Int. J. Cancer: **113,** 835–840 (2005)

Dietary intervention in prostate cancer patients: PSA response in a randomized double-blind placebo-controlled study

Ries Kranse[1], Pieter C. Dagnelie[2], Monique C. van Kemenade[3], Frank H. de Jong[4], Jan H.M. Blom[5], Lilian B.M. Tijburg[6], Jan A. Weststrate[6] and Fritz H. Schröder[1]*

[1]*Department of Urology, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands*
[2]*Department of Epidemiology, Maastricht University, Maastricht, The Netherlands*
[3]*Department of Nutrition, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands*
[4]*Department of Internal Medicine, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands*
[5]*Department of Urology, St. Franciscus Gasthuis, Rotterdam, The Netherlands*
[6]*Unilever Health Institute, Vlaardingen, The Netherlands*

The objective of this study was to show or to exclude an effect of dietary supplement on rising prostate-specific antigen (PSA) levels. We have studied the effect of a dietary supplement (verum, administered for 6 weeks) containing plant estrogens, antioxidants, including carotenoids, selenium and other putative prostate cancer inhibiting substances in a randomized placebo-controlled double-blind crossover study in 37 hormonally untreated men with prostate cancer and increasing PSA levels. Outcome measures were changes in the rates of change of serum concentrations of total and free PSA and changes in male sex hormone levels. Male sex hormone levels were significantly lower during the verum phase (DHT: −0.11 nmol/L, $p = 0.005$; testosterone: −1 nmol/L, $p = 0.02$). Total PSA doubling time was unaffected. Free PSA, which increased during the placebo phase (average doubling time of 68 weeks), decreased during the verum period (average half-life of 13 weeks; $p = 0.02$). In those men in whom the free androgen index decreased (21 out of 32), a significant decrease in the slopes of both total and free PSA was observed ($p = 0.04$). Overall total PSA doubling times did not increase significantly during verum. However, the study demonstrates that this dietary intervention reduces DHT and testosterone levels and increases free PSA doubling time (and total PSA doubling time in a relevant subgroup). If future studies confirm that these observations translate into a slowing of disease progression, a dietary intervention may become an attractive option for prostate cancer treatment and prevention.

Key words: prostate cancer; diet; prostate-specific antigen slope; doubling time; soy; antioxidants

Epidemiologic data strongly suggest that environmental factors play a role in prostate cancer progression. Prostate cancer incidence and mortality rates in Japan and other Asian countries are much lower than in Western countries. Around 1985, before the prostate-specific antigen (PSA) era, age-standardized prostate cancer incidence rates were roughly 60–90 per 100,000 per year in the United States *versus* 7 per 100,000 per year in Osaka, Japan.[1] Interestingly, autopsy studies have demonstrated that latent prostate cancer of the focal noninvasive type is as common in Japan as it is in Western countries.[2,3] When Japanese men move to California, their risk for prostate cancer increases 4-fold.[4] This suggests that environmental factors, most probably diet, play a central role in promoting progression of prostate cancer from the latent to the clinical stage. Diet may affect the tumori genesis directly or indirectly by affecting hormone levels (prostate cancer is a hormone-sensitive tumor). Soy, a major constituent of the Asian diet, is rich in phytoestrogens.[5] Estrogens have been, and still are, in use as treatment for advanced prostate cancer.

Within Europe, low prostate cancer incidence and mortality rates are observed in Mediterranean countries.[6,7] Low southern European rates have been suggested to be related to the consumption of tomatoes, which contain high levels of antioxidants, notably lycopene.[8,9] It is noteworthy that many isoflavonoids (present in the Eastern diet) and lignans (present in the fruits and vegetables of the Mediterranean diet, but also in cereals such as rye) are phytoestrogens. Many of these phytoestrogens exhibit antioxidant properties in addition to their estrogenic/antiestrogenic effects.[8–10]

The working hypothesis of the present study was that a cocktail of phytochemicals might affect male sex hormone levels and, simultaneously, the rates of increase of PSA and free PSA levels, which are markers for progression of prostatic cancer.[11] This hypothesis and the choice of the components of the dietary supplement were based on evidence from epidemiologic studies. Increased use of some of the components such as selenium[12,13] and vitamin E[14] have been shown to correlate with a decreased incidence of prostate cancer in prospective studies. Evidence has also been presented that (iso)flavonoids (green tea) and phytosterols may have a preventive effect.[15–17] In this study of a dietary supplement, PSA levels may relate to tumor mass and, as far as the slope of PSA is concerned, with proliferative activity. However, since various substances have been shown to interfere with PSA *per se*, therapeutic efficacy cannot be derived from this study.[18] Total PSA in serum analytically consists of free PSA (fPSA) and PSA bound to α-1-antichymotrypsin (ACT).[19,20] Free PSA was recently shown to relate to several molecular forms of PSA, of which one I-PSA may be more specific for cancer than for benign prostatic hyperplasia (BPH) or benign prostatic tissue.[21]

Material and methods

This is a double-blind randomized placebo-controlled 2-arm crossover intervention study of a dietary supplement in prostate cancer patients with rising PSA and without systemic treatment.

Planned study population and inclusion and exclusion criteria

Based on the sample size calculation, 15–20 patients were to be included in each treatment arm of this study. Participants were patients of the Departments of Urology of the Erasmus Medical Center and the St. Franciscus Gasthuis and were recruited during the fall of 1998.

Eligible for inclusion were men of any age with confirmed rising PSA levels >0.1 ng/ml and no clinical evidence of (recurrent) prostate cancer (PC) after radical prostatectomy, radiotherapy, or under watchful waiting. Prior to entry, PSA levels of >0.1 ng/ml had to be measured on >2 occasions at a >3-month interval. In patients studied after radiotherapy or during watchful waiting, rising PSA levels were established by visual inspection of PSA levels over time. Exclusions include any hormone or other systemic or radiotherapy after definitive treatment.

Grant sponsor: Unilever Research Laboratories, Vlaardingen, The Netherlands.
***Correspondence to:** Erasmus Medical Center Rotterdam, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands. Fax: +31-10-463-3968. E-mail: e.vandenberg@erasmusmc.nl
Received 16 December 2003; Accepted after revision 30 June 2004
DOI 10.1002/ijc.20653
Published online 21 October 2004 in Wiley InterScience (www.interscience.wiley.com).

Intervention and timing

Participants were given a dietary supplement (verum) in the form of a water-dissolvable powder to be taken 3 times a day and 20 g of margarine per day. The experimental supplement contained a number of experimental ingredients (Table I), while the control supplements (placebo) margarine and drink contained none of these. Compliance was asserted by monitored dietary records and compliance markers. Source details of the compounds are available on request.

The time schedule and the details of interventions and diagnostic steps are indicated in Figure 1. Participants were requested not to change diet during the study (except from taking the supplements).

Outcome measures

The primary endpoint of the study was the slope of the rise in PSA (dPSA) compared between placebo and verum. dPSA was defined as the slope of the regression line through all weekly ^{2}log-transformed PSA measurements during the concerned period. Total PSA (tPSA) and fPSA were analyzed separately and in a similar fashion. Secondary endpoints were plasma levels of luteinizing hormone (LH), testosterone (T), 5α-dihydrotestosterone (DHT) and sex hormone binding globulin (SHBG) compared between intervention and placebo. The free androgen index (FAI) was defined as the ratio of testosterone and SHBG levels. Compliance with the use of the supplement was evaluated by measuring plasma concentrations of the phytosterols daidzein and genestein for the beverage and β-carotene for the use of margarine.

Statistical considerations and power

The sample size calculation was based on variations observed in the slope of PSA after radical prostatectomy. Details relating to this sample size calculation were reported earlier.[11] It was estimated that a 70% reduction in PSA slope could be detected with a power of 90% with 20 subjects per treatment arm. It was therefore decided to include 30–40 patients in the study.

Stopping rules

Participants taking <90% of all supplements were to be excluded from the analysis. Participants experiencing clinical progression or an unexpected rise of PSA were immediately informed. If, upon confirmation, treatment was to be changed, the patient was allowed to complete the dietary intervention but was excluded from the statistical analysis. This applied to all interventions that are known to interfere with PSA levels.

TABLE I – COMPOSITION OF THE DIETARY SUPPLEMENT (AMOUNTS PER DAY)

Component
Margarine (20 g)
Vitamin E (50 mg α-tocopherol)
Phytosterols (1.5 g)[1]
Selenium (0.2 mg organic selenium in 0.5 g bakers yeast)
Placebo beverage (3 servings of 200 ml/day)
Caffeine similar to supplement
Beverage (3 servings of 200 ml/day)
Green tea[2]
Isoflavones[3]
100 mg phytoestrogens
60 mg genestein
40 mg daidzein
Carotinoids
10 mg luteine
10 mg lycopene
10 mg palm carotenoids (including some α-carotinoids)

[1]Concentrates from soy bean oil distillates amounting to 2–3 times the amount in Eastern diet (Henkel, La Grange, GA) esterified with fatty acids from sunflower oil.–[2]Hot water extract from green tea, freeze-dried amounts as in 6 cups of green tea.–[3]Soy extract, 40% isoflavones (ADM). Full fat margarine was used (70% margarine with 70% essential fats). Green tea solids contained 50 mg caffeine per g extract. The placebo beverage was supplemented with the same amount of caffeine. Lycopene, β-carotene and lutein were added in a water-dispersable form.

Loss to follow-up and adverse events

In case of suspected supplement-related adverse events, the code was to be broken. Patients lost to follow-up and dropouts were to be included in the analysis only for completed study periods.

Ethical approval

Ethical approval was obtained from the institutional medical ethical committee. All participants gave written informed consent.

Dietary records

All participants were seen weekly by a dietician (M.C.v.K., Ineke Kersten). The frequency of dietary records and other follow-up procedures is indicated in Figure 1. Adverse events were systemically recorded.

Biochemical measurements

Serum was separated immediately after blood collection and stored at −80°C; processing of the samples was conducted blinded to the treatment group. Total PSA and free PSA in serum were measured by use of the Immulite assay of Diagnostic Products Corporation (DPC, Los Angeles, CA). Since free PSA levels are typically quite low, an extremely sensitive assay (detection limit 0.01 ng/ml) was chosen for the present study.

Testosterone (coated tube radioimmunoassay; DPC), 5α-dihydrotestosterone (enzyme-immune assays provided by Diagnostics Biochem Canada, London, Ontario, Canada), LH and SHBG (Immulite kits; DPC) were determined in samples collected every odd week. Interassay variations for these assays were 4.8% for LH, 9.0% for SHBG, 8.1% for testosterone and 8.4% for DHT. Since only free testosterone is biologically active, the FAI (defined as the molar ratio of T and SHBG) was determined and used as a secondary endpoint together with the DHT index (DHTI, obtained by dividing DHT levels by SHBG levels). All blood samples were drawn between 08:00 and 13:00 o'clock. Quality control was performed by assaying samples from the same serum pool repeatedly within every assay run.

Antioxidants including vitamin E in plasma and the phytoestrogens daidzein, genestein, equol (a metabolite of daidzein) and enterolactone (a lignan not present in the verum as a negative control) were measured by mass spectrometry. These determinations were carried out as previously described.[22] Vitamin E was analyzed according to the techniques previously described.[23]

Statistical analysis

All measured laboratory data were first plotted as a function of time for visual inspection. Simple descriptive statistics (mean, median, standard deviations) and nonparametric statistical tests (Wilcoxon's signed-rank test) were used to study the serum levels of the antioxidants, phytoestrogens and metabolites of phytoestrogens.

All PSA values were ^{2}log-transformed to account for the exponential increase of PSA over time. Slopes for free and total PSA and hormone levels were determined for each period. The PSA and free PSA slopes were estimated by fitting a straight line through their ^{2}log-transformed values. The doubling time can be calculated as 1/PSA slope if the slope is positive (*i.e.*, if PSA increases over time). The half-life can be calculated identically if the slope is negative (*i.e.*, if PSA decreases over time). The term "response" is used below both for hormone levels and for PSA/free PSA slopes.

The changes in tPSA and fPSA doubling times and in hormone levels were studied by means of analysis of variance. During each period, the observed change is assumed equal to the sum of average levels, a treatment effect, a period effect, an interaction between the period and the treatment effect and some residual variance. The treatment effect estimates the response that can be attributed to the effect of taking the supplement only. The period

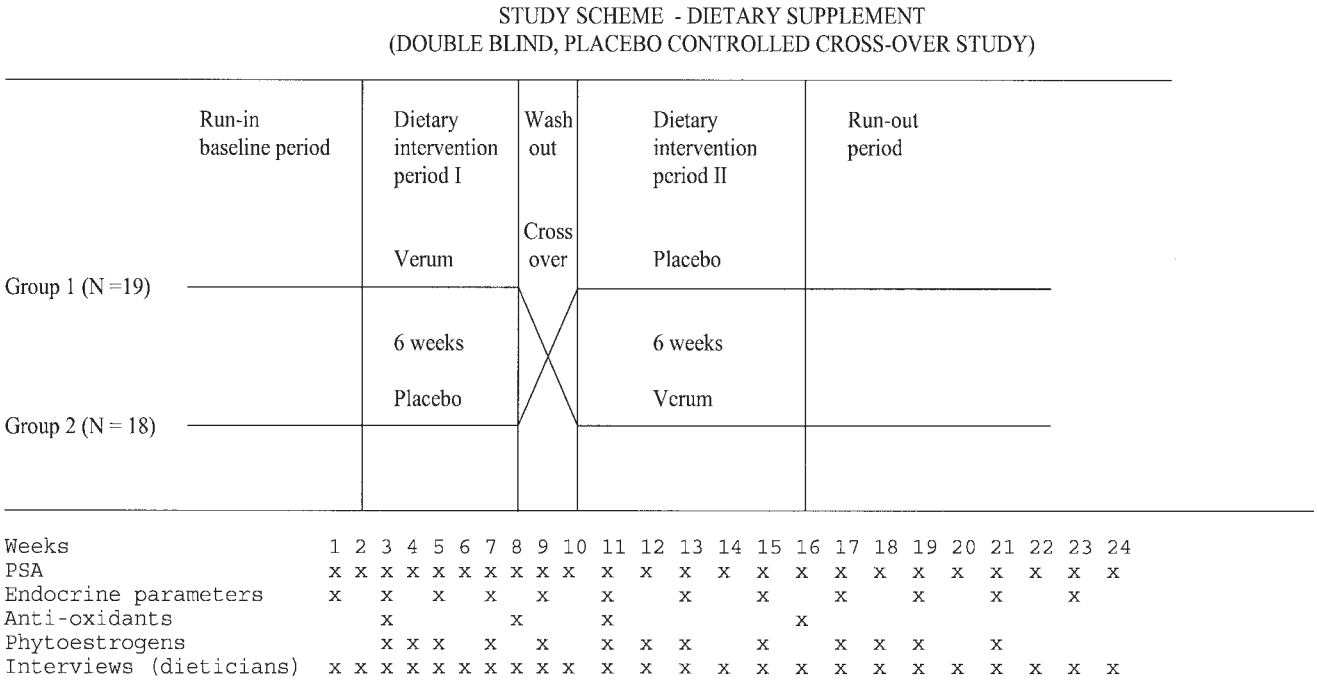

FIGURE 1 – Study flow chart. Seven day food records were recorded at baseline, during the verum period in each subject and during the placebo period in each individual.

effect estimates changes attributed to the study period. The interaction term (or carryover effect) was evaluated in the model and subsequently removed because no significant effect was found. The 3 described variables, treatment effect, period effect, interaction and residual variation, were estimated by means of a linear regression procedure. In addition, the method proposed by Senn[24] for analyzing crossover studies was applied. The resulting data are not given in detail for space considerations.

Spearman's rank correlation coefficients were calculated to explore the relation between differences in PSA or free PSA slopes from the verum compared to the placebo phase of the study and simultaneous differences in male sex hormone levels (T, DHT, SHBG, LH, FAI and DHTI). Two-tailed statistics and 95% confidence intervals are reported throughout this article; p-values < 0.05 were considered statistically significant.

Results

Assignment and participants

Thirty-seven eligible patients consented and were assigned by individual randomization to either of the 2 treatment arms (Fig. 2). Color coding of verum and placebo (orange and blue) was used to achieve double blinding. Twenty-six participants had previously undergone a radical prostatectomy, 6 had been treated with radiotherapy, whereas the remaining 5 patients were managed by watchful waiting with their primary tumor *in situ*. At baseline, the following descriptive statistics were recorded (range, median value): age, 54–81, 70 years; weight, 58–107, 81 kg; PSA, 0.13–87.3, 3.24 ng/ml; testosterone, 10.1–32.1, 14.4 nmol/L; DHT, 0.86–5.92, 1.68 nmol/L. Eighty-three percent of the patients had clinical stage T1 or T2; in 60% of the patients who underwent a radical prostatectomy grade 1 or grade 2 tumors were found. All participants were recruited within a 2-week period.

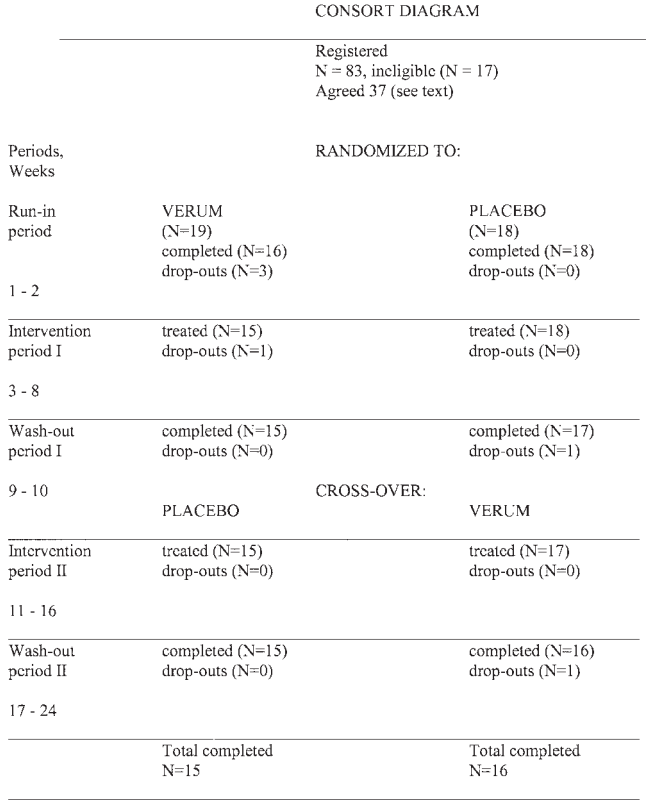

FIGURE 2 – Consort diagram of recruitment.

Dropouts

Figure 2 gives a detailed account of recruitment, randomization, dropouts and time periods for those who completed the study. Other reasons for refusals (n = 14) included distance, inconvenience of frequent visits, vacation plans and unavailability for primary contacts. Six men who were eligible, registered and randomized declined for various reasons. There were no serious adverse events; one patient reported abdominal pains and decided

to discontinue. He stopped during the washout period and was excluded because of lack of blood samples. No participants experienced clinical progression during the trial period; the code was broken for all 32 eligible participants at the time of completion of the study. Since the supplement was not available on the market, men who wished to continue on a similar regimen were given dietary advice. Severe violations in dietary intake were not seen.

Table II lists the serum levels of the food compounds for the different phases in the study. As expected on the basis of the chemical composition of the verum supplement, the serum levels of the 6 compounds listed in Table II were significantly higher during the intervention period as compared to the placebo period. For the carotenoids a 2-fold and for the phytoestrogens daidzein and genestein approximately 100-fold increases in serum levels were observed. The levels of enterolactone ($p = 0.04$) and equol ($p = 0.09$) also increased. Neither fat nor energy intake as measured by the 7-day food records changed over time; a slight (400 g in median weight) but statistically significant increase in weight was observed.

Table III shows the changes in total and free PSA kinetics between verum and placebo periods. Table IV summarizes changes in PSA doubling times. The doubling time of total PSA increased from an average of 41 weeks during the placebo period to 44 weeks during the verum period. This treatment effect was not statistically significant. If the analysis of variance was repeated for only those participants (21 men out of 32) in whom a decrease in free androgen index levels was observed, a significant treatment effect for total PSA slopes was found. In these men, the average PSA doubling time was 36 weeks during the placebo phase and 115 weeks during the verum phase of the study ($p = 0.04$; Table IV).

The results of the analysis of variance described above are not given in detail for reasons of space. The 2 statistical methods used to analyze the data yielded identical results (analysis of variance and Senn's method[24]). The analysis showed a significant treatment effect for free PSA (slope decreased from a doubling time of 68 weeks to a half-life of 13 weeks; $p = 0.02$), testosterone (decreased 0.98 nmol/L; $p = 0.02$), DHT (decreased 0.11 nmol/L; $p = 0.005$) and FAI (decreased 0.01; $p = 0.07$; Table III).

Differences in the slopes of total PSA between the verum and placebo phase were significantly correlated with simultaneous differences in the free androgen index (Spearman's rank correlation = 0.47; $p = 0.01$). Differences in the free PSA slopes were

TABLE II – OVERVIEW OF THE SERUM LEVELS OF THE BIOACTIVE FOOD COMPOUNDS DURING PLACEBO VERSUS VERUM INTERVENTION IN A CROSSOVER DESIGN

Food compound (unit of measurement)	Placebo, mean, median, standard deviation	Verum, mean, median, standard deviation	*p*-value[1]
α-β-carotene (nmol/l)	608, 515, 410	1,369, 1,248, 768	< 0.0001
α-tocopherol (vitamin E) (μmol/l)	29, 29, 7	33, 32, 8	< 0.0001
lutein (nmol/l)	122, 115, 47	260, 239, 101	< 0.0001
Lycopene (nmol/l)	305, 296, 140	650, 649, 238	< 0.0001
Daidzein (ng/ml)	8.0, 7.0, 5.7	534, 520, 233	< 0.0001
Genestein (ng/ml)	17, 13, 15	1,589, 1,470, 739	< 0.0001
Enterolactone (ng/ml)	9, 5.8, 10.5	11, 9.4, 9.8	0.04
Equol (ng/ml)	0.34, 0.10, 0.79	52.1, 0.15, 103.1	0.09

Six weeks of intervention separated by 2 weeks of washout ($n = 32$). The 2-week washout period may be too short for some of the nutrients, specifically selenium and vitamin E.
[1]All *p*-values were obtained by means of Wilcoxon's signed-rank test.

TABLE III – AVERAGE RESPONSES, TREATMENT EFFECTS (ASSESSED EFFECT OF THE INTERVENTION) AND PERIOD EFFECTS (EFFECT ATTRIBUTED TO THE PERIOD)

Parameters	Mean response, 2-sided *p*-value	Treatment effect, 2-sided *p*-value	Period effect, 2-sided *p*-value
Total PSA slope[2] log (ng/ml)/(week)			
$n = 32$	0.024 ($p < 0.001$)	−0.0018 ($p = 0.84$)	0.0014 ($p = 0.87$)
$n = 21$ (FAI ≤ 0)	0.0184325 ($p = 0.01$)	−0.0194612 ($p = 0.04$)	−0.0121827 ($p = 0.19$)
Free PSA slope[2] log (ng/ml)/(week)			
$n = 32$	−0.032 (NS)	−0.093 ($p = 0.02$)	0.14 ($p < 0.001$)
$n = 21$ (FAI ≤ 0)	−0.0291954 ($p = 0.29$)	−0.1023862 ($p = 0.05$)	0.1068415 ($p = 0.04$)
Testosterone (ng/ml)	15.5 ($p < 0.001$)	−0.98 ($p = 0.018$)	−0.13 ($p = 0.75$)
Free androgen index (no dimension)	0.20 ($p < 0.001$)	−0.0095 ($p = 0.07$)	−0.0061 ($p = 0.23$)
Dihydrotestosterone (ng/ml)	1.90 ($p < 0.001$)	−0.11 ($p = 0.005$)	−0.059 ($p = 0.12$)
Dihydrotestosterone index (no dimension)	0.026 ($p < 0.001$)	−0.0014 ($p = 0.13$)	−0.0017 ($p = 0.07$)
SHBG (ng/ml)	83.4 ($p < 0.001$)	−2.25 ($p = 0.23$)	2.39 ($p = 0.21$)
LH (ng/ml)	7.01 ($p < 0.001$)	−0.11 ($p = 0.73$)	−0.34 ($p = 0.30$)

A first and second period are discerned in the study; half of the men received the verum during the first period, the other half during the second period.

TABLE IV – PSA DOUBLING TIMES (DT) OR PSA HALF-LIFES (HL) IN WEEKS AND THEIR 95% CONFIDENCE INTERVALS SEPARATED FOR TOTAL AND FREE PSA AND VERUM VERSUS PLACEBO PERIODS

Marker	Subgroup	Doubling time during verum (95% CI)	Doubling time during placebo (95% CI)	*p*-value of the difference in doubling times
Total PSA	All men ($n = 32$)	44 (32 to 71)	41 (30 to 63)	0.84
	Men with decreased FAI ($n = 21$)	115 (53 to −678)	36 (26 to 56)	0.042
Free PSA	All men ($n = 32$)	−13 (−25 to −9)	68 (19 to −43)	0.02
	Men with decreased FAI ($n = 21$)	−12 (−20 to −8)	45 (14 to −40)	0.049

$n = 21$ relates to those men who had a decrease in the free androgen index during verum.

significantly correlated with differences in the total PSA slopes and with differences in LH levels and DHT (details are on file).

A significant period effect was observed in one arm of the study for free PSA only. Free PSA slopes also decreased significantly from placebo to verum in the 21 men with a decrease in FAI and plasma testosterone levels. In these men, the doubling time was 45 weeks during placebo and changed to a half-life of 12 weeks during verum ($p = 0.049$). During the verum period, DHT levels and testosterone levels decreased significantly; the FAI decreased at borderline significance.

Discussion

Prompted by the epidemiologic evidence referred to above, we have studied the effect of a dietary supplement on PSA and free PSA in a placebo-controlled crossover study. The study utilized innovative methodology to establish the potential efficacy of new preventive measures or agents for treatment of prostate cancer. We show that by using PSA slopes for comparison, a small sample size and a short time period can be utilized provided PSA progression has been established and quantified.

No significant treatment effect was seen in relation to total PSA. This may be in part due to the heterogeneity of the patient material, which is reflected in a great variability of the PSA levels seen during the run-in period[25] and to the relatively short intervention period. At the same time, a significant treatment effect on free PSA slopes/doubling time was seen. Free PSA levels, which increased during the placebo phase with a doubling time of 68 weeks, decreased during verum treatment with an average half-life of 13 weeks. The significant period effect, which was observed in the analysis of free PSA only, may be explained by the presence of spontaneously (*i.e.*, nonintervention-related) decreasing free PSA levels in a number of men (despite increasing total PSA levels) combined with the lower limit of the ultrasensitive free PSA assay used (lower limit of detection and resolution 0.01 ng/ml). In such men, the lower limit of detection is more likely reached in the second period of the study than in the first (the longer the time interval of spontaneous decrease, the lower the levels of free PSA will become). If free PSA levels are below the detection limit, the assay measures a steady 0.01 ng/ml corresponding to a free PSA slope of zero. Thus, in men with spontaneously decreasing free PSA levels and a low free PSA level at the baseline (many of the postradical prostatectomy patients), a period effect is likely to occur. If, in addition, verum affects free PSA slopes, a mix of a period and a treatment effect results.

Ratio of free and total PSA

The quotient of free and total PSA, FT ratio, has been introduced as a tool to differentiate BPH from prostate cancer (*i.e.*, to differentiate between and not within individuals; low FT ratios correspond to an increased prostate cancer risk).[26,27] The use of the FT ratio within patients, *e.g.*, to assess preoperatively the pathologic stage or monitor disease progression, is less well established.[28,29] In our study, no FT ratio treatment effect was found (data not shown).

We found that T and DHT levels decreased significantly by 0.98 nmol/L (6%) and by 0.11 nmol/L (6%) during the verum period when compared to the placebo phase. Simultaneously, a significant increase of tPSA doubling time from 36 to 115 weeks ($p = 0.04$) was seen in those 21 men whose free androgen index decreased during verum. In parallel, the free PSA levels, which increased during the placebo phase with a doubling time of 68 weeks, decreased during verum supplementation with an average half-life of 13 weeks ($p = 0.049$). When percent free testosterone calculated on the basis of the law of mass action[30] was used instead of the free androgen index, our conclusions did not change qualitatively (the correlation coefficient for the relationship between FAI and percent free testosterone was 0.82).

Differences in plasma testosterone levels and in the free androgen index between the placebo and the verum period were positively correlated with simultaneous differences in the total PSA slopes. Also, the increase in free PSA showed a marginally significant negative correlation with differences in LH and free DHT index. It has been observed by others that, within certain PSA ranges, the levels of free PSA correlate with LH.[31] Similar differences in androgen levels were seen in a comparison of Dutch and Japanese men[32] and in a study of healthy Finnish men put temporarily on a vegetarian diet.[33] Obviously, these small changes may only be indicative of other mechanisms that impact on PSA levels.

This study in part confirms our working hypothesis that during the verum period a significant decrease in fPSA is seen. During verum, testosterone and DHT levels decreased significantly, FAI levels decreased at borderline significance levels, while LH levels were unaffected. A significant decrease in the slope of fPSA and tPSA is seen in men in whom the free androgen index decreased during verum. At this point, we may only speculate about which substance in the verum cocktail may have caused the effects. As we have observed a hormone response, we think it is reasonable to assume that the phytoestrogens are at least in part involved. Treatment effects in fPSA and tPSA slopes correlated positively with each other and with treatment effects in the free androgen index. Based on the relatively small testosterone treatment effect, the fact that a consistent free and total PSA response is only observed in men with a decreasing FAI during verum and the absence of an LH effect, we hypothesize that the observed treatment effect may be related to effects on peripheral testosterone synthesis from androstenedione. Phytoestrogens block the activity of the enzyme 17β-hydroxysteroid dehydrogenase type 5[34] that converts androstenedione into testosterone.[35] The conversion of androstenedione into estrone regulated by aromatase and the conversion of estrone to the biologically active estradiol by 17β-hydroxysteroid dehydrogenase type 1, however, might remain unaffected by the intervention. Intervention thus decreases testosterone levels and increases estrogen levels (since more androstenedione is available for conversion into estradiol). The "decreased testosterone level induced LH increase" may therefore be compensated by the "estradiol increase induced LH decrease," leaving overall LH levels during testosterone unaffected as observed. Considering the setup of this study, judgment concerning a possible effect of the antioxidants is impossible.

The question whether the impact on fPSA and tPSA slopes seen correlates with a stabilization or decrease in tumor mass remains unresolved. This issue can be dealt with by using human prostate cancer lines transplantable in nude mice. In this setting, PSA and tumor mass can be studied simultaneously as previously suggested.[11,18]

Acknowledgements

This study would not have been possible without the work and dedication of Bertrand van Zelst, Ellen van den Berg, Ineke Kersten, Mathilde Dijk, Dr. Bert Blijenberg, Monique Roobol, Dr. Wim Hop and Dr. Theo Stijnen. Karin van Alphen provided patient and effective secretarial support.

References

1. Boyle P, Maisonneuve P, Napalkov P. Geographical and temporal patterns of incidence and mortality from prostate cancer. Urology 1995;46(3 Suppl A):47–55.
2. Yatani R, Chigusa I, Akazaki K, Stemmermann GN, Welsh RA, Correa P. Geographic pathology of latent prostatic carcinoma. Int J Cancer 1982;29:611–6.
3. Yatani R, Shiraishi T, Nakakuki K, Kusano I, Takanari H, Hayashi T, Stemmermann GN. Trends in frequency of latent prostate carcinoma in Japan from 1965–1979 to 1982–1986. J Natl Cancer Inst 1988;80:683–7.
4. Shimizu H, Ross RK, Bernstein L, Yatani R, Henderson BE, Mack TM. Cancers of the prostate and breast among Japanese and white

immigrants in Los Angeles County. Br J Cancer 1991;63: 963–6.
5. Bingham SA, Atkinson C, Liggins J, Bluck L, Coward A. Phyto-oestrogens: where are we now? Br J Nutr 1998;79:393–406.
6. Griffiths K, Adlercreutz H, Boyle P, Denis L, Nicholson R, Morton M. Nutrition and cancer. Oxford: ISIS Medical Media, 1996.
7. Khlat M. Cancer in Mediterranean migrants: based on studies in France and Australia. Cancer Causes Control 1995;6:525–31.
8. Giovannucci E. Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. J Natl Cancer Inst 1999; 91:317–31.
9. Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, Willett WC. Intake of carotenoids and retinol in relation to risk of prostate cancer. J Natl Cancer Inst 1995;87:1767–76.
10. Adlercreutz H, Mazur W. Phyto-oestrogens and Western diseases. Ann Med 1997;29:95–120.
11. Schroder FH, Kranse R, Barbet N, Hop WC, Kandra A, Lassus M. Prostate-specific antigen: a surrogate endpoint for screening new agents against prostate cancer? Prostate 2000;42:107–15.
12. Duffield-Lillico AJ, Dalkin BL, Reid ME, Turnbull BW, Slate EH, Jacobs ET, Marshall JR, Clark LC. Selenium supplementation, baseline plasma selenium status and incidence of prostate cancer: an analysis of the complete treatment period of the Nutritional Prevention of Cancer Trial. Br J Urol Int 2003;91:608–12.
13. Duffield-Lillico AJ, Reid ME, Turnbull BW, Combs GF Jr, Slate EH, Fischbach LA, Marshall JR, Clark LC. Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: a summary report of the Nutritional Prevention of Cancer Trial. Cancer Epidemiol Biomarkers Prev 2002;11: 630–9.
14. Heinonen OP, Albanes D, Virtamo J, Taylor PR, Huttunen JK, Hartman AM, Haapakoski J, Malila N, Rautalahti M, Ripatti S, Maenpaa H, Teerenhovi L, et al. Prostate cancer and supplementation with alpha-tocopherol and beta-carotene: incidence and mortality in a controlled trial. J Natl Cancer Inst 1998;90:440–6.
15. Liao S, Umekita Y, Guo J, Kokontis JM, Hiipakka RA. Growth inhibition and regression of human prostate and breast tumors in athymic mice by tea epigallocatechin gallate. Cancer Lett 1995;96: 239–43.
16. Awad AB, Fink CS. Phytosterols as anticancer dietary components: evidence and mechanism of action. J Nutr 2000;130:2127–30.
17. Thomas JA. Diet, micronutrients, and the prostate gland. Nutr Rev 1999;57:95–103.
18. Thalmann GN, Sikes RA, Chang SM, Johnston DA, von Eschenbach AC, Chung LW. Suramin-induced decrease in prostate-specific antigen expression with no effect on tumor growth in the LNCaP model of human prostate cancer. J Natl Cancer Inst 1996;88:794–801.
19. Lilja H, Christensson A, Dahlen U, Matikainen MT, Nilsson O, Pettersson K, Lovgren T. Prostate-specific antigen in serum occurs predominantly in complex with alpha 1-antichymotrypsin. Clin Chem 1991;37:1618–25.
20. Malm J, Lilja H. Biochemistry of prostate specific antigen, PSA. Scand J Clin Lab Invest 1995;221(Suppl):15–22.
21. Stenman UH, Leinonen J, Alfthan H, Rannikko S, Tuhkanen K, Alfthan O. A complex between prostate-specific antigen and alpha 1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: assay of the complex improves clinical sensitivity for cancer. Cancer Res 1991;51:222–6.
22. Hargreaves DF, Potten CS, Harding C, Shaw LE, Morton MS, Roberts SA, Howell A, Bundred NJ. Two-week dietary soy supplementation has an estrogenic effect on normal premenopausal breast. J Clin Endocrinol Metab 1999;84:4017–24.
23. Roodenburg AJ, Leenen R, van het Hof KH, Weststrate JA, Tijburg LB. Amount of fat in the diet affects bioavailability of lutein esters but not of alpha-carotene, beta-carotene, and vitamin E in humans. Am J Clin Nutr 2000;71:1187–93.
24. Hansen B, de Ridder B. The AB/BA cross-over: how to perform the two-stage analysis if you can't be persuaded that you shouldn't. Rotterdam: Erasmus University, 1996. 93–100.
25. Komatsu K, Wehner N, Prestigiacomo AF, Chen Z, Stamey TA. Physiologic (intraindividual) variation of serum prostate-specific antigen in 814 men from a screening population. Urology 1996;47: 343–6.
26. Bangma CH, Kranse R, Blijenberg BG, Schroder FH. The value of screening tests in the detection of prostate cancer: II, retrospective analysis of free/total prostate-specific analysis ratio, age-specific reference ranges, and PSA density. Urology 1995;46:779–84.
27. Bangma CH, Kranse R, Blijenberg BG, Schroder FH. The value of screening tests in the detection of prostate cancer: I, results of a retrospective evaluation of 1726 men. Urology 1995;46:773–8.
28. Veltri RW, Miller MC, Mangold LA, O'Dowd GJ, Epstein JI, Partin AW. Prediction of pathological stage in patients with clinical stage T1c prostate cancer: the new challenge. J Urol 2002;168:100–4.
29. Meyer A, Jung K, Lein M, Rudolph B, Schnorr D, Loening SA. Factors influencing the ratio of free to total prostate-specific antigen in serum. Int J Cancer 1997;74:630–6.
30. Sodergard R, Backstrom T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. J Steroid Biochem 1982; 16:801–10.
31. Sumi S, Umeda H, Yano M, Koga F, Arai K, Imai T, Hosoya Y, Maeda S, Honda M, Suzuki T, Yoshida K. Studies on changes in the ratio of free to total PSA after endocrine treatment of prostate carcinoma. Hinyokika Kiyo 1996;42:937–41.
32. de Jong FH, Oishi K, Hayes RB, Bogdanowicz JF, Raatgever JW, van der Maas PJ, Yoshida O, Schroeder FH. Peripheral hormone levels in controls and patients with prostatic cancer or benign prostatic hyperplasia: results from the Dutch-Japanese case-control study. Cancer Res 1991;51:3445–50.
33. Hamalainen E, Adlercreutz H, Puska P, Pietinen P. Diet and serum sex hormones in healthy men. J Steroid Biochem 1984;20:459–64.
34. Krazeisen A, Breitling R, Moller G, Adamski J. Phytoestrogens inhibit human 17beta-hydroxysteroid dehydrogenase type 5. Mol Cell Endocrinol 2001;171:151–62.
35. Andersson S, Moghrabi N. Physiology and molecular genetics of 17 beta-hydroxysteroid dehydrogenases. Steroids 1997;62:143–7.