npg

ORIGINAL ARTICLE

Isoflavone supplements stimulated the production of serum equol and decreased the serum dihydrotestosterone levels in healthy male volunteers

M Tanaka[1], K Fujimoto[1], Y Chihara[1], K Torimoto[1], T Yoneda[1], N Tanaka[1], A Hirayama[1], N Miyanaga[2], H Akaza[2] and Y Hirao[1]

[1]Department of Urology, Nara Medical University, Kashihara, Japan and [2]Department of Urology, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Japan

The aim of this study was to evaluate the effect of supplementing healthy men with soy isoflavones on the serum levels of sex hormones implicated in prostate cancer development. A total of 28 Japanese healthy volunteers (18 equol producers and 10 equol non-producers) between 30 and 59 years of age were given soy isoflavones (60 mg daily) supplements for 3 months, and the changes in their sex hormone levels were investigated at the baseline and after administration. The serum and urine concentrations of daidzein, genistein, and the levels of equol in the fasting blood samples and 24-h stored urine samples were also measured. All 28 volunteers completed the 3-month supplementation with isoflavone. No changes in the serum levels of estradiol and total testosterone were detected after 3-month supplementation. The serum levels of sex hormone-binding globulin significantly increased, and the serum levels of free testosterone and dihydrotestosterone (DHT) decreased significantly after 3-month supplementation. Among the 10 equol non-producers, equol became detectable in the serum of two healthy volunteers after 3-month supplementation. This study revealed that short-term administration of soy isoflavones stimulated the production of serum equol and decreased the serum DHT level in Japanese healthy volunteers. These results suggest the possibility of converting equol non-producers to producers by prolonged and consistent soy isoflavones consumption.

Introduction

Epidemiological studies have shown that the incidences of malignancies, such as prostate cancer, breast cancer and colon cancer, are commonly high in the Western Europe and United States of America, but low in the Asian populations who consume large amounts of soy bean foods.[1] Soy bean products are rich in isoflavones, such as genistein and daidzein. Isoflavones have been suggested as the principal chemical constituents responsible for the potential preventive effect of soy bean against prostate cancer.[2] Possible mechanisms have been proposed for the anti-tumor activity of soy isoflavones against prostate cancer, including estrogen-like effects,[3] prevention of oxidative DNA damage,[4] reduction in cancer cell proliferation[5] and inhibition of angiogenesis.[6]

It has been argued for long whether the lower incidences of prostate cancer among the Asian people are because of the inhibitory effects of isoflavones (contained abundantly in soy beans) and equol (directly metabolized from daidzein by the intestinal bacterial microflora) against prostatic carcinogenesis. Equol has a weak phytoestrogen activity.[7] Especially, equol and iso-flavones have a binding affinity to estrogen receptor β.[8] In addition, equol can bind to the sex hormone-binding globulin (SHBG),[9,10] and inhibit the growth of prostate cells in vitro.[11,12] Therefore, equol can act as an anti-androgen and inhibit the development of sex hormone-dependent tumors, such as mammary gland cancer and prostate cancer.[12] Earlier, we reported that some people can metabolize daidzein into equol, whereas others cannot, and showed that the intake quantity of isoflavones and the proportions of equol producers differed significantly among the races and age stratifications.[13]

In this intervention study, to elucidate the biological impact of isoflavones on equol-producing ability and sex hormonal variation in association with prostate carcinogenesis, we investigated whether the constant daily consumption of isoflavone supplements influenced the

serum levels of isoflavones, equol and sex hormonal biomarkers in healthy Japanese men.

Participants and methods

A total of 28 Japanese healthy volunteers (18 equol producers and 10 equol non-producers), between 30 and 59 years of age were given soy isoflavones (60 mg per day) for 3 months, and the changes in the serum levels of sex hormones, cholesterol and isoflavones were measured at the baseline, and at 1 month and 3 months later. This protocol was designed with reference to earlier studies.[14–18] This study was conducted to determine the persistence of diet-induced effects on isoflavone metabolism and disposition. Whether a volunteer is an equol producer or non-producer was determined as in our earlier study, in which we conducted a food survey on the daily intake of soybean isoflavone and measured the serum concentrations of isoflavone and equol in all participants.[13] Five tablets of Isofla A (Fuji Oil Co. Ltd, Osaka, Japan) were administered immediately before breakfast and dinner twice a day to all volunteers. In total, 10 tablets per day contained 60 mg isoflavone, 2.4 g carbohydrate, 80 mg protein, 70 mg lipid, 100 mg calcium, 60 mg magnesium, 30 IU vitamin D3, and 3 mg vitamin E. Isoflavone (60 mg) consisted of 19.1 mg daidzin, 3.5 mg genistin, 10.4 mg glycitin, 8.1 mg malonyl daidzin, 2.2 mg malonyl genistin, 3.4 mg malonyl glycitin, 7.3 mg acetyl daidzin, 1.9 mg acetyl genistin, 3.6 mg acetyl glycitin, 0.2 mg daidzein, 0.1 mg genistein and 0.3 mg glycitein.

The institutional reviewer board approved this study, and a written informed consent was obtained from all the volunteers. Each volunteer was given packages of Isofla A tablets with a log at the baseline visit. At 1 month and 3 months later, the residual tablets, used packages and log for counting the residual tablets were collected from all the volunteers to check the intake adherence. The intake of isoflavones from daily foods varied among the volunteers, but they maintained the same life style, including diet, exercise, work and sleep patterns, while being enrolled in this study.

Blood samples at 0800 hours before breakfast (after 10-h fasting) and 24-h urine samples were taken at the baseline, and at 1 month and 3 months after starting isoflavone administration. For all volunteers, blood samples were taken before breakfast, and the separated sera were stored at $-20\,^\circ$C or lower. Urine was taken for 24 h in dark plastic bottles containing 2 g ascorbic acid, and kept in a cool place during storage. The urine was mixed well and a sample was frozen directly after measuring the volume. These serum and urine samples were subsequently transported on dry ice to the laboratory of SRL Co. Ltd (Tokyo, Japan). The sample size of all volunteers for detecting the serum isoflavone concentration was not calculated, but it was regulated by the feasibility for each investigator.

The concentrations of genistein, daidzein and equol in the serum and urine samples were measured by reversed-phase high-performance liquid chromatography-multiple reaction ion monitoring mass spectrometry.[19] The serum concentrations of cholesterols (total, high-density lipoproteins and low-density lipoproteins),

estradiol, SHBG, total and free testosterone and dihydrotestosterone (DHT) were measured at the same time points at SRL Co. Ltd.

In addition to comparing the serum concentration of isoflavones in the two participant groups, we also compared the participants on the basis of their capability of equol production. The non-producers were defined as having a serum equol concentration below the limit of detection by the present assay system, that is, 0.5 ng ml^{-1}.

Statistical analyses were carried out using non-parametric Wilcoxon's test and χ^2-test. A P-value of <0.05 was considered as representing a statistically significant difference.

Results

The demographic characteristics of the healthy volunteers are shown in Table 1. Between the equol producers and the non-producers, there was a significant difference in age. All 28 volunteers completed the 3-month isoflavone administration as scheduled. No statistically significant adverse events were reported by the study participants. Diarrhea was the most frequently reported adverse event and occurred in 3 (11%) of the 28 participants. Two participants had diarrhea of grade 1 according to the Common Terminology Criteria for Adverse Events (v3.0) several times during the consecutive 2 days, and one complained of grade 1 diarrhea once only. No participant discontinued the study regimen or withdrew from the study because of these adverse events. The mean adherence rate was more than 99% during the whole study period. During the 3 months of Isofla A administration, all participants showed no marked change in their life styles.

No significant difference was noticed in the total cholesterol between the mean serum levels at the baseline and the end of study period. Although the mean high-density lipoprotein-cholesterol level at 3 months increased significantly when compared with that of the baseline, the mean low-density lipoprotein-cholesterol significantly decreased during 3-month isoflavone administration (Table 2). No significant changes in the mean serum levels of estradiol and total testosterone after 3-month administration were noticed when compared with the baseline (Table 2). However, the mean level of SHBG significantly increased after 3 months of isoflavone administration, and the mean serum levels of free testosterone and DHT decreased

Table 1 Demography of healthy male volunteers

	Equol non-producers (n = 10)	Equol producers (n = 18)	
Age (years)	36.7 ± 5.2	43.2 ± 7.8	P < 0.05
Height (cm)	170.8 ± 7.3	169.5 ± 6.9	ns
Weight (kg)	64.8 ± 4.1	67.8 ± 3.9	ns
BMI (kg m^{-2})	22.2 ± 0.6	23.6 ± 0.7	ns
Former or current smoker	50.0%	55.6%	ns
Family history of cancer	40.0%	44.4%	ns
Family history of benign prostatic hyperplasia	30.0%	31.1%	ns

Abbreviations: BMI, body mass index; ns, non-significant.

Table 2 Variations of the mean serum levels of cholesterols and sex hormones after isoflavone supplementation

	Before supplementation	1 month later	3 months later	
Serum cholesterol				
Total cholesterol (mg per 100 ml)	209.0 ± 35.5	206.7 ± 30.7	204.5 ± 31.7	ns
HDL-cholesterol (mg per 100 ml)	55.4 ± 11.8	57.3 ± 10.0	59.8 ± 11.4	$P < 0.05$
LDL-cholesterol (mg per 100 ml)	132.2 ± 34.9	127.0 ± 30.9	118.4 ± 30.1	$P < 0.05$
Serum sex hormones				
Estradiol (pg ml^{-1})	25.0 ± 5.6	25.9 ± 5.6	25.2 ± 6.5	ns
Sex hormone-binding globulin (nmol l^{-1})	52.2 ± 19.8	47.1 ± 15.1	61.2 ± 19.9	$P < 0.05$
Dihydrotestosterone (ng ml^{-1})	0.96 ± 0.27	0.78 ± 0.23	0.79 ± 0.23	$P < 0.05$
Free testosterone (pg ml^{-1})	74.9 ± 3.5	71.8 ± 11.9	70.9 ± 11.2	$P < 0.05$
Total testosterone (pg ml^{-1})	541.0 ± 125.0	569.0 ± 108.0	576.0 ± 135.0	ns

Abbreviations: HDL, high-density lipoproteins; LDL, low-density lipoproteins; ns, non-significant.

Table 3 Variations of the mean serum and urine levels of genistein, daidzein and equol after isoflavone supplementation

	Before supplementation	1 month later	3 months later	
Serum isoflavone				
Genistein (ng ml^{-1})	81.7 ± 80.0	108.0 ± 106.4	97.6 ± 97.8	$P < 0.05$
Daidzein (ng ml^{-1})	40.5 ± 48.4	132.7 ± 109.7	133.8 ± 107.0	$P < 0.01$
Equol (ng ml^{-1})	21.0 ± 44.8	41.3 ± 69.7	64.7 ± 125.9	$P < 0.01$
Urine isoflavone				
Genistein (nmol per day)	23.9 ± 20.5	88.7 ± 66.7	103.1 ± 73.7	$P < 0.01$
Daidzein (nmol per day)	35.8 ± 36.2	41.7 ± 28.7	50.1 ± 41.6	$P < 0.05$
Equol (nmol per day)	12.7 ± 19.8	36.4 ± 40.5	37.9 ± 61.4	$P < 0.05$

after 3-month administration when compared with the baseline (Table 2).

The mean serum concentration of isoflavones and equol at the baseline, and at 1 month and 3 months after isoflavone administration are shown in Table 3. Genistein increased more significantly in the urine than in the serum. Daidzein and equol showed marked increases in the serum as well as in the urine after 3-month administration. Two equol non-producers became equol producers after 3 months of isoflavone administration. Of these two volunteers, one had serum equol levels of <0.5 ng ml^{-1}, <0.5 ng ml^{-1} and 1.1 ng ml^{-1}, whereas the other had <0.5 ng ml^{-1}, 1.1 ng ml^{-1} and 24.1 ng ml^{-1} at the baseline, 1 month, and 3 months after isoflavone administration, respectively.

The mean serum levels of cholesterol, SHBG, estradiol, testosterone and its metabolites were analyzed in the equol producers and non-producers (Table 4). The mean high-density lipoprotein-cholesterol level significantly increased and the mean low-density lipoprotein-cholesterol level showed a significant decrease after 3-month isoflavone administration in the equol producers. Neither the equol producers nor the non-producers showed any changes in the mean serum level of estradiol during the study period. Equol producers showed a significant increase in the mean SHBG level and a significant decrease in the mean DHT level 3 months later. The free testosterone level showed a significant decrease in the equol producers, but not in the non-producers, whereas the total testosterone level showed no significant increase in both groups.

Discussion

Prostate cancer has recently become an increasingly important public health issue in Japan. Despite the extensive studies on the pathogenesis and clinical behavior of prostate cancer, the etiological origins or the host-environmental risk factors, which promote its progression, have not yet been well elucidated.[20] In fact, the African-American men have the highest morbidity rates of prostate cancer in the world, whereas the Asian men natives to their countries, such as the Japanese, Korean and Chinese, have the lowest rates.[21]

However, the incidence of prostate cancer in Japan has recently increased as the diets and life styles became westernized. Although latent or clinically insignificant prostate cancer is detected at a high rate—similar to that in the American men—in autopsy studies on the Asian men, the morbidity rate of clinically significant prostate cancer is 80-fold higher in USA.[22] This suggests that the same dietary factors may also promote the progression of latent or microscopic prostate cancer to clinically significant and metastatic prostate cancer.[23] Some epidemiological studies indicated that the level of dietary soy consumption may be linked to a decreased risk of prostate cancer.[24] The standard intake of isoflavones by the American men was commonly a couple of milligrams per day. The ninefold decrease in prostate cancer mortality among the Japanese men as compared with that in USA may be attributed, in part, to the high soy protein content in the Japanese diet.[25]

Isofla A tablets are commercially sold in Japan as a supplement, and the most dominant flavonoid was an

Table 4 Variations of the mean serum levels of cholesterols and sex hormones in equol producers and non-producers after isoflavone supplementation

	Before supplementation	1 month later	3 months later	
Total cholesterol (mg per 100 ml)				
Equol producers	209.2 ± 40.1	205.6 ± 30.2	204.1 ± 34.3	ns
Equol non-producers	208.5 ± 27.3	208.7 ± 33.0	205.2 ± 28.3	ns
HDL-cholesterol (mg per 100 ml)				
Equol producers	53.1 ± 10.3	56.1 ± 10.7	57.7 ± 11.6	$P < 0.05$
Equol non-producers	59.5 ± 13.8	59.5 ± 8.8	63.5 ± 10.4	ns
LDL-cholesterol (mg per 100 ml)				
Equol producers	134.9 ± 38.4	126.3 ± 31.5	117.1 ± 30.8	$P < 0.05$
Equol non-producers	127.4 ± 28.9	128.7 ± 33.0	120.8 ± 30.3	ns
Estradiol (pg ml^{-1})				
Equol producers	24.7 ± 4.7	25.7 ± 5.1	24.6 ± 5.4	ns
Equol non-producers	25.5 ± 7.2	26.3 ± 6.7	27.0 ± 7.6	ns
Sex hormone-binding globulin (nmol l^{-1})				
Equol producers	50.1 ± 17.2	45.9 ± 14.9	60.7 ± 22.1	$P < 0.05$
Equol non-producers	56.1 ± 24.3	49.3 ± 15.8	62.0 ± 16.3	ns
Dihydrotestosterone (ng ml^{-1})				
Equol producers	0.93 ± 0.25	0.77 ± 0.24	0.75 ± 0.18	$P < 0.05$
Equol non-producers	1.03 ± 0.32	0.82 ± 0.22	0.86 ± 0.23	ns
Free testosterone (pg ml^{-1})				
Equol producers	74.6 ± 3.8	74.0 ± 4.0	70.1 ± 4.6	$P < 0.05$
Equol non-producers	75.4 ± 3.0	67.8 ± 19.2	73.6 ± 2.6	ns
Total testosterone (pg ml^{-1})				
Equol producers	529.0 ± 119.0	564.0 ± 107.0	541.0 ± 114.0	ns
Equol non-producers	561.0 ± 140.0	578.0 ± 114.0	638.0 ± 153.0	ns

Abbreviations: HDL, high-density lipoproteins; LDL, low-density lipoproteins; ns, non-significant.

isoflavone glycoside daidzin. The second dominant flavonoid glycitin is an isoflavone glycoside present in foods containing soybean. The metabolite of glycitin synthesized by intestinal microflora is glycitein, which scavenges the intracellular reactive oxygen species. The other ingredients are malonyl or acetyl glycosides and isoflavone aglycones. Diverse bacterial strains of human origin have specific β-glucosidase and β-glycuronidase activity in the metabolism of dietary isoflavones from glycosides to aglycones. Aglycone and sulfate of genistein and daidzein have estrogen-like activity and so does equol.

Genistein reportedly inhibits the growth of androgen-dependent and androgen-independent prostate cancer cell lines[26] and inhibits the development of prostate-related cancers in Wistar rats.[27] The mechanism of action by which genistein exerts this anti-tumor angiogenesis is not well understood. Enough scientific data have accumulated to allow clinical intervention of soy protein in human trials as an adjuvant supplementation for the treatment of prostate cancer, but the optimal and effective doses of soy protein are not yet known. In this study, we also assessed the effects of the daily consumption of isoflavone supplements on the serum high-density lipoprotein-cholesterol and low-density lipoprotein-cholesterol levels, and measured the isoflavone concentrations in the blood and urine of healthy men. Importantly, our results, as well as those of an earlier study,[14] supported the hypothesis that the continuous intake of isoflavones is likely to play a promising role in chemoprevention against cardiovascular disease.

In a recent study, it was found that isoflavones could increase the production of SHBG in the liver and bind to biologically active testosterone. Consequently, lowering the free testosterone levels and its bioavailability to the target prostate cells should theoretically halt cancer cell proliferation, inhibit tumor progression and reduce the tumor volume in accordance with the changes in the prostate-specific antigen.[16] In our study, the SHBG level significantly increased, whereas the free testosterone level and DHT level decreased after 3-month isoflavone administration as compared with the baseline. This result is in consensus with several earlier studies and a recent review on the effects of isoflavone.[11,12,28]

On the other hand, there are some limitations of the product used in our study. The interactions of isoflavones and other nutrients included in Isofla A tablets were not considered. The other ingredients, such as vitamin D or E, may have some effect on SHBG, DHT or testosterone levels, although our earlier study showed that the intake amounts of vitamin D and E were not risk factors for the development of prostate cancer in Japanese men.[29] Furthermore, supplementation is usually a high-dose and unbalanced administration unlike the physiological condition, and we donot know whether the supplemental isoflavones behave exactly the same as isoflavones from the actual food, particularly because the intestinal microflora variability or genetic differences in isoflavone metabolism may vary among the individuals or the races. It is still a question how the food substances work in the actual food in comparison with their action in the supplemental form.

In our earlier case–control study, the ability of producing equol or equol itself closely correlated with the lower incidence of prostate cancer.[30] At 3 months after soy isoflavone supplementation, serum equol was detected in two healthy volunteers among the equol non-producers group. This suggests the possibility of converting equol non-producers to producers by prolonged and consistent isoflavones consumption. Moreover, prolonged and consistent isoflavones consumption could potentially delay the onset of prostate cancer by interfering with carcinogenesis. A public health initiative now may exist to identify non-toxic therapies for cancer. Several clinical trials evaluated the role of various nutritional supplementations in the treatment of localized prostate cancer. On the basis of updated scientific evidences, numerous nutritional strategies could be used for clinical nutrition interventional trials on the use of individual supplements or dietary modification versus the incorporation of multiple nutritional strategies. As prostate cancer grows relatively slowly in comparison with other malignancies, the potential impact of nutritional intervention may spare patients from undergoing a variety of toxic treatments for prostate cancer, and therefore improve their quality of life. The results of our study warrant further preclinical and clinical trials focusing on the role of isoflavone in chemoprevention against prostate cancer.

Conclusion

This study revealed that short-term administration of soy isoflavones to Japanese healthy volunteers increased the SHBG level, and decreased the free testosterone and DHT levels. Moreover, equol was detected in the serum of two healthy volunteers among the equol non-producers group. These results suggest the possibility of converting equol non-producers to producers by prolonged and consistent soy isoflavones consumption, and that a diet based on soy isoflavones will be useful in preventing prostate cancer development.

Conflict of interest

Acknowledgements

References

1 Adlercreutz H, Honjo H, Higashi A, Fotsis T, Hämäläinen E, Hasegawa T *et al*. Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. *Am J Clin Nutr* 1991; **54**: 1093–1100.

2 Zhou JR, Gugger ET, Tanaka T, Guo Y, Blackburn GL, Clinton SK. Soybean phytochemicals inhibit the growth of transplantable human prostate carcinoma and tumor angiogenesis in mice. *J Nutr* 1999; **129**: 1628–1635.

3 Knight DC, Eden JA. A review of the clinical effects of phytoestrogens. *Obstet Gynecol* 1996; **87**: 897–904.

4 Wei H, Cai Q, Rahn RO. Inhibition of UV light-and Fenton reaction –induced oxidative DNA damage by the soybean isoflavone genistein. *Carcinogenesis* 1996; **17**: 73–77.

5 Shao ZM, Alpaugh ML, Fontana JA, Barsky SH. Genistein inhibits proliferation similarly in estrogen receptor-positive and negative human breast carcinoma cell lines characterized by p21WAF1/CIP1 induction, G2/M arrest and apoptosis. *J Cell Biochem* 1998; **69**: 44–54.

6 Fotsis T, Pepper M, Adlercreutz H, Fleischmann G, Hase T, Montesano R *et al*. Genistein, a dietary-derived inhibitor of *in vitro* angiogenesis. *Proc Natl Acad Sci USA* 1993; **90**: 2690–2694.

7 Price KR, Fenwick GR. Naturally occurring oestrogens in foods-a review. *Food Addit Contam* 1985; **2**: 73–106.

8 Petersen DN, Tkalcevic GT, Koza-Taylor PH, Turi TG, Brown TA. Identification of estrogen receptor β2, a functional variant of estrogen receptor β expressed in normal rat tissues. *Endocrinology* 1998; **139**: 1082–1092.

9 Déchaud H, Ravard C, Claustrat F, de la Perrière AB, Pugeat M. Xenoestrogen interaction with human sex hormone-binding globulin (hSHBG). *Steroids* 1999; **64**: 328–334.

10 Martin ME, Haourigui M, Pelissero C, Benassayag C, Nunez EA. Interactions between phytoestrogens and human sex steroid binding protein. *Life Sci* 1996; **58**: 429–436.

11 Hedlund TE, Johannes WU, Miller GJ. Soy isoflavonoid equol modulates the growth of benign and malignant prostatic epithelial cells *in vitro*. *Prostate* 2003; **54**: 68–78.

12 Lund TD, Munson DJ, Haldy ME, Setchell KD, Lephart ED, Handa RJ. Equol is a novel anti-androgen that inhibits prostate growth and hormone feedback. *Biol Reprod* 2004; **70**: 1188–1195.

13 Fujimoto K, Tanaka M, Hirao Y, Nagata Y, Mori M, Miyanaga N *et al*. Age-stratified serum levels of isoflavones and proportion of equol producers in Japanese and Korean healthy men. *Prostate Cancer Prostatic Dis* 2008; **11**: 252–257.

14 Urban D, Irwin W, Kirk M, Markiewicz MA, Myers R, Smith M *et al*. The effect of isolated soy protein on plasma biomarkers in elderly men with elevated serum prostate specific antigen. *J Urol* 2001; **165**: 294–300.

15 Hussain M, Banerjee M, Sarkar FH, Djuric Z, Pollak MN, Doerge D *et al*. Soy isoflavones in the treatment of prostate cancer. *Nutr Cancer* 2003; **47**: 111–117.

16 Kumar NB, Cantor A, Allen K, Riccardi D, Besterman-Dahan K, Seigne J *et al*. The specific role of isoflavones in reducing prostate cancer risk. *Prostate* 2004; **59**: 141–147.

17 Maskarinec G, Morimoto Y, Hebshi S, Sharma S, Franke AA, Stanczyk FZ. Serum prostate-specific antigen but not testosterone levels decrease in a randomized soy intervention among men. *Eur J Clin Nutr* 2006; **60**: 1423–1429.

18 Kumar NB, Krischer JP, Allen K, Riccardi D, Besterman-Dahan K, Salup R *et al*. Safety of purified isoflavones in men with clinically localized prostate cancer. *Nutr Cancer* 2007; **59**: 169–175.

19 Coward L, Kirk M, Albin N. Analysis of plasma isoflavones by reversed-phase HPLC-multiple reaction ion monitoring-mass spectrometry. *Clin Chim Acta* 1996; **247**: 121–142.

20 Carter HB, Coffey DS. The prostate: an increasing medical problem. *Prostate* 1990; **16**: 39–48.

21 Hsing AW, Tsao L, Devesa SS. International trends and patterns of prostate cancer incidence and mortality. *Int J Cancer* 2000; **85**: 60–67.

22 Yatani R, Chigusa I, Akazaki K, Stemmermann GN, Welsh RA, Correa P. Geographic pathology of latent prostatic carcinoma. *Int J Cancer* 1982; **29**: 611–616.

23 Wynder EL, Rose DP, Cohen LA. Nutrition and prostate cancer: a proposal for dietary intervention. *Nutr Cancer* 1994; **22**: 1–10.

24 Oishi K, Okada K, Yoshida O, Yamabe H, Ohno Y, Hayes RB *et al*. A case–control study of prostatic cancer with reference to dietary habits. *Prostate* 1988; **12**: 179–190.

25 Messina M, Barnes S. The role of soy products in reducing risk of cancer. *J Natl Cancer Inst* 1991; **83**: 541–546.

26 Peterson G, Barnes S. Genistein and biochanin A inhibit the growth of human prostate cancer cells but not epidermal growth factor receptor tyrosine autophosphorylation. *Prostate* 1993; **22**: 335–345.

27 Pollard M, Wolter W, Sun L. Prevention of induced prostate-related cancer by soy protein isolate/isoflavone-supplemented diet in Lobund-Wistar rats. *In Vivo* 2000; **14**: 389–392.

28 Perabo FG, Von Löw EC, Ellinger J, von Rücker A, Müller SC, Bastian PJ. Soy isoflavone genistein in prevention and treatment of prostate cancer. *Prostate Cancer Prostatic Dis* 2008; **11**: 6–12.

29 Nagata Y, Sonoda T, Mori M, Miyanaga N, Okumura K, Goto K et al. Dietary isoflavones may protect against prostate cancer in Japanese men. *J Nutr* 2007; **137**: 1974–1979.

30 Akaza H, Miyanaga N, Takashima N, Naito S, Hirao Y, Tsukamoto T et al. Comparisons of percent equol producers between prostate cancer patients and controls: case-controlled studies of isoflavones in Japanese, Korean and American residents. *Jpn J Clin Oncol* 2004; **34**: 86–89.