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## REVIEW

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# Role of Mammalian Lignans in the Prevention and Treatment of Prostate Cancer

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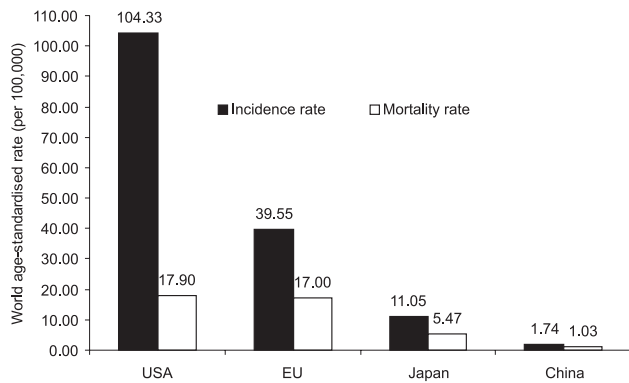
**Abstract:** Prostate cancer is poised to become the most prevalent male cancer in the Western world. In Japan and China, incidence rates are almost 10-fold less those reported in the United States and the European Union. Epidemiological data suggest that environmental factors such as diet can significantly influence the incidence and mortality of prostate cancer. The differences in lifestyle between East and West are one of the major risk factors for developing prostate cancer. Traditional Japanese and Chinese diets are rich in foods containing phytoestrogenic compounds, whereas the Western diet is a poor source of these phytochemicals. The lignan phytoestrogens are the most widely occurring of these compounds. *In vitro* and *in vivo* reports in the literature indicate that lignans have the capacity to affect the pathogenesis of prostate cancer. However, their precise mechanism of action in prostate carcinogenesis remains unclear. This article outlines the possible role of lignans in prostate cancer by reviewing the current *in vitro* and *in vivo* evidence for their anticancer activities. The intriguing concept that lignans may play a role in the prevention and treatment of prostate cancer over the lifetime of an individual is discussed.

### Incidence and Epidemiology of Prostate Cancer

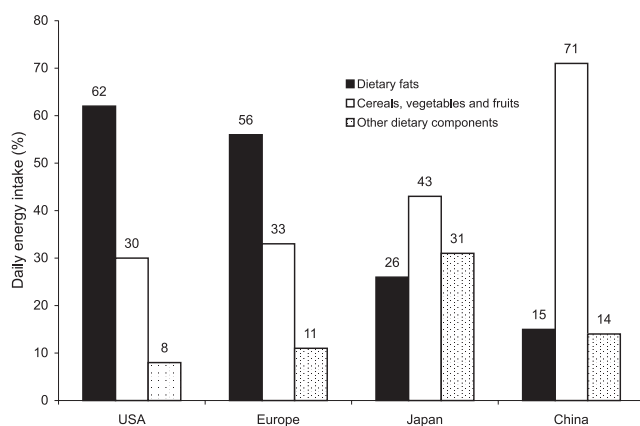
Globally, prostate cancer is the third most common cancer in men. The incidence of prostate cancer globally has been increasing by 1.7% per year for the last 15 yr (1). Marked differences in world age-standardized incidence and mortality rates of prostate cancer exist among the United States, European Union, Japan, and China (1). Figure 1 illustrates approximately a 60-fold difference in incidence rates and a 17-fold difference in mortality rates between the United States and China (1).

The etiology of prostate cancer is complex and multifactorial. Primary risk factors are age, diet, and genetic defects (2). Prostate cancer is usually only observed in males over the age of 50 yr. The World Health Organization estimates that 30% of all cancers are primarily a result of dietary factors (2). Familial prostate cancer occurs in approximately 10% of cases, twice the rate of hereditary breast cancer, and it develops at an earlier age than nonfamilial prostate cancer (2). Defects in the normal hormonal interactions with the prostate are a common feature of prostate cancer. The role of genes and growth factors in the development and progression of prostate cancer is beyond the scope of this article and is discussed elsewhere (3,4). Epidemiological studies have shown that, although the incidence of noninvasive prostate cancer in Asian societies is similar to that in Western society, the incidence of invasive cancer and associated mortality is lower (5). Migrant populations are exposed to novel environmental factors such as diet. Incidence rates of prostate cancer in migrants from Asian countries living in the United States tend to match those of the Caucasian and African-American populations over several generations (6). For example, Chinese male migrants from Shanghai to the United States report a 27-fold increase in cases of prostate cancer, after several generations, compared with those remaining in Shanghai (6).

These data strongly suggest that diet, not genetics, plays a more central role in the pathogenesis of prostate cancer. There is some evidence suggesting that the differences in diet between Eastern and Western cultures are responsible for the dramatic difference in the incidence of prostate cancer (5,6). There is a distinct difference in the dietary patterns between Eastern and Western cultures as shown in Fig. 2. This figure highlights the conspicuous differences in daily energy sources between Eastern and Western cultures (7). The Western diet, typified by the United States and Europe, is high in fat and low in cereals, vegetables, and fruit. The Japanese diet is much lower in daily fat intake and the Chinese diet is very



**Figure 1.** World age-standardized incidence and mortality rates (cases per 100,000) of prostate cancer in the United States, European Union, Japan, and China. Global mean age-standardized incidence and mortality rates are 21.3% and 7.95%, respectively (data compiled from Ref. 1).



**Figure 2.** Dietary sources of daily energy intake, expressed as a percentage, for the United States, Europe, Japan, and China. Dietary fats include edible fats, added fats, meats, and dairy products. Other dietary components include added salts, added sweeteners, alcohol, pulses, nuts, and seeds (adapted from Ref. 7).

high in cereals, vegetables, and fruits. Phytoestrogens are found in high amounts in some cereals, vegetables, and fruits. It is generally accepted that a high-fiber, low-fat diet is beneficial in the prevention of many cancers, including prostate cancer. Men consuming a traditional high-fiber, low-fat Eastern diet have up to 10 times less incidence of prostate cancer than those men consuming a Western diet. Vegetarian men have lower rates of prostate cancer than men who consume an omnivorous diet. Vegetarian and Asian men have very low incidences of invasive prostate cancer compared with Western men (5). The traditional Eastern diet is rich in phytoestrogen compounds, especially those derived from soy. However, most cereals, fruits, and vegetables are richer in lignan phytoestrogens than the other forms of phytoestrogens. Given the wider distribution of lignans in foods than the other phytoestrogens, and the greater potential dietary consumption, the role of lignans in modulating the risk of developing prostate cancer is an interesting avenue of research. The role of lignans in prevention and treatment of prostate cancer is the focus of this article.

## The Role of Androgens and Estrogens in Prostate Cancer

The role of androgens in prostate homeostasis and disease has been recently reviewed (8). Maintenance of correct prostatic function occurs via the interaction of androgens and growth factors, which influence gene expression at specific points in the cell cycle. There is some controversial evidence to suggest that estrogens influence prostatic function as prostate cells express both forms of the estrogen receptor (9–11). Estrogens are steroid hormones derived from androgens, in the male, in peripheral tissues by the aromatase enzyme. The  $\alpha$  estrogen receptor ( $ER\alpha$ ) is reported to be absent in metastatic prostate cancer (10). Loss of expression of the  $\beta$  estrogen receptor ( $ER\beta$ ) occurs during the progression of prostate cancer and affects the pathogenesis of the disease (11,12). This suggests that  $ER\beta$  regulates proliferation of prostate cells; indeed, adenoviral restoration of  $ER\beta$  function inhibits invasiveness and proliferation (13). Phytoestrogens have a slightly higher affinity for the  $ER\beta$  receptor (9).

Estradiol-17 $\beta$  stimulates hepatic production of sex hormone-binding globulin. This protein binds both estrogens and androgens, regulating the levels of free hormones in the plasma. Estrogens can directly interact with the estrogen receptor and regulate estrogen-responsive gene expression. Theoretically, estrogens in the male serve to modulate the level of free androgens in the plasma by promoting the concentration of sex hormone-binding globulin and by influencing the hypothalamus to produce less testosterone. This has the effect of moderating the influence of androgens in prostate development.

Endocrine therapy is a viable treatment option for prostate cancer. Suppression of circulating testosterone levels in the plasma occurs by inhibiting the pituitary–gonadal axis using either anti-androgenic or estrogenic compounds. This inhibition of sex hormone function results in tumor regression. However, these treatments are only effective in tumors retaining hormone sensitivity; advanced prostate tumors are often insensitive to hormonal influences. The shift between androgen dependence and insensitivity is a key step in the pathogenesis of the disease. The problem of late diagnosis of prostate cancer means that, by the time endocrine therapy is administered, many of the tumor cells have developed androgen insensitivity and can proliferate irrespective of androgenic influence. Those cells retaining androgen sensitivity can still proliferate at a reduced capacity due to the residual presence of adrenal androgens. Any effects of reduced tumor volume are therefore only palliative and only delay the deterioration by a few months or years.

## Lignan Phytoestrogens

### Introduction

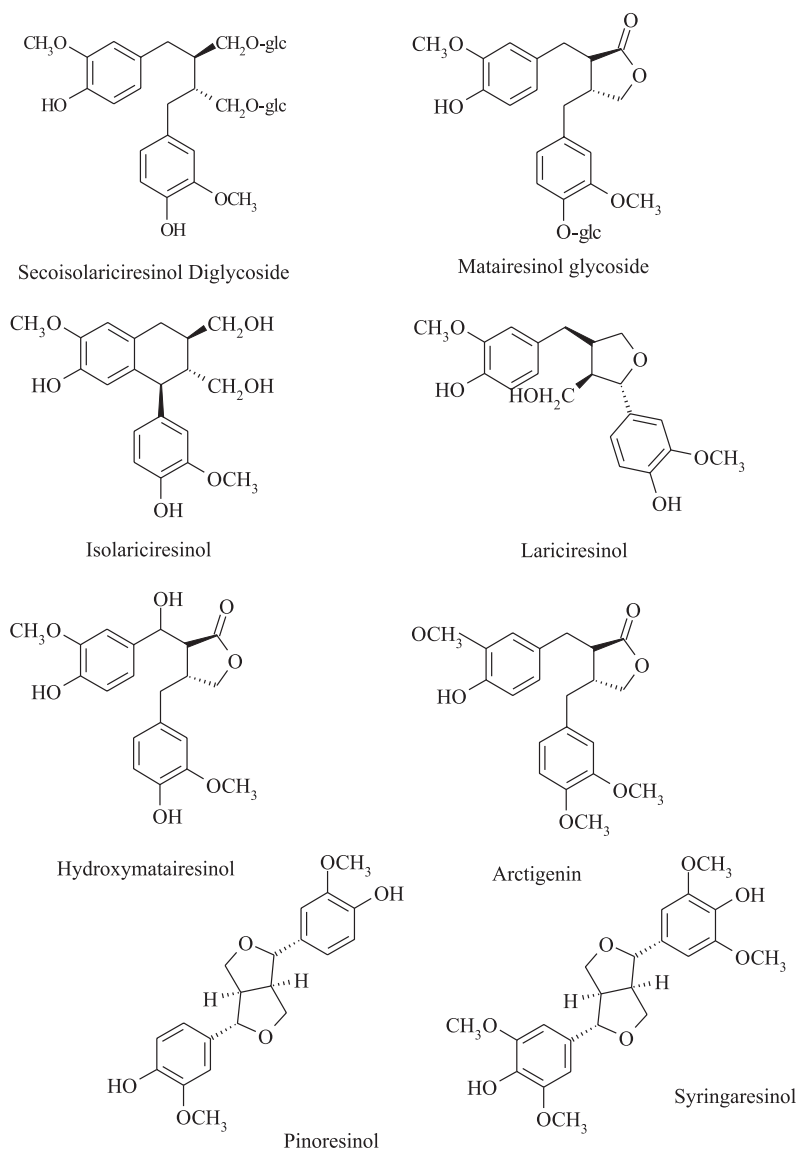
Epidemiological data suggest that differences in diet between Eastern and Western cultures may account for the dra-

matic difference in incidence and mortality rates of prostate cancer (1,7). Traditional Asian diets are rich in foods such as cereals, fruits, and vegetables that contain high levels of phytoestrogenic compounds such as isoflavones and lignans (7). The mammalian lignans enterolactone and enterodiol were first characterized in both human and vervet monkey reproductive cycles over 25 yr ago (14–17). Lignans are present in a range of biological fluids in several diverse populations, suggesting a biological role for these compounds (18–20). Morton et al. reported that the prostate fluid levels of enterolactone of Chinese, Portuguese, and British men were significantly higher than their plasma levels (19). This suggests that lignans accumulate in prostatic fluid and may achieve significantly higher concentrations than those reported in plasma. The reason for this accumulation is unknown and implies that lignans could play a role in prevention and treatment of prostate cancer.

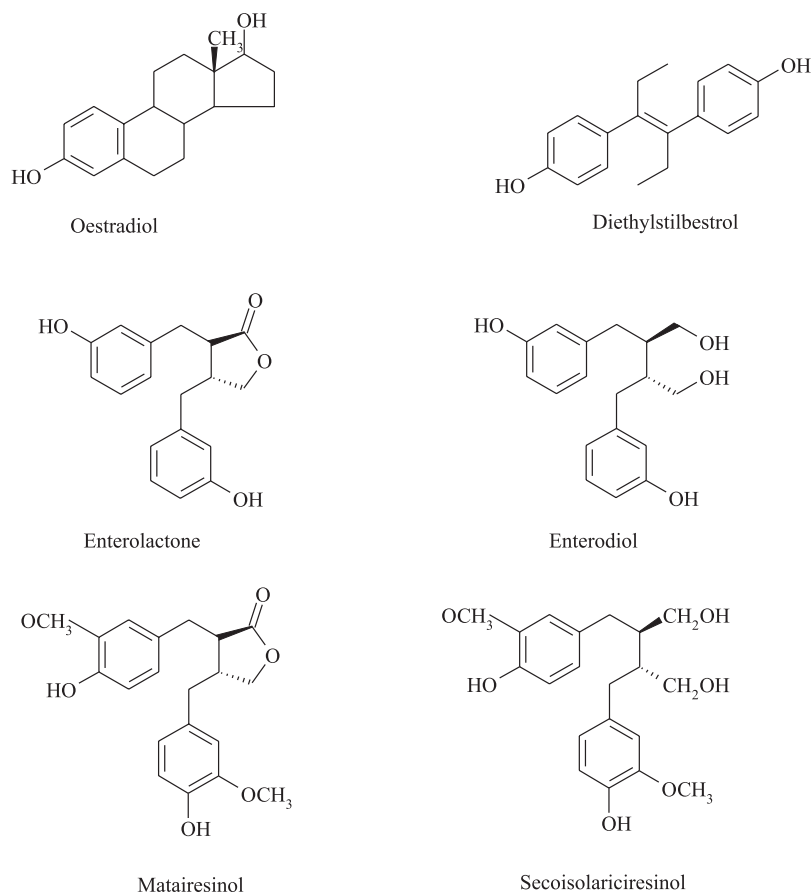
## Chemistry and Analytical Methods

Mammalian lignans are diphenolic compounds containing a 2,3-substituted di-1,4-benzylbutane structure (14,17, 20). The hydroxyl group at the meta positions of both aromatic rings distinguishes them from their plant precursors. Due to their structural similarity with natural estrogen, they could exert estrogenic activity (20). Figure 3 shows several known plant precursors of the mammalian lignans. Figure 4 illustrates the similarities between the mammalian lignans, a synthetic estrogen, and estradiol.

Isolation and analysis of lignans in both foods and biological matrices are difficult due to the similarity of structures and chemical properties. Current analytical methods include high-performance liquid chromatography with ultraviolet detection, gas chromatography with mass spectrometric detection (GC-MS), liquid chromatography with mass spectrometric detection, and time-resolved fluoroimmunoassay



**Figure 3.** Known dietary precursors of the mammalian lignans enterolactone and enterodiol (adapted from Refs. 21 and 22).



**Figure 4.** Structural similarities between selected phytoestrogens, a synthetic estrogen, and estradiol (adapted from Refs. 21 and 22).

(TR-FIA). Each of these techniques has advantages and disadvantages reviewed elsewhere (21,22).

### Dietary Sources

Lignans occur in most cereals, fruits, and vegetables. Tables 1 and 2 list the quantity of selected lignans in several common foods and drinks (23–29). Linseed (flaxseed) is the richest natural source of lignans. The principal dietary lignans consumed are isolariciresinol, pinoresinol, secoisolariciresinol diglycoside, secoisolariciresinol, hydroxymatairesinol (HMR), matairesinol glycoside, and

matairesinol (30). Secoisolariciresinol and matairesinol are the main precursors of enterolactone and enterodiol (30).

The paucity of data on the dietary consumption of lignans prevents accurate estimation of dietary exposure. Recent dietary intake assessments have focused on the isoflavones rather than the lignans. The VENUS database, funded by the European Union as part of the Phytohealth project, is a compilation of literature and unpublished data and contains limited data on lignan content of several foods and is accessible online (31,32). There are currently two methodologies for

**Table 1.** Concentrations of Mammalian Lignans ( $\mu\text{g}/100$  g sample) in a Range of Foods<sup>a</sup>

Food	Enterolactone	Enterodiol
Oil seeds	3,718	16,743
Dried whole legumes	321	241
Legume hulls	270	101
Whole cereals	244	115
Dried seaweeds	217	684
Cereal brans	216	270
Whole cereals	244	115
Vegetables	78	66
Fruits	48	35

<sup>a</sup>: Compiled from Ref. 23.

**Table 2.** Concentrations of Plant Lignans (mg/100 g dry sample) in a Range of Foods and Drinks<sup>a</sup>

Food or Drink	Secoisolariciresinol	Matairesinol	Reference
Flaxseed	369.9	1.087	24
Kidney bean	0.07	0	29
Barley bran	0.063	0	26
Wheat bran	0.11	0	26
Rye	0.471	0.065	26
Broccoli	0.414	0.023	26
Green pepper	0.117	0.007	27
Strawberry	1.5	0.781	27
Cranberry	1.51	0	26
Earl Grey black tea	1.59	0.197	28
Japanese green tea	2.46	0.186	28

<sup>a</sup>: Compiled from Refs. 24 and 26–29.

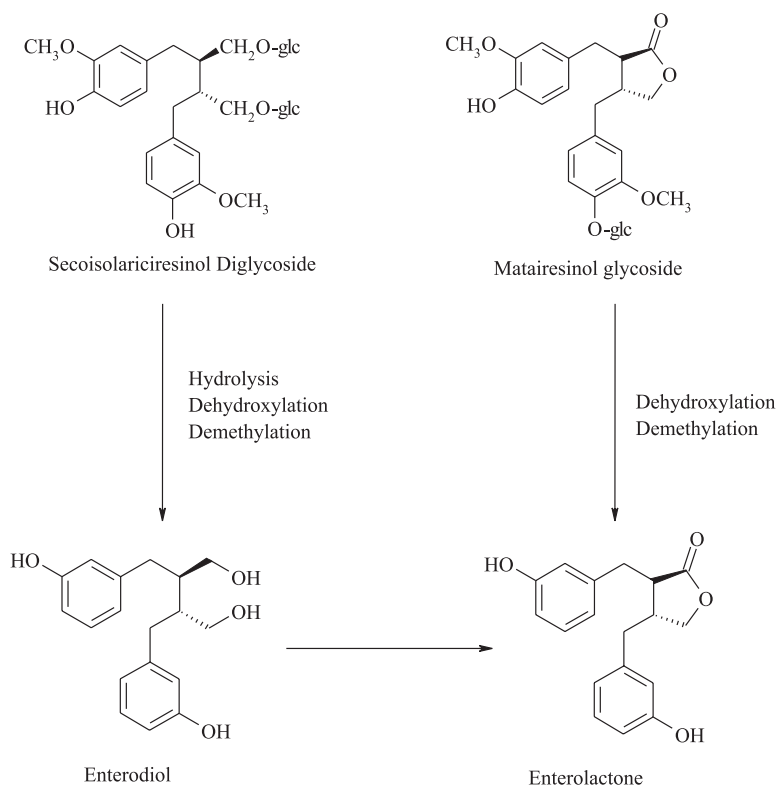
determining the lignan content of foods. Thompson et al. use indirect *in vitro* fermentation with colonic microflora to assess production of enterolactone and enterodiol from plant lignans (23). Mazur et al. utilize isotope-dilution gas-chromatography mass spectrometry (IDGCMS) to measure the levels of plant lignans in foods (24). A review and comparison of these methods are available (25). Measurements of plasma levels and urinary excretion are more widely used to assess dietary exposure to lignans.

### Pharmacokinetics

Enterolactone and enterodiol are the primary lignans observed in human biological fluids. The bioavailability of these lignans has been recently reviewed (33). Intestinal bacteria produce enterolactone and enterodiol from their plant precursors (34–36). Figure 5 illustrates a proposed schematic of the production of enterolactone and enterodiol by intestinal microflora. Other plant lignans, such as pinoresinol, syringaresinol, lariciresinol, arctigenin, and HMR, can also be metabolized into enterolactone and enterodiol but at lower levels than secoisolariciresinol or matairesinol (30). Due to the virtually negligible levels of plant lignans in the plasma, the intestinal metabolism of these lignans into their mammalian forms seems essential for absorption across the gut wall (33).

There are very limited studies on the pharmacokinetics of lignans in men. Most studies have used foods containing known precursors to assess the adsorption, distribution, metabolism, and excretion (ADME) profile of lignans (37–40).

The concentration of enterolactone and enterodiol in biological fluids varies considerably by geographical region and analytical method. Kilkkinen et al. measured the serum levels of enterolactone in 2,380 Finnish men aged 25–64 yr, participating in a national nutrition survey, using TR-FIA and found that the median serum enterolactone concentration was 13.8 nmol/l (37). Another study by Kilkkinen et al. reported mean enterolactone serum levels of 15.9 nmol/l in 214 Finnish men in a nested case-control study, again using TR-FIA (38). Jacobs et al. reported mean enterolactone concentrations of 6.2 nmol/l, measured using TR-FIA, in five American men fed a diet high in whole grains (39). Juntunen et al. fed a diet rich in rye bread, a rich source of lignans, to 18 Finnish men aged 41–43 yr and measured (using TR-FIA) a mean serum enterolactone concentration of 25.6 nmol/l (40). However, Morton et al. measured both serum and prostatic fluid levels of enterolactone and enterodiol in Portuguese, British, and Chinese men using GC-MS (19). There was no difference in the mean plasma level of enterolactone among Portuguese and British men (both 3.27 nmol/l); however, Chinese men had 5.2 nmol/l. Portuguese man had much lower levels of enterodiol in their plasma than Chinese men (0.30 compared with 1.4 nmol/l). The plasma levels of enterodiol for British men were not reported. The plasma levels measured by Morton et al. are lower than the other studies reported here due to the different analytical technique used and the samples were from men without any dietary intervention. The mean concentrations of enterolactone and enterodiol in prostatic fluid (based on a sample volume of



**Figure 5.** The production of enterolactone and enterodiol from secoisolariciresinol diglycoside and matairesinol by human intestinal bacteria (adapted from Ref. 36).

50–200 µl), measured by Morton et al., are of particular interest (19). In Portuguese men, the enterolactone levels were 2,720–10,860 nmol/l and the enterodiols levels were 223–892 nmol/l. The concentrations of enterolactone and enterodiols in British men were 340–1,360 and 43–172 nmol/l, respectively. Chinese men had enterolactone and enterodiols levels of 515–2,060 and 26.5–106 nmol/l, respectively (19).

There are extensive ADME studies using radiolabeled secoisolariciresinol diglycoside in rats (41,42). These rodent studies, although useful, focused on either colon or breast cancer. Differences in dietary consumption, interindividual variation in gut microflora, and the use of oral antibiotics also affect the pharmacokinetics of lignans (36,38,43).

A very recent study investigated the pharmacokinetics of mammalian lignans in men and women (44). In this study, subjects (six men and six women, consuming a low-lignan diet 1 wk before and during the study) drank a solution of secoisolariciresinol diglycoside in water (1.31 µM/kg of body weight) prior to breakfast, with plasma and urinary samples collected at defined intervals (44). The advantage of this approach, compared with using dietary intervention with lignan-rich foods, is that a number of standard pharmacokinetic parameters can be measured for the first time. Enterolactone was detected in the plasma 8 h after consumption of the lignan solution (44). In males, enterolactone had an absorption half-life of 8.4 h, reached its maximum plasma concentration of 42 nmol/l after 24.1 h, had an elimination half-life of 15.1 h, and a maximum retention time of 43.2 h (44). In the male subjects, enterodiols had an absorption half-life of 3.4 h, reached its maximum plasma concentration of 65 nmol/l after 17.8 h, had an elimination half-life of 4.6 h, and a maximum retention time of 23.9 h (44). This means that enterodiols are absorbed slower than enterolactone but have a higher maximum plasma concentration. In addition, enterodiols are excreted faster than enterolactone with a lower retention time. Although this information is useful there are some limitations: the subject size was small (only six men and six women), and it would have been interesting if the authors had been able to sample prostatic fluid in the male subjects. This may have provided further evidence for the accumulation of mammalian lignans in prostatic fluid as measured by Morton et al. (19).

The mammalian lignans are absorbed directly into the circulation and are metabolized by the hepatic phase II enzymes to form mono- and disulfate conjugates. These sulfate conjugates are the most common form of mammalian lignans in the circulation, whereas the urinary form of lignans is mainly monoglucuronides. Interindividual variation in both gut microflora and hepatic detoxification enzymes almost certainly plays a role in the occurrence and bioactivity of mammalian lignans (33).

### Anticancer Effects

Lignans exert a range of endocrine and nonendocrine biological effects with relevance to cancer risk. The possible role of lignans in disease, particularly cancer, was implied by

the finding that urinary lignan excretion in omnivores and in women with breast cancer was lower than that in vegetarians with a lower risk of cancer (45,46). The Japanese have a low incidence of cancer, such as that of the prostate, but high urinary levels of isoflavone phytoestrogens (47). The precise mechanisms by which the lignans exert their biological effects remain unclear. Lignans, by virtue of their structural similarity to estrogen, exert endocrine effects such as modulation of steroid metabolism and plasma binding of both androgens and estrogens (48–57). However, Adlercreutz et al. determined that the concentrations of mammalian lignans required to compete with the binding of estradiol to the nuclear estrogen type II receptor were 100–1,000 times higher than natural estrogens (55). The nonendocrine effects of lignans reported include inhibition of proliferation, antioxidant activity, modulation of angiogenesis, modulation of cellular growth factors, and genotoxicity (58–63).

This section reviews both the direct and indirect endocrine and nonendocrine anticancer effects of the lignans as shown in a range of *in vitro* and *in vivo* studies.

### In Vitro Studies on Lignans

The *in vitro* evidence, outlined subsequently, strongly suggests that lignans play a protective role in the prevention and treatment of prostate cancer. The daily energy intakes for the Western diet, outlined in Fig. 2, indicate very high levels of fat in the diet compared with Japanese and Chinese diets. The 1997 WCIF/AICR report on food and the prevention of prostate cancer identifies total fats, saturated fats, meats, and dairy products as possible dietary risk factors for prostate cancer (7). High fat levels result in altered hormonal levels, including elevated androgen levels. As lignans possess steroid metabolism and activity-modulating properties, they could serve to counteract some of the effects of hormonal imbalance due to high dietary fat intake.

**Antiproliferative activity:** A recent study by Lin et al. (64) investigated the effect of enterolactone and enterodiols on the growth of PC-3, DU-145, and LNCap human prostate cancer cell lines. Proliferation was measured using a fluorometric assay with propidium iodide as the fluorophore. Over a dose range of 10–100 µM, enterolactone inhibited the growth of all cell lines, whereas enterodiols only inhibited the PC-3 and LNCap cells. Enterolactone was also more potent ( $IC_{50} = 57 \mu M$ ) than enterodiols ( $IC_{50} = 100 \mu M$ ).

Initial results from the principal author's laboratory suggest that mammalian lignans inhibit the cell cycle of PC-3 human prostate cancer cells, resulting in significantly increased cell doubling times (65). An article on the cell cycle effects of lignans in three prostate cancer cell lines (PC-3, LNCap, and DU-145) is currently being prepared for publication from this laboratory. Our findings indicate that both mammalian lignans (enterolactone and enterodiols) and one of the plant lignans (matairesinol) inhibit the proliferation of prostate cancer *in vitro* (66).

**Steroid metabolism and activity:** Lignans inhibit the activity of human aromatase, 17 $\beta$ -hydroxysteroid dehydrogenase, and 5 $\alpha$ -reductase (48–57). The lignans appear unique among the phytoestrogens in their ability to modulate steroid activity via the aromatase enzymes. In plasma, the phytoestrogens are transported to their target tissues by carrier proteins, such as  $\alpha$ -fetoprotein, sex hormone-binding globulin, and human sex steroid-binding protein. These proteins normally play a role in maintaining the levels of free hormone in plasma. Sex steroid-binding protein has been found in prostate cells (57).

Martin et al. investigated the ability of several phytoestrogens, including enterolactone and enterodiol (dose range 2–200 nM), in affecting the binding of sex steroid-binding protein with either estradiol or testosterone (48). In the case of estradiol to sex steroid-binding protein, enterolactone was more potent at inhibition than enterodiol or genistein. Enterolactone was more effective at inhibiting the binding of testosterone to sex steroid-binding protein than genistein, and enterodiol showed negligible inhibition (48). Schottner et al. reported that matairesinol, secoisolariciresinol, enterolactone, and enterodiol inhibit the binding of 5 $\alpha$ -dihydrotestosterone with human sex hormone-binding globulin (49). Adlercreutz et al. reports that serum levels of 6  $\mu$ M enterolactone are sufficient to inhibit the activity of human aromatase (50). Adlercreutz et al. report that serum levels of 0.5–50.0  $\mu$ M enterolactone and enterodiol are sufficient to inhibit binding of testosterone and estrogen to sex hormone-binding globulin (51). Garreau et al. reported that enterolactone was more effective than enterodiol at inhibiting the binding of  $\alpha$ -fetoprotein to either estrogen or testosterone (52). Brooks and Thompson have investigated the ability of enterolactone and enterodiol to inhibit the activity of aromatase and 17 $\beta$ -hydroxysteroid dehydrogenase in MCF-7 human breast cancer cells (53). This has the effect of reducing the amount of estradiol produced. A 50- $\mu$ M concentration of enterolactone significantly inhibits estradiol production (53). Evans et al. report that an enterolactone and enterodiol concentration of 100  $\mu$ M significantly inhibited the activity of 5 $\alpha$ -reductase and 17 $\beta$ -hydroxysteroid dehydrogenase in genital skin fibroblasts (54). Adlercreutz et al. determined that enterolactone concentrations of 0.5–10  $\mu$ M stimulated the synthesis of sex hormone-binding globulin (55).

Mammalian lignans compete with estrogens and testosterone for binding of plasma proteins; in effect, they are dietary modulators of the physiochemical activity of the sex hormones.

**Antioxidant capacity:** There is evidence in the literature to suggest that several lignans display antioxidant properties (58,59,67). Kangas et al. examined the antioxidant and antitumor properties of HMR in vitro and in vivo by investigating its ability to affect lipid peroxidation and superoxide scavenging compared with Trolox (a synthetic vitamin E derivative) and enterolactone (67). HMR was several orders of magnitude more effective at superoxide scavenging and at in-

hibiting lipid peroxidation than enterolactone. However, enterolactone was significantly more effective (IC<sub>50</sub> = 1  $\mu$ mol/l) than HMR (IC<sub>50</sub> = 10  $\mu$ mol/l) at inhibiting an oxidative burst in human monocytes (67).

Prasad investigated the ability of several lignans and vitamin E to inhibit the chemiluminescence of zymosan-activated polymorphonuclear leukocytes (58). In this model system, enterolactone was the most potent antioxidant (IC<sub>50</sub> = 1 mg/ml) compared with enterodiol (IC<sub>50</sub> = 2.5 mg/ml), secoisolariciresinol diglycoside, secoisolariciresinol, and vitamin E (IC<sub>50</sub> = 5 mg/ml) (58).

Kitts et al. studied the antioxidant activity of secoisolariciresinol diglycoside, enterodiol, and enterolactone by assessing their ability to inhibit lipid peroxidation and both specific and nonspecific Fenton reactant-induced hydroxyl scavenging (59). Ten- and 100  $\mu$ M concentrations of the lignans inhibited lipid peroxidation. The results of this study indicate that, in the assay models used, enterolactone and enterodiol are significantly more potent antioxidants than secoisolariciresinol diglycoside at both 10 and 100  $\mu$ M.

Niemeyer and Metzler investigated the antioxidant potential of enterolactone, enterodiol, matairesinol, and secoisolariciresinol in vitro using the ferric reducing/antioxidant power assay (60). Secoisolariciresinol and matairesinol (50–400  $\mu$ mol/l) were found to be more potent than ascorbic acid (10–800  $\mu$ mol/l) and significantly more potent than enterolactone and enterodiol (1–2  $\mu$ M) (60).

**Antigenotoxic activity:** Kulling et al. used cell-free microtubule assembly and various selected endpoints in cultured V79 Chinese hamster cells to assess the genotoxicity of enterolactone, enterodiol, matairesinol, and secoisolariciresinol (61). Diethylstilbestrol (aneuploidogen) and 4-nitroquinoline-*N*-oxide (clastogen) were used as positive controls. None of the four lignans had any activity in any of the assays used. In contrast, other phytoestrogens such as genistein have been shown to have in vitro genotoxic activity in the V79 cell line (61). Therefore, it is concluded that the lignans tested do not possess any aneuploidogenic or clastogenic properties in the model system used. However, the authors of this article referred to the lack of information on the genotoxicity of any oxidative metabolites of these lignans.

**Anti-angiogenic activity:** The process of tumor angiogenesis is a critical event in the pathogenesis of the many cancers, including prostate cancer. Vascular endothelial growth factor (VEGF) is associated with the angiogenic process and is the focus of much of the research into anti-angiogenic treatments. A recent article has examined the role of angiogenesis in prostate cancer (62). Studies pertaining to the influence of phytoestrogens on angiogenesis have focused on the isoflavones, particularly genistein. There have been no studies to date on the role of lignans in angiogenesis in prostate cancer.

Fotsis et al. investigated the phytoestrogen content of 24-h urine samples collected from subjects consuming soy-rich

vegetarian diet. Of the six fractions collected, one contained enterolactone, enterodiol, and matairesinol (63). Enterolactone, over a concentration range of 0–100  $\mu$ M, was found to inhibit the growth of endothelial cells, supplemented with basic fibroblast growth factor, derived from bovine brain tissue. However, this study focused on the anti-angiogenic potential of genistein, an isoflavone found in soy, and not that of enterolactone or the other lignans measured in one of the fractions.

However, a recent study using MDA-MB-435 human breast cancer cells transplanted into mice fed a 10% flaxseed-supplemented diet for 8 wk found a significant decrease in the tumor volume, incidence of metastases, and levels of VEGF (68).

**Summary of in vitro studies:** The studies reviewed previously suggest that both plant and mammalian lignans possess beneficial anticancer effects in vitro. However, the effective doses used in the in vitro studies are in the micromolar range, significantly higher than the plasma levels reported by several authors (37–40,44). These supraphysiological doses used may not be achievable in vivo through dietary consumption of foods rich in lignans. The observation by Morton et al. regarding the significantly higher prostatic fluid levels of mammalian lignans compared with plasma levels may, if confirmed in future studies, clarify the role of dietary consumption of lignans in prostate cancer. The anticancer effects reported can only be indicative of possible effects of lignans in prostate cancer. Despite the evidence described previously, very few of the studies used prostate cell lines. The validity of the in vitro effects reported with respect to prostate cancer requires clarification and support from in vivo studies.

The evidence for antiproliferative effects of lignans in prostate cancer is limited to two published studies (64,65). Therefore, only possible antiproliferative properties can be ascribed to lignans. Several reports suggest that lignans inhibit the activity of critical aspects of sex steroid metabolism and activity (48–57). These studies are limited by the fact that they were mainly performed in breast cancer research or female subjects. As prostate cancer is also a hormone-dependent cancer, it is feasible to infer that the effects reported may be seen in this type of cancer. There is only one article on lignans and steroid metabolism in prostate cancer (54). In the investigations into the antioxidant potential of lignans, half of the studies indicate that plant lignans are more potent than mammalian lignans (60,67), whereas the others suggest that it is the opposite situation (58,59). The antioxidant potency of the lignans tested appears to depend on which assay is chosen. Lignans were not genotoxic in the sole study in this area (61). However, the authors did concede that the oxidative metabolites of enterolactone and enterodiol may or may not possess genotoxicity (61). This study used Chinese hamster cells, a fibroblast cell line. The reason the authors chose this cell line is unclear and may be due to its suitability for the endpoints selected. It is questionable as to the relevance of these data to prostate cancer. The anti-angiogenic

effects of the lignans reported by Fotsis et al. (63) were measured in endothelial cells derived from bovine brain tissue. The validity of these results with respect to prostate cancer is a pertinent issue. The recent study by Dabrosin et al. shows that dietary intervention with flaxseed affects several indicators of angiogenesis in breast cancer but not necessarily prostate cancer (68).

### **In Vivo Studies on Lignans**

A number of limited animal and human studies support the reported in vitro anticancer effects of lignans outlined previously. Lignans are not widely available in purified form and are difficult to synthesize; therefore, many of the in vivo studies have used flaxseed or rye, which are rich sources of lignans. It is difficult to ascribe any observed in vivo effects of flaxseed and rye to the lignans alone as many other potentially bioactive compounds are present in these foods. The majority of the in vivo studies have been in murine and rodent models; only a few dietary interventions with lignans have used human subjects. Despite the limitations, in vivo research has provided experimental evidence for the potential role of lignans in the prevention and treatment of prostate cancer. Tables 3 and 4 provide a summary of the current in vivo research into the anticancer effects of lignans.

**Animal studies:** Bylund et al. transplanted 70 athymic mice (BALB/cABom strain) with approximately 8 million human LNCap prostate cancer cells 3 days before dietary intervention with 1 of 7 diets (69). The control diet (CC) consisted of cornstarch, sucrose, low-fat milk powder, corn oil, and lard supplemented with cellulose. The experimental rye bran diets consisted of the control diet supplemented with rye bran (RB diet), with rye bran extracted with ethyl acetate (EXRB diet), and with a higher fat content (HFRB diet). Rye bran is known to contain lignans such as secoisolariciresinol and matairesinol (26). In addition, a soy-based diet (SCC) consisted of the CC diet with soy replacing the milk powder. The remaining two diets were derived from the CC diet supplemented with ethyl acetate extracts from rye bran (CCEE diet) and ethyl acetate extracts of alkylresorcinols extracted from rye bran (CCA diet) (69). Six of the diets were controlled for fat levels (12.7 g/100 g of diet) and energy density (377.5 kcal/100 g). The HFRB diet had a fat content 18.1 g/100 g and an energy density of 399.4 kcal/100 g of diet. After 5 wk on dietary intervention, the RB and SCC diets were significantly different from the control diet in terms of tumor volume. After 6 wk, these two diets also had lower prostate specific antigen (PSA) levels than the control diet (69). At sacrifice (9 wk) the mice fed the RB and CCEE diets had significantly lower tumor take rates, tumor volumes, and PSA levels and higher necrotic and apoptotic levels compared with the control diet (69). The HFRB results were not significantly different from the control diet. This suggests that the phytoestrogens present in these diets (both lignans and isoflavones) could be responsible for the growth restriction of the tumor observed. However, both rye-based and soy di-



**Table 3.** Summary of the In Vivo Animal Studies Into the Effects of Lignans on Prostate Cancer<sup>a,b</sup>

Model	Intervention	Duration (wk)	Effect of Intervention	Reference
BALB/cABom mice ( <i>n</i> = 70)	Transplant of LNCap cells with 1 of 7 distinct rye- and soy-based diets	9	Reduced tumor take rates, tumor mass, and PSA levels	69
TRAMP mice ( <i>n</i> = 135)	5% (by weight) flaxseed-supplemented diet	20 and 30	Increased necrosis and apoptosis Decreased tumor mass	70
Dunning R3327 rat ( <i>n</i> = 125)	33% soy flour diet 33% rye bran diet 33% heat-treated rye bran diet 33% rye endosperm diet	24	Lower Gleason grade observed Decreased tumor mass observed in all diets after 16 wk; most significant decrease in tumor mass was measured for the rye bran diet	72
BALB/cABom mice	0.15% or 0.30% HMR diet	9	Both diets had lower tumor take rates and tumor volumes than the control mice	73

*a:* Adapted from Refs. 69, 70, 72, and 73.

*b:* Abbreviations are as follows: PSA, prostate specific antigen; TRAMP, transgenic adenocarcinoma mouse prostate; HMR, hydroxymatairesinol.

**Table 4.** Summary of the In Vivo Human Studies Into the Effects of Lignans on Prostate Cancer<sup>a,b</sup>

Model	Intervention	Duration	Effect of Intervention/Study	Reference
Human ( <i>n</i> = 25)	30 g/day flaxseed diet	3 wk	Decreased total serum cholesterol, total testosterone, and free androgen index Patients with a Gleason grade of 6 or less had reduced PSA levels Apoptosis and proliferation rates dependent on length of intervention	74
Human ( <i>n</i> = 10)	295 g/day rye bran bread diet	3 wk	Increase in serum plasma enterolactone levels Increase in apoptosis rates	75
Human ( <i>n</i> = 26)	50 g soy bread diet 50 g soy bread + 20 g linseed diet Wheat bread diet	27.4 (±3.6) days	Increase in total PSA Increase in total androgen index	76
Human ( <i>n</i> = 214)	Nested case-control study of Finnish men involved in the Alpha-Tocopherol, Beta-Carotene study	6 yr	Decrease in free to total PSA ratio No relationship between lignan consumption and prostate cancer risk No relationship between serum enterolactone concentration and prostate cancer risk	38
Human ( <i>n</i> = 136)	105-item dietary questionnaire	10 yr	No relationship between prostate cancer risk and a diet of fruit and vegetables or a diet of red meat and starch	77

*a:* Adapted from Refs. 38 and 74–77.

*b:* Abbreviation is as follows: PSA, prostate specific antigen.

ets contain other bioactive compounds in addition to phytoestrogens.

Lin et al. studied the effect of a 5% (by weight) flaxseed-supplemented diet on 135 male transgenic adenocarcinoma mouse prostate (TRAMP) mice for 20 and 30 wk, respectively (70). These transgenic mice possess a rat probasin promoter that regulates the prostate-specific expression of the SV40 T-antigen (71). The advantage of this study is that TRAMP mice do not depend on exogenous hormones or transplanted tumors to be a suitable animal model. The experimental and control diets were equivalent in terms of calories, carbohydrates, protein, and fat content. All of the control mice and 97% of the intervention mice developed prostate cancer; however, there was a marked difference in

the tumor mass and characteristics of the tumors. The intervention mice had a significantly lower average tumor mass and percentage tumor mass to body mass ratio than those found in the control mice after 20 and 30 wk of dietary intervention. Despite the fact that the Gleason grading system is used to assess the aggressiveness of human prostate cancers, the authors of the article used this system to measure the aggressiveness of the mouse tumors at 30 wk. The intervention mice had a significantly lower Gleason score than the control mice. The level of cellular proliferation as measured by the KI-67 nuclear antigen decreased significantly. The apoptotic index, measured using the deoxynucleotidyl transferase-mediated dUTP-digoxigenin nick end labeling (TUNEL) technique, increased significantly in the intervention mice.

Zhang et al. have reported on the restriction of tumor growth in 125 Dunning R3327 rats transplanted with prostate tumors (72). The rats consumed one of the experimental diets for 24 wk. The control diet was fiber-free (FF). The experimental diets were FF diet with 33% soy flour (SD diet), 33% rye bran (RB diet), 33% heat-treated rye bran (HRB diet), and 33% rye endosperm (RE), respectively. The rats consuming the RB, HRB, and SD diets showed a significant decrease in tumor volume compared with rats on the FF diet (72). The RB diet was the most effective at suppressing tumor growth, of all the diets, after 24 wk despite different energy intakes among the diets. To account for differences in energy intake by rats consuming the RB diet, a second experiment was performed. The same prostate tumors from the first experiment were transplanted into 150 fresh rats, and tumor development was examined with dietary intervention with six isocaloric diets (72). The control diets were FF and cellulose supplemented FF (FC). The RB diet from the first experiment was used unchanged. The other diets were FF diet with 33% enzyme-treated (0.2%  $\beta$ -glucanase and xylanase) rye bran (RS diet), 33% rye bran plus 10% soy flour (RS diet), and 3.3% linseed (flaxseed) (LS diet). The rats were fed the diets for 18 wk and the tumor volume was assessed at sacrifice. In this experiment, only the RB diet significantly inhibited the tumor mass of the rats. Enzymatic treatment and the addition of soy flour negated the effect of rye bran. The LS diet was observed to have no effect on tumor growth.

Bylund et al. have recently reported the anticancer properties of HMR on prostate cancer in mice (73). This is the first in vivo study to use a purified lignan. HMR was chosen as it is available in sufficient quantities from the Norway spruce (*Picea abies*) and is structurally similar to matairesinol (73). In addition, HMR is metabolized into enterolactone (30,34). This study used 36 athymic male mice of the BALB/cABom strain. These mice were implanted with human LNCap prostate cancer cells 3 days prior to dietary intervention. The interventions were a control diet, a 0.15% HMR diet, and a 0.3% HMR diet for 9 wk. All diets were low fat and isocaloric. The effect of 0.15% and 0.30% HMR on the tumor take rates and growth of the implanted LNCap cells were the primary endpoints utilized. The results show that both interventions with HMR have significant anticancer properties (73). At sacrifice, the mice fed the HMR-supplemented diets had lower tumor take rates and tumor volumes than the con-

trol mice (73). An increase in the level of nongrowing tumors was seen as well as an increase in the apoptotic index (measured by the TUNEL technique) (73). The 0.30% HMR diet also inhibited the proliferation of the LNCap tumors (measured by the KI-67 nuclear antigen) (73). This study supports the hypothesis that lignans may play a role in the early stages of prostate tumorigenesis.

**Human studies:** There are limited studies available on the effect of lignans on human prostate cancer. Demark-Wahnefried et al. performed a pilot study with 25 men awaiting prostatectomy (74). The men consumed a low-fat (20%) diet supplemented with 30 g/day of flaxseed. The average duration of subjects on the diet was 34 days. The endpoints used in the study were alterations in the levels of PSA, testosterone, and free androgen index and total serum cholesterol, and the tumors assessed for apoptotic and proliferation changes. In subjects with a Gleason score of 6 or less, there was a significant decrease in PSA levels. The level of proliferation and apoptosis depended on the number of days on the supplemented diet. The results of this study are summarized in Table 5.

Bylund et al. investigated the effect of a short-term intervention with rye bran bread in men diagnosed with prostate cancer (75). The subjects had localized prostate tumors and were not suffering from any other chronic or acute diseases. The diets consisted of soft and crisp rye bran bread ( $n = 10$ ) and a control soft and crisp wheat bread diet ( $n = 8$ ) (75). The rye group consumed 689 kcal of soft and crisp rye bread (284 nmol/l secoisolariciresinol and 267 nmol/l of matairesinol) daily. The control group consumed 701 kcal of soft and crisp wheat bread (52 nmol/l secoisolariciresinol and 11 nmol/l of matairesinol) daily. The intervention was for 3 wk. At analysis, the rye bread group had significantly higher levels of apoptosis (measured by the TUNEL technique) and proliferation (measured by the KI-67 nuclear antigen). Plasma levels of enterolactone were elevated, as were urinary excretion levels of enterolactone, enterodiols, and matairesinol. No changes in serum levels were reported for testosterone, estradiol, sex hormone-binding globulin, or PSA.

Dalais et al. recently investigated the effects of a diet rich in phytoestrogens in men awaiting radical prostatectomy (76). Twenty-six men with prostate-localized prostate cancer were divided into three groups and fed one of three diets. The con-

**Table 5.** Effects of 30 g/day Flaxseed-Supplemented Diet on Human Subjects Awaiting Prostatectomy<sup>a,b</sup>

Parameter	Day 0	Day 34	Mean Change	P Value
PSA (ng/ml)	8.14	8.50	0.36	0.58
Gleason sum (7–10)	11.48	15.08	3.6	0.13
Gleason sum (2–6)	7.09	6.43	6.43	0.10
Total testosterone index (ng/dl)	422.1	360.1	–62	0.002
Free androgen index (%)	36.3	29.3	–7	0.01
Total serum cholesterol (mg/dl)	200.8	174.3	–26.5	<0.001

a: Adapted from Ref. 74.

b: Abbreviation is as follows: PSA, prostate specific antigen.

trol diet was wheat-based bread, and the experimental diets were supplanted with either 50 g of soy grit or a mixture of 50 g soy and 20 g of linseed (flaxseed). The subjects consumed four slices of bread for 23–27 days. No changes in serum levels were reported with any of the diets for testosterone, dihydrotestosterone, or sex hormone-binding globulin. Those men fed a diet containing 50 g of soy had significantly decreased total PSA and increased free androgen levels and free to total PSA levels. However, these effects were not reported in the diet of 50 g of soy and 20 g of linseed. The altered free androgen and PSA levels observed agree with the findings of Demark-Wahnefried (74).

Kilkinen et al. investigated the hypothesis that enterolactone is protective against prostate cancer risk in Finnish men (38). They performed a 6-yr nested case-control study of 214 men diagnosed with prostate cancer from the 29,000 men involved in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention study. A slight negative correlation between serum enterolactone concentration and both smoking and body mass index was found as well as a slight positive correlation with both age and number of smoking years. However, no correlation was found between either lignan consumption or serum enterolactone levels and prostate cancer risk.

Tseng et al. examined dietary patterns among 136 men diagnosed with prostate cancer that were involved in the United States National Health and Nutrition Examination Survey and the Epidemiological Follow-up Study during the periods 1971–1975 and 1982–1994 (77). The responses from the dietary questionnaires were identified as one of three groups: vegetable-fruit, red meat-starch (red meats, potatoes, cheese, and salty snacks), and Southern (cornbread, grits, sweet potatoes, beans, and rice). No link between the vegetable-fruit and red meat-starch groups and prostate cancer risk was reported. The Southern diet showed a slight reduction in prostate cancer risk. The authors speculate that this reduction may be due to a higher exposure to sunlight and therefore higher levels of vitamin D.

**Summary of in vivo animal and human studies:** The in vivo animal studies seem to support the in vitro observations of protective effects (48–67). All of the animal studies reported a significant decrease in tumor mass after dietary intervention with lignan-rich foods. However, the few in vivo human studies available are inconsistent. Two of the five studies reported beneficial results in men consuming a lignan-rich diet (74,75) and another found that soy and linseed together had no effects (76), whereas the remaining two found no link between serum enterolactone levels and prostate cancer risk (38,77). The other human studies found no association between serum enterolactone levels and prostate cancer risk in men consuming a more varied diet (38,75).

There are a number of discrepancies with the in vivo data with respect to the effect of lignans in prostate cancer. Several of the dietary interventions used foods rich in lignans, such as flaxseed (70,72,74,76) and rye bread (69, 72, 75), or in isoflavones, such as soy (69,76). It is not possible to ascribe the in vivo anticancer effects reported to the lignans

alone. There are many other bioactive components in the foods used that may influence prostate pathogenesis either on their own or synergistically with the lignans. The exact nutritional and lignan content of the diets were not clear or measured in plasma. It is reported that lignans accumulate at much higher concentrations in prostatic fluid than in plasma (19). The level of fat in the diets affects the interpretation of the results. A high-fat diet is generally accepted to increase the risk of prostate cancer by altering the balance of sex hormones such as testosterone (7). Reducing the level of fat in the diet reduces the risk of prostate cancer (78). Only the study by Zhang et al. included a high-fat control (72). The lack of a high-fat control in many of the diets also prevents any biological effects reported from being ascribed solely to the lignans. Many of the studies used a small sample size and a short dietary intervention. It is conceivable that long-term exposure to lignans may be required for any protective effects to be observed.

The serum enterolactone levels reported by Kilkinen and others are discrete measurements at specific time points (38,77). They did not measure the level of serum enterolactone over a defined time. They also did not measure the levels in prostatic fluid. If the hypothesis that lifetime consumption of lignan affects the pathogenesis of prostate cancer is valid, then serum concentrations are not valid measurements of lignan consumption and risk of prostate cancer as lignans can achieve much higher prostatic levels than found in the plasma (19).

Although the animal and human in vivo studies support many of the reported in vitro effects of lignans, there is an acute lack of knowledge regarding the anticancer effects of pure lignan compounds.

## Conclusions

Prostate cancer is becoming the most common male cancer in the Western world, and epidemiological evidence suggests that dietary modification is a potential method for prevention and treatment of the disease. As with most cancers, an unacceptably high number of cases of prostate cancer are diagnosed at an advanced stage. With the possibility that dietary intervention may not be sufficient to achieve the effective biological levels reported in vitro, it may be that lignans are more suitable as a pharmacological treatment. Indeed, there is a lack of evidence that lignans may be preventative against prostate cancer; it may be the case that lignans are more suitable in conjunction with current treatments for prostate cancer. The side effects of the current treatments are a source of considerable psychological stress for patients. The perceived “loss of masculinity” associated with the more advanced treatments almost certainly affects the quality of life for the patient. Other more effective treatment options are required. It is theoretically possible that intervention with lignans in men with prostate cancer will reduce the need for radical procedures such as prostatectomy or reduce the chance of relapse.

However, the exact molecular mechanism of action of the lignans is unclear as our understanding is hampered by the lack of experimental evidence. Future research should focus specifically on the *in vitro* effects of lignans, especially pure lignans, on established prostate cancer cell lines. The pharmacokinetics of the lignans requires considerable clarification prior to any meaningful *in vivo* work. Any future *in vivo* studies should also use purified lignans, account for the role of fat in prostate cancer, include measurement of lignans in prostatic fluid, and be of sufficient sample size to be statistically significant. This will clarify if lignans possess any value in the prevention and treatment of prostate cancer. In addition, this will determine whether dietary or pharmacological intervention is the most effective method of using lignans in the development of novel treatments for prostate cancer.

### Acknowledgments and Notes

The principal author would like to acknowledge the University of Ulster for the Vice Chancellor's Research Scholarship award. Address correspondence to Mark J. McCann, Northern Ireland Centre for Diet and Health (NICHE), Centre for Molecular Biosciences (CMB), University of Ulster, Coleraine, Northern Ireland BT52 1SA. Phone: (0044) 028 7032 3068. FAX: (0044) 028 7032 3023. E-mail: mj.mccann@ulster.ac.uk.

Submitted 6 May 2004; accepted in final form 4 May 2005.

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