Emerging Evidence on the Role of Soy in Reducing Prostate Cancer Risk

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Soyfoods are a unique dietary source of isoflavones, which have both hormonal and non-hormonal effects relevant to prostate cancer prevention. In vitro, the main soybean isoflavone, genistein, inhibits prostate cancer cell growth; in animals, most but not all studies show isoflavone-rich soy protein and isolated isoflavones inhibit prostate tumor development. Currently, although only limited epidemiologic data indicate soy intake reduces prostate cancer risk, results from a pilot intervention trial suggest isoflavones may be beneficial to prostate cancer patients. For several reasons, men concerned about their prostate health may consider incorporating soy into their diet.

Key Words: soy, isoflavones, genistein, prostate, cancer, review

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Introduction

Worldwide cancer of the prostate is the fourth most common cancer and sixth most common cause of cancer death. There are striking differences in prostate cancer rates among regions in the world; for example, prostate cancer mortality rates are three times higher in developed countries than in developing countries. Mortality rates are known to be especially low in Asia. Age-adjusted mortality rates per 100,000 men for Singapore and Hong Kong for the period 1973 to 1977 were 1.9 and 2.1, respectively, compared with 15.0 and 21.6 for France and Sweden, respectively. Because of the high socioeconomic status of Japan, the low prostate cancer mortality rate of 2.4 per 100,000 men is particularly noteworthy.

Migration data indicate that the variation in international prostate cancer rates is not due to genetic differences. Age-adjusted prostate cancer incidence rates per 100,000 for the period 1978 to 1981 for men in Shanghai were 1.5 as opposed to 15.1 and 48.4 for Chinese and non-Chinese men in the United States, respectively. Among the Chinese, therefore, rates were tenfold higher for those living in the United States than for those in their native homeland, although the United States non-Chinese rate was still threefold higher than the Chinese American rate. Similarly, age-adjusted prostate cancer incidence rates for Japanese men born in the United States are reported to be four times higher than for native Japanese men. Furthermore, Shimizu et al. found that native Japanese men who migrated to the United States, even relatively late in life, experienced marked increases in prostate cancer risk such that their ultimate risk was similar to non-Japanese men born in the United States. This observation indicates that lifestyle factors later in life have an important influence on prostate cancer risk.

Despite the low mortality rate, autopsy data reveal that many Japanese men do develop prostate cancer. Furthermore, a recent analysis suggested that the incidence of prostate cancer in Japan may be significantly underestimated. Based on prostate-specific antigen levels, Shibata et al. estimated that only 19% of prostate cancers in Japan are detected compared with 69% for Japanese American men. Because the mortality rates are so low, if the true incidence of prostate cancer in Japan is indeed much higher than commonly perceived, one might deduce that cases of prostate cancer in Japan do not progress to the more advanced form of the disease.

Substantial data indicate that dietary differences among cultures significantly contribute to variations in prostate cancer rates. One food that is under investigation for its chemopreventive properties in the prostate is soy. This is partially a result of the low rates of prostate cancer mortality in Asia but also because soy is essentially a unique dietary source of isoflavones. The U.S. National Cancer Institute (NCI) has been actively investigating isoflavones since 1991; in 1998, the Chemoprevention Branch of the NCI judged genistein, the main isoflavone in soybeans, to be a key chemopreventive...
agent. Currently the NCI is funding phase I and phase II trials examining the relationship between soy and prostate cancer. Recently, the International Prostate Health Council, a European group of experts, concluded that isoflavones prevent the progression of the latent form of prostate cancer to the more advanced stages of this disease.\(^9\) The American Cancer Society includes eating soyfoods as one of seven recommendations for reducing prostate cancer risk.\(^1\)

Because prostate cancer is a disease of older men, even modestly delaying the onset and/or the progression of prostate cancer will substantially reduce the morbidity, mortality, and economic burden of this disease in high-risk countries such as the United States. Soy could be of particular benefit to U.S. African Americans, who have the highest rate of prostate cancer mortality in the world. The purpose of this short review is to evaluate the in vitro, animal, epidemiologic, and clinical data most relevant to the role of soy, especially isoflavones, in preventing and treating prostate cancer.

### Isoflavones

Isoflavones are a subclass of a larger and ubiquitous group of nutraceuticals called flavonoids. In comparison with most flavonoids, however, isoflavones have a very limited distribution in the plant kingdom. Soybeans are the only nutritionally relevant natural dietary source of isoflavones. The three soybean isoflavone aglycones are genistein, daidzein, and glycitein. In the soybean itself, these three isoflavones are present as the glycosides, genistin, daidzin, and glycitin. The glycoside can also be esterified with either malonyl or acetic acid such that there are a total of 12 different isoflavone isomers. Generally, soyfoods contain somewhat more genistein than daidzein and relatively little glycitein (<10% of total isoflavone content).

Given the similarity in chemical structure between isoflavones and estrogen, it is not surprising that isoflavones bind to estrogen receptors. Compared with 17β-estradiol, however, isoflavones have a relatively low binding affinity for estrogen receptor alpha (ERα) as the IC\textsubscript{50} (concentration of a compound decreasing maximum \(^{3}H\)estradiol binding by 50%) is generally at least two orders of magnitude higher. Nevertheless, even this low binding affinity suggests isoflavones hold the potential to exert physiologic effects in vivo because serum isoflavone levels in people who eat soyfoods reach the low micromolar range, which is approximately 1000-fold higher than endogenous estrogen levels.\(^1\)

Furthermore, isoflavones have a relative binding affinity for ERβ that is only slightly lower than that of 17β-estradiol; some studies also suggest these two compounds are equally potent at triggering transcriptional activity when bound to ERβ.\(^1\) This is because binding to the estrogen receptor is only one factor determining the ability to affect estrogen-regulated genes; the conformational change induced by the ligand and the resulting interaction of the ligand-receptor complex with coregulators and coactivators is critically important. In part because of their preferential binding to ERβ, isoflavones are often regarded as natural selective estrogen receptor modulators, similar to the drugs tamoxifen and raloxifene.

Independent of any direct hormonal effects, isoflavones, especially genistein, influence signal transduction in vitro and in vivo. Genistein inhibits the activity of many enzymes and cellular factors that control the growth and differentiation of cells,\(^1\) and in some experimental systems, isoflavones exhibit antioxidant activity.\(^1\) The effects of genistein on signal transduction explain why this isoflavone inhibits the growth of a wide range of cancer cells in vitro.

### In Vitro Anticancer Effects of Isoflavones

Genistein inhibits the growth of both androgen-dependent (LNCaP)\(^1\),\(^1\) and androgen-independent (DU-145, PC-3, PC3-M, PSL-10)\(^1\),\(^1\) prostate cancer cells in vitro in a dose-dependent manner. Most studies indicate that the concentration required to inhibit cell growth by 50% (IC\textsubscript{50}) is generally approximately 25 μM, although Davis et al. reported an IC\textsubscript{50} of only 5 μM for LNCaP cells.\(^2\) Clearly, even in people who eat substantial amounts of soy, the IC\textsubscript{50} for growth inhibition exceeds the serum concentration of genistein, especially when most of the genistein in serum and tissue is conjugated and therefore likely to be biologically inactive.\(^2\)

Kyle et al. found that prolonged (11 days) exposure to genistein lowered the IC\textsubscript{50} two- to threefold in comparison with shorter (3 days) exposure;\(^2\) this led researchers to question the relevance of the in vitro IC\textsubscript{50} and to suggest that genistein might be more potent in vivo than in vitro, a sentiment echoed by other researchers as well.\(^2\) Furthermore, Peterson and Barnes observed that compared with unstimulated cells, the IC\textsubscript{50} values for epidermal growth factor–stimulated LNCaP and DU-145 cells were reduced by approximately 32% and 85%, respectively.\(^1\) In several studies, daidzein also inhibited cell growth, although, in most cases, much higher concentrations were required; however, Davis et al. found that in PC3 cells daidzein and genistein had similar growth inhibitory effects.\(^2\)

In addition to those findings cited above, Santibanez et al. found that independent of effects on cell growth, genistein (30 μM) caused a threefold inhibition of the invasive capacity of PC-3 cells.\(^2\) Geller et al. showed that genistein in doses of 1.25 to 10 μg/mL (to convert μg/mL to μM, multiply by 3.7) decreased the growth of human-patient benign prostatic hypertrophy and prostate
cancer in histoculture in a dose-dependent manner. Genistein has also been shown to enhance the ability of radiation to kill prostate cancer cells in vitro.22

Finally, genistein reduces prostate-specific antigen (PSA) secretion in LNCaP cells.19,25 PSA is a serine protease synthesized by the prostate and mammary epithelial cells that is used as a tumor marker for detecting and monitoring treatment response in prostate cancer patients. Although genistein similarly inhibits the growth of LNCaP and VeCaP cells, low (1–6 μM) genistein concentrations reduce PSA secretion in LNCaP cells, whereas much higher (>10 μM) concentrations are required to inhibit PSA secretion in VeCaP cells.25

**Animal Studies**

A number of different models, each with advantages and disadvantages, have been employed to study prostate cancer in animals. Pollard and colleagues have contributed significantly to the understanding of the role of diet, in general, and soya and isoflavones, in particular, in the development of prostate cancer in the Lobund-Wistar (L-W) rat. The L-W rat is the only known rat strain that is inherently predisposed to spontaneously metastasizing hormone-influenced adenocarcinomas in the prostate. Tumors develop in the dorsolateral and anterior lobes and the seminal vesicles, which is why these growths are referred to as P-SV tumors. On average, approximately 30% of L-W rats develop tumors spontaneously in 26 months. This model is likely to be the most sensitive to mildly potent chemopreventive agents such as those found in foods.

Tumor induction in L-W rats can be enhanced by a single intravenous injection (30 mg/kg body weight) of methylnitrosourea (MNU), such that approximately 30% of L-W rats will develop tumors by 12 months. Implants of testosterone propionate (TP) will further increase the percentage of tumor-bearing rats, and a combination of MNU and TP leads to a 90% incidence rate in 10 months.33 However, this latter approach may overwhelm the mildly preventive effects of dietary agents.

As noted in Table 1, Pollard and Luckert showed that L-W rats fed soy protein–containing diets have a reduced incidence of P-SV tumors in comparison with rats fed casein when diets are started both prior to, and after, MNU administration, although the effects of soy are more pronounced when fed prior to the carcinogen.34 Tumor latency (i.e., the time it takes on average for the first tumor to be detected) was increased only in the diet containing isoflavone-rich isolated soy protein (ISP+) and primarily only when fed prior to carcinogen administration.34 In three other experiments, Pollard and colleagues found that in L-W rats, ISP+ markedly reduced tumor incidence in comparison with both that which is typical for control animals and in comparison with animal groups fed a natural-ingredient diet containing soymeal.35–37 These effects were observed in the case of both spontaneously developing tumors and in rats given MNU; furthermore, tumor inhibition was noted even when the ISP+ diet was fed beginning when the rats were 12 months old.37 The superior anticancer effects of ISP+ in comparison with soymeal are surprising because the isoflavone content of these products is similar. It appears that constituents in soymeal block the ability of isoflavones to suppress tumor development, perhaps by blocking the ability of isoflavones to lower serum testosterone levels.

Recently, a transgenic mouse model of prostate cancer was developed. Using this model to study the effects of genistein, Mentor-Marcel et al. fed mice diets containing 0, 100, 250, or 500 mg/kg from 5 to 30 weeks of age.38 They found a statistically significant decrease in the incidence of advanced prostate lesions. Serum genistein concentrations increased in a dose-dependent manner reaching a high of approximately 400 nmol/L, which is well within the range observed in people who eat soyfoods.

Seven studies listed in Table 1 examined the effects of soy or isoflavones on prostate tumors induced by inoculating animals with cancer tissue or cancer cell lines; of these seven, three reported pronounced inhibitory effects,29,39,40 two reported modest protective effects,41,42 one reported no effects22 and one reported that ISP increased tumor volume.43 Unarguably, the most impressive results come from the two studies by Zhou et al.29,40 In one, they found that the combination of soy protein and isoflavones dose-dependently suppressed tumor formation in severe combined immune-deficient mice subcutaneously inoculated with LNCaP cells.29 In this study, histologic examination of tumor tissue showed that tumor cell proliferation was only modestly reduced, whereas there was a huge increase in apoptosis and a marked reduction in microvessel density. Serum levels of the angiogenic protein insulin-like growth factor-I were also reduced in mice fed soy protein and isoflavones. In the other study, which utilized a similar experimental design, soy phytochemical concentrate (SPC), an isoflavone-rich product, not only significantly inhibited tumor development by itself, but the combination of SPC and green tea extract synergistically inhibited prostate tumorigenicity, final tumor weight, and metastases to lymph nodes in vivo.40 Inhibition of tumor progression was associated with reduced tumor cell proliferation and tumor angiogenesis. The combination of SPC and green tea synergistically inhibited final tumor weight and metastasis.

Two studies in Table 1 induced tumors by chemical carcinogen; in one an isoflavone mixture significantly decreased the combined incidence of tumors in the pros-
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<td>Pollard34</td>
<td>L-W rat/i.p. MNU + TP</td>
<td>Diets fed beginning 7 days prior to MNU</td>
<td>Incidence (%) Latency (mos)</td>
<td>Data represent combined effects from two (pre-MNU) and three (post-MNU) experiments, respectively. Rats consumed 3.8 mg and 0.22 mg/day genistein in high- (ISP+) and low-soy protein isolate (ISP−)–containing diets, respectively. Trials terminated 14 months after MNU. No statistics reported.</td>
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<tr>
<td></td>
<td></td>
<td>Casein</td>
<td>40/52 (74)</td>
<td>7.8</td>
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<td></td>
<td></td>
<td>20% ISP−</td>
<td>10/24 (41.6)</td>
<td>7.3</td>
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<td></td>
<td></td>
<td>20% ISP+</td>
<td>8/24 (33.3)</td>
<td>10.0</td>
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<tr>
<td></td>
<td>Diets fed 21 days post MNU</td>
<td>Casein</td>
<td>40/52 (74)</td>
<td>7.8</td>
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<tr>
<td></td>
<td></td>
<td>20% ISP−</td>
<td>20/33 (60)</td>
<td>9.3</td>
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<td></td>
<td></td>
<td>20% ISP+</td>
<td>18/35 (51)</td>
<td>10.6</td>
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<tr>
<td>Pollard35</td>
<td>L-W rat/i.p. MNU given at 3 months</td>
<td>L-485 (30% soymeal) L-474 (ISP)</td>
<td>Incidence (%)</td>
<td>Rats on L-485 and L-474 diets consumed ~4.00 and ~3.85 mg genistein/day. Serum testosterone 70% lower in rats fed L-474. No statistics reported.</td>
</tr>
<tr>
<td>Pollard36</td>
<td>L-W rat/Spontaneous</td>
<td>L-485 (soymeal) L-474 (ISP)</td>
<td>Incidence (%)</td>
<td>Serum testosterone 64% lower on rats fed L-474. Rats on both diets consumed about 4–5 mg genistein/day. Control diets normally lead to 30% tumor incidence.</td>
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<tr>
<td>Pollard37</td>
<td>L-W rat/Spontaneous</td>
<td>L-485 (soymeal) L-474 (ISP)</td>
<td>Incidence (%)</td>
<td>Serum testosterone 60% lower on L-474. Genistein intake similar on the two diets. No statistics reported.</td>
</tr>
<tr>
<td>Mentor-Marcel38</td>
<td>TRAMP mice/Spontaneous</td>
<td>Genistein mg/kg diet</td>
<td>Incidence (%) of advanced lesions</td>
<td>Diets were fed from week 5–6 to week 28–30. No effects on body weight, epididymides, testes, or kidneys. Differences in tumor incidence significant ($P = 0.041$).</td>
</tr>
<tr>
<td>Naik22</td>
<td>Copenhagen rat/MAT-Lylucells injected s.c.</td>
<td>Genistein (mg/kg/day) Drinking water</td>
<td>Tumor weight (% relative to controls)</td>
<td>Genistein given days 4–13 in drinking water. No significant differences.</td>
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Table 1. Effects of Soy Protein and Isoflavones on Prostate Tumor Development in Rodents (Cont’d)

<table>
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<th>Study</th>
<th>Model</th>
<th>Treatment</th>
<th>Protein Source</th>
<th>Isoflavone Source</th>
<th>Tumor Volume (cm³) at 3 wk</th>
<th>Notes</th>
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<tr>
<td>Zhou²⁹</td>
<td>SCID mice/LNCaP cells injected s.c.</td>
<td>20% protein +/- varying concentrations of SPC g/100 g diet</td>
<td>Casein ± 0.0</td>
<td>ISP ± 0.0</td>
<td>2.32 ± 0.31</td>
<td>Soy phytochemical concentrate (SPC) was an alcohol extract of soy flour containing 1.22 mg, 0.64 mg, and 0.21 mg (aglycone) genistein, daidzein, and glycitein/g, respectively. Beginning in order listed in column 3, the diets contained 0, 244, 159, 403, 794, and 1038 mg genistein/kg, respectively. Factor analysis indicated there was a significant effect of SPC (&lt;0.05) but not protein.</td>
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<tr>
<td>Zhou⁴⁰</td>
<td>SCID mice inoculated intraprostatically with 2 x 10⁶ LNCaP cells</td>
<td>Soy phytochemical concentrate (SPC) containing diet significantly decreased tumorigenicity compared with control. The combination of black tea and SPC synergistically inhibited tumor formation.</td>
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<tr>
<td>Aronson⁴²</td>
<td>CB17 beige SCID mice/LNCaP cells injected s.c.</td>
<td>Fat level, protein type, +/- isoflavones (IF)</td>
<td>High-fat + casein</td>
<td>High-fat + ISP + IF</td>
<td>0.86 ± 0.21</td>
<td>On a caloric basis, the low- and high-fat diets were ≈12% and 42% fat. The low-fat + ISP + IF group was significantly different from other groups (P &lt; 0.05). No difference in latency or PSA levels. Testosterone levels 70% lower in LF-ISP-IF but not significant. Diets fed prior to inoculation. (The IF diets were 0.18% by weight IF.)</td>
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<tr>
<td>Schleicher³⁹</td>
<td>L-W rat/K1 cells injected s.c.</td>
<td>Genistein s.c. 50 mg/kg every 12 h beginning at time of cell injection</td>
<td>DMSO</td>
<td>Gen</td>
<td>3.77 ± 0.23</td>
<td>Rats killed 31 d after injections. Tumor invasion and metastasis was significantly decreased. Body weight, prostate, and seminal vesicle weight significantly decreased. Differences in tumor weight significant (P = 0.0007).</td>
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<tr>
<td>Landstrom⁴¹</td>
<td>Copenhagen × Fisher FI/s.c. transplanted Dunning R3327 PAP prostate tumors</td>
<td>Fiber free (FF)</td>
<td>Soy flour (SD)</td>
<td>Rye bran (RB)</td>
<td>Heat-treated rye bran (HRB)</td>
<td>At weeks 14 and 16, but not weeks 18–24, tumor incidence was lower in the SD, RB, and HRB groups versus the FF group. After adjustment for body weights, tumor volumes were significantly lower in the SD, RB, and HRB versus the FF group. Tumor growth rates in the SD, RB, and HRB groups were significantly lower at 12–14 wks but not thereafter. No effect of diet on serum testosterone or testis weight.</td>
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<td>First Author and Reference</td>
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<td>Cohen43</td>
<td>Copenhagen rat/AT-1 cells injected s.c.</td>
<td>Casein 20% Soy protein 0%</td>
<td>% increase from baseline 633</td>
<td>Diets fed beginning day of inoculation. AT-1 cells are a rapidly growing androgen-independent cell line derived from the Dunning 3727 transplantable prostate tumor. Ten percent and 20% ISP diets significantly different from control.</td>
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<td>Onozawa44</td>
<td>F344 rat/3.2’-dimethyl-4-amino biphenyl (DMAB) + testosterone</td>
<td>Control (C) C + IF mixture (100 ppm) C + IF mixture (400 ppm)</td>
<td>Incidence (%) in prostate and seminal vesicles 21/35 (60) 17/49 (35) 13/45 (29)</td>
<td>Differences between isoflavone groups and control statistically significant. Isoflavone mixture was 74.2% genistein and 20.7% daidzein. Difference between C and low-IF mixture ( P &lt; 0.05 ) and high-IF mixture ( P &lt; 0.01 ) was significant.</td>
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<tr>
<td>Kato45</td>
<td>F344 rat/repeated s.c. injections of DMAB</td>
<td>Control (C) C + 0.1% Genistin C + 0.1% Daidzin</td>
<td>Ventral carcinomas Inc(%) No/cm² mm²/cm² 46 3.2 0.81</td>
<td>Diets fed throughout experiment. The number of tumors/cm² in the genistin and daidzin groups was significantly ( P &lt; 0.05 ) different from the control group.</td>
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</table>

L-W = Lobund-Wistar, i.p. = intraperitoneal, TP = testosterone propionate, s.c. = subcutaneous, MNU = methylnitrosourea, ISP = isolated soy protein, DMAB = 3.2’-dimethyl-4-amino biphenyl, SPC = soy phytochemical concentrate, TRAMP = transgenic mouse model of prostate cancer, SCID = severe combined immune-deficiency, PSA = prostate specific antigen, IF = isoflavones.
tate and seminal vesicles, and in the other the isoflavones genistin and daidzin each suppressed the incidence of ventral carcinomas. Of the studies listed in Table 1, all but two showed that soy or isoflavones exerted at least modest suppressive effects on prostate carcinogenesis. In one of these, Naik et al. failed to show that intraperitoneal administration of genistein affected the growth of MAT-Lyu cells injected subcutaneously in Copenhagen rats. In the other study by Cohen et al., diets comprised of either 10 or 20% ISP led to a doubling of tumor size in Copenhagen rats injected subcutaneously with AT-1 cells compared with rats fed diets containing either no soy or 5% ISP. Because this study and the study by Naik et al. involved the same strain, they raise the possibility that soy is not effective in the Copenhagen rat although very modest protective effects were observed by Landstrom et al. in the Copenhagen × Fisher FI rat.

A more likely explanation for the lack of results in the study by Naik et al. is the relatively small amount of genistein the rats received. Rats given the highest (0.428 mg/kg) dose would have been exposed to only approximately 0.1 mg genistein/day. By comparison, in the study by Zhou et al. in which a dose-dependent decrease in tumor growth in response to dietary isoflavones was observed, mice ingested as much as 5 mg isoflavones/day. Assuming approximately half the isoflavone content was genistein and that the isoflavone absorption efficiency was 30%, animals were exposed to as much as 0.5 mg genistein/day. The failure to see tumor suppression in the study by Cohen may have been due to the type of cell used to induce tumors. The AT-1 cell is a rapidly growing androgen-independent cell line derived from the Dunning 3727 transplantable prostate tumor, which has no detectable dihydrotestosterone receptors. These cells are viewed as providing a useful model for advanced (hormone refractory) prostate cancer. On the basis of their results, Cohen et al. issued a cautionary statement about the use of soy in human studies involving advanced hormone-refractory prostate cancer.

Finally, one of the most provocative animal studies didn’t involve the development of prostate tumors but rather examined the impact of genistein on epidermal growth factor receptor (EGFR) levels in the dorsolateral prostate of L-W rats. EGF is considered a potent mitogen for the prostate. Rats were fed 0.025- to 1.0-mg genistein/g diet. There was a dose-dependent decrease in EGFR levels with the highest dose of genistein inhibiting the expression of the EGFR by 50%. Free genistein concentrations in the serum and prostate of rats given this dose were only 137.4 nmol/L and 156.1 pmol/g, respectively. These concentrations are at least 100-fold lower than the concentrations required to inhibit the growth of prostate cancer cells in vitro. This study therefore indicates not only that genistein affects signal transduction in vivo but that the in vitro data may underestimate the anticancer effects of this isoflavone. Other research by this same group shows that dietary genistein fed to male Sprague-Dawley rats for just two weeks reduced mRNA expression of the androgen receptor and ERα and ERβ in the dorsolateral prostate.

**Epidemiologic Studies**

**Ecologic**

Hebert et al. found a strong inverse relationship between soy product intake and prostate cancer mortality among 42 countries for which soy intake data were available. Although impressive, the value of this observation is unclear because only Korea and Japan consumed appreciable amounts of soy; of the other 40 countries, 19 consumed no soy, 16 derived ≤5 kcal/day from soy, and five derived between 9 and 36 kcal/day. By contrast with these findings, in an ecologic study involving 47 prefectures in Japan, Nagata failed to find a relationship between total soy or isoflavone intake and prostate cancer mortality. However, the narrow range of soy intake among the prefectures may have hindered the ability to identify protective effects; the lowest and highest prefectures consumed 21 and 35 mg isoflavones/day, respectively.

**Case-control Studies**

None of the six case-control studies listed in Table 2 found that soy intake was associated with a statistically significant decreased risk of prostate cancer; all but one study, however, was relatively small. Three of these studies were conducted in North America and one each in China, Japan, and Taiwan. In the Japanese study only the intake of miso was reported, whereas the Taiwanese study only reported soymilk intake. In the Chinese study, cases did consume less soy than controls but the results were not statistically significant (P = 0.016). The authors of this study commented that the lack of variation in soy consumption contributed to the lack of statistically significant effects.

The results from all three North American studies suggested soy intake was protective and, although none reported statistically significant differences, two of the studies reported P values for various indicators of soy intake that were close to significance. The ability of western epidemiologic studies not specifically involving subjects of Asian ethnicity to provide insight into the possible health effects of soy consumption is extremely limited, however, because soy intake is so low. For example, in the study by Strom et al., median isoflavone intake was only approximately 100 μg/day, compared with an average Asian daily intake of approximately 35
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<td>Hebert\textsuperscript{47}</td>
<td>Ecologic, age-adjusted mortality rates per 100,000 men aged 45–75</td>
<td>59 countries (soy intake data for 42 countries), FAO disappearance data from years 1979–1981</td>
<td>Mean daily per capita calories from soy was 8; range was 0–94 calories</td>
<td>Greater calories from cereals, nuts, oil seeds, fish, and soy protective, effect size/kcal from soy 4 times greater than any other factor</td>
<td>Only Korea and Japan consumed appreciable amounts of calories from soy, mortality data were for a period 10 years later than dietary data</td>
</tr>
<tr>
<td>Nagata\textsuperscript{48}</td>
<td>Ecologic, Japan</td>
<td>47 prefectures, 3-day dietary records, data from the National Household Survey. Intake data for years 1980–1985</td>
<td>Mean isoflavone intake, 28.8 mg/day, minimum and maximum intake, 21.3 mg and 35.7, respectively</td>
<td>Adjusted Pearson correlation coefficients for intake and mortality: isoflavones, 0.24; protein, 0.19; total soy products, 0.20, no measure of soy intake significant</td>
<td>Author commented that the range of intake might have been too narrow to see significant effects, mortality data for year 1995</td>
</tr>
<tr>
<td>Mills\textsuperscript{57}</td>
<td>Prospective, USA (non-Hispanic, Seventh-day Adventists)</td>
<td>Follow-up period, 1976–1982, 14,000 men, 180 cases, food-frequency questionnaire</td>
<td>Specific data on soy not provided, intake categories for VPP were &lt;1×/week, 1–4×/week, and ≥5×/wk</td>
<td>Age-adjusted relative risks for tertiles two and three were 0.83 (0.59–1.16) and 0.67 (0.40–1.12), trend P = 0.10</td>
<td>VPP include meat substitutes such as soy products and gluten-based products, 18 cases and 13,060 person-years in the high-VVP category, high intake of beans, lentils, and peas was also protective (P = 0.01)</td>
</tr>
<tr>
<td>Severson\textsuperscript{54}</td>
<td>Prospective, United States (Hawaii)</td>
<td>Follow-up period, 1965–1986, 7999 Japanese men, food-frequency questionnaire and 24 h dietary recall, only 23 foods considered, diet assessment mostly aimed at determining extent of westernization of diet</td>
<td>Miso and tofu intake tertiles were ≤1×/week, 2–4×/week, and ≥5×/week</td>
<td>Age-adjusted relative risk ± 95% CI of incidence for miso soup and tofu first through third tertiles of intake were: 1.00, 1.19 (0.50–1.76); and 1.24 (0.51–3.04), respectively, and 1.00, 0.78 (0.53–1.14), and 0.35 (0.08–1.43), respectively. For tofu intake, trend P = 0.054</td>
<td>Only 174 cases, number of cases and controls in the third tertile of tofu intake was only 2 and 165, respectively</td>
</tr>
<tr>
<td>Hirayama\textsuperscript{56}</td>
<td>Prospective, Japan</td>
<td>17-year follow-up period, 3,849,637 person-years of observation, intake data based on interview using food-frequency questionnaire</td>
<td>Miso soup intake categories: daily, occasionally, rarely/none</td>
<td>Relative risks ±90% CI for age-adjusted prostate cancer death for high miso intake, 1.45 (1.09–1.94)</td>
<td>Miso was the only soy product assessed, small number of cases (n = 183)</td>
</tr>
<tr>
<td>Study</td>
<td>Type</td>
<td>Country, Population Details</td>
<td>Measure</td>
<td>Methods</td>
<td>Findings</td>
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<tr>
<td>Jacobsen⁵⁵</td>
<td>Prospective, USA</td>
<td>Follow-up 1976–1982, 12,395 men, 147 cases, 1976–1992, 2276 men, 78 cases</td>
<td>Only soymilk intake reported, intake categories: never, &lt;daily, daily, and &gt;1/day</td>
<td>Adjusted relative risks ±95% CI for incidence for intake quartiles one through four were 1.00, 0.9 (0.5–1.4), 0.7 (0.4–1.4), and 0.3 (0.1–0.9), P value for linear trend, 0.02, when the two highest categories were merged, the relative risk was 0.6 (0.3–1.0)</td>
<td>Soymilk was protective in subgroup analysis of men who denied having BPH (P = 0.02) or prostate surgery (P = 0.007), soymilk intake was unrelated to the risk of advanced cancer cases, only 223 men in the high-soymilk category</td>
</tr>
<tr>
<td>Oishi⁵⁰</td>
<td>Case-control, Japan</td>
<td>100 cases, 100 controls with BPH and 100 HC, intake based on interviews using food-frequency questionnaire</td>
<td>Absolute data on intake not provided, subjects categorized as having either high or ordinary intake.</td>
<td>Relative risks ±95% CI, cases versus BPH: high intake: 1.29 (0.57–2.92), ordinary intake: 1.47 (0.66–3.25), cases versus HC: high intake: 0.64 (0.31–1.34), ordinary intake: 1.40 (0.62–3.15)</td>
<td>Miso soup intake unrelated to risk, no data on the intake of other soy products was provided</td>
</tr>
<tr>
<td>Lee⁵²</td>
<td>Case-control, China</td>
<td>133 cases, 265 neighborhood controls, food-frequency questionnaire, 140 foods (27 food groups)</td>
<td>Weekly soy intake of 50 g units, 9.9 cases 11.7 controls</td>
<td>For incidence soy intake cases versus controls, P = 0.16 for trend</td>
<td>Authors commented lack of significant effect may have been due to lack of variation in soy consumption</td>
</tr>
<tr>
<td>Sung⁵¹</td>
<td>Case-control, Taiwan</td>
<td>90 cases, 180 hospital controls, intake assessed by food-frequency questionnaire</td>
<td>Soymilk categories were consumed or not consumed</td>
<td>Odds ratio ±95% CI for incidence for consumption versus no consumption 0.95 ± (0.45–2.00)</td>
<td>Soymilk only soy product reported</td>
</tr>
<tr>
<td>Strom⁵³</td>
<td>Case-control, United States</td>
<td>83 cases, 107 controls, food-frequency questionnaire</td>
<td>Median and range of genistein (G) and daidzein (D) intakes (μg/day), cases: G, 19.8 (0–970); D, 14.2 (0–4383), controls: G, 29.7 (0–947), D, 22.8 (0–20,950)</td>
<td>Adjusted odds ratio ±95% CI controls versus cases, genistein: 0.71 (0.39–1.30), P = 0.26; daidzein: 0.57 (0.31–1.05), P = 0.07</td>
<td>Soy intake based on estimated isoflavone intake almost negligible</td>
</tr>
<tr>
<td>Kolonel⁴⁹</td>
<td>Case-control, United States</td>
<td>1619 cases, 1618 all controls, 847 normal controls (normal prostate specific antigen levels), food-frequency questionnaire</td>
<td>Quintile range for total soy product intake (g/day)</td>
<td>Adjusted odds ratio ±95% CI for Q1 versus Q1 plus P value for trend, all cases/all controls, 0.62 (0.44–0.89), P = 0.06, all cases/normals controls, 0.64 (0.42–0.98), P = 0.17, advanced cases/all controls, 0.59 (0.35–1.00), P = 0.13</td>
<td>Legume intake (excluding and including soybeans) was significantly protective in most comparisons, legumes included miso, soybeans, tofu, aburage, 12 types of beans and peas, and black-eyed peas</td>
</tr>
<tr>
<td>Villeneuve⁵⁸</td>
<td>Case-control, Canada</td>
<td>1623 cases, 1623 controls, dietary questionnaire</td>
<td>Categories for tofu or soybeans: none, some, unknown</td>
<td>Adjusted odds ratio ±95% CI for some versus none 0.8 (0.6–1.1), P for trend = 0.29</td>
<td></td>
</tr>
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</table>

FAO = Food and Agriculture Organization, BPH = benign prostatic hyperplasia, HC = hospital controls, VPP = vegetarian protein products, CI = confidence interval.
mg.\textsuperscript{48,59} In the largest of the three North American case-control studies, which was conducted in Hawaii, soyfood intake was inversely (odds ratio for fifth versus first quintile, 0.62; $P$ for trend = 0.06) related to prostate cancer, but the effect was not quite statistically significant.\textsuperscript{39} This study is intriguing because 37\% ($n = 3237$) of subjects were Chinese or Japanese who consumed relatively high amounts of soy, although the cutoff for the highest quintile of soyfood intake was still only $>39$ g/day. Furthermore, the intake of legumes excluding soyfoods was equally protective; it is therefore difficult to conclude that this study is supportive of soy having direct anticancer effects.

**Prospective Studies**

Four prospective studies are included in Table 2; two\textsuperscript{54,55} of these reported inverse relationships between soy intake and risk, one did not (only miso intake was reported),\textsuperscript{55} and the other found the consumption of vegetarian protein products was marginally protective (odds ratio third versus first tertile, 0.67, $P = 0.10$), but soy would only be one food within this group.\textsuperscript{57} In the large Japanese prospective study by Hirayama, miso intake was actually associated with an increased risk of prostate cancer, but there were only 183 deaths owing to prostate cancer over the 17 years of follow-up.\textsuperscript{56}

The two studies that provide the most support for the hypothesis that soy intake reduces prostate cancer are the prospective studies involving Japanese men residing in Hawaii,\textsuperscript{54} and Seventh-day Adventists (SDA) in California.\textsuperscript{55} In the former study, Severson et al. found that tofu intake, but not miso intake, was associated with a dose-dependent decrease in prostate cancer risk, although the results for the first versus the third tertile (relative risk, 0.35; $P = 0.054$) of intake did not quite reach statistical significance. The tertiles for tofu intake in this study were $<$1/week, 2–4×/week, and $\geq$5×/week.\textsuperscript{54} In the SDA study by Jacobsen et al., frequent (i.e., more than once a day) consumption of soymilk (the only soy product reported) was associated with a marked reduction in risk (relative risk fourth versus first quartile of intake, 0.3; $P$ value for linear trend = 0.03). Intake categories for this study were (0, $<$1/week, 1×/week, and $\geq$1×/day).\textsuperscript{55} These findings for soymilk obviously differ from the lack of protective effects for soy milk reported by Sung et al. in their Taiwanese case-control study.\textsuperscript{51} Furthermore, this cohort appears to be the same one in which Mills et al. found that legume (i.e., beans, lentils, and peas) intake was protective against prostate cancer.\textsuperscript{58} Furthermore, neither the study by Severson et al.\textsuperscript{54} or Jacobsen et al.\textsuperscript{55} reported total soy intake. Moreover, in both of these studies, the number of men with prostate cancer was quite small. Thus, while suggestive of protective effects, the studies by Severson et al.\textsuperscript{54} and Jacobsen et al.\textsuperscript{55} should be viewed with considerable skepticism.

**Clinical Studies**

Although studies examining the relationship between serum testosterone levels and prostate cancer risk have produced somewhat mixed results, prostate cancer is regarded as a hormone-dependent cancer.\textsuperscript{60} Seven short-term trials have examined the impact of isolated isoflavones (derived from soy or red clover), isoflavone-rich soy protein, or soy foods on reproductive hormones in young and older men. Men in these studies, which lasted from approximately 3 to 12 weeks in duration, were exposed to between 40 and 130 mg isoflavones/day. None of these studies reported significant effects on serum testosterone or dihydrotestosterone levels.\textsuperscript{61–66} The lack of changes in serum levels, however, does not preclude changes in hormonal balance with the prostate itself. In a small cross-sectional study in Japan, Nagata et al. found that total and free testosterone concentrations were inversely correlated with soy product intake after controlling for the covariates, although these correlations were of only borderline significance ($r = -0.25$, $P = 0.05$ and $r = -0.25$, $P = 0.06$, respectively) and the study involved only 69 men.\textsuperscript{67} No relationship between soy milk intake and serum levels of testosterone, free testosterone, androstanediol glucuronide, sex hormone–binding globulin, or luteinizing hormone was noted in a cross-sectional analysis of 696 British men with a wide range of soy intakes.\textsuperscript{68}

Four studies examined the impact of soy or isoflavones on PSA levels.\textsuperscript{66,69–71} Urban et al. found that the consumption of 40 g soy protein/day, which provided approximately 70 mg isoflavones, had no effect on serum PSA levels in 34 elderly men over a 6-week period.\textsuperscript{69} Not only was the duration of this study relatively short, however, but PSA levels were only mildly elevated: the median value was approximately 6.0 ng/mL. Nevertheless, confirming these results, Jenkins et al. failed to find that the consumption of approximately 44 g/day soy protein for 3 to 4 weeks had an impact on serum total or free PSA in 46 healthy middle-aged men with a range of starting PSA values.\textsuperscript{70} The lack of effect of soy on PSA persisted for 3 months in a subgroup of men who continued to consume a lower level of soy. Similarly, in 19 prostate cancer patients who consumed 160 mg of isoflavones per day derived from red clover for 7 to 54 days (median, 20 days), PSA levels remained unchanged (pre- and post-treatment means were 10.9 μg/L and 11.3 μg/L, respectively).\textsuperscript{66} By contrast to the findings from these three studies, however, a small pilot study conducted by the Karmanos Cancer Institute at Wayne State University found that in approximately 50 to 70\% (results varied according to the cancer classification of the men) of the 41 men with treated but uncontrolled prostate cancer, the
linear rise in PSA levels prior to the study was significantly decreased in response to the daily consumption of 120 mg isoflavones derived from soybeans for 6 months.71

In addition to these four studies, a case report indicated that prostate cancer tissue taken from a man who, for one week prior to surgery, consumed daily 160 mg of isoflavones derived from red clover, had undergone significant apoptosis.72 The author of this report speculated that the isoflavones may have induced apoptosis. As mentioned previously, isoflavones have been shown to induce apoptosis in prostate cells in vitro19 and in prostate tissue in animals.29 In an effort to examine the finding from this case report, Jarred et al. conducted a nonrandomized, nonblinded trial with historically matched controls in which prostate cancer patients consumed 160 mg/day of red clover–derived dietary isoflavones for a median of 20 days.66 Apoptosis in radical prostatectomy specimens from 18 treated patients was significantly higher than in the 18 control subjects (P = 0.0018), specifically in regions of low-to-moderate–grade cancer (Gleason grade 1–3). Jarred et al. suggested that dietary isoflavones might halt the progression of prostate cancer, potentially contributing to the lower incidence of clinically significant disease in Asian men.

Isoflavone levels in prostatic fluid are higher in men from soyfood-consuming countries than from countries where soy is not consumed, and isoflavones are concentrated in the prostatic fluid by approximately twofold relative to the serum.73 Thus, the prostate gland is exposed to high concentrations of isoflavones in men who eat soyfoods.

Finally, a few clinical studies have examined the possible anticancer effects of soy or isoflavones in general, although the extent to which the results of these studies are specific to prostate cancer is unclear. In men consuming 60 mg isolated isoflavones/day for 3 weeks, mean levels of 5-hydroxymethyl-2′-deoxyuridine (an oxidation product) in DNA from nucleated blood cells decreased by 61%.74 Mitchell and Collins found that in normal males the consumption of one liter of soymilk/day (probable isoflavone intake ≥100/day) for 4 weeks significantly decreased oxidative damage to DNA bases detected by the comet assay.75 In a 3-week study, Davis et al. investigated the in vivo effect of soy isoflavone supplementation (60 mg/day) on NF-kappa B activation induced by tumor necrosis factor alpha (TNF-α) in vitro in peripheral blood lymphocytes of six healthy men and found supplementation protected from TNF-α-induced NF-kappa B activation.76

Possible Mechanisms for Anticancer Effects

Both estrogens and androgens likely play a role in the development of prostate cancer;60 high-dose estrogen has been used in the treatment of prostate cancer.77 As noted previously, however, neither soy nor isoflavones appear to affect serum testosterone levels in men. Moreover, genistein is equally effective at inhibiting the growth of androgen-dependent and androgen-independent prostate cancer cells in vitro18 and therefore anti-androgen effects are not necessary for growth inhibition. Similarly, Kyle et al. showed that the lack of estrogen receptors did not prevent genistein from inhibiting the growth of PC3-M cells in vitro.23 Still, the preferential binding affinity of isoflavones for ERβ should not be overlooked given the interest in the role of ERβ in prostate carcinogenesis.78–80

Although genistein inhibits the activity of tyrosine protein kinases (a group of mitogenic enzymes) in vitro,14 Barnes and Peterson showed early on that the growth inhibitory effect of genistein against LNCaP cells was not related to phosphorylation inhibition.18 Similarly, Santibañez found that although high concentrations of genistein inhibited the growth of PC-3, DU-145, and LNCaP cells, phosphorylation was decreased only in the PC-3 cells.21

Several investigators have found that genistein induces apoptosis; this has been observed for LNCaP19 and PC3-M cells.23 Shen et al. observed no change in apoptosis in response to genistein concentrations of 5 to 20 μM, and yet growth inhibition occurred in this range; at 40 μM, however, there was a 44% increase in apoptosis.20 Kyle attributed growth inhibition entirely to apoptosis induction because he noted no effect of genistein on the cell cycle; this disagrees with the observations of others.20 Davis et al. reported that the growth inhibitory effects of genistein were accompanied by a G2/M cell cycle arrest30 and Shen et al. observed that in response to genistein there was a dose-dependent decrease in the percentage of LNCaP cells in the S-phase, with a concomitant increase in the percentage of cells in the G0/G2 phase.20

Research also indicates that genistein directly influences peptides that control the growth of cells. For example, Bhatia and Agarwal found that in DU-145 cells, high concentrations (>50 μM) of genistein impaired erbB1-Shc-ERK1/2 signaling24 and Davis et al. found that genistein down-regulates cyclin B and upregulates p21WAF1.30 Shen et al. also found that genistein increased expression of p21WAF1 and p27KIP1 in LNCaP cells.20 These two negative cell-cycle regulators, which act as cyclin-dependent kinase inhibitors, have recently been linked to prostate carcinogenesis. Bergan et al. found that genistein induced cell adhesion of PC3-M cells, a process that was enhanced when cells were plated in the absence of fibronectin. Compounds that stimulate cell attachment are considered potential chemopreventive agents be-
cause attachment of epithelial cells to each other and to the extracellular matrix an important factor in the control of cell division and metastasis.\textsuperscript{81} One must recognize, however, that there are many pathways through which genistin may inhibit prostate cancer cell growth; Li and Sarkar recently demonstrated that in PC3 cells, 832 genes showed a greater than twofold change after genistin treatment.\textsuperscript{82} Finally, recent work indicates that genistin may increase prostate tissue vitamin D levels, which offers another mechanism by which soy can potentially decrease prostate cancer risk.\textsuperscript{83}

Conclusion

Clearly, there are both hormonal and non-hormonal mechanisms by which soy, because it is a rich source of isoflavones, can suppress the development and/or growth of prostate tumors. Genistin inhibits the growth of androgen-dependent and androgen-independent prostate cancer cells in vitro, and although the genistin concentrations required to inhibit growth exceed those found in the serum of animals or men consuming soy or exposed to isoflavones, the animal data generally show that isoflavones inhibit prostate tumor development and growth.\textsuperscript{29} The epidemiologic data are very limited and inconsistent, but the findings from the two prospective studies conducted in the United States are intriguing.\textsuperscript{54,55} Arguably, the most impressive results are those from the Karmanos Cancer Institute, which showed that isoflavone supplements decreased the linear rise in PSA levels in men with treated but uncontrolled prostate cancer.\textsuperscript{71} Although this was a pilot study that involved a small number of subjects, these findings are impressive for two reasons. First, beneficial effects were observed even though conventional medical treatment was ineffective. These men would be the least likely group in which to expect a favorable response to dietary treatment. Second, the amount of isoflavones given to these men was only modestly (see below) higher than the amount recommended for the generally healthy adult population and was certainly not more than three times the average Japanese daily intake.\textsuperscript{48,59} Ordinarily, very high amounts of any pharmacologic or dietary agent are needed when attempting to treat, rather than to prevent, a disease.

Prostate cancer affords many opportunities for clinical interventions. More studies examining the effects of soy and isoflavones on serum PSA levels are needed. It will also be important to determine the effects of these agents on prostate tissue in men with prostate cancer. Until such studies are conducted, no firm conclusions about the role of soy and isoflavones in prostate cancer can be made. Nevertheless, the existing data indicate that men concerned about their prostate health may consider incorporating soy into their diet. This may be warranted because, at the very least, soyfoods are a good source of high-quality protein that may help to reduce the risk of coronary heart disease and osteoporosis. On the basis of Asian intake, and a large amount of clinical and epidemiologic data, consuming 15 g soy protein and 50 mg isoflavones per day is a reasonable goal that is both achievable and likely to be efficacious for those diseases for which soy is proven to be beneficial. These amounts are provided by approximately two servings of traditional soyfoods.

Substituting 15 g soy protein for 15 g animal protein would cause the current U.S. dietary ratio of animal to plant protein to fall from 2:1 to a more desirable 1:1, the ratio it was in the early 1900s. At this level of intake, soy protein would still represent less than 20% of the average protein intake of U.S. adults; soyfoods would therefore function as just one other good source of high-quality protein.

11. American Cancer Society. Available at: ???. Accessed ??.
13. An J, Tzagarakis-Foster C, Scharschmidt TC, Lomri


