicity on cell growth. Therefore the possible cytotoxic properties of fatty acids remain an issue of debate.
7.3 Future Directions

The broad approach used in these studies to investigate the association between ALA and prostate cancer is comprehensive and of merit in attempting to completely understand the nature of this relationship. However, what our studies consistently lacked was power. Our approach represents a start towards understanding the association, but needs to be repeated. Additional careful prospective and case-control studies, clinical trials using prostate cancer biomarkers, and in vitro research are required to provide further evidence to answer the question of ALA’s role in prostate cancer development more definitively. In terms of public health, assessment of communities and nations in transition from saturated fat to ALA-rich vegetable oils should be monitored. An example of the importance of monitoring population dietary patterns is the debate whether mandatory folic acid prevents or promotes cancer, as described by Dr. Young-In Kim [165-167]. Although folic acid is generally regarded as safe, there are concerns that folic acid supplementation may enhance the development and progression of already existing, undiagnosed premalignant lesions. Due to this concern, Kim suggests careful monitoring of the United States population that has been exposed to a significant increase in folate intake as a result of folic acid fortification [167]. Therefore, in nations, such as Poland, that have overwhelmingly switched from animal to vegetable fats over the period of one decade [168], careful monitoring is required due to the public health concern over the potential cancer-promoting effect of ALA. Of note is that with the switch from saturated to ALA-rich vegetable fats, there has been a significant fall in deaths from ischaemic heart disease, which has been hypothesized to be a result of this change in dietary fat [168], making the elucidation of the effect of ALA on prostate cancer more pressing. In terms of clinical data, our data are the first in the field. So, future large randomized controlled trials studying CHD, or other diseases, and use an ALA intervention need to be undertaken so that additional secondary analyses using cancer biomarkers, such as serum PSA, can be conducted. Lastly, the mechanism by which ALA may stimulate or reduce prostate cancer is unknown. Additional in vitro studies are required to investigate the cellular mechanisms behind this association, with perhaps particular attention to the kinome and intracellular triggers for apoptosis or cell growth.
8. Conclusion
8 Conclusion

We have demonstrated the following:

1. From the meta-analysis, dietary ALA intake was not significantly associated with an increased risk of prostate cancer and after a sensitivity analysis among the prospective studies, ALA was even found to have a significant protective effect.

2. From the randomized controlled trial, there were no cases of prostate cancer among participants in the ALA group after study completion, no significant difference in change in PSA between ALA and control treatments, and no relation between changes in PSA and changes in serum ALA.

3. From the cell culture studies, there was no significant stimulation of prostate cancer cells with study serum from either the ALA or control treatment and no difference between treatments, and inhibition of prostate cancer cell growth by ALA compared to other fatty acids in a dose-dependent manner.

The overall conclusion is that we could find no clear evidence for an association between ALA and increased prostate cancer risk.
9 References


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