Clinical Investigations of the Metabolic Effects of High Fiber Foods in Relation to Prostate Cancer

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
Graduate Department of Nutritional Sciences
University of Toronto

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Clinical Investigations of the Metabolic Effects of High Fiber Foods in Relation to Prostate Cancer

Master of Science, 1998
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ABSTRACT

The aim was to investigate the effects of soluble and insoluble fiber in a mixed diet and lignans in flaxseed on serum prostate specific antigen (PSA). Compared to high insoluble fiber diets, high soluble fiber diets significantly reduced serum PSA by 0.07 ± 0.03 ng/ml (p=0.035) in healthy men but did not affect the serum levels of steroid sex hormones. Fecal lithocholic acid output was increased (40 ± 10 mg/day, p=0.001) and was positively correlated with the decrease in serum PSA (r=0.57, p=0.035). Furthermore, the high fiber diets resulted in changes in total fatty acids that were related to serum PSA, although there was no significant difference in total fatty acid concentrations between the diets.

Flaxseed supplementation reduced the rate of rise in PSA in men with prostate cancer, although non-significantly. Serum lycopene and thiol concentrations were lower on flaxseed without reaching statistical significance, despite no change in lycopene intake.
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Nauman Tariq
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<tr>
<td>ACT</td>
<td>$\alpha_1$-Antichymotrypsin</td>
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<td>ALA</td>
<td>$\alpha$-Linolenic Acid</td>
</tr>
<tr>
<td>AMG</td>
<td>$\alpha_2$-macroglobulin</td>
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<tr>
<td>CE</td>
<td>Cholesteryl Ester</td>
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<tr>
<td>GHRH</td>
<td>Growth Hormone Releasing Hormone</td>
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<td>GnRH</td>
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<td>Follicle Stimulating Hormone</td>
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<td>Free Testosterone</td>
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<td>PL</td>
<td>Phospholipid</td>
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<td>PAP</td>
<td>Prostatic Acid Phosphatase</td>
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<td>PSA</td>
<td>Prostate Specific Antigen</td>
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<td>TG</td>
<td>Triglyceride</td>
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1.0 Introduction

In Canada, among men, prostate cancer is the most frequently diagnosed cancer, and the second most common cause of death due to cancer (National Cancer Institute of Canada 1997). Various factors including serum sex hormones (Labrie et al. 1997; Andersson et al. 1993; Haapianen et al. 1986) and serum ALA (α-Linolenic Acid) levels (Gann 1994) and dietary fat and particularly ALA intake may relate to prostate cancer (Giovannucci et al. 1993; Armstrong and Doll 1975, Dolecek and Granditis 1991). Since dietary fiber has been shown to modulate sex hormone levels or activity (Adlercreutz 1990; Goldin et al. 1982) and increase fecal sterol losses as bile acids (Jenkins et al. 1993), dietary fiber may alter the risk of prostate cancer through increased fecal sex hormone loss. Lignans found in flaxseed and other phytoestrogens have been shown to block sex hormone action (Adlercreutz and Mazur 1997; Barnes et al. 1994; Shutt and Cox 1972) and may also alter the risk of prostate cancer. The effects of diet on prostate cancer can be assessed by monitoring serum PSA (Prostate Specific Antigen) levels. PSA seems to be useful serum marker for predicting and monitoring prostate cancer (Gann et al. 1995).

The hypotheses therefore were that (i) fiber increases sex hormone excretion as marked by an increase in fecal steroid loss and thereby reduces serum PSA, (ii) fiber alters serum fatty acid metabolism, and changes in serum fatty acids levels reduce serum PSA, and (iii) lignans in flaxseed, which affect breast and colon cancer in laboratory
animal studies (Serraino and Thompson 1992a; 1992b) possibly by blocking sex hormone
receptors suppresses prostate tumor growth and decreases the rate of rise of serum PSA in
men diagnosed with biopsy proven prostate cancer.

The aims therefore were (i) to assess the effects of dietary fiber on serum PSA, sex hormone levels and fecal steroid excretion, (ii) to determine whether a change in serum fatty acids was seen with dietary fiber which related to altered PSA levels and (iii) to assess whether flaxseed reduced the rate of rise of serum PSA. We also wished to determine whether flaxseed effected serum thiol levels as a marker of oxidative stress in men diagnosed with biopsy proven prostate cancer, since oxidative DNA damage may be one of the factors associated with the development of prostate cancer.
CHAPTER 2. LITERATURE REVIEW

2.0 Epidemiology

The incidence of prostate cancer has surpassed that of lung cancer, making this malignancy the most commonly diagnosed cancer and the second leading cause of cancer deaths in Canadian men (National Cancer Institute of Canada 1997). Geographical variations in risk, such as the fourfold to sixfold higher prostate cancer rates in Japanese-American men living in the United States compared with Japanese men in Japan (Shibata et al. 1997) suggest that environmental risk factors such as diet play an important role in the etiology of prostate cancer. The causes of prostate cancer are not well understood, but they presumably involve the interaction of factors such as hormones and diet (Haas and Sakr 1997). Diet can influence hormone levels, fatty acid profile and antioxidant status and thereby control the development of the disease (Serra-Majem et al. 1993). Hormones, particularly androgens, affect normal and possibly malignant growth of the prostate gland.

Both the causes and the treatment of prostate cancer are under debate. Prostate cancer incidence rises with age (Kosary et al. 1995). The incidence of prostate cancer ranges between 40% to 80% by the eighth decade of life in men (Pienta and Esper 1993; Dhom 1983). Death due to prostate cancer in different areas of North America range in males from 12% to 38% (National Cancer Institute of Canada 1997; Merrill and Brawley 1997; Smart 1997). The marked difference between incidence and mortality has posed a problem in terms of treatment. The problem is further compounded by the increased rate of clinically diagnosed disease over the last 6 years since the use of serum prostate
specific antigen (PSA) screening (Stamey et al. 1989). This now routine serum test is identifying many individuals in whom a diagnosis would not otherwise have been made.

The Japanese experience supports the concept that there are two extremes of the disease. At one end of the spectrum, poorly differentiated neoplastic cells are invasive, grow rapidly and metastasize, and at the other end of the spectrum, well differentiated cells that are slow growing and undergo apoptosis may result in no significant tumor progression. Thus, prostate cancer death rates was low at $3.3 \times 10^5$/year for Japanese men (Tominaga et al. 1993) compared to $14.2 \times 10^5$/year for white North Americans (Tominaga et al. 1993). “Latent” prostate carcinoma (noted incidentally at post-mortem) was $9,250/10^5$ in Japanese compared to $8,050/10^5$ in white North American men (Furusato 1990; Yatani et al. 1988; Guileyardo et al. 1980). These data emphasize the large disparity between the occurrence of malignant cells in the prostate and death from prostate carcinoma.

African-American men have 1.5 to 3.3 times higher prostate cancer incidence (Polednak and Flannery 1992) and 2 to 3 times higher mortality (Powell 1997) compared to American white men. They also have higher testosterone levels than American white men (Ross et al. 1986) and serum androgen levels may be higher in African-American women during pregnancy leading to a greater exposure of the fetus to androgens during the development of the prostate (Henderson et al. 1988). The androgen exposure in African-Americans, which is higher than in the other groups, is thought to result in increased cellular proliferation, leading to a higher risk of malignant transformation (Henderson et al. 1982).
Furthermore, African-American and American white men have higher 5α-reductase levels as compared with native Japanese and Chinese men. The enzyme 5α-reductase converts testosterone to dihydrotestosterone, the latter being more biologically active. The difference may contribute to the lower incidence of prostate cancer observed in Japanese men (Jenkins et al. 1992; Ross et al. 1992). Low androgen levels are also found in Japanese and Chinese men probably because total and free testosterone (FT) serum levels are generally decreased with high-fiber and low-fat diets (Howie and Shultz 1985).

Environmental factors such as exposure to cadmium (van der Gulden 1995; Elghany et al. 1990) and sunlight (Hanchette and Schwartz 1992) may be important in determining the risk factors of prostate cancer. Particularly compelling are the studies of migrant populations that have implicated diet in the development of prostate cancer (Giles and Ireland 1997; Muir et al. 1991; Adlercreutz 1990; Flanders 1984; Waterhouse et al. 1982; Dunn 1975). For Japanese men, it has been possible to examine prostate cancer incidence in the migrants themselves (Tsugane et al. 1989; Kolonel et al. 1980). Compared with the incidence in Japanese men, the incidence among first-generation Japanese men in Brazil is more than doubled, and in the incidence among first-generation Japanese men living in Hawaii is increased more than ten-fold (Severson et al. 1989). Nomura and Kolonel found that when migrants from countries with low prostate cancer rates go to countries with higher rates they tend to experience greater risk compared with their compatriots who do not migrate (Nomura and Kolonel 1991).

Autopsy data suggests that there is little international variation in histological evidence of asymptomatic prostate cancer during life despite substantial differences in
the incidence of clinical disease (Nomura and Kolonel 1991). Despite the differences in clinically manifest prostate cancer, and consequently in the incidence rates, cancers which are restricted to the prostate gland and have remained undiagnosed in life ("latent" or "histological" prostate cancer) are found at autopsy with about the same frequency in Japanese men as in white males in the United States, Canada, England and Austria (Wynder et al. 1971). This observation was refined by Akazaki and Stemmerman (Akazaki and Stemmerman 1973) who noted that the prostate cancer mortality rate for Japanese in Hawaii is closer to that of the American white population than to the rate for native Japanese. They went on to perform a histological study of latent carcinoma of the prostate. They found no differences in the prevalence rates between the two groups, but larger lesions suggestive of rapid growth rates were more common among migrants to Hawaii.

Data such as these have drawn attention to international differences in disease rates and the environmental factors, which might be responsible since migration studies, have implicated geography rather than genetic differences. In common with other nutrition related cancers, breast and colon, fat and meat intake have been shown in international comparisons to be positively associated with prostate cancer risk (Rose et al. 1986). These associations have been implicated for prostate cancer in cohort studies (LeMarchand et al. 1994; Giovannucci et al. 1993). Of most recent interest has been the implication of plant-derived sex hormones, lignans and isoflavones (Adlercreutz 1990) and the carotenoid lycopene (Giovannucci et al. 1995) which also appear to protect against the development of prostatic carcinoma.
2.1 Dietary Fiber

There is a suggestion in the literature that dietary fiber may be helpful in preventing or treating prostate cancer by influencing sex hormone and bile acid metabolism, recirculation and excretion (Rao 1996; Carter 1993; Adlercreutz 1990; Ross et al. 1990; Shultz and Howie 1986). As already described, sex steroids, particularly androgens, have long been implicated in the pathogenesis of prostate cancer (Figure 2.1). Some specific component of fiber may protect against breast or prostate cancer through their steroid-hormone-binding capability (Ross et al. 1990; Whitten and Shultz 1988; Shultz and Howie 1986). A high intake of dietary fiber by premenopausal women increases fecal wet and dry weight, which correlates positively with all three unconjugated estrogens and total estrogen in feces (Goldin et al. 1982). Goldin and others (1982) proposed that the reason for reduced intestinal reabsorption and increased elimination of estrogens by the fecal route in subjects consuming much fiber seems to be the larger fecal bulk and decreased concentration of intestinal β-glucuronidase which reduces hydrolysis of the biliary steroid conjugates, an event necessary for reabsorption. Some fibers also have the property of binding sex hormones, particularly non-polar estrogens (Whitten and Shultz 1988; Shultz and Howie 1986).

Dietary fiber also seems to influence bile acid metabolism by partial interruption of the enterohepatic circulation by altering the intestinal metabolism and increasing the fecal excretion of these compounds (Jenkins et al. 1993; Adlercreutz 1990). Increased bile acids loss in the feces may be a marker of increased steroid loss on a diet high in fiber. A low-fat and/or high-fiber diet affects sex hormone metabolism also in men by
decreasing testosterone and FT (Howie and Shultz 1985; Hamalainen et al. 1984; Hamalainen et al. 1983). Overall reduction in the bioavailability of the sex hormones suggests that a fiber-enriched diet could theoretically reduce the risk of hormone-dependent cancers.

The US Department of Agriculture and Department of Health and Human Services (US Department of Agriculture 1985), The American Heart Association (Nutrition Committee and the Council on Arteriosclerosis 1984), the Food and Nutrition Board of the National Research Council (Food and Nutrition Board 1982), and the Committee on Diet, Nutrition and Cancer (1980) have recommended diets higher in dietary fiber.

2.2 Endocrine Control

The prostate gland is under endocrine control during its growth and development. Testicular hormones play a major role not only in the normal development, but also in the pathophysiology of the prostate. A normal function of the hormonal axis beginning with a rhythmic release of GHRH and GnRH by the hypothalamus followed by a pulsatile secretion of LH and FSH from the pituitary maintains a normal testosterone secretion from the testes and is responsible for normal prostate growth and function. Serum testosterone levels regulate the release of hypothalamic and pituitary hormones by negative feedback inhibition. Androgen receptors are found on the epithelium overlying the stroma of the prostate. Surgical or chemical castration frequently causes tumor to
Figure 2.1  Hormones affecting prostate growth.
regress and it has been observed that eunuchs rarely develop prostate cancer (Ross and Schottenfeld 1996; Huggins and Hodges 1941).

Androgens are involved in the pathogenesis of prostate cancer (Gann et al. 1996) but estrogens also seem to be involved in the development of prostatic disease. There may be a possible protective effect of endogenous estrogens (Haapianen et al. 1986; Rannikko et al. 1983). Estradiol (estradiol-17ß, E2) is a steroid hormone with a molecular mass of 272.3 daltons, which circulates predominately protein-bound. In addition to estradiol, other natural steroidal estrogens include estrone and their conjugates. In men estrogens are secreted principally by the adrenals and by the testes. The prostate obtains estrogen, not only from peripheral sources, but also through aromatase activity within its own stroma and from hydrolysis of the abundant estrone sulphate in the plasma to produce free estrone by stromal sulfatase activity.

Clinical and experimental studies have demonstrated both direct and indirect effects of estrogens on the prostate and this has led to the rationale behind the favorable response to estrogen therapy in hormone-sensitive prostatic carcinoma. Pharmacological doses of synthetic estrogens, such as diethylstilbestrol (DES), have proved to be an effective therapy for advanced prostate cancer.

The direct effect of estrogens includes stimulation of the fibromuscular stroma and induction of metaplasia of prostatic epithelium. Estrogens, androgen and progesterone receptors have been identified in diseased prostates. Using immunoelectron microscopy, the presence of estrogen receptors was detected in the peripheral portions of the nuclei, the nuclear membrane and in internal chromatin in neoplastic prostate
Srinivasan et al. 1995). Sinha and other (1973) have demonstrated that cells of the prostatic epithelium bind estrogen.

The indirect effect of estrogens is generally believed to be mediated primarily via suppression of the hypothalmo-hypophyseal axis, thus reducing the circulation androgens. That is, estrogen suppresses the secretion of gonadotrophins. Thus, secretion of testicular androgens is repressed, leading to involution of the prostate. In addition, estrogen, mediated by sex hormone binding globulin, participates with androgen in setting the pace of prostatic growth and function (Farnsworth 1996).

Androgens and estrogens are synthesised from cholesterol, which is either produced from acetate or accumulated from circulating LDL. One major pathway involves the transformation of cholesterol to progesterone, 17α-hydroxyprogesterone and then to androstenedione while the other pathway involves the transformation of cholesterol to pregnenolone, 17α-hydroxypregnenolone, dehydroepiandrosterone and then to androstenedione. The pregnenolone from the second pathway may also be converted to progesterone while 17α-hydroxypregnenolone may be converted to 17α-hydroxyprogesterone. In the presence of aromatase enzyme, the androstenedione is metabolized either directly to estrone or indirectly to estradiol after its conversion to testosterone (Figure 2.2).

Estrogens and testosterone undergo enterohepatic circulation. That is, they are excreted in the bile. Fecal bile acid loss is positively correlated to fecal excretion of estrogen and possibly testosterone levels (Goldin et al. 1982). As noted earlier, dietary fiber is known to increase fecal bile acid output and may affect fecal hormone excretion.
Figure 2.2: Biosynthesis of Testosterone and Estrogen
and alter prostate cancer incidence, disease progression and mortality. Although the role of endogenous steroids in the etiology of prostate cancer is not fully defined, there is evidence that suggests the likelihood that a vegetarian diet may result in a different hormonal milieu (Howie and Shultz 1985; Hamalainen et al. 1983).

2.3 Dietary Fat

Hypotheses relating the risk of prostate cancer to a high intake of fat arose originally from observations on the international rates of mortality from prostate cancer (Berg 1975; Wynder et al. 1971). Several studies found a positive correlation between prostatic cancer occurrence and mortality and the per capita consumption of fat (Giles and Ireland 1997; Stemmerman et al. 1985; Kolonel et al. 1981; Blair and Fraumeni 1978; Armstrong and Doll 1975; Berg 1975; Howell 1974), as well as strong positive correlation between the mortality rates for prostate cancer and those for other forms of cancer suspected as being associated with fat intake, e.g. breast and ovary (Boyle and Zaridze 1993).

In general there is a strong positive correlation between fat consumption and energy intake (Simopoulos et al. 1987). Rose et al found only a weak positive association between prostate cancer mortality rates and estimates of daily caloric intake (Rose et al. 1986). When they considered the sources of energy, the results followed those observed for dietary fat; a strong positive correlation was seen when only calories of animal origin were included (r = 0.68), whereas for vegetable-derived calories the relationship was negative (r = -0.44).
Armstrong and Doll (1975) showed that prostate cancer mortality rates for different countries were highly positively correlated with estimates of total fat consumption. Using nutritional data published from the Food and Agriculture Organisation of the United Nations and international cancer mortality statistics prepared by Kurihara and others (1984), Rose and Connolly (1992) also showed that there is a strong positive correlation ($r = 0.704$) between available fat from animal sources from 28 countries, expressed as a percent of total calories per capita per day and the corresponding age-adjusted prostate cancer mortality rates. However, a relationship is completely absent when only fats from vegetable sources are examined.

Positive association between risk of prostate cancer and animal fat consumption have also been reported in a prospective study of Seventh Day Adventist (Mills et al. 1989) and in a prospective study in Hawaii (Le Marchand et al. 1994). However, other prospective studies in Hawaii failed to find this association (Severson et al. 1989; Stemmerman et al. 1985). It has also been suggested that there is an inverse association between the risk of prostate cancer and intake of total fat, animal fat, monounsaturated fat, and particularly saturated fat (odds ratio = 0.69, 95 percent confidence interval = 0.40 – 1.18, $p=0.05$) (Ghadirian et al. 1996).

The US Health Professionals Follow-up Study (Giovannucci et al. 1993) examined prospectively the relationship between prostate cancer and dietary fat, including specific fatty acids and dietary sources of fat using data from the Health Professionals Follow-up Study. This study had a prospective cohort of 51 529 U.S. men, aged 40 through 75 at recruitment and free from cancer, who completed a validated food-frequency questionnaire in 1986 and who had been followed for four years. Total fat
consumption was positively related to risk of advanced prostate cancer (age- and energy-adjusted relative risk RR = 1.79, with 95% confidence interval [CI] = 1.04-3.07, for high versus low quintile of intake; P [trend] = 0.06). This association was due primarily to animal fat (RR = 1.63; 95% CI = 0.95-2.78; P [trend] = 0.08), but not vegetable fat. Red meat represented the food group with the strongest positive association with advanced cancer (RR = 2.64; 95% CI = 1.21-5.77; P = 0.02). Fat from dairy products (with the exception of butter) or fish was unrelated to risk. Saturated fat, monounsaturated fat, and ALA, were positively associated with advanced prostate cancer risk; only the association with ALA persisted when saturated fat, monounsaturated fat, linoleic acid, and ALA were modelled simultaneously (multivariate analysis RR = 3.43; 95% CI = 1.67-7.04; P [trend] = 0.002). The results from this study support the hypothesis that animal fat, especially fat from red meat, is associated with an elevated risk of prostate cancer. After adjusting for calorie intake, low ALA consumption from animal fat but not vegetable fat was directly related to lower relative risk of prostate cancer (Giovannucci et al. 1993). It may be that ALA in meat may be a marker of some other factor in meat responsible for the association with prostate cancer.

In summary, ecological (Mills et al 1994; Rose et al 1986), case-control (Bairati et al 1998; Rohan et al. 1995; Ross et al. 1987; Heshmat et al. 1985; Graham et al. 1983), and cohort (Le Marchand et al 1994; Giovannucci 1993; Mills et al 1989) studies have suggested that there is a positive association between dietary fat and particularly fat from meat and the risk for prostate cancer. Vegetarians tend to consume greater amounts of fiber than do non-vegetarians (Howie and Shultz 1985; Goldin et al. 1982). Compared with persons consuming an omnivorous Western diet, vegetarians have a lower mortality
rate for certain cancers, including prostate cancer (Philips et al. 1983; Philips 1975). This diet may alter hormone concentrations, resulting in a lower incidence of prostate cancer.

2.4 Phytoestrogens and Lignans

Soy is a dietary compound widely consumed in Japan; a nation with one of the lowest incidences of prostate cancer in the world (Adlercreutz et al. 1991). Soy is thought to possess broad anticancer properties that may be attributed to compounds collectively called phytoestrogens (Adlercreutz and Mazur 1997). Phytoestrogens including flaxseed lignans have been measured in human saliva, plasma, urine, prostatic fluid and in semen (Adlercreutz et al. 1993, Kelly et al. 1993; Adlercreutz et al. 1991; Finlay et al. 1991; Adlercreutz et al. 1986; Dehennin et al. 1982). Lignans are phytoestrogens found in high concentrations in flaxseed, and have some similarities in structure in structure and function to soy phytoestrogens. Hormone-dependent cancers may be responsive to flaxseed phytoestrogens as demonstrated in studies of laboratory animals (Serraino and Thompson 1992a; 1992b).

2.4.1 Isoflavones

The presence of non-steroidal substances with estrogenic activity in certain plants and foodstuffs of plant origin has been recognized for some time and many hundreds of plants manifest some degree of estrogenic activity (Price and Fenwick 1985; Bradbury and White 1954). The soy isoflavonoids, daidzein, genistein, coumestrol and equol possess weak estrogenic activity (Bickoff 1961; Pope and Wright 1954), although anti-estrogenic properties have been described (Waters and Knowler 1982). As weak
estrogens, the isoflavonoids compete with estradiol for binding to the nuclear estrogen receptor (Barnes et al. 1994; Markiewicz et al. 1993; Tang and Adams 1980; Martin et al. 1978; Shutt and Cox 1972) and have been shown to inhibit human prostate cancer cell lines (Peterson and Barnes 1993).

2.4.2 Lignans

Mammalian lignans, enterolactone and enterodiol, are compounds that resemble endogenous sex hormones and have also been demonstrated to bind to the estrogen receptor, with much greater affinity and much lesser potency than estradiol (Setchell and Adlercreutz 1988; Rao et al. 1978; Shutt and Cox 1972). The partial agonist and antagonist action of lignans on the estrogen receptor may help to decrease the androgen:estrogen ratio in the serum of prostate cancer patients (Verdeal 1980). Because of the similarities between estrogen and the other sex hormones, it is feasible that these components of flaxseed may also bind to and act on androgen and progesterone receptors as agonists or antagonists, leading to decreased testosterone binding and/or balancing the androgen:estrogen ratio. Research is needed on the effects of these compounds on sex hormones in vivo. However, one explanation for the action of plant sex hormones in inhibiting tumor growth is that they block the action of endogenous sex hormones on hormone-dependent tumor cells and so induce apoptosis.

Soy and flaxseed phytoestrogens also stimulate the synthesis of sex hormone binding globulin (SHBG) in the liver (Mousavi and Adlercreutz 1993; Adlercreutz et al. 1987). Higher plasma levels of SHBG and decreased serum concentrations of FT have been reported for both vegetarian men (Belanger et al. 1989), compared to an omnivorous
group, and also for Japanese and Chinese men (Vermeulen 1993). Studies by Rubin et al. (1974) and Vermeulen et al. (1974; 1972) have shown that for the young adult male, approximately 57% of plasma testosterone is specifically and avidly bound to SHBG, about 40% is less tightly bound to serum albumin and a smaller amount, about 1% is bound to corticosteroid binding globulin. Of the total testosterone in plasma, only 2% are free and this biologically active fraction passively diffuses into the prostate target cells. Therefore, any increase in SHBG, following ingestion of phytoestrogens may result in a decreased concentrations of the growth promoting hormone, testosterone.

Lignans and isoflavonoids have also been shown to inhibit both 5α-reductase and 17β-hydroxysteroid dehydrogenase (Evans et al. 1995). The enzyme 17β-hydroxysteroid dehydrogenase catalyzes the reversible interconversion of 17β-hydroxy and 17-keto steroids, i.e. the conversion of testosterone and to androstenedione and vice-versa, and inhibition of this enzyme may have a significant influence on the metabolism of both androgens and estrogens (Figure 2.1).

Lower levels of 5α-reductase have been reported by Ross et al. (1992) in young Japanese men when compared with their Western counterparts. It is possible that the high consumption of phytoestrogens from plant material in the Japanese diet results in a significant effect on the biological availability and metabolism of androgens such as FT and DHT and contribute to the lower incidence of prostate cancer observed in the Japanese population.

Feeding studies have demonstrated that flaxseed reduces the chemically induced tumor burden in rats in the case of breast cancer (Serraino and Thompson 1992a) and also in the case of colon cancer (Serraino and Thompson 1992b). Increasing consumption of
the phytoestrogen compounds in flaxseed may thus be a means of prevention and a more “palatable” treatment for prostate cancer.

2.5 Diet and Oxidative Stress

Oxidative stress arises from both endogenous and exogenous sources. Despite antioxidant defence mechanisms, cell damage from free radicals can stimulate mutagenesis through oxidative DNA damage (Dreher and Junod 1996). All components of DNA can be attacked by the hydroxyl radical, OH\(^-\), a highly reactive oxygen-centered radical. Singlet \(O_2\) attacks guanine preferentially. The mechanisms of free radical formation are shown in Figure 2.3. Antioxidant defence systems scavenge and minimise the formation of oxygen-derived species, but they are not 100% effective (Halliwell 1994). Hence diet-derived antioxidants may be particularly important in diminishing cumulative oxidative damage.

The multistep carcinogenic process is regulated by the interactions between pro- and anti-oxidants. Oxidative stress may be positively associated with breast, colon, and prostate cancer (Hietanen et al. 1994). Antioxidants include nutrients such as vitamins (e.g. vitamin C, E and beta-carotene), trace elements that are components of enzymes performing antioxidant functions (e.g. Se, Cu, Zn, Mn), phenolic acids, lignans, phytoestrogens, thiols and lycopene that is a non-provitamin A carotenoid. Thiol groups in cystine containing proteins and glutathione become oxidised and bind one another through a sulphur bridge. Thus, low serum thiol levels represent increased oxidative stress.
Radical formation by electron transfer by the addition of a single electron to a molecule:

\[(i)\quad A + e^- \rightarrow A^-\]

Radical formation by homolytic fission of a covalent bond of a molecule, with each fragment retaining one of the paired electrons:

\[(ii)\quad X:Y \rightarrow X^- + Y^+\]

Radical formation by heterolytic fission in which the electrons of the covalent bond are retained by only one of the fragments of the parent molecule:

\[(iii)\quad X:Y \rightarrow X^- + Y^+\]

Figure 2.3 Mechanisms of Free Radical Formation
Free radicals may be involved in each of the three stages of carcinogenesis: initiation, promotion and progression (Guyton and Kensler 1993). Antioxidants might be expected to reduce the risk of this disease by scavenging or preventing the production of reactive free radicals or by enhancing the activity of the enzymes that can detoxify carcinogens including hydroxyl radicals and reactive oxygen (Bankson et al. 1993). Antioxidants may reduce DNA damage and this facet of metabolism has also been considered to be protective against cancer development or progress of initiated cells (Satoh and Sakagami 1996; Laughton et al. 1991).

Selenium is an essential element for humans. Glutathione peroxidase, an enzyme that protects the cells from oxidative damage by catalysing the destruction of $\text{H}_2\text{O}_2$ and lipid hydroperoxides by reduced glutathione, contains selenium as a prosthetic group. Ecological (Schrauzer et al. 1977) and cohort (Willett et al. 1983) studies have shown an inverse association between selenium intake and various human cancers including prostate cancer (Vaughan and McTiernan 1986; Webber et al. 1985;).

Vitamin E is an antioxidant that has been demonstrated to have a wide range of anticancer properties. These properties include protection against carcinogenesis (Statland 1992) and inhibition of tumour progression (Gerber et al. 1997). The precise mechanistic pathway of vitamin E's beneficial effects is largely unknown.

Lycopene concentrations in the prostate range from 0 to 2.58 nmol/g and in serum between 0.6 to 1.9 nmol/mL (Clinton et al. 1996). Giovannucci and others (1995) found an inverse association between dietary lycopene intake and risk of prostate cancer however not all studies have shown the same relationship between dietary intake (Le
Marchand et al. 1991) and serum levels of lycopene (Hsing et al. 1990) and risk of prostate cancer.

Reactive oxygen species and reactive nitrogen species are formed in the human body (Figure 2.4). Endogenous antioxidant defenses are inadequate to scavenge them completely, so that ongoing oxidative damage to DNA, lipids, proteins and other molecules can be demonstrated and may contribute to the development of cancer. Antioxidants may help to scavenge oxidants or oxidized compounds. This could result in a cancer-protective effect via a decrease in oxidative and other damage to DNA in humans. Hence diet-derived antioxidants may be particularly important in protecting against prostate cancer.

2.6 Prostate Anatomy and Histology

In humans, the prostate consists of three anatomical zones, termed peripheral, transition, and central (McNeal 1968). In practice, distinctions between the three zones are often based predominantly on geographical location within the prostate because the anatomical boundaries are relatively subtle in the absence of disease.

The peripheral zone comprises 65% of the prostate volume. It extends around the posterolateral peripheral aspects of the gland from its apex to its base. Its histological appearance is characterized by small, small simple acinar spaces lined by tall columnar secretory epithelial cells. As is true for the entire gland, the acini are embedded in a smooth muscle stroma. The secretory ducts leading away from the peripheral zone empty into the distal 1.5 cm of posterolateral prostatic urethra at 1- to 2-mm intervals. The main ducts give rise to several branches that arborize into secretory lobules.
(i) Formation of superoxide radical (\( \cdot O_2^- \)), hydrogen peroxide (\( H_2O_2 \)), hydroxyl radical (\( \cdot OH \)) and water stepwise, univalent reductions of molecular oxygen.

\[
\begin{align*}
  \text{O}_2 & \rightarrow \cdot \text{O}_2^- \rightarrow \text{H}_2\text{O}_2 \rightarrow \cdot \text{OH} \rightarrow \text{H}_2\text{O} \\
\end{align*}
\]

(ii) Formation of nitric oxide (\( \text{NO}^- \)) from L-arginine, and of peroxynitrate (\( \text{ONOO}^- \)) and hydroxyl radical from nitric oxide and superoxide radical.

\[
\begin{align*}
  \text{L-arg} & \rightarrow \text{NO}^- \rightarrow \text{ONOO}^- \rightarrow \cdot \text{OH} \\
\end{align*}
\]

Figure 2.4 Formation of Reactive Oxygen and Nitrogen Species
The central zone is a cone shaped region that contains 25% of the prostatic volume. The ducts of the central zone join the urethra at the verumontanum. The central zone surrounds the ejaculatory ducts. Histologically the central zone is identified by the presence of relatively large acini of irregular contour lined by low columnar to cuboidal epithelium. The smooth muscle stroma of the central zone appears more compact than that of the peripheral zone.

The transition zone of the prostate comprises 5-10% of the prostatic volume. The transition zone is composed of two bilaterally symmetrical lobes found on the sides of the proximal prostatic urethra. This zone is separated from the other two zones described above by a narrow band of muscular stroma that extends in an arch from the posterior urethra at the midprostate to the anteriormost aspects of the gland. The ducts of the transition zone empty into the urethra bilaterally at the base of the verumontanum. Histologically, the transition zone acini resemble those of the peripheral zone; however, the surrounding stroma is more compact, similar to that of the central zone.

2.7 Prostate Specific Antigen

Prostate Specific Antigen (PSA) was first identified by Hara and others (1971) and purified by Wang and others (1979). PSA is a 35 kDa single-chain glycoprotein composed of 93% amino acids and 7% saccharides (Papsidero et al. 1981). PSA was originally referred to as an antigen because rabbit antiserum raised against the crude extract of human prostatic tissue contained antibodies to a prostatic tissue-specific protein and using this antiserum a protein was detected in prostate but not in other human tissues (Wang et al. 1979).
Smaller amounts of PSA have since been found in cancerous (Diamandis et al. 1994; Yu et al. 1994) and non-cancerous breast tissues (Yu et al. 1995a), in an ovarian cancer (Yu et al. 1995b), in milk (Yu and Diamandis 1995c), in amniotic fluid (Yu and Diamandis 1995d) and in salivary duct carcinoma (James et al. 1996).

PSA slowly forms stable complexes in vitro with several of the extracellular protease inhibitors such as ACT (α1-Antichymotrypsin) and AMG (α1-Macroglobulin) (Christenssson et al. 1990). The ACT complex is the predominant form of PSA in serum. PSA in complex with AMG is not usually detected by most immunoassays, because this complex is generally engulfed by AMG, resulting in loss of most of the accessible PSA immunoreactivity. On average, 15 - 20% of the total PSA concentration in serum occurs in the free noncomplexed form (Lilja et al. 1991).

PSA synthesis is under the control of androgen and progesterone receptors, which belong to the nuclear receptor subfamily (Wahli and Martinez 1991). PSA, a serine protease, is produced primarily by the glandular epithelial cells of the prostate and secreted into the seminal fluid (Lilja 1985; Lilja et al. 1988; Wang et al. 1981). Once bound by its respective hormone, the hormone receptor complex is activated through phosphorylation, and diffuses into the nucleus to bind to a specific hormone-response element on the PSA gene, found on chromosome 19 (Bulbul et al. 1992; Beato 1989). The gene is transcribed to mRNA, then translated to PSA (Bulbul et al. 1992, Beato 1989). PSA lyses seminal coagulum (Lilja 1985). Its half-life in serum is 2.2 to 3.2 days (Oesterling et al. 1988; Stamey et al. 1987).

The serum concentrations of PSA are increased in various disease states of the prostate, especially in cancer of the prostate (Oesterling 1991). PSA represents a useful
tumor marker for monitoring progression and response to treatment among patients with prostate cancer (Smart 1997; Gann et al. 1995). Serum PSA level is directly proportional to tumor volume (Table 2.1) (Aarnink et al. 1996; Noldus and Stamey 1996; Bosch et al. 1995; Monda et al. 1995; Blackwell et al. 1994; Walz et al. 1992; Collins et al. 1993; Oosterom et al. 1989). That is, as prostate volume increases, serum PSA increases. Thus, the rate of change in serum PSA (PSA velocity) can be used to detect and monitor prostate cancer growth since serum PSA positively correlates with cancer volume and be used as a tool in the clinical management of prostate disease (Cadeddu et al. 1993). Serial measurements of PSA potentially provide an insight into the natural history of prostate cancer and tumor behavior (Pearson et al. 1994). In addition, the ratio free PSA/total PSA is higher in the case of prostate cancer than in the case of benign prostatic hypertrophy (Espana 1997; Filella 1997; Morote 1997; Ravery and Boccon-Gibod 1997; Wolff 1997). Therefore, determination of the percentage of free PSA enhances the discrimination between benign prostatic hypertrophy and prostate cancer.

Japanese men have significantly lower serum PSA levels and mortality rate compared to similarly aged white men (Oesterling et al. 1995; Tominaga et al. 1993). Similarly, African-American men have significantly higher serum PSA and a mortality rate two to three times greater than Caucasian American men (Powell 1997) do. However, most importantly, as discussed below, PSA levels in the low to normal range can also indicate the future risk of prostate cancer onset (Gann et al. 1995).

The Physicians’ Health Study (Gann et al. 1995), is an ongoing randomized trial that enrolled 22 071 men aged 40 to 84 years in 1982. In this study, the sensitivity and specificity of PSA for each year of follow-up and for aggressive and non-aggressive
### Table 2.1  Correlation Between Serum PSA Levels and Prostate Tumor Volume

<table>
<thead>
<tr>
<th>Reference</th>
<th>r-value</th>
<th>p-value</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aamink et al. 1996</td>
<td>0.85</td>
<td>&lt;0.001</td>
<td>243</td>
</tr>
<tr>
<td>Noldus and Stamey 1996</td>
<td>0.50</td>
<td>&lt;0.001</td>
<td>259</td>
</tr>
<tr>
<td>Bosch et al. 1995</td>
<td>0.58</td>
<td>&lt;0.001</td>
<td>502</td>
</tr>
<tr>
<td>Monda et al. 1995</td>
<td>0.51</td>
<td>&lt;0.001</td>
<td>100</td>
</tr>
<tr>
<td>Blackwell et al. 1994</td>
<td>0.56</td>
<td>&lt;0.001</td>
<td>311</td>
</tr>
<tr>
<td>Walz et al. 1994</td>
<td>0.33</td>
<td>&lt;0.001</td>
<td>253</td>
</tr>
<tr>
<td>Collins et al. 1993</td>
<td>0.56</td>
<td>&lt;0.001</td>
<td>1627</td>
</tr>
<tr>
<td>Oosterom et al. 1989</td>
<td>0.55</td>
<td>&lt;0.001</td>
<td>290</td>
</tr>
</tbody>
</table>
cancer was assessed. Compared with men with PSA levels less than 1.0 ng/ml, those with PSA levels between 2.0 and 3.0 ng/ml had a relative risk for prostate cancer of 5.5 (95% confidence interval, 3.7 to 9.2). Prostate-specific antigen levels less than the usual cutoff of 4.0 ng/ml were positively associated with further substantial increase in risk compared with the lowest levels (Gann et al. 1995).

The clinical cut-off point of PSA concentrations as non-cancerous prostatic carcinoma (including benign prostatic hypertrophy, prostatitis, and prostatic infarction) is unclear. Some authorities consider it to be as high as 15 ng/ml (Mettlin 1993). Patients with biopsy proven prostate cancer are unlikely to have extracapsular disease or metastasis if PSA is less than 20 ng/ml. PSA values of 40 ng/ml and above are usually indicative of metastatic prostate cancer, however, non-metastatic disease has been seen in some cases with PSA values of greater than 80 ng/ml. PSA is a helpful negative predictor of prostate cancer, with an accuracy rate of 94% to 96% (Mettlin 1993).

To measure serum PSA we used the Immulite Analyzer (Diagnostic Products Corporation, Los Angeles, CA, 90045-5597) and the IMx assay (Abbott Laboratories IMx® System, Abbott Park IL). Both these assays measure PSA in the free form and PSA complexed with ACT.

2.8 Summary

Differential exposure to environmental factors is linked to the risk of prostate cancer. This conclusion follows from the wide geographical variations in incidence within single ethnic/racial groups, and especially, from the remarkable changes in incidence observed on first generation migrants. Susceptibility to environmental risk
factors may vary across racial and ethnic groups. Such differences in susceptibility could be related to the dietary pattern across ethnic/racial groups. Dietary fiber, fat and phytoestrogen intake may be important factors in the etiology of prostate cancer. The therapeutic potential of diet can be assessed using serum PSA as a marker of prostate activity in the normal and diseased prostate.
3.0 Introduction

Prostate cancer is currently the most frequently diagnosed cancer and the second leading cause of cancer death among men in the United States (Merrill and Brawley 1997). Autopsy data indicate that latent, microscopic prostate cancer is present in at least 30% of men older than 50 years (Chodak et al. 1994). It has been proposed, in common with other hormone-dependent cancers, that dietary components which reduce sex hormone levels or activity may reduce the endocrine stimulus to hormone-dependent tumors and hence the risk of cancer development. Recent interest has focussed on plant phenolic compounds, flavanoids, isoflavanoids and lignans, which may block hormone action (Adlercreutz and Mazur 1997; Adlercreutz 1995; Messina et al. 1994).

Furthermore high fiber diets have been shown to increase fecal steroid loss as bile acids in both men and women (Jenkins et al. 1993) and to increase fecal output of estrogens in premenopausal women (Goldin et al. 1982). These data raise the question of whether soluble fiber diets, by increasing fecal steroid losses, reduce serum testosterone levels or the ratio of FT:estradiol and whether these changes reduce the endocrine stimulus to the prostate, as reflected in lower PSA levels.

Changes in serum PSA and sex hormone levels were assessed in men on high soluble and insoluble fiber diets. Lower hormone levels on either diet would support a possible link between high fiber consumption and subsequent development of prostate cancer, as has been suggested for breast cancer (Colditz et al. 1995). Bile acid output in
the feces of these subjects has been reported previously as part of a larger study on the effects of fiber on blood lipids (Jenkins et al. 1993).

3.1 Methods

3.1.1 Subjects and Study Protocol

Fourteen healthy men who had previously been found to have mild to severe dyslipidemia were studied. Thirteen of the subjects were Caucasian and one subject was West Indian. They were predominately of normal weight (BMI = 26.0 ± 0.9 kg/m², range 21.7 to 34.1 kg/m²), and their mean age was 49 ± 3 years (range 29 to 64 years). The serum PSA levels of these subjects (range of 0.2 to 2.4 ng/ml) were within the normal age related reference range of 0 to 2.5 ng/ml (40-49 years) (Partin et al. 1996; Oesterling et al. 1993). These subjects had not been pre-screened for serum PSA. After following a National Cholesterol Education Step 2 diet for at least two months, the subjects were randomly assigned to two metabolically controlled diets, each of four-month duration. One diet was high in soluble fiber and the other high in insoluble fiber. Eight individuals were given the soluble fiber diet first and 6 individuals were given the insoluble fiber diet first. The metabolic diets were separated by a two-month return to an ad libitum Step 2 diet (total fat <30 percent of calories; saturated fat <7 percent of calories; and cholesterol <200 mg daily). None of the subjects had clinical or biochemical evidence of diabetes, hepatic disease, renal disease, benign prostatic hypertrophy or prostate cancer.

Before the study began and at weeks 2, 4, 8, 12, 14, and 16 of each metabolic diet, fasting blood samples were taken for estradiol, FT and serum PSA levels. PSA levels were reported for the group of 14 subjects. The subjects were also divided into two
groups, one with higher and the other with lower starting PSA values. Subjects with higher starting PSA levels were thought to be at greater risk of developing prostate cancer. Three-day fecal collections were made on an outpatient basis at the end of week 16 of both metabolic diets, placed on frozen carbon dioxide in a polystyrene container, and returned by courier to the laboratory, where the samples were weighed and stored at -20°C before freeze-drying. The University of Toronto ethics committee approved the study protocol. Informed consent was obtained from all subjects.

At each clinic visit, dieticians assessed subjects’ diet compliance using menus on which each food was checked when eaten. Additional items were recorded in a blank column opposite the prescribed diet. Body weight was measured at each clinic visit and the results were used to adjust prescribed intake. All foods were packed at a central location and delivered weekly by courier to the subjects’ homes.

3.1.2 Diets

The two vegetarian metabolic diets shared a common core of foods, to which high soluble or insoluble fiber foods or prepared dishes were added. Our aim was to provide 2-week repeating menus with 20 percent or less of the dietary calories as fat, 20 percent as protein, and 60 percent or more as available carbohydrate (with 25 to 30 g of fiber per 1000 kcal), and less than 50 mg of dietary cholesterol daily.

The foods high in soluble fiber were barley, dried lentils, peas and beans in precooked form (as instant soups, in cans or glass jars, or as frozen dinners such as kidney-bean chili), oat bran and a commercially available breakfast cereal enriched with
psyllium. The foods high in insoluble fiber included wheat-bran breakfast cereals, high-fiber crackers, and a high-fiber bread containing fine ground wheat bran and added gluten, to balance the vegetable protein in both diets. On both diets very low intakes of fat and cholesterol were achieved through the use of low-fat dairy foods (skim milk, low-fat yogurt, cottage cheese, and skim-milk cheese) and vegetable protein products (including soybean products, tofu and food containing wheat gluten).

We assessed energy requirements using standard Lipid Research Clinics Tables with adjustment for the subjects' physical activity and seven-day dietary record. Diets were devised using a database in which the majority of foods had been analyzed in our laboratory with Association of Official Analytical Chemists methods for macronutrients (AOAC official methods of analyses 1980) and fiber (Prosky et al. 1988). The fatty acid profile was determined by gas chromatography (Cunnane and Armstrong 1990). Food-composition tables of the U.S. Department of Agriculture were used (Watt and Merrill 1963) for foods that had not been analyzed directly. The percentage figures for soluble and insoluble fiber were derived from tables (Anderson and Bridges 1988) and were applied to our values for total dietary fiber to give absolute amounts. Certain products that were not listed in the tables were analyzed specifically for soluble and insoluble fiber (Prosky et al. 1988).

3.1.3 Analyses

The Abbott IMx® PSA assay, a Microparticle Enzyme Immunoassay (MEIA), was used for the quantitative measurement of PSA in human serum (Abbott Laboratories
IMx® System, Abbott Park, IL) (Garg et al. 1995). The IMx® System measures total PSA, which includes PSA in the free form, and PSA complexed with ACT.

For the quantitative measurement of estradiol, the double antibody estradiol $^{125}$I radioimmunoassay of Diagnostic Products Corporation (DPC®) was used (Diagnostic Products Corporation, Los Angeles, CA) (Schioler et al. 1988). FT in serum was measured using the DCP's® Coat-A-Count® FT procedure (Diagnostic Products Corporation, Los Angeles, CA) (Wheeler 1995). Serum estradiol and FT was analysed at Saint Michael's Hospital Hormone Assay Laboratory using a radioimmunoassay based on the competitive binding of labelled ($^{125}$I-) and unlabelled antigen (in standards or samples) to their specific antibody (Wide et al. 1973).

Fecal acidic and neutral sterols were measured in finely ground freeze-dried feces from three day collections after extraction, thin-layer chromatography, methylation and trimethylsylation followed by gas-liquid chromatography with a DB-1 column (J&W Scientific, Folsom, CA), with 5β-cholinic acid and 5α-cholestane, respectively, as internal standards (Anderson et al. 1984).

3.1.4 Statistical Analysis

Data are expressed as mean ± SEM. Power analysis to determine the sample size to demonstrate a difference in serum PSA levels was not calculated since this study was originally designed to investigate the effects of high fiber foods on serum lipid levels (Jenkins et al. 1993). Treatment values for serum PSA, FT and estradiol represent the mean of the 2 to 16 week values. The response variables were found to conform to
normal distribution using the Wilk-Shapiro test (PROC UNIVARIATE/SAS) (SAS/STAT User’s Guide (ed. 6) 1990). The significance of treatment effect was assessed by way of Analysis of CoVariance (CANOVA) in SAS (PROC MIXED) with diet as the main effect, random subject variable to alert the procedure to the fact that it is a crossover design (random, mixed model) and a continuous effect given by the baseline value (SAS/STAT User’s Guide (ed. 6) 1990). The strength of linear association between treatment endpoints was assessed by Pearson product-moment correlation (PROC CORR/SAS) (SAS/STAT User’s Guide (ed. 6) 1990).

3.2 Results

The diets were well accepted, and compliance was good, as judged by close agreement between the macronutrient profile of the prescribed diets and the diets recorded as consumed (Jenkins et al. 1993). The macronutrient profiles of the diets are shown in Table 3.1.

3.2.1 Prostatic Specific Antigen

Baseline serum PSA levels (week 0) on the soluble fiber diet (0.97 ± 0.17 ng/mL) and on the insoluble fiber diet (1.02 ± 0.17 ng/mL) were not significantly different (p=0.165, n=14) (Table 3.2). On the metabolic diet periods the mean PSA on the soluble fiber diet (0.96 ± 0.17 ng/mL) was significantly lower than the mean PSA on the insoluble fiber diet (1.03 ± 0.19 ng/mL), a difference of -0.07 ± 0.03 ng/mL (p=0.035, n = 14) (Table 3.3).
Table 3.1  Mean Daily Macronutrient Intakes and Profiles of the Metabolic Diets$^{1,2}$

<table>
<thead>
<tr>
<th>Macronutrient Profile</th>
<th>Soluble Fiber Diet</th>
<th>Insoluble Fiber Diet</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calorie Intake (kcal)</td>
<td>2369 ± 115$^3$</td>
<td>2245 ± 129</td>
<td>0.024</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>49.8 ± 2.7</td>
<td>48.9 ± 2.8</td>
<td>0.384</td>
</tr>
<tr>
<td>(% of calorie intake)</td>
<td>18.9 ± 0.5</td>
<td>19.7 ± 0.6</td>
<td>0.080</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>10.1 ± 0.5</td>
<td>10.0 ± 0.6</td>
<td>0.759</td>
</tr>
<tr>
<td>(% of calorie intake)</td>
<td>3.9 ± 0.1</td>
<td>4.1 ± 0.2</td>
<td>0.052</td>
</tr>
<tr>
<td>Monounsaturated Fat (g)</td>
<td>14.1 ± 1.1</td>
<td>13.8 ± 1.1</td>
<td>0.485</td>
</tr>
<tr>
<td>(% of calorie intake)</td>
<td>5.3 ± 0.2</td>
<td>5.6 ± 0.3</td>
<td>0.198</td>
</tr>
<tr>
<td>Polyunsaturated Fat (g)</td>
<td>21.6 ± 1.2</td>
<td>21.2 ± 1.3</td>
<td>0.419</td>
</tr>
<tr>
<td>(% of calorie intake)</td>
<td>8.2 ± 0.2</td>
<td>8.5 ± 0.2</td>
<td>0.004</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>114.4 ± 7.0</td>
<td>110.3 ± 7.3</td>
<td>0.133</td>
</tr>
<tr>
<td>(% of calorie intake)</td>
<td>19.2 ± 0.4</td>
<td>19.5 ± 0.4</td>
<td>0.025</td>
</tr>
<tr>
<td>Available Carbohydrate (g)</td>
<td>363.0 ± 16.9</td>
<td>336.9 ± 20.3</td>
<td>0.012</td>
</tr>
<tr>
<td>(% of calorie intake)</td>
<td>61.4 ± 0.7</td>
<td>60.0 ± 0.9</td>
<td>0.039</td>
</tr>
<tr>
<td>Total Dietary Fiber (g)</td>
<td>56.6 ± 3.1</td>
<td>65.2 ± 4.0</td>
<td>0.001</td>
</tr>
<tr>
<td>(g/1000 kcal)</td>
<td>23.9 ± 0.7</td>
<td>29.2 ± 1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Soluble Fiber (g)</td>
<td>17.9 ± 1.0</td>
<td>11.7 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(g/1000 kcal)</td>
<td>(7.6 ± 0.2</td>
<td>5.3 ± 0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insoluble Fiber (g)</td>
<td>38.5 ± 2.2</td>
<td>53.2 ± 3.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(g/1000 kcal)</td>
<td>16.3 ± 0.6</td>
<td>23.7 ± 0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dietary Cholesterol (mg)</td>
<td>24.4 ± 3.1</td>
<td>28.6 ± 4.5</td>
<td>0.192</td>
</tr>
<tr>
<td>(mg/1000 kcal)</td>
<td>10.5 ± 1.4</td>
<td>13.3 ± 2.4</td>
<td>0.083</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>1.8 ± 0.6</td>
<td>2.3 ± 0.8</td>
<td>0.327</td>
</tr>
<tr>
<td>(% of calorie intake)</td>
<td>0.6 ± 0.2</td>
<td>0.8 ± 0.3</td>
<td>0.276</td>
</tr>
</tbody>
</table>

17-day food records

2n=7

3mean ± SEM for multiple measurements from 2 to 16 weeks
Table 3.2 Baseline Serum PSA and Serum Hormones and Fecal Steroids for 14 Subjects\(^1\) on Insoluble Fiber, Soluble Fiber Diets and the Baseline Differences\(^2\) Between Diets

<table>
<thead>
<tr>
<th>Variable</th>
<th>Soluble Fiber Diet</th>
<th>Insoluble Fiber Diet</th>
<th>Baseline</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum PSA (ng/mL)</td>
<td>0.97 ± 0.17(^3)</td>
<td>1.02 ± 0.17</td>
<td>-0.05 ± 0.03</td>
<td>0.165</td>
</tr>
<tr>
<td>Serum PSA &gt; 0.8 (n=7) (ng/mL)</td>
<td>1.36 ± 0.27</td>
<td>1.44 ± 0.24</td>
<td>-0.08 ± 0.05</td>
<td>0.190</td>
</tr>
<tr>
<td>Serum PSA &lt; 0.8 (n=7) (ng/mL)</td>
<td>0.58 ± 0.08</td>
<td>0.60 ± 0.07</td>
<td>-0.02 ± 0.04</td>
<td>0.639</td>
</tr>
<tr>
<td>Serum Free Testosterone (pmol/L)</td>
<td>67 ± 5</td>
<td>70 ± 5</td>
<td>-3 ± 3</td>
<td>0.291</td>
</tr>
<tr>
<td>Serum Estradiol (pmol/L)</td>
<td>72 ± 9</td>
<td>78 ± 8</td>
<td>-6 ± 5</td>
<td>0.204</td>
</tr>
<tr>
<td>Free Testosterone/Estradiol</td>
<td>1.16 ± 0.20</td>
<td>1.04 ± 0.17</td>
<td>0.12 ± 0.05</td>
<td>0.042</td>
</tr>
<tr>
<td>Fecal Steroid Excretion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Bile Acids (mg/day)</td>
<td>293 ± 56</td>
<td>244 ± 48</td>
<td>-49 ± 35</td>
<td>0.194</td>
</tr>
<tr>
<td>Cholic (mg/day)</td>
<td>27 ± 12</td>
<td>25 ± 16</td>
<td>2 ± 6</td>
<td>0.665</td>
</tr>
<tr>
<td>Chenodeoxycholic (mg/day)</td>
<td>14 ± 6</td>
<td>15 ± 9</td>
<td>1 ± 5</td>
<td>0.840</td>
</tr>
<tr>
<td>Deoxycholic (mg/day)</td>
<td>151 ± 30</td>
<td>122 ± 21</td>
<td>29 ± 23</td>
<td>0.239</td>
</tr>
<tr>
<td>Lithocholic (mg/day)</td>
<td>93 ± 14</td>
<td>74 ± 12</td>
<td>19 ± 12</td>
<td>0.162</td>
</tr>
<tr>
<td>Ursodeoxycholic (mg/day)</td>
<td>8 ± 2</td>
<td>8 ± 3</td>
<td>0 ± 3</td>
<td>0.978</td>
</tr>
<tr>
<td>Total Neutral Sterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (mg/day)</td>
<td>622 ± 74</td>
<td>633 ± 111</td>
<td>11 ± 120</td>
<td>0.927</td>
</tr>
<tr>
<td>Coprostanone (mg/day)</td>
<td>57 ± 9</td>
<td>62 ± 14</td>
<td>-5 ± 12</td>
<td>0.706</td>
</tr>
<tr>
<td>Coprostanol (mg/day)</td>
<td>358 ± 59</td>
<td>389 ± 75</td>
<td>31 ± 86</td>
<td>0.729</td>
</tr>
<tr>
<td>Cholesterol Excretion (mg/day)</td>
<td>206 ± 56</td>
<td>183 ± 46</td>
<td>23 ± 46</td>
<td>0.611</td>
</tr>
</tbody>
</table>

\(^1\)n=14 unless indicated otherwise

\(^2\)Soluble Fiber Diet (0) – Insoluble Fiber Diet (0)

\(^3\)mean ± SEM
Table 3.3  Serum PSA and Serum Hormones\(^1\) and Fecal Steroids for 14 Subjects\(^2\) on
Insoluble Fiber, Soluble Fiber Diets and the Treatment Differences\(^3\) Between
Diets

<table>
<thead>
<tr>
<th>Variable</th>
<th>Soluble Fiber Diet</th>
<th>Insoluble Fiber Diet</th>
<th>Treatment Difference(^3)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum PSA (ng/mL)</td>
<td>0.96 ± 0.17(^*)</td>
<td>1.03 ± 0.19</td>
<td>-0.07 ± 0.03</td>
<td>0.035</td>
</tr>
<tr>
<td>Serum PSA &gt; 0.8 (n=7) (ng/mL)</td>
<td>1.35 ± 0.27</td>
<td>1.48 ± 0.29</td>
<td>-0.13 ± 0.03</td>
<td>0.008</td>
</tr>
<tr>
<td>Serum PSA &lt; 0.8 (n=7) (ng/mL)</td>
<td>0.58 ± 0.08</td>
<td>0.58 ± 0.08</td>
<td>0 ± 0.03</td>
<td>0.899</td>
</tr>
<tr>
<td>Serum Free Testosterone (pmol/L)</td>
<td>63 ± 5</td>
<td>66 ± 5</td>
<td>-3 ± 3</td>
<td>0.300</td>
</tr>
<tr>
<td>Serum Estradiol (pmol/L)</td>
<td>66 ± 8</td>
<td>69 ± 7</td>
<td>-3 ± 2</td>
<td>0.159</td>
</tr>
<tr>
<td>Free Testosterone/Estradiol</td>
<td>1.14 ± 0.17</td>
<td>1.11 ± 0.16</td>
<td>0.03 ± 0.05</td>
<td>0.550</td>
</tr>
<tr>
<td>Fecal Steroid Excretion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Bile Acids (mg/day)</td>
<td>341 ± 56</td>
<td>203 ± 35</td>
<td>138 ± 28</td>
<td>0.001</td>
</tr>
<tr>
<td>Cholic (mg/day)</td>
<td>19 ± 5</td>
<td>10 ± 2</td>
<td>9 ± 3</td>
<td>0.020</td>
</tr>
<tr>
<td>Chenodeoxycholic (mg/day)</td>
<td>12 ± 4</td>
<td>4 ± 1</td>
<td>7 ± 3</td>
<td>0.021</td>
</tr>
<tr>
<td>Deoxycholic (mg/day)</td>
<td>183 ± 30</td>
<td>105 ± 20</td>
<td>79 ± 14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lithocholic (mg/day)</td>
<td>121 ± 21</td>
<td>81 ± 14</td>
<td>40 ± 10</td>
<td>0.003</td>
</tr>
<tr>
<td>Ursodeoxycholic (mg/day)</td>
<td>6 ± 2</td>
<td>3 ± 1</td>
<td>3 ± 2</td>
<td>0.102</td>
</tr>
<tr>
<td>Total Neutral Sterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (mg/day)</td>
<td>457 ± 73</td>
<td>456 ± 69</td>
<td>1 ± 63</td>
<td>0.981</td>
</tr>
<tr>
<td>Coprostanone (mg/day)</td>
<td>36 ± 13</td>
<td>52 ± 13</td>
<td>-16 ± 10</td>
<td>0.170</td>
</tr>
<tr>
<td>Coprostanol (mg/day)</td>
<td>299 ± 66</td>
<td>286 ± 64</td>
<td>13 ± 45</td>
<td>0.913</td>
</tr>
<tr>
<td>Cholesterol Excretion (mg/day)</td>
<td>122 ± 29</td>
<td>118 ± 29</td>
<td>4 ± 24</td>
<td>0.877</td>
</tr>
</tbody>
</table>

\(^1\)Treatment values for serum PSA, FT and estradiol represent the mean of the 2 to 16
week values.

\(^2\)n=14 unless indicated otherwise

\(^3\)Soluble Fiber Diet (Wk16) – Insoluble Fiber Diet (Wk 16)

\(^4\)mean ± SEM
When subjects with baseline PSA levels above 0.8 ng/mL were assessed separately, the PSA concentrations were $1.35 \pm 0.27$ ng/mL on soluble fiber and $1.48 \pm 0.29$ ng/mL on insoluble fiber, a treatment difference of $0.13 \pm 0.03$ ng/mL (p=0.008, n=7). (Table 3.3). However, in those subjects (n=7) with baseline PSA concentrations below 0.8 ng/ml no significant difference was observed.

3.2.2 **Serum Hormone Levels**

No differences were seen in baseline levels of serum FT ($3 \pm 3$ pmol/L, p = 0.291) or serum estradiol ($7 \pm 5$ pmol/L, p = 0.204) between treatments. However, the ratio of FT:estradiol was significantly higher on the soluble fiber diet ($1.16 \pm 0.20$) compared to the ratio on the insoluble fiber diet ($1.04 \pm 0.17$), p = 0.042 (Table 3.2). On the insoluble fiber diet, serum estradiol levels fell significantly from $78 \pm 8$ pmol/L at week 0 to $69 \pm 7$ pmol/L, a difference of $9 \pm 3$ pmol/L, p=0.004. However, no significant treatment differences were noted between the soluble and the insoluble fiber diets in serum estradiol ($3 \pm 2$ pmol/L, p = 0.159), serum FT ($3 \pm 3$ pmol/L, p = 0.300) or the ratio of FT:estradiol ($0.03 \pm 0.05$, p=0.547). Nor did the treatment difference in PSA show significant associations with the treatment difference in either sex hormone or the ratio of FT:estradiol.

3.2.3 **Fecal Bile Salts and Neutral Sterols**
Baseline levels (week 0) of fecal bile acid and neutral sterol excretion on the soluble and insoluble fiber diets were similar (Table 3.2). At week sixteen fecal bile acid excretion was significantly higher on the soluble fiber diet than on the insoluble fiber diet for total bile acids (138 ± 28 mg/day, p=0.001), cholic acid (9 ± 3, p=0.020), chenodeoxycholic acid (7 ± 3, p=0.021), deoxycholic acid (79 ± 14, p<0.001) and lithocholic acid (40 ± 10 mg/day, p=0.003). (Table 3.3). The treatment difference in lithocholic acid excretion was significantly positively correlated with the treatment difference in serum PSA levels (r=0.566, p=0.035) as shown in Figure 3.1.

No differences were observed in neutral sterol excretion (total neutral sterols, cholesterol, coprostanone, and coprostanol) between the two dietary treatments after sixteen weeks (Table 3.3) and differences in fecal losses of neutral sterols were not related to differences in serum PSA levels.

3.3 Discussion

We found those diets high in soluble fiber significantly reduced serum PSA levels compared to diets high in insoluble fiber. Serum sex hormone levels were not related to the lower PSA levels on the soluble fiber diet. However, fecal lithocholic acid output, which was significantly increased on the diets high in soluble fiber compared to the diets high in insoluble fiber, was significantly correlated to the treatment difference in serum PSA.

Gann and others (1995) found that men with serum PSA levels between 1.01 to 1.50 ng/mL were at twice the risk of developing prostate cancer compared with men in
Figure 3.1 Correlation Between Differences in Serum PSA and Fecal Lithocholic Acid Output

1 Change Represents Soluble Fiber Diet (Mean Wk 2 to Wk 16 Values) - Insoluble Fiber Diet (Mean Wk 2 to Wk 16 Values)

2 Change Represents Soluble Fiber Diet (Wk 16) - Insoluble Fiber Diet (Wk 16)
the reference category of 1.0 ng/mL or less. We also found that those men with a mean PSA of 1.42 ng/mL were more responsive to dietary intervention than men with a mean PSA of 0.58 ng/mL. All the PSA levels of our subjects were within the normal age related reference range (PSA less than 2.5 ng/mL, 40-49 years of age). However, in view of the high prevalence of prostate cancer in older men and the presence of nests of malignant cells in 30% of men over the age of 50 years (Chodak 1994), it is possible that the reduction in serum PSA may have involved cells either at higher risk or on the path to malignant transformation and responsible for the higher overall PSA level. If studies had been carried out in men with prostate cancer, the question arises as to whether a greater reduction in PSA levels would have been observed. In this respect, it is of interest that high fiber vegetarian diets have been reported to possibly delay the progression of prostatic carcinoma (Carter et al. 1993).

Sex hormones are implicated in the pathogenesis of hormone-dependent cancer of the breast and prostate (Yoo et al. 1997; Gann et al. 1996; Andersson et al. 1993). Pharmacological reduction of androgen activity is one of the treatment options for prostate cancer (Goethuys et al. 1997; Labrie et al. 1997; Cox and Crawford 1995; Schelhammer et al. 1995; Blackledge 1993; Delaere and van Thillo 1991; Newling 1990; Sogani and Whitmore 1988; Narayana et al. 1981). Our aim was to see whether soluble fiber reduced androgenic activity as indicated by lower serum PSA levels in healthy subjects.

Soluble fiber is known to increase fecal steroid elimination including fecal bile acid excretion (Spiller 1996). Experimental evidence also suggests that diet can influence serum sex hormone levels and their fecal excretion (Adlercreutz 1990; Goldin
and Gorbach 1988). Increased dietary fiber and lower animal protein intake have been shown to increase fecal estradiol, estrone, and estriol losses in vegetarian women compared to omnivorous women (Goldin et al. 1982). Although we did not measure fecal sex hormones, the increase in fecal bile acid output provides evidence for increased fecal steroid elimination. Fecal β-glucuronidase activity was lower in the vegetarians than in the omnivores (1.0 μg per minute per milligram of fecal protein, as compared with 1.8 μg per minute per milligram; p=0.05) (Goldin et al. 1982). The authors suggested that the dietary fiber effects on gut flora resulted in increased fecal estrogen elimination and thus a reduced enterohepatic circulation of estrogens. Pusateri and others (1990) found a significant relationship between high dietary fiber intakes, fecal fiber elimination and significantly increased fecal estrogens and testosterone losses in men despite no significant reduction in plasma levels. We found that soluble fiber resulted in an increased fecal bile acid output compared to the diet high in insoluble fiber. We also noted that the increase in fecal lithocholic acid excretion was significantly positively correlated with lower serum PSA levels. However no treatment difference was seen in serum sex hormones. The question remains as to whether an increased fecal loss of testosterone would reduce PSA production and prostate cellular activity in the absence of a change in plasma testosterone levels.

We conclude that soluble fiber may produce a modest reduction in PSA levels. The mechanism could be positively related to increased fecal sterol losses suggested by the positive association between changes in serum PSA and fecal lithocholic acid excretion despite no change in serum sex hormones. The long-term implications of diet
changes in prostate cancer in relation to fiber remain to be explored in subjects with higher PSA levels or with established prostatic disease.
CHAPTER 4. HIGH FIBER FOODS, FATTY ACIDS AND PROSTATE CANCER

4.0 Introduction

We found that serum PSA levels were reduced on diets high in soluble fiber compared to diets high in insoluble fiber by $0.07 \pm 0.03$ ng/ml, $p=0.035$ (Table 3.3). No changes in serum sex hormones accounted for the reduction in serum PSA although increased output of fecal bile acids was observed ($+138 \pm 28$ mg/day, $p<0.001$) on soluble fiber which, in part might indicate increased sex steroid elimination. Since FT has been implicated in the etiology of prostate cancer, increased sex steroid elimination may positively relate to lower serum PSA levels. However, in the absence of a clear reason for the lower PSA concentrations on the soluble fiber, alternative explanations were explored.

Epidemiological studies suggest that dietary fat (Dijkman and Debruyne 1996; Waterhouse and Muir 1982; Armstrong and Doll 1975) and animal products, especially red meat, may be implicated in the progression of prostate cancer (Giovannucci et al. 1993; Kolonel et al. 1988). Case control studies have also found a positive association between total and saturated fat intake and risk of developing prostate cancer (West et al. 1991; Slattery et al. 1990; Ross et al. 1983; Graham et al. 1982).

In addition, dietary fatty acids have been suggested to play a role in prostate cancer development (Gann et al. 1994; Giovannucci et al. 1993). Prospective cohort studies suggest low plasma CE (Cholesteryl Ester) ALA levels may be associated with reduced risk of advanced prostate cancer. In vitro, arachidonic acid (Ghosh and Myers 1997) and linoleic acid (Rose et al. 1991) have been shown to stimulate growth of the
PC-3 prostate cancer cell line while n-3 polyunsaturates (PUFA) inhibited PC-3 cell growth due to an arrest in proliferation rather than toxicity (Rose et al. 1991). Other studies have suggested that fatty acids that have 14-22 carbon chains and one to six double bonds in the cis configuration might act as 5α-reductase inhibitors (Neiderprum et al. 1995; Liang and Liao 1992). The enzyme, 5α-reductase, is involved in the conversion of testosterone to dihydrotestosterone, the active metabolite. The relative inhibitory potencies of unsaturated fatty acids are, in decreasing order: gamma-linolenic acid > cis-4,7,10,13,16,19-docosahexaenoic acid = cis-6,9,12,15-octatetraenoic acid = arachidonic acid =ALA > linoleic acid > palmitoleic acid > oleic acid > myristoleic acid (Liang and Liao 1992). The relative inhibitory potencies of saturated fatty acids are, in decreasing order: capric acid (10:0) > myristic acid (14:0) > hendecanoic acid (11:0) > lauric acid (12:0) > tridecylic acid (13:0) (Neiderprum et al. 1995).

We have therefore assessed the fatty acid profiles in the CE, PL (Phospholipid) and TG (Triglyceride) fractions of serum from men with PSA levels in the normal range to determine whether possible fiber related effects on fatty acid metabolism accounted for the differences in serum PSA with special reference to possible changes in ALA metabolism. The subjects in whom these assessments were made had taken part in a study investigating the lipid lowering effects of soluble versus insoluble fiber diets (Jenkins et al. 1993).

4.1 Methods

4.1.1 Subjects and Study Protocol
The subject and study protocol is described in section 3.1.1. Blood for fatty acid analysis was collected at the beginning and end of each metabolic diet while the subjects were fasting. Blood was collected in test tubes that were free of additives and 1 mL of serum was allotted for fatty acid analysis.

4.1.2 Diets

The profiles of the diets are described in section 3.1.2 on.

4.1.3 Analyses

The method for PSA analysis is described in section 3.1.3.

Fatty acids in food and serum were analyzed by Ms Mary-Ann Ryan at the University of Toronto Department of Nutritional Sciences under the supervision of Dr S.C. Cunnane. Seven-day composite diets from the soluble and insoluble phases were homogenized and freeze-dried for fatty acid analysis. The material was ground and analyzed by the method of Ulberth and Henninger: One Step Extraction/Methylation Method for Determining the Fatty acid Composition of Processed Foods (JAOCS, 1992). Individual fatty acids in food were separated with a 30m X 0.25 mm inner diameter fused silica column coated with 0.25 µ DB-23 (J & W Scientific, Folson, CA). 1 µg samples were injected at a column temperature of 50°Celsius. Two temperature ramps were used to reach a final temperature of 230°Celsius. Helium at 1 Bar was the carrier gas. Gas-liquid chromatography separation was done on a Hewlett Packard GC with 7673A autosampler and Hewlett Packard 3393A integrator. Fatty acids were identified by
comparisons of their retention times with authentic standards (Supelco C, Bellefonte PA; Nu Chek Prep, Elysian MN).

Serum fatty acids were extracted and total PL, TG and CE were separated by thin layer chromatography on Whatman 20 X 20 cm K6F silica gel 60 plates (layer thickness 250 μm (Cunnane and Armstrong 1990). Heptadecanoic acid was added before extraction as internal standard. CE were saponified and the fatty acid methyl esters were prepared by the method of Morrison and Smith (1964). Gas-liquid chromatography separation was done on a Hewlett Packard 5890 gas chromatograph equipped with a flame ionization detector, Hewlett Packard 3393A integrator. Individual fatty acids were separated and identified by comparison with authentic standards as described above. Percentage fat in the dry serum sample was calculated by means of the heptadecanoic acid internal standard and the sample weight.

4.1.4 Statistical Analysis

Serum PSA and fatty acid concentrations are expressed as mean ± SEM. The statistical methods used are the same as those described in section 3.1.4

4.1 Results

The diet compliance was good as judged by close agreement between the macronutrient profile of the prescribed diets (Table 3.1) and the profile observed. The fatty acid profiles of the metabolic diets were similar (Table 4.1).
Table 4.1  Fatty Acid Profile of 7-day Metabolic Diet Composites$^{1,2,3}$

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Soluble Fiber Diet</th>
<th>Insoluble Fiber Diet</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td>3.3 ± 0.4$^{4,5}$</td>
<td>3.3 ± 0.1</td>
<td>1.000</td>
</tr>
<tr>
<td>14:0</td>
<td>4.2 ± 0.9</td>
<td>4.2 ± 1.0</td>
<td>0.804</td>
</tr>
<tr>
<td>16:0</td>
<td>41.7 ± 3.1</td>
<td>43.8 ± 2.8</td>
<td>0.146</td>
</tr>
<tr>
<td>17:0</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>1.000</td>
</tr>
<tr>
<td>18:0</td>
<td>15.1 ± 1.5</td>
<td>15.4 ± 1.1</td>
<td>0.737</td>
</tr>
<tr>
<td>20:0</td>
<td>1.6 ± 0.4</td>
<td>1.6 ± 0.4</td>
<td>0.789</td>
</tr>
<tr>
<td>22:0</td>
<td>2.4 ± 0.8</td>
<td>2.4 ± 0.7</td>
<td>0.836</td>
</tr>
<tr>
<td>24:0</td>
<td>0.9 ± 0.3</td>
<td>0.9 ± 0.2</td>
<td>0.761</td>
</tr>
<tr>
<td>Sum of Saturates</td>
<td>69.7 ± 6.4</td>
<td>72.1 ± 5.8</td>
<td>0.412</td>
</tr>
<tr>
<td>14:1n-5</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.500</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.2</td>
<td>0.215</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>82.1 ± 10.8</td>
<td>81.9 ± 9.0</td>
<td>0.940</td>
</tr>
<tr>
<td>18:1n-7</td>
<td>4.2 ± 0.8</td>
<td>4.0 ± 0.4</td>
<td>0.852</td>
</tr>
<tr>
<td>20:1n-9</td>
<td>1.4 ± 0.3</td>
<td>1.4 ± 0.3</td>
<td>0.391</td>
</tr>
<tr>
<td>22:1n-9</td>
<td>0.5 ± 0.2</td>
<td>0.7 ± 0.3</td>
<td>0.270</td>
</tr>
<tr>
<td>Sum of Monounsaturates</td>
<td>89.3 ± 11.2</td>
<td>89.1 ± 9.8</td>
<td>0.941</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>134.5 ± 16.1</td>
<td>145.5 ± 12.3</td>
<td>0.118</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>11.1 ± 1.5</td>
<td>10.5 ± 1.1</td>
<td>0.425</td>
</tr>
<tr>
<td>Sum of Polyunsaturates$^{5}$</td>
<td>145.6 ± 16.4</td>
<td>156.1 ± 12.3</td>
<td>0.133</td>
</tr>
</tbody>
</table>

$^{1}$7-day metabolic diet composites

$^{2}$n=14

$^{3}$No detectable trans fatty acids

$^{4}$(milligrams of fatty acids / 100 grams of food)

$^{5}$mean ± SEM

$^{6}$No detectable 20:4n-6, 20:5n-3, 22:6n-3
4.2.1  Fatty Acids in Serum

4.2.1.1 Treatment Differences

Fatty acid levels in μg/dL and percentage of total fatty acids in the CE, PL and TG fraction after 16 weeks on the soluble and insoluble fiber diets are shown in Figure 4.1 and 4.2, respectively. Total fatty acids in the CE, PL and TG fractions were similar on the soluble and insoluble fiber diets (Table 4.2). The total saturated fatty acids (%) in CE and TG were significantly higher on the soluble fiber diet than on the insoluble fiber diet, while the reverse was seen with total polyunsaturated fatty acids in mg/dL and also expressed as the percentage of total fatty acids in CE and TG respectively (Table 4.2).

No differences were observed in serum ALA levels in the CE, PL or TG fractions on the soluble and insoluble fiber diet. Eicosapentaenoic acid (%) in CE was significantly higher on the soluble fiber diet than on the insoluble fiber diet (Table 4.2). Docosahexaenoic acid (μg/dl) in serum TG was significantly higher on the soluble fiber diet than on the insoluble fiber diet (Table 4.2).

The only fatty acid to show changes in all fractions was linoleic acid. Linoleic acid (μg/dl and %) in CE, and PL fractions was significantly lower on the soluble fiber diet compared to the insoluble fiber diet (Table 4.2). Significantly lower percentage of linoleic acid on the soluble fiber diet was also seen in the TG fraction but not when expressed in μg/dl (Table 4.2). However, no treatment differences were observed with arachidonic acid levels in the CE, PL and TG fractions on the soluble and insoluble fiber diets.
Table 4.2  Fatty Acids in Serum Lipid Fractions<sup>1,2</sup>

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Cholesteryl Esters&lt;sup&gt;3&lt;/sup&gt;</th>
<th>p-value</th>
<th>Phospholipids</th>
<th>p-value</th>
<th>Triglycerides</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:3n-3</td>
<td>(µg/dL) 1.7 ± 2.0&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.429</td>
<td>-0.3 ± 0.7</td>
<td>0.631</td>
<td>-3.1 ± 7.6</td>
<td>0.692</td>
</tr>
<tr>
<td></td>
<td>(%) 0.1 ± 0.1</td>
<td>0.055</td>
<td>0.0 ± 0.0</td>
<td>0.577</td>
<td>-0.1 ± 0.2</td>
<td>0.625</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>(µg/dL) 6.4 ± 4.0</td>
<td>0.131</td>
<td>-0.8 ± 3.0</td>
<td>0.784</td>
<td>-0.8 ± 1.5</td>
<td>0.616</td>
</tr>
<tr>
<td></td>
<td>(%) 0.3 ± 0.1</td>
<td>0.029</td>
<td>0.1 ± 0.1</td>
<td>0.662</td>
<td>0.0 ± 0.0</td>
<td>0.326</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>(µg/dL) -1.0 ± 1.5</td>
<td>0.538</td>
<td>4.7 ± 2.3</td>
<td>0.840</td>
<td>1.5 ± 0.5</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>(%) -0.0 ± 0.1</td>
<td>0.816</td>
<td>0.2 ± 0.2</td>
<td>0.223</td>
<td>0.1 ± 0.1</td>
<td>0.126</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>(µg/dL) -134.4 ± 37.5</td>
<td>0.003</td>
<td>-70.2 ± 24.3</td>
<td>0.013</td>
<td>-26.6 ± 43.3</td>
<td>0.550</td>
</tr>
<tr>
<td></td>
<td>(%) -2.5 ± 0.7</td>
<td>0.003</td>
<td>-1.8 ± 0.0</td>
<td>0.029</td>
<td>-4.1 ± 1.6</td>
<td>0.021</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>(µg/dL) 0.7 ± 9.8</td>
<td>0.942</td>
<td>-11.8 ± 7.8</td>
<td>0.153</td>
<td>0.1 ± 2.1</td>
<td>0.956</td>
</tr>
<tr>
<td></td>
<td>(%) 0.2 ± 0.2</td>
<td>0.223</td>
<td>0.4 ± 0.5</td>
<td>0.425</td>
<td>0.1 ± 0.1</td>
<td>0.313</td>
</tr>
<tr>
<td>Sum of Polyunsaturates (µg/dL)</td>
<td>-123.5 ± 51.9</td>
<td>0.033</td>
<td>-76.1 ± 36.8</td>
<td>0.059</td>
<td>-25.4 ± 53.4</td>
<td>0.642</td>
</tr>
<tr>
<td></td>
<td>(%) -1.6 ± 0.6</td>
<td>0.016</td>
<td>-0.1 ± 1.5</td>
<td>0.952</td>
<td>-3.9 ± 1.6</td>
<td>0.035</td>
</tr>
<tr>
<td>Sum of Monounsaturates (µg/dL)</td>
<td>-1.8 ± 23.0</td>
<td>0.938</td>
<td>-15.1 ± 9.5</td>
<td>0.137</td>
<td>6.0 ± 73.9</td>
<td>0.983</td>
</tr>
<tr>
<td></td>
<td>(%) 1.1 ± 0.5</td>
<td>0.068</td>
<td>0.4 ± 0.4</td>
<td>0.351</td>
<td>1.6 ± 1.3</td>
<td>0.222</td>
</tr>
<tr>
<td>Sum of Saturates (µg/dL)</td>
<td>-0.3 ± 11.2</td>
<td>0.976</td>
<td>-72.5 ± 50.9</td>
<td>0.178</td>
<td>6.0 ± 73.9</td>
<td>0.937</td>
</tr>
<tr>
<td></td>
<td>(%) 0.5 ± 0.2</td>
<td>0.004</td>
<td>-0.9 ± 1.8</td>
<td>0.636</td>
<td>2.2 ± 1.1</td>
<td>0.045</td>
</tr>
<tr>
<td>Total Fatty Acids (µg/dL)</td>
<td>-125.6 ± 80.1</td>
<td>0.141</td>
<td>-163.7 ± 82.5</td>
<td>0.069</td>
<td>-17.5 ± 193.5</td>
<td>0.930</td>
</tr>
</tbody>
</table>

<sup>1</sup>Soluble Fiber Diet (Wk16) - Insoluble Fiber Diet (Wk16); <sup>2</sup>n=14; <sup>3</sup>mean ± SEM; <sup>4</sup>Percentage of Total Fatty Acids within the Serum Lipid Fraction
Figure 4.1 Fatty acid levels (ug/dL) after 16 weeks on the soluble fiber diet compared with levels after 16 weeks on the insoluble fiber diet.
Figure 4.2  Percentage of total fatty acids in the CE, PL, and TG fractions after 16 weeks on the soluble fiber diet compared with levels after 16 weeks on the insoluble fiber diet.
4.2.2.1 Prostate Specific Antigen

PSA data are presented in section 3.2.1.

4.2.2.2 Correlations with PSA

The treatment differences in the sum of saturated, monounsaturated, polyunsaturated and total fatty acids in the TG fraction (Soluble Fiber Diet (Wk16) – Insoluble Fiber Diet (Wk 16)) were correlated positively with the treatment differences in serum PSA (Table 4.3) (Figure 4.3). Likewise, treatment differences in TG ALA, eicosapentaenoic acid, docosahexaenoic acid, linoleic and arachidonic acid correlated with the treatment difference in serum PSA as shown in Table 4.3 and Figure 4.3. However, the glycerol component of serum TG was negatively correlated with serum PSA (r = -0.60, p=0.024). No other significant correlations were observed between the treatment differences in the sum of saturated, monounsaturated, polyunsaturated, total and in individual fatty acids in the CE and PL fractions with the treatment differences in serum PSA. The correlations of the treatment differences between serum PSA with total fatty acids in the CE (r=0.38, p=0.174), PL (r=0.32, p=0.263) and TG (0.67, p=0.010) fractions are shown in Figure 4.4.

4.3 Discussion

In our studies, no association was found between serum ALA in the CE and PL fractions and PSA after four months on the soluble fiber diet compared to levels after
Table 4.3

Correlation of the Treatment Difference\(^1\)\(^2\) between Serum TG Fatty Acids\(^2\) and PSA\(^3\)

<table>
<thead>
<tr>
<th>TG Fatty Acid</th>
<th>Correlation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:3(n-3)</td>
<td>0.553</td>
<td>0.040</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>0.623</td>
<td>0.017</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>0.579</td>
<td>0.030</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>0.479</td>
<td>0.083</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>0.655</td>
<td>0.011</td>
</tr>
<tr>
<td>Sum of Saturates</td>
<td>0.604</td>
<td>0.022</td>
</tr>
<tr>
<td>Sum of Monounsaturates</td>
<td>0.595</td>
<td>0.025</td>
</tr>
<tr>
<td>Sum of Polyunsaturates</td>
<td>0.562</td>
<td>0.037</td>
</tr>
<tr>
<td>Total FA</td>
<td>0.661</td>
<td>0.010</td>
</tr>
</tbody>
</table>

\(^1\)Soluble (Wk16) - Insoluble (Wk16) Fiber Diet

\(^2\)\(n = 14\)

\(^3\)\(\mu g/dl\)

\(^4\)\(ng/ml\)
4.3 -0.2
-0
1
-0.2
0.2
PSA (ng/mL)

3 -0.2
-0.1
0
0.1 0.2
PSA (ng/mL)

-0.3 -0.2 -0.1 0 O 0.1 0.2
PSA (ng/mL)

-0.3 -0.2 -0.1 0 O 0.1 0.2
PSA (ng/mL)

-0.3 -0.2 -0.1 0 O 0.1 0.2
PSA (ng/mL)

-0.3 -0.2 -0.1 0 O 0.1 0.2
PSA (ng/mL)

MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids
SFA = saturated fatty acids
*Changes in Fatty Acids Represent Soluble Fiber Diet (Wk16) - Insoluble Fiber Diet (Wk16) Fiber Diet
and Changes in PSA Represent Soluble Fiber Diet (Mean Wk 2 to Wk 16 Values) - Insoluble Fiber Diet
(Mean Wk 2 to Wk 16 Values); n=14

Figure 4.3 Correlation of the Treatment Differences* Between Serum Triglyceride Fatty Acids and PSA
Figure 4.4  Correlation of the Treatment Differences* Between Serum Total Fatty Acids and PSA

*Changes in Fatty Acids Represent Soluble Fiber Diet (Wk16) - Insoluble Fiber Diet (Wk16) Fiber Diet and Changes in PSA Represent Soluble Fiber Diet (Mean Wk 2 to Wk 16 Values) - Insoluble Fiber Diet (Mean Wk 2 to Wk 16 Values); n=14
four months on the insoluble fiber diet. ALA levels in the TG fraction were not significantly different on the soluble fiber diet compared to the insoluble fiber diet, but the treatment difference in TG ALA was positively correlated with the treatment difference in serum PSA. No association was found between changes in ALA and serum PSA that could be accounted for by the PSA association with TG total fatty acids (r=0.66, p=0.010) after partial correlation controlling for the total fatty acids.

Prospective studies of the Doctors and Health Professionals Cohort identified a positive association between serum ALA and prostate cancer (Gann et al. 1994). The relative risk of prostate cancer was elevated twofold to threefold for men in successively higher quartiles of plasma ALA levels compared with those whose levels were below the detection threshold (Gann et al 1994). Associations have also been made between saturated fat and red meat and prostate cancer (Giovannucci et al. 1993). The MRFIT study, on the other hand, suggested that ALA consumption might reduce the risk of death from all forms of cancer (Dolecek and Granditis 1991). It may be that ALA is a marker of both total fat consumption and fatty red meat intake since ALA from animal sources but not of vegetable origin has been implicated as a risk factor for prostate cancer (Gann et al. 1994). The degree to which non-fat related aspects of diet might have played a part in determining the serum lipid profiles and have a relation with diseases cannot be determined from our studies since no changes in ALA were observed. However, as shown in table Table 4.1 and Table 4.2, lacto-vegetarian diets, high in either soluble or insoluble fiber, but with similar dietary fatty acid profiles did not alter serum ALA levels but did result in dissimilar serum fatty acid profiles, especially in relation to linoleic acid.
Our results pose at least two questions. To what extent can non-lipid components of the diet influence fatty acid metabolism and the composition of serum and presumably tissue membrane lipids? Can these effects influence the association between dietary fatty acids, serum fatty acids and prostate cancer risk (Giovannucci et al. 1993)?

Various aspects of diet, other than fatty acid composition, may influence fatty acid metabolism and hence ultimately serum fatty acid profiles (Ackman and Cunnane 1992). Trace element deficiency or excess may alter desaturation and chain elongation. Trace elements were not measured in our studies but insoluble fibers together with associated phytates may increase losses of zinc and magnesium (Reinhold et al. 1976). In the present study even though dietary metal ion intake may have remained in the normal range, over a period of four months, an effect on fatty acid metabolism may have taken place. In this respect, it is of interest that the longer chain ω-3 fatty acids EPA (%) and DHA (%) were higher on the soluble fiber diet, in the CE and TG fractions respectively. Likewise, soluble fiber foods tend to block the post-prandial nutrient and endocrine responses. Although the effects of slowing carbohydrate absorption have already been studied in relation to serum fatty acids in acute studies (Cunnane et al 1995), it is possible that this aspect of a fiber diet may influence serum fatty acids in the long term since higher serum linoleic acid levels were observed on insoluble fiber compared to soluble fiber, although this finding might also be partly explained by the non-significantly higher linoleic acid intake on the insoluble fiber diet.

Finally, increased bile acid output on soluble fiber may alter the relative amounts of different fatty acids absorbed. Studies have been carried out to assess linoleic acid losses on insoluble fiber and outputs are very small amounting to 0.2 g/day (Jenkins and
Cunnane, unpublished). No data are available on losses of linoleic acid on soluble fiber diets where bile acid outputs are larger than on the insoluble fiber diets to account for the lower serum linoleic acid levels in the CE, PL, and TG fractions observed here.

We conclude that changing the proportion of soluble and insoluble fiber in the diet may alter the serum fatty acid profile with lower linoleic acid levels on soluble fiber. It is possible that part of the consistent changes in linoleic acid in CE, PL and TG fractions may relate to the ratio of n-6/n-3 dietary fatty acid intakes. One of the findings of interest emerged with the correlation of the treatment differences in serum TG fatty acids and PSA. However, these effects do not appear to explain the lower PSA levels observed on the soluble fiber despite the epidemiological data linking fatty acids and prostate cancer. No treatment differences were noted in ALA to explain the lower PSA levels but the significant association between the treatment difference in TG total fatty acids and serum PSA may relate to factors other than soluble and insoluble fiber intake and needs further investigation.

Our study demonstrated a number of differences in absolute or percentage amounts of fatty acids predominantly in the cholesteryl ester and triglyceride fractions. The most consistent change observed in all fractions was the lower percentage linoleic on the soluble fiber diet. However, of note was the lack of any significant differences in ALA between treatments and the significant positive correlation between the treatment difference in TG ALA and PSA.
CHAPTER 5. EFFECT OF FLAXSEED ON PROSTATE CANCER

5.0 Introduction

We found that soluble fiber lowers serum PSA compared to insoluble fiber (Table 3.3). To gain some insights into the mechanism by which soluble fiber reduced serum PSA we measured fecal bile acids and neutral sterols, as possible markers of fecal steroid excretion. We found that lower serum PSA levels positively related to increased fecal lithocholic acid excretion (Figure 3.1). Since dietary fat, including ALA, have been implicated in the etiology of prostate cancer (Giovannucci et al. 1993), we also examined the effect of dietary fiber on serum fatty acids. We found that serum ALA levels were not significantly different between the soluble and insoluble fiber diets (Table 4.2), but the treatment differences between serum individual and total TG fatty acids and PSA (Table 3.1) were positively correlated. Finally, to explore the effects of possible dietary hormone blocking agents and antioxidants in relation to progression of prostate cancer, we studied the effect of defatted (cold-pressed) flaxseed, incorporated into muffins, on the rate of rise of serum PSA in men with prostate cancer together with serum markers of oxidative stress. Studies have indicated that plant foods, which are rich sources of isoflavones, flavanoids, and lignans, may prevent cancer development especially of breast and prostate because of their potential hormone blocking action (Giles and Ireland 1997; Morton et al. 1997; Adlercreutz 1995; Thompson 1994). Since hormone blockade is used in the treatment of sex hormone dependant cancers of breast and prostate (Crawford 1989; Early Breast Cancer Trialists’ Collaborative Group 1988), we felt it appropriate to see whether lignans may reduce serum prostate specific antigen (PSA) levels in established prostate cancer as an
indication of hormone blockade. PSA is a prominent prognostic indicator used in identification and monitoring progress of prostatic disease (Gann et al. 1995). We studied flaxseed since it has been shown to produce lignans at a level of 119 times that of wheat bran, its nearest counterpart (Thompson et al. 1991).

Flaxseed has several components that have attracted attention as possibly playing a role in cancer prevention. These include ALA, dietary fiber and lignans, all of which are found in especially high concentration in flaxseed (Thompson et al. 1991). Plant phenolics, isoflavones, flavanoids, and lignans are also potential antioxidants which may reduce DNA damage and this facet of metabolism has also been considered to be protective against cancer development or progress of initiated cells (Satoh and Sakagami 1996; Laughton et al. 1991). In addition, consumption of antioxidants including vitamin E, selenium and the carotenoid, lycopene, have been positively associated with reduced incidence and mortality from prostate cancer (Giovannucci et al. 1996; Willett et al. 1983). We therefore measured serum fasting thiols and lycopene as indicators of change in oxidative stress. Under oxidative stress thiol groups in cystine containing proteins and glutathione become oxidised and bind one another through a sulphur bridge. Thus, low serum thiol levels represent increased oxidative stress.

The aim of this study therefore was to determine whether a reduction in the rate of rise of serum PSA as a marker of prostatic disease activity (Gann et al. 1995) could be achieved with the ingestion of flaxseed and whether this was associated with changes in serum lycopene or thiol levels as measures of oxidative stress.
5.1 Methods

5.1.1 Subjects

Urologists at the University of Toronto referred patients. All subjects had undergone radiotherapy for biopsy proven prostate cancer. Subjects that showed a consistent rise in serum PSA levels were asked to join the study. The subjects had PSA levels rising at a rate of $0.5 \pm 0.1\%$ per day and were candidates for hormonal ablation or orchiectomy.

The 7 study volunteers had an average BMI of $25.3 \pm 1.4$ (range 20.0 to 29.1 kg/m$^2$), histological Gleason grade of $7 \pm 1$ (range 5 to 9), and mean age of $71 \pm 1$ years (range 66 to 76 years). They had been diagnosed with prostate cancer for an average of $3 \pm 1$ years.

5.1.2 Protocol

This was a 3-phase trial with 2-month control periods preceding and following the two or three month treatment period. The treatment period was a trial of defatted flaxseed supplementation, given in the form of muffins. Body weight, blood pressure and 12h overnight fasting blood samples were obtained initially for complete blood count, eosin sedimentation rate, liver and renal function tests, blood lipids, PAP and PSA. Immediately prior to starting the flaxseed treatment phase, at the end of the flaxseed treatment phase and two months following the flaxseed treatment phase, subjects provided seven-day food records. Throughout the course of the study, subjects were seen at bi-weekly intervals and fasting blood was obtained. Serum was analyzed for PSA,
PAP, thiol and lycopene levels. Fasting body weight and seated blood pressure were taken.

5.1.3 Diets

Throughout the study protocol, volunteers were instructed to follow an NCEP Step 2 diet (<30% of calories from fat, <7% Saturated Fat, <200 mg cholesterol) emphasizing increased vegetable consumption in line with those nutritional principles which appear beneficial in relation to prostate cancer (Giovannucci et al. 1995; LeMarchand et al. 1994; Giovannucci et al. 1993; Ross et al. 1987; Rose et al. 1986). Prior to flaxseed supplementation, subjects were provided with photocopies of their pre-flaxseed control diet records with suggestions on foods to exchange for the flaxseed muffins in order to avoid increasing caloric intake or changing the macronutrient profile of the diet.

Seven-day diet histories were assessed for macronutrient profile and dietary fiber using a database derived from USDA data (Watt and Merrill 1963) in which additional foods had been analyzed in our laboratory with Association of Official Analytical Chemists methods for macronutrients (AOAC, 1980) and fiber (Prosky et al. 1988). The macronutrient profiles of the diets consisting of the flaxseed and pre- and post-flaxseed control phases are shown in Table 5.1.

5.1.4 Flaxseed Supplements

The macronutrient profile of the flaxseed muffin is shown in Table 5.2. The flaxseed supplement was developed to deliver a daily dose of 21 g of partially defatted flaxseed in
Table 5.1  Mean Daily Macronutrient Intakes and Profile of the Control 1 (Pre-Flaxseed), Flaxseed (Treatment) and Control 2 (Post-Flaxseed) Diets $^{1,2,3}$

<table>
<thead>
<tr>
<th>Macronutrients</th>
<th>Control Diet 1 (Pre-Flaxseed)</th>
<th>Flaxseed Diet (Treatment)</th>
<th>Control Diet 2 (Post-Flaxseed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calorie Intake</td>
<td>$1920 \pm 116^d$</td>
<td>$1759 \pm 242$</td>
<td>$1678 \pm 139$</td>
</tr>
<tr>
<td>Total Fat (g/day)</td>
<td>$48.0 \pm 6.1$</td>
<td>$40.8 \pm 8.1$</td>
<td>$40.8 \pm 8.1$</td>
</tr>
<tr>
<td>(% of calorie intake)</td>
<td>$22.6 \pm 2.9$</td>
<td>$21.8 \pm 4.2$</td>
<td>$21.8 \pm 4.2$</td>
</tr>
<tr>
<td>Saturated Fat (g/day)</td>
<td>$12.1 \pm 2.1$</td>
<td>$11.0 \pm 2.7$</td>
<td>$10.5 \pm 2.9$</td>
</tr>
<tr>
<td>(% of calorie intake)</td>
<td>$5.7 \pm 0.9$</td>
<td>$5.6 \pm 1.2$</td>
<td>$5.5 \pm 1.3$</td>
</tr>
<tr>
<td>Monounsaturated Fat (g/day)</td>
<td>$20.0 \pm 3.0$</td>
<td>$15.3 \pm 2.6$</td>
<td>$16.1 \pm 4.0$</td>
</tr>
<tr>
<td>(% of calorie intake)</td>
<td>$9.4 \pm 1.4$</td>
<td>$7.9 \pm 1.0$</td>
<td>$8.6 \pm 2.1$</td>
</tr>
<tr>
<td>Polyunsaturated Fat (g/day)</td>
<td>$10.9 \pm 2.0$</td>
<td>$8.1 \pm 1.2$</td>
<td>$9.6 \pm 1.5$</td>
</tr>
<tr>
<td>(% of calorie intake)</td>
<td>$5.1 \pm 1.0$</td>
<td>$4.4 \pm 0.7$</td>
<td>$5.2 \pm 0.8$</td>
</tr>
<tr>
<td>Protein (g/day)</td>
<td>$75.2 \pm 7.3^a$</td>
<td>$73.2 \pm 10.5^{ab}$</td>
<td>$55.3 \pm 5.6^b$</td>
</tr>
<tr>
<td>(% of calorie intake)</td>
<td>$15.7 \pm 1.2^a$</td>
<td>$16.6 \pm 0.9^a$</td>
<td>$13.1 \pm 0.8^b$</td>
</tr>
<tr>
<td>Available Carbohydrate (g/day)</td>
<td>$283.7 \pm 24.8$</td>
<td>$260.9 \pm 42.3$</td>
<td>$259.6 \pm 23.8$</td>
</tr>
<tr>
<td>(% of calorie intake)</td>
<td>$59.1 \pm 3.8$</td>
<td>$59.6 \pm 4.3$</td>
<td>$62.5 \pm 4.9$</td>
</tr>
<tr>
<td>Total Dietary Fiber (g/day)</td>
<td>$33.1 \pm 4.7$</td>
<td>$37.0 \pm 6.5$</td>
<td>$30.8 \pm 3.6$</td>
</tr>
<tr>
<td>(g/1000 calories)</td>
<td>$17.3 \pm 2.4$</td>
<td>$21.1 \pm 3.7$</td>
<td>$18.4 \pm 2.2$</td>
</tr>
<tr>
<td>Dietary Cholesterol (mg/day)</td>
<td>$114.6 \pm 32.4$</td>
<td>$97.2 \pm 33.4$</td>
<td>$72.7 \pm 32.2$</td>
</tr>
<tr>
<td>(mg/1000 calories)</td>
<td>$63.3 \pm 19.5$</td>
<td>$53.4 \pm 18.1$</td>
<td>$41.5 \pm 19.3$</td>
</tr>
<tr>
<td>Alcohol (g/day)</td>
<td>$7.4 \pm 3.5$</td>
<td>$8.4 \pm 4.2$</td>
<td>$7.2 \pm 4.5$</td>
</tr>
<tr>
<td>(% of calorie intake)</td>
<td>$2.6 \pm 1.1$</td>
<td>$2.7 \pm 1.3$</td>
<td>$2.6 \pm 1.3$</td>
</tr>
</tbody>
</table>

$^1$Data with different letter designations significantly different at $p<0.05$

$^2$7 day food records

$^3$n=7

$^4$mean ± SEM
<table>
<thead>
<tr>
<th>Macronutrient Profile</th>
<th>Moisture (g/100 g wet weight)</th>
<th>38.61</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ash (g/100 g wet weight)</td>
<td>2.51</td>
</tr>
<tr>
<td></td>
<td>Protein (g/100 g wet weight)</td>
<td>9.22</td>
</tr>
<tr>
<td></td>
<td>Fat (g/100 g wet weight)</td>
<td>2.06</td>
</tr>
<tr>
<td></td>
<td>Total Carbohydrate (g/100 g wet weight)</td>
<td>47.59</td>
</tr>
<tr>
<td></td>
<td>Fiber (g/100 g wet weight)</td>
<td>9.79</td>
</tr>
<tr>
<td></td>
<td>Available Carbohydrate (g/100 g wet weight)</td>
<td>37.80</td>
</tr>
<tr>
<td></td>
<td>Total Calories (calories/100 g wet weight)</td>
<td>246</td>
</tr>
</tbody>
</table>
2 muffins where each muffin weighed approximately 50 grams. Based on our preliminary studies, 21 g of defatted flaxseed was the maximum daily intake that the individuals diagnosed with prostate cancer were prepared to consume daily for two to three month duration. The two flaxseed muffins provided between 60 to 70 mg/day of the plant lignan secoisolariciresinol-diglycoside in the 21 g of defatted flaxseed (Thompson et al. 1991).

To assess compliance, subjects were instructed to return uneaten muffins on their bi-weekly clinic visits and to record when and how many flaxseed test muffins were eaten on their dietary records.

5.1.5 Analysis

Serum PSA was analysed in duplicate using the Abbott IMx assay (Abbott Laboratories IMx System, Abbott Park, IL) (Garg et al. 1995) as described in section 3.1.3. The IMx® System measures total PSA, PSA in the free form and PSA complexed with ACT. The coefficient of variation of serum PSA is 5.1%.

Prostatic-acid-phosphatase was analysed using the Stratus® PAP Fluorometric Enzyme Immunoassay (Giegel et al. 1982) for the quantitative measurement of PAP in serum at St. Michael’s Hospital Department of Biochemistry. The coefficient of variation of serum PAP is 11.8%.

Serum protein oxidation was estimated by measuring the loss of reduced thiol (-SH) groups using 5,5’-dithio-bis (2-nitrobenzoic acid) (DTNB). Serum samples were incubated with DTNB for 15 min and centrifuged at 10 000g. Absorbance was measured at 412 nm (Hu et al. 1994). Serum lycopene was extracted using hexane/methylene chloride (5:1) and
quantified on reverse phase HPLC column with methanol : acetonitrile : methylene chloride:
water (7:7:2:0.16) as mobile phase using UV/Vid detector at 472 nm (Stahl et al. 1992).
The coefficient of variation of serum lycopene and thiols is 4.0% and 1.6% respectively.

5.1.6 Statistical Analysis

All data are expressed as mean ± SEM. Serum PSA was also expressed as the daily percentage change. The significance of difference in biochemistry, anthropometric, and dietary data between phases was assessed by the general linear model procedure in SAS (SAS/STAT User’s Guide, 1990).

5.2 Results

The compliance in eating the flaxseed muffins was 94.6 ± 2.6%. The mean PSA changes over time for the pre- and post-flaxseed periods were compared with the change in PSA on the flaxseed treatment. The daily percent change in serum PSA levels (PSA velocity) was lower, but not significantly, on the flaxseed supplementation phase of the study (0.25 ± 0.11 %/day) compared to the mean control (0.73 ± 0.26 %/day) (Figure 5.1), (p=0.060).

Absolute levels of serum PSA were significantly positively correlated with the absolute levels of serum PAP (r=0.914), p=0.001 and Gleason score (r=0.816), p=0.001. PSA velocity was also significantly positively correlated to the absolute levels of serum PAP (r=0.819), p=0.001 and Gleason score (r=0.896), p=0.001.
Figure 5.1  Effect of Flaxseed Muffins on the Rate of Rise of Serum PSA (ng/ml)

\[ \text{Flex} \]

\[ \text{Control} \]

\[ \text{Daily Percentage Rise in Serum PSA} \]

\[ \text{p} = 0.060; \ u = 7 \]
As shown in Table 5.3, body weight, blood pressure, serum PAP and serum lycopene and thiol levels did not differ significantly between the treatment and the mean of pre- and post-treatment control phases.

5.3 DISCUSSION

We explored the possible use of flaxseed supplementation of 2 to 3 months in duration on men diagnosed with prostate cancer. The PSA velocity, expressed as daily percentage change in serum PSA, tended to be reduced, though not significantly, on the flaxseed muffin test phase compared to the mean of the control phases by $0.49 \pm 0.21$ %/day. No antioxidant effects of flaxseed supplementation were observed.

The therapeutic potential of flaxseed to lower serum PSA levels may relate to its phytoestrogen content. Soy consumption and the phytoestrogens they contain have become the focus of research aimed at explaining the low death rate from prostate cancer in soy eating parts of the world, such as Japan and China (Adlercreutz et al. 1995; Messina et al. 1994; Wynder et al. 1991; Yu et al. 1991). International comparisons of disease rates have focused attention on the environmental factors that might be responsible since epidemiology and migration studies have implicated geography rather than genetic differences (Tominaga et al. 1993; Furusato 1990; Yatani et al. 1988; Rose et al. 1986; Guileyardo et al. 1980). Prostate cancer cells in tissue culture have shown that soy materials containing the sex hormone analogues diadzein, genistein and equol have significantly inhibited the growth of human prostate cancer cell lines (Barnes et al. 1994). Higher concentrations of phytoestrogens, diadzein and equol, found in the plasma and prostatic fluid of men from Hong Kong and higher concentrations of the lignan enterolactone, found in the prostatic fluid of men from Portugal compared to levels in
Table 5.3  Body Weight, Blood Pressure, Antioxidant Status, PAP and PSA at the End of the Control 1 (Pre-Flaxseed), Flaxseed (Treatment), and Control 2 (Post-Flaxseed) Phases$^{1,2}$

<table>
<thead>
<tr>
<th></th>
<th>Control 1</th>
<th>Flaxseed (Treatment)</th>
<th>Control 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (kg)</td>
<td>72.9 ± 4.0$^3$</td>
<td>72.1 ± 3.6</td>
<td>72.5 ± 3.6</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>24.8 ± 1.4</td>
<td>24.5 ± 1.3</td>
<td>24.6 ± 1.3</td>
</tr>
<tr>
<td>Systolic BP$^4$</td>
<td>141 ± 3</td>
<td>137 ± 3</td>
<td>136 ± 4</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>85 ± 2</td>
<td>82 ± 2</td>
<td>84 ± 3</td>
</tr>
<tr>
<td>Thiol (μmol/L)</td>
<td>260.6 ± 14.9</td>
<td>263.0 ± 15.0</td>
<td>304.3 ± 13.2</td>
</tr>
<tr>
<td>Lycopene (nmol/L)</td>
<td>215.7 ± 54.9</td>
<td>195.9 ± 40.7</td>
<td>265.4 ± 45.1</td>
</tr>
<tr>
<td>PAP (I/L)</td>
<td>1.5 ± 0.3</td>
<td>2.0 ± 0.6</td>
<td>2.2 ± 0.7</td>
</tr>
<tr>
<td>PSA (ng/ml)</td>
<td>30.0 ± 11.1</td>
<td>37.2 ± 14.2</td>
<td>45.0 ± 18.2</td>
</tr>
</tbody>
</table>

$^1$Data not significantly between the Control 1, Flaxseed (Treatment), Control 2 phases

$^2$n=7

$^3$mean ± SEM

$^4$BP=Blood Pressure
men living in Britain may, in part, be responsible for lower incidence of prostate cancer in men from Asia and also some Mediterranean countries (Morton et al. 1997). In our study, a higher intake of flaxseed lignans than was ingested may have significantly reduced the rate of rise in serum PSA.

Like the isoflavonoids in soy, flaxseed also contains high concentrations of a precursor sex hormone analogue, secoisolariciresinol-diglycoside. In a preliminary study with two men, we first assessed the effects of flaxseed on FSH, LH, and FT, since the phytoestrogens may also exert agonist and antagonist activity at the level of the pituitary, in short term studies of 1 month duration before proceeding to longer studies of 2-3 month flaxseed supplementation.

The preliminary study showed no major changes in serum FSH, LH and FT levels with dietary flaxseed supplementation. If anything, a rise rather than a fall was seen in FT. To determine whether flaxseed supplementation may reduce the rate of rise of serum PSA, a longer flaxseed supplementation phase was thought to be required.

Increased intake of antioxidants (vitamin E, selenium, and lycopene) has been positively associated with reduced mortality or incidence of prostate cancer. In addition the presence of prostate cancer has been positively associated with the increased proportion of DNA adducts resulting from DNA oxidation (Nevalainen et al. 1993). The benefits of antioxidants may therefore lie in preventing progressive DNA damage over time. In addition, assuming a constant intake, antioxidant concentrations will tend to reflect their level of utilization in neutralizing oxidative stress. If this was so, antioxidant concentrations might be expected to rise on flaxseed feeding, if flaxseed lignans had
antioxidant properties (Prasad 1997; Thompson 1994). In our study, serum lycopene and thiol concentrations tended to be lower on flaxseed, despite no change in lycopene intake.

Lycopene is the most efficient scavenger of singlet oxygen among the common carotenoids (Di Mascio et al. 1989) and is the predominant carotenoid in the prostate (Kaplan et al. 1987). Giovannucci and others found that dietary lycopene from tomato sauce, tomatoes, pizza and strawberries was significantly positively associated with lower prostate cancer risk (Giovannucci et al. 1995). We found no change in lycopene intakes and no alteration in serum lycopene concentrations on flaxseed to suggest a sparing of lycopene utilization. Plasma sulphydryl (SH) groups are susceptible to oxidative damage and are used as a marker of oxidative stress (Frei et al. 1988). Flaxseed supplementation did not have a significant effect on the oxidation of serum thiols. We therefore have no evidence for an antioxidant effect of lignans in partially defatted flaxseed.

However, it is also possible that established tumor might respond differently to antioxidants. The decrease in protein thiol groups as markers of oxidative stress may be seen as an undesirable effect since increased oxidative stress may damage proteins, cellular membranes and genetic materials (Ames et al. 1993). On the other hand, generation of oxygen radicals appears to be involved in the initiation of apoptosis (Delia et al. 1997) and forms part of the natural defensive processes against transformed or foreign cells (Dormandy 1978; Hassett and Cohen 1989).

We do not know whether our dosage level with flaxseed was optimal and whether flaxseed and soy phytoestrogens analogues have the same spectrum of activities. In vitro the soy phytoestrogens genistein and biochanin A have been shown to inhibit the growth of human prostatic cancer cell lines (LNCaP and DU-145) (Peterson and Barnes 1993).
Genistein is also effective in inhibiting growth of breast (Peterson and Barnes 1991), colon (Clarke et al. 1989), and liver (Mousavi and Adlercreutz 1993) cancer cells and appears to be effective with or without stimulation by growth factors (Peterson and Barnes 1993). Genistein has been found to act in vitro in an additive or synergistic manner with other anti-tumor agents to inhibit cancer cell growth and to induce differentiation (Clarke et al. 1989; Watanabe et al. 1989; Katzenellenbogen et al. 1977). Interestingly, genistein is equally effective in both estrogen dependent (MCF-7) and estrogen-independent breast cancer cell lines (Peterson et al. 1991), suggesting that phytoestrogens may inhibit carcinogenesis by mechanism(s) independent of the sex hormone receptors.

It has been observed that estrogens can have blocking actions on androgen and progesterone receptors (Yu et al. 1994) but such effects of soy isoflavonoids have not been reported. Only recently has interest been stimulated in flaxseed in vitro and receptor studies similar to those reported for soy have not been carried out. However, feeding studies have demonstrated that flaxseed reduces the chemically induced tumor burden in rats in mammary (Serraino and Thompson 1992a) and colon carcinogenesis studies (Serraino and Thompson 1992b), although not all studies have noted this effect (Gilbert 1987). In addition, the structural similarity between plant phytoestrogens and diethylstilbestrol, a synthetic estrogen used in the treatment of prostatic carcinoma, has further encouraged investigation of the impact of flaxseed lignans on prostate cancer.

A vegetarian diet (Giovannucci et al. 1993) high in lycopenes (Giovannucci et al. 1995) and low in fat (LeMarchand et al. 1991) may reduce the risk of the development and retard the progression of prostatic disease. Whether flaxseed has any effect on
prostate cancer or its biomarker PSA, remains to be determined on larger number of subjects. The current study does not preclude a modest effect that may be important in the long term. If flaxseed is found to have an effect in larger studies, it is unlikely to be related to changes in endogenous sex hormone levels or depressed antioxidant status induced by flaxseed feeding.

Flaxseed may be effective in reducing the PSA velocity in men diagnosed with prostate cancer. The number of subjects needed to demonstrate a 0.49 ± 0.05 %/day difference as significant is 13 with alpha at 0.05 and beta at 80%. If there is an effect of flaxseed it is therefore likely to be modest by comparison with drugs such as finesteride that block hormone action and are currently used in the treatment prostate cancer (Crawford et al. 1989). The effect of flaxseed in combination with hormone antagonists needs to be explored. In addition, it remains to be determined whether the amount of flaxseed ingestion demonstrates a dose response relationship with serum PSA levels.
CHAPTER 6

6.0 General Discussion

Since soluble fiber was observed to decrease serum PSA levels compared to insoluble fiber, we wanted to determine whether the beneficial effects of soluble fiber was due to its effects on steroid elimination and serum fatty acid levels. In the first study, the lack of significance between serum hormone levels on the two metabolic diets, one high in soluble fiber and the other high in insoluble fiber, did not rule out the possibility of significant differences in fecal steroid hormone excretion. Pusateri and others (1990) found significant relationships between dietary and fecal fiber components and fecal, but not plasma, steroid hormones. Although serum levels of FT and estradiol were not significantly altered, fecal excretion of these hormones may have been increased if, however, turnover rather than serum levels are important. In that case, increased fecal steroid hormone loss may provide a possible explanation for the reduction of serum PSA on soluble fiber.

The beneficial effects of soluble fiber may be related to its ability to alter serum fatty acid pattern and hence prostate cancer risk since dietary fiber altered the serum fatty acid pattern in men despite similar intakes of fatty acids on the metabolically controlled soluble and insoluble fiber diets. No relation was found between serum ALA levels and serum PSA. Our findings did not support the findings of Gann et al. (1994).

Flaxseed may be effective in reducing the rate of rise of serum PSA in men diagnosed with prostate cancer but there was insufficient power to detect a significant difference. Oxidative stress was measured as a possible explanation for the effects of defatted flaxseed on prostate cancer, however, the sample size was too small to
conclusively determine the effects of flaxseed in men diagnosed with prostate cancer. A flowchart of research finding is shown in Figure 6.1.

6.1 Conclusion

The aim of the first study was to assess the effects of dietary fiber on serum PSA, sex hormone levels and fecal steroid excretion in healthy middle-aged men. Compared with insoluble fiber, soluble fiber was found to significantly decrease serum PSA. Dietary fiber was shown to increase fecal excretion of fecal total bile acids and the reduction in serum PSA on soluble fiber compared to insoluble fiber was significantly positively correlated to increased fecal lithocholic acid excretion perhaps as a marker of fecal steroid excretion.

The aim of the second study was to determine the effects of dietary fiber on serum fatty acid profile and to determine whether a change in serum fatty acids altered the risk of developing prostate cancer in healthy middle-aged men. Low levels of serum ALA have been positively associated with reduced risk of prostate cancer (Giovannucci et al. 1993). However, no association was found between serum PSA and ALA in the CE and PL after four months on the soluble fiber diet compared to levels after four months on the insoluble fiber diet.

The aim of the third study was to assess the impact of longer-term flaxseed supplementation on serum PSA and oxidative stress in men diagnosed with biopsy proven prostate cancer. The data suggested that flaxseed supplementation decreased serum PSA levels but greater power was needed to show an effect. No antioxidant effects of flaxseed were observed.
Effect of Diet on Prostate Cancer

- Effects of Soluble vs. Insoluble Dietary Fiber in men without prostate cancer
  - Decrease in Serum PSA on Soluble Fiber Compared with Insoluble Fiber
  
  1. Related to increased fecal excretion of lithocholic acid but not to serum sex hormone levels

- Effects of Flaxseed in men diagnosed with prostate cancer
  - Non-significant Tendency for Lower Serum PSA levels with Flaxseed Supplements

  1. Not related to antioxidant effects

2. Related to triglyceride but not cholesterol ester nor phospholipid total fatty acids

Figure 6.1 Flowchart of Research Findings
6.2 Future Directions

Measuring sex hormone excretion in the feces of men consuming diets high in soluble fiber and diets high in insoluble fiber may provide a possible explanation for the reduction of serum PSA in healthy men on high soluble fiber diets, since sex hormones are considered to play a role in the etiology of prostate cancer (Labrie et al. 1997; Gann et al. 1996; Andersson et al. 1993; Boyle and Zaridze 1993; Adlercreutz 1990; Henderson et al. 1982; Berg 1975; Huggins and Hodges 1941).

Randomized clinical intervention trials designed to alter TG fatty acid profile should be targeted at groups at risk for prostate cancer since we have shown that certain TG fatty acids correlated positively with serum PSA. The association between TG fatty acids and PSA warrants further investigation with respect to elucidating the mechanisms by which fatty acids influence prostate function.

The potential therapeutic effects of flaxseed supplementation, in men diagnosed with prostate cancer, need further exploration. The number of subjects needed to demonstrate a significant reduction in the rate of rise of serum PSA of 0.49 ± 0.21 %/day is 13, based on the data generated in the flaxseed supplementation study, with alpha at 0.05 and beta at 80%. Urologists at the University of Toronto referred potential study candidates who had PSA levels that were rising slowly enough to warrant dietary intervention rather than aggressive treatment. Subjects were then followed for at least 2 months and those men who showed a consistent rise in serum PSA levels over this period were asked to start flaxseed supplementation. Some of the potential candidates for the flaxseed supplementation study, whose PSA levels we had been monitoring were advised by the study urologists to undergo more aggressive treatment options such as
orchiectomy and hormonal ablation. Other potential subjects did not show a consistent rise in serum PSA even 4 years after initial recruitment into the study and thus were not given flaxseed supplementation. Despite the difficulties in subject recruitment, a larger sample size may provide evidence for the efficacy of flaxseed supplementation for men diagnosed with prostate cancer.

Measuring lignans in the urine of men given flaxseed supplementation may help to determine the relation of these compounds to slowing or halting the progression of prostate cancer. Prostate biopsy specimens from men given flaxseed supplementation may be analyzed to assess the effects of flaxseed diet on oxidative stress, DNA damage and Gleason score. Cell lines from prostate cancer tissues may be exposed to lycopene and phytoestrogens, including lignans, and analyzed to determine effects on cell growth and methylation, anaplastic change and transformation.

Future prospective studies need to be conducted to determine whether a combination of intervention strategies rather than individual factors, including high soy, flaxseed, lycopene intake and a meat free diet, would be most effective in reducing the risk of developing or slow down the progression of prostate cancer with dietary therapy.

6.3 Summary

1. Soluble fiber significantly reduced serum PSA levels in healthy men compared with insoluble fiber. The reduction in serum PSA related positively to increased fecal lithocholic acid excretion possibly as a marker of increased fecal steroid excretion but without a change in serum sex hormone levels.
2. Dietary fiber changed serum fatty acid profile independent of fatty acid intake but this did not explain the reasons for the reduction in serum PSA on soluble fiber. However, treatment differences (Soluble (Wk 16) – Insoluble (Wk 16) Fiber Diet) in TG fatty acids related to treatment differences in serum PSA.

3. Flaxseed tended to reduce serum PSA but larger studies are required to demonstrate a significant PSA effect. Flaxseed did not alter oxidative stress.
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