Influence of Selenium-enriched Yeast Supplementation on Biomarkers of Oxidative Damage and Hormone Status in Healthy Adult Males: A Clinical Pilot Study[1]

Karam El-Bayoumy,[2] John P. Richie, Jr., Telih Boyiri, Despina Komninou, Bogdan Prokopczyk, Neil Trushin, Wayne Kleinman, Julie Cox, Brian Pittman, and Steven Colosimo

American Health Foundation, Valhalla, New York 10595

Abstract

The mechanisms responsible for the protective role of selenium against the development of prostate cancer remain to be determined (L. C. Clark *et al.,* J. Am. Med. Assoc., *276:* 1957–1963, 1996). In the present study, we tested the hypothesis that selenium supplementation reduces oxidative stress. A secondary aim was to determine whether selenium-induced changes in testosterone (T) metabolism may also be involved. To this end, we conducted a double-blind, randomized, placebo-controlled trial of 247 μg selenium/day administered p.o. in the form of Se-enriched yeast. Study subjects were 36 healthy adult males, 11 blacks and 25 whites, 19–43 years of age. Supplementation occurred over the first 9 months, after which all subjects were placed on placebo for an additional 3 months. Blood and urine were collected at baseline and after 3, 9, and 12 months. In the selenium group, plasma selenium levels were 2-fold higher than baseline values after 3 and 9 months and returned to 136% of baseline after 12 months ($P < 0.0001$), whereas in the placebo group, levels were unchanged. A 32% increase in blood glutathione (GSH) levels was observed after 9 months in the selenium group only ($P < 0.05$). This change coincided with a 26% decrease in protein-bound GSH (bGSH) and a 44% decrease in bGSH:GSH ratios ($P < 0.05$). The changes in GSH and bGSH were highly correlated with changes in plasma selenium concentrations and may reflect a decrease in oxidative stress. No changes were observed in either group for plasma T, dihydrotestosterone (DHT) or DHT:T ratios, suggesting that selenium had no effect on the α-reductase involved in the conversion of T to DHT. A small but significant decrease in prostate-specific antigen levels was observed after 3 and 9 months ($P < 0.001$), and this difference disappeared after 12 months. Future trials will test the above hypothesis in prostate cancer patients and in subjects at high risk for prostate cancer.

Introduction

Several epidemiological studies, experimental research, and a recent clinical intervention trial supported the hypothesis that enhanced selenium status reduces the risk of cancer, including that of cancer of the prostate (1–12). One of the most exciting clinical trials in the United States supported the protective effect of selenium-enriched yeast against cancer of the prostate (13, 14). This protective effect was confirmed in a recent follow-up investigation of this trial (15). However, the mechanisms responsible for the protective effect remain largely unknown. The outcome of this trial prompted two new clinical trials: Prevention of Cancers by Intervention with Selenium Trial (PRECISE), in three European countries and the Selenium and Vitamin E Cancer Prevention Trial (SELECT) in the United States (16–18).

Prostate cancer presents a major clinical and public health challenge in the United States. Adenocarcinoma of the prostate is now the most frequently diagnosed malignancy in adult males and the second most frequent cause of death due to cancer in males; it is estimated that 189,000 new cases of prostate cancer will be diagnosed in the year 2002 and that 30,200 men will die from this disease (19). Earlier estimates indicate that a 50-year-old American male has a 40% chance of developing prostate cancer during his lifetime, a 10% chance of being diagnosed with it, and a 2–3% chance of dying from this disease (20). Today the probability of developing invasive cancer of the prostate by age group is as follows: birth to 39 years, less than 1 in 10,000; 40–59 years, 2.08 or 1 in 48; 60–79 years, 12.5 or 1 in 8; and overall (birth to death), 16.67 or 1 in 6 (19).

Although the etiology of prostate cancer remains poorly understood, a number of hypotheses have been put forth. The most commonly considered risk factors are briefly mentioned here. Studies in migrant populations pointed to the role of environmental factors in the development of prostate cancer (21–23). The role of Western diet, especially fat, has been suggested, but its role in prostate cancer development has not been consistent in the literature (24–28). Available data suggest the protective role of dietary components (such as fruits, vegetables, whole grains, and soy) against the development of prostate cancer (29–31).

The involvement of genetic factors (family component) has been suggested (32–35). Androgen metabolism may be implicated because the androgen receptor gene is highly polymorphic in humans (36). An association between increased

levels of T[3] and an increased incidence and/or risk of prostate cancer has been seen in some studies (37–41); however, another view argues against this finding/prevalent opinion (42). Differences in activity of α-reductase, an enzyme that converts T to DHT, have been correlated with ethnic patterns of prostate cancer (43). Higher levels of T in African-American men than in their Caucasian counterparts may explain the increased risk of prostate cancer of the former group (44). In general, cell division and proliferation in the prostate are controlled by the levels of these hormones (45–49). Because DHT has a higher binding affinity to the androgen receptor and to DNA than T, it may serve as a promoter of prostate cancer (50).

On the basis of the available knowledge, there is an obvious and urgent need to systematically investigate how a nutritional supplement such as Se-enriched yeast modulates the most commonly considered risk factors in the development of prostate cancer not only in cancer patients or in high-risk populations but, more importantly, in healthy males, as intended in the Selenium and Vitamin E Cancer Prevention Trial (17). In the present study, we examined the effect of Se-enriched yeast supplementation in healthy males on blood levels of GSH, the major cellular antioxidant, as an indicator of antioxidant capacity, and on bGSH as a marker of oxidative stress. The levels of 8-OHdG in urine were also measured as an indicator of oxidative damage to DNA. We further examined the effect of supplementation of Se-enriched yeast on serum androgen (T and DHT) levels. Finally, to examine possible prostate-specific changes, we determined the effect of Se-enriched yeast on the levels of PSA.

Materials and Methods

Study Design and Subject Characteristics. To examine possible mechanisms of action for the protective effect of Se-enriched yeast, we have conducted a randomized, double-blinded, placebo-controlled clinical trial of Se-enriched yeast supplementation. The study design was approved by the institutional review board of the American Health Foundation. Our goals were to examine the effects of selenium supplementation in healthy subjects on (*a*) bound and free GSH levels, (*b*) levels of urinary 8-OHdG, (*c*) T metabolism as determined by assessment of T:DHT ratios in serum, and (*d*) circulating PSA levels.

Study Design. Subjects were healthy adult males (19–43 years of age). All subjects were randomized into either the Se-enriched yeast (200 μg Se/day) arm or the placebo arm. Baseline data were collected on demographics, lifestyle habits, and usual dietary practices. Se-enriched yeast and regular yeast (placebo) were obtained from Nutrition 21 (San Diego, CA).

Blood and urine were collected at baseline and at 3, 9, and 12 months. Subjects from both groups were placed on placebo at 9 months, and final blood and urine samples were collected at 12 months. Specimen collection consisted of two 10-ml venous blood samples taken at 10 a.m. $\pm$ 0.5 h to avoid problems of circadian fluctuations. Spot urine sample was collected in amber bottles containing ascorbic acid to prevent artifactual oxidation and ethanol to inhibit bacterial growth. Compliance was monitored by pill count as documented in a diary and log book, which were provided to each participant, and by measuring plasma selenium concentrations (see "Re-

---

[3] The abbreviations used are: T, testosterone; bGSH, protein-bound glutathione; DHT, dihydrotestosterone; GSH, glutathione; PSA, prostate-specific antigen; 8-OHdG, 8-hydroxydeoxyguanosine; HPLC, high-performance liquid chromatography; MPA, metaphosphoric acid; SM, selenomethionine.

Table 1 Gradient program

Time (min)	% A	% B	% C	Curve
Initial	100	0	0	*
0.5	99	1	0	11
18.0	95	5	0	6
19.0	91	9	0	6
29.5	83	17	0	6
35	0	60	40	11
38	100	0	0	11

* Not applicable.

sults"). It should be noted that it takes 6–9 months for selenium concentration in serum to reach a steady state (14). At the 12 month point, blood and urine samples were collected to determine whether the effect of selenium supplementation on parameters measured in this study is reversible. Each participant was telephoned at 4-week intervals to assess any problems he may have encountered and also as a reminder to follow the study regimen.

Quantification of SM and Se-methylselenocysteine in Selenium Yeast Capsules. Selenium-enriched yeast (lot number 70228) and regular yeast (placebo; lot number 70229) used in this study as supplied by Nutrition 21 were analyzed for selenium content by Par Laboratories Inc. (Charlotte, NC). The results indicated that the selenium-enriched yeast contains 247 μg/capsule selenium, whereas regular yeast contained <5 μg/capsule. In addition, we specifically quantified the levels of SM and Se-methylselenocysteine in the selenium-enriched yeast capsules using a Waters AccQ·Fluor reagent kit (Waters Corp., Milford, MA) according to previously published methods (51, 52). Two capsules of selenium-enriched yeast were randomly selected. The contents of each capsule were removed and weighed, and approximately 8.0 mg of yeast from each sample were hydrolyzed with 1.5 ml of 6 N HCl at 110°C for 26 h *in vacuo*. The resulting solutions were freeze-dried, and the residues were redissolved in 0.2 ml of 20 mM HCl. For quantitative assessment of SM, 20 μl of selenium yeast hydrolysate were placed in an autosampler vial, followed by the addition of 60 μl of AccQ·Fluor borate buffer and 20 μl of reconstituted AccQ·Fluor reagent. After incubation for 1 min at room temperature, the samples were heated for 10 min at 55°C.

Aliquots of 10 μl were analyzed by HPLC on a reverse phase Waters AccQ·Tag 3.9 $\times$ 150-mm column. The HPLC system consisted of G Waters 600 Multisolvent Delivery System and a Hitachi L7485 fluorescence spectrophotometer. The mobile phase was composed of three solvents: AccQ·Tag eluent A (solvent A), acetonitrile (solvent B), and water (solvent C). The column was maintained at 37°C with a flow rate of 1 ml/min. The gradient program used for the analysis of SM is tabulated in Table 1.

Under these conditions, SM eluted at approximately 35.5 min. For the quantitative assessment of Se-methylselenocysteine, the gradient program of 100% A to 83% A and 17% B in 51 min (curve 6) was used. Under these conditions, Se-methylselenocysteine eluted at 40.5 min. SM and Se-methylselenocysteine were quantified based on standard curves constructed for the analyses. SM and Se-methylselenocysteine accounted for 42% and 25%, respectively, of the total Se incorporated into the yeast.

Sample Processing. All biological samples were coded immediately with an identifying number and blinded to the laboratory with regard to the race or group status of the subject. Blood and

Table 2   Comparison of study populations by age, race, smoking status, and body mass index

	Placebo	Selenium
No. of subjects		
Recruited	26 (100%)	26 (100%)
Dropped out	7 (27%)	9 (35%)
Finished study	19 (73%)	17 (65%)
Age (yrs)	31.1 ± 4.2	30.7 ± 4.4
Race		
Black	6 (31.6%)	5 (29.4%)
White	13 (68.4%)	12 (70.6%)
Smoking status		
Smokers	0	0
Nonsmokers	19	17
BMI (kg/m^2)^a	25.2 ± 4.13	24.2 ± 4.57
Compliance^b (%)	>95	>95

^{a} BMI, body mass index.
^{b} Based on capsule count.

urine were placed on ice immediately after collection and processed within 1 h. An aliquot of whole blood (0.2 ml) was removed and processed by deproteinization with 4 volumes of ice-cold 50 g/liter MPA. After 10–20 min, acid extracts were obtained by centrifugation at 13,000 × g for 2 min and stored at −70°C until analysis. The remaining acid-insoluble pellet was stored at −80°C until analysis for bGSH content. Remaining whole blood samples were centrifuged at 2,100 × g for 15 min at 4°C, and the plasma was carefully removed and aliquoted into labeled tubes, which were sealed and put into storage at –70°C. Urine samples were aliquoted into labeled tubes and also stored at –70°C.

Measurement of Biomarkers. GSH was determined using our previously described micromethod (53), which is based on the 5,5′-dithiobis(2-nitrobenzoic acid)/enzymatic recycling procedure of Tietze (54) and Owens and Belcher (55). Bound GSH was determined in MPA-insoluble pellets. After washing three times by resuspension in 5% MPA and centrifugation, the pellets were resuspended in 8 M urea containing 1 mM EDTA and incubated for 10 min at 4°C. After addition of a few drops of octanol to prevent foaming, potassium borohydride was added to a final concentration of 35 mg/ml. After incubation for 30 min at 4°C, the solution was acidified with 10% MPA containing 1 mM EDTA, and the pH was adjusted to 2.5–3.0. The resulting mixture was filtered through a 0.45 μm filter and analyzed for GSH as described above.

Plasma total selenium levels were determined by atomic absorption spectrophotometry as described previously (56, 57). Total serum T was determined by RIA using the DPC Coat-a-Count kit (Diagnostic Products Corp., Los Angeles, CA). Serum DHT was analyzed by direct ELISA kit (Diagnostics Biochem Canada, Inc., London, Ontario, Canada). Total PSA was analyzed by the ELISA procedure (Tandem E PSA) of Hybritech (San Diego, CA). Urinary 8-OHdG levels were measured at baseline and after 9 months using HPLC with electrochemical detection by ESA Laboratories, Inc. (Chelmsford, MA). Urine creatinine was determined using a Vitros Ektachem 500 clinical chemistry analyzer.

Statistical Analysis. Biochemical levels of interest (selenium, glutathione, and so forth) measured over time (baseline and 3, 9, and 12 months) were compared between selenium-treated and placebo subjects using repeated-measures ANOVA (58).
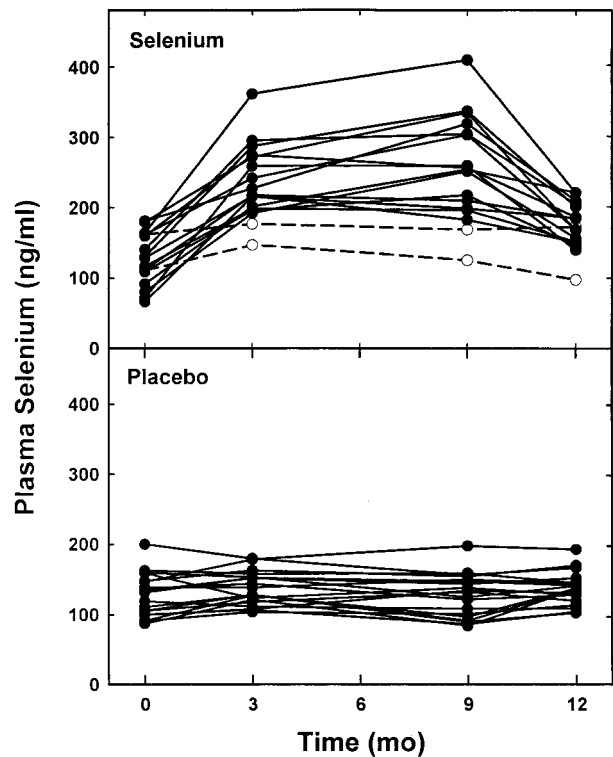

Fig. 1. Individual plasma selenium levels. Blood samples were obtained from subjects in both placebo (n = 19) and Se-enriched yeast supplement (n = 17) groups at baseline and after 3, 9, and 12 months. In the selenium group, Se-enriched yeast was replaced with placebo for the final 3-month washout period. Plasma was isolated and analyzed for selenium content as described in the text.

Results

Subject characteristics and compliance based on capsule count are described in Table 2. A high degree of compliance was also indicated based on plasma selenium measurements (Fig. 1). An increase in plasma Se was observed after 3 and 9 months in all but two subjects in the Se-enriched yeast group. In contrast, Se levels were unchanged in all subjects from the placebo group. Since two subjects in the Se-enriched yeast group appeared to be noncompliant based on blood Se levels as well as pill count data and diary records, all analyses were conducted both with and without these two subjects. In every case, removal of these subjects had no effect on the results obtained. All data presented include the two subjects in question. The average increase over baseline after 3 + 9 months in the Se-enriched yeast group was 2-fold (Fig. 2). After an additional 3-month washout period, plasma selenium levels in the Se-enriched yeast-treated group were reduced from 3- and 9-month values but were not completely back to baseline levels. Selenium levels at 3, 9, and 12 months were significantly higher in individuals in the selenium arm than in those in the placebo arm (P < 0.001).

Blood GSH levels were examined in all subjects as an indicator of systemic antioxidant capacity (Fig. 3). Baseline values for GSH levels for all subjects were within the normal range for men of this age group (53, 59). No differences between placebo and Se-enriched yeast groups were observed at baseline and after 3 months. However, at 9 months, GSH levels were 19% (P < 0.005) greater in the Se-enriched yeast-treated group than in controls. This increase disappeared after the 3-month washout period. In contrast, the levels of bGSH
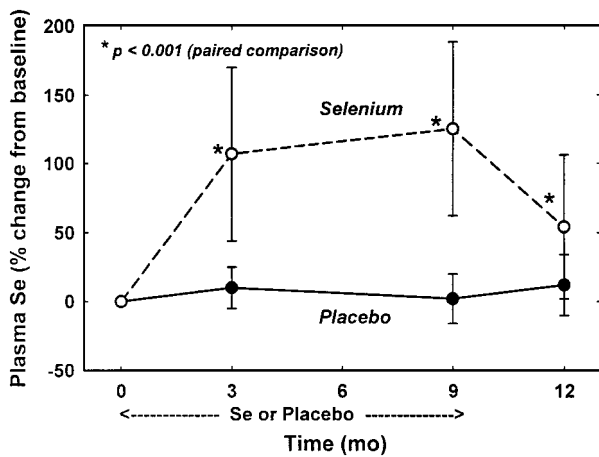
Fig. 2. Supplementation with Se-enriched yeast enhances plasma selenium levels. Blood selenium levels were assessed in study subjects as described in the Fig. 1 legend. Levels are expressed as the percentage change from baseline values. Mean baseline concentration values were 128 (placebo group) and 127 ng/ml (Se-enriched yeast group). *Points* and *bars* are group means and SD, respectively.

Fig. 3. Supplementation with Se-enriched yeast alters blood glutathione and glutathiolation. Blood samples, obtained as described in Fig. 1, were processed and analyzed for GSH and bGSH as described in text and expressed as percentage change from baseline values. Mean baseline concentration values for GSH were 0.836 (placebo group) and 0.780 μmol/ml (Se-enriched yeast group); those for bGSH were 0.156 (placebo group) and 0.173 μmol/ml (Se-enriched yeast group); and those for bGSH:GSH ratio were 0.198 (placebo group) and 0.238 (Se-enriched yeast group). *Points* and *bars* are group means and SD, respectively.

were significantly lower in the Se-treated group at 9 months compared with the placebo group. No differences in bGSH were observed at other time periods. The increases in GSH levels after 9 months were highly correlated with increases in plasma selenium concentrations in Se-enriched yeast-supplemented individuals ($r = 0.71$; $P < 0.001$). Similarly, decreases in bGSH over this same time period were also highly correlated with changes in plasma selenium ($r = -0.75$; $P < 0.001$).

Mean urinary 8-OHdG levels were unchanged between baseline and 9 months for both placebo and Se-enriched yeast groups. For the Se-enriched yeast group, mean ± SD values were 3.16 ± 1.28 and 3.10 ± 1.29 ng/mg creatinine for baseline and 9 month samples, respectively. For the placebo group, mean ± SD values were 4.18 ± 4.78 and 4.94 ± 6.34 ng/mg creatinine for baseline and 9-month samples, respectively.

The baseline levels of T were within the normal range of this age group (Fig. 4). No differences were observed in T levels for both the selenium and placebo groups between baseline and any of the time points. DHT levels were also unchanged in both groups throughout the study period. DHT levels were 18–20% of T levels, and the ratio of DHT:T in each subject was remarkably constant throughout the study.

Fig. 5 shows the effect of Se-enriched yeast on plasma PSA levels. As expected, baseline levels were low in these healthy subjects. A significant decrease in PSA levels was observed after 3 and 9 months ($P < 0.001$); after 12 months, no significant difference in PSA levels was seen.

For each of the biomarkers analyzed, no differences between races were observed. Whereas the mean baseline DHT level in blacks (mean ± SD, 961 ± 401 mg/ml) was 20% greater than that in whites (mean ± SD, 797 ± 259 mg/ml), the difference was not statistically significant ($P = 0.08$).

Discussion

Investigating the effects of a chemopreventive nutritional supplement in healthy people, and in high-risk individuals and cancer patients, by means of biomarkers that would signal the progression of disease, represents an important approach to elucidating mechanisms of action and prevention (10, 11, 18). In the present study, we tested the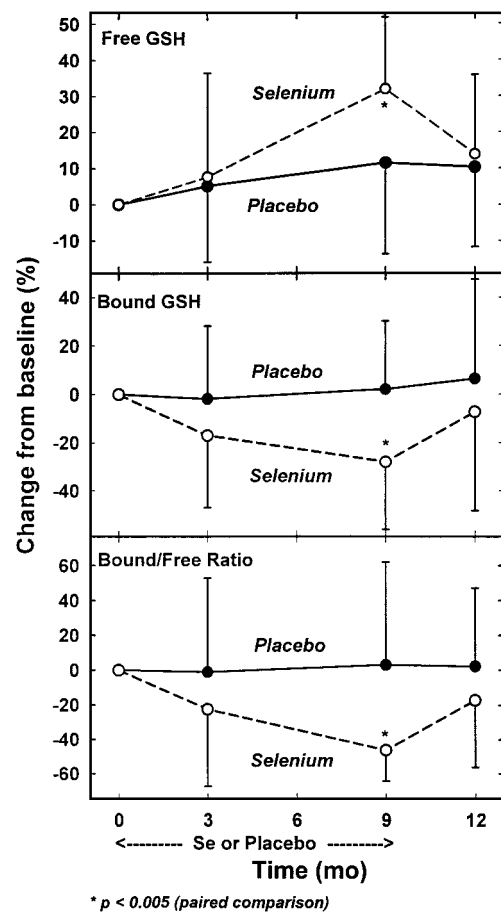 hypothesis that the protective role of Se-enriched yeast in the development of prostate cancer (13–15) involves inhibition of oxidative stress. Initially we have tested this hypothesis in healthy males; the same hypothesis should be tested in future studies in prostate cancer patients or in high-risk populations (*e.g.,* those with abnormal PSA levels).

The selenium-enriched yeast is a complex matrix; however, 67% of the selenium content in the yeast was accounted for by SM and Se-methylselenocysteine, whereas the remaining 33% remained structurally unidentified. Nevertheless, selenium-enriched yeast has been shown to be protective against the development of prostate cancer (13–15). Whereas SM represents the major selenium-containing component in selenium-enriched yeast, it is either weak or lacks any protective effects in several preclinical investigations including prostate cancer models (5, 9). Whether SM or another form of selenium is responsible for cancer chemoprevention by selenium-enriched yeast in humans remains unknown.

In this study we recruited both African Americans and Caucasians; the former subjects have the highest prostate cancer rates in the world and have a prostate cancer rate nearly
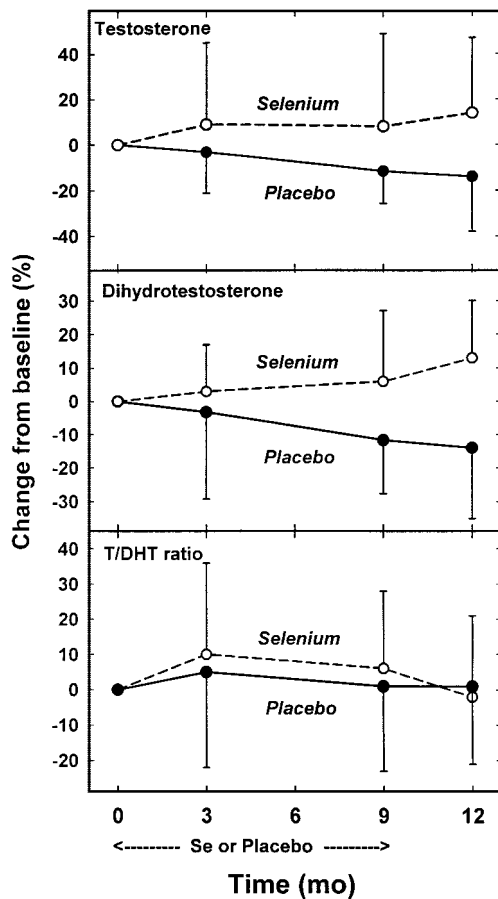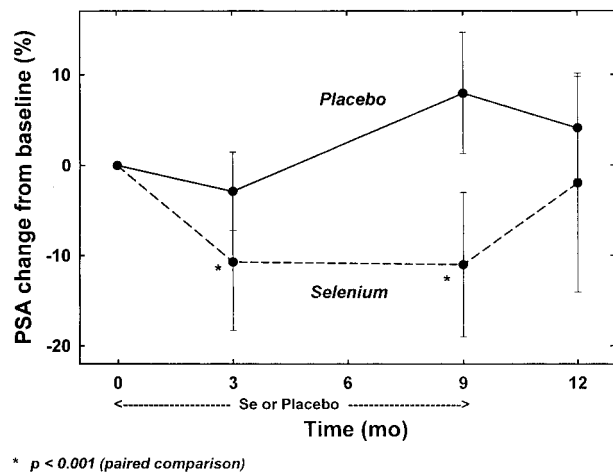
Fig. 4. Supplementation with Se-enriched yeast has no effect on plasma androgens. Plasma samples, obtained as described in the Fig. 1 legend, were analyzed for T and DHT content as described in text and expressed as percentage change from baseline values. Mean baseline concentration values for T were 5.05 (placebo group) and 4.63 ng/ml (Se-enriched yeast group); those for DHT were 0.894 (placebo group) and 0.797 ng/ml (Se-enriched yeast group); and those for the T:DHT ratio were 6.32 (placebo group) and 6.49 (Se-enriched yeast group). *Points* and *bars* are group means and SD, respectively.

* *p* < 0.001 *(paired comparison)*

Fig. 5. Supplementation with Se-enriched yeast decreases plasma PSA levels. Plasma samples, obtained as described in Fig. 1, were analyzed for total PSA content as described in text and expressed as percentage change from baseline values. Mean baseline concentration values for PSA were 0.53 (placebo group) and 0.72 ng/ml (Se-enriched yeast group). *Points* and *bars* are group means and SD, respectively.

twice that of white Americans in the United States (10, 17). Smokers and heavy alcohol users were excluded because of potential confounding effects on several factors including the generation of oxidative damage (60) and the metabolism of male hormones (reviewed in Ref. 61). Moreover, smoking as an etiological factor in the development of prostate cancer remains inconclusive (61).

Oxidative stress is the result of an imbalance favoring prooxidants that will lead to macromolecular damage. It has long been known that selenium can act as an antioxidant among many other functions (9). In this study we showed that Se-enriched yeast supplementation enhanced the formation of reduced GSH in blood and that the degree of GSH induction was highly correlated with enhancements in blood selenium levels. As the most abundant and ubiquitous intracellular antioxidant, GSH plays a central role in protecting macromolecules against free radical attack and oxidative damage and maintaining redox status within a cell (62). In addition, as a substrate for the selenium-containing enzyme glutathione peroxidase, GSH protects against peroxide-induced cellular damage. Because blood GSH may serve as a marker of susceptibility to oxidative

damage, the present results would indicate that long-term supplementation with Se-enriched yeast can reduce the levels of oxidative damage through induction of GSH. In addition, enhancement of GSH may also protect against carcinogenesis affecting other relevant pathways because GSH also functions in the maintenance of immune function, detoxification of carcinogens and co-carcinogens, and regulation of cell proliferation and apoptosis (63–66).

GSH in blood and tissues is also bound to proteins through the process of glutathiolation, *i.e.,* the formation of mixed disulfides of GSH with cysteine residues in proteins. Protein glutathiolation is known to alter the activity of a number of key enzymes and metabolic pathways and is thought to be an important posttranslational regulatory mechanism for many proteins (67). Because glutathiolation occurs through the reaction of oxidized glutathione with protein thiols through a thiol/disulfide exchange reaction, and oxidized glutathione formation is dependent upon levels of oxidative stress, bGSH levels have been proposed as a marker of oxidative stress (68). In the present study, we observed a significant decrease in bGSH in the Se-enriched yeast group concomitant with an increase in GSH levels. Like GSH, bGSH changes were highly correlated with changes in blood selenium levels. A substantial 50% decrease also occurred in bGSH:GSH ratios after 9 months. These decreases in bGSH and bGSH:GSH ratios are suggestive of a decrease in oxidative stress induced by selenium supplementation. This progressive change in favor of antioxidation provides support for the hypothesis that a reduction in oxidative damage is involved in the mechanism of selenium chemoprevention. However, because 8-OHdG levels were unchanged by Se-enriched yeast supplementation, it is likely that this mechanism does not involve a reduction in the production of oxidative damage to DNA in the form of 8-OHdG. It is also possible that changes may occur in the target tissue that are not reflected by urinary indicators.

Whereas numerous investigations have examined the effects of selenium supplementation on GSH peroxidase activities, to our knowledge there have been no studies examining effects on free and bGSH levels. In one previous report, sup-

plementation of HIV-infected patients with SM (100 μg Se/day) resulted in a substantial increase in blood GSH levels after 12 months; however, baseline levels were >80% lower than those in noninfected individuals (69). In the present report, selenium supplementation was effective at increasing GSH levels in individuals with normal baseline values.

A second aim of this study was to examine the effect of Se-enriched yeast on T metabolism. Our values of T are comparable with those reported previously in the same age groups (41). It was reported that mean T levels in black college students were significantly higher (about 19%) than those in whites (41); however, in the present study, levels of T were similar in blacks and whites. Whereas mean DHT levels were 20% greater in blacks than whites at baseline, this difference was not statistically significant ($P = 0.08$). Taken together, our results suggest that selenium supplementation has little effect on the conversion of T to DHT. However, the activity of α-reductase responsible for this conversion may vary between healthy individuals and those at different stages of this disease. Although finasteride, an α-reductase inhibitor, demonstrated preclinical preventive efficacy in rat models for prostate cancer, it may not be effective in preventing human prostate cancer in clinical trials (reviewed in Ref. 11). Nevertheless, in future trials, we will determine the effect of Se-enriched yeast on the activity of this enzyme by measuring T, DHT, and the ratio of DHT:T in patients with abnormal PSA (>4 ng/ml) as well as in those who have prostate intraepithelial neoplasia but refuse any treatment or surgery. Trials using finasteride showed that, in addition to inhibiting α-reductase, this drug also inhibited PSA levels (70–72).

Clinicians use PSA to screen for prostate cancer. Among biopsied men without histological evidence of prostate cancer, African Americans have a significantly higher PSA level and PSA density (serum PSA:prostate volume ratio) than white men in the same age group (73). The results of this study clearly showed that Se-enriched yeast significantly decreased PSA levels after 3 and 9 months of supplementation. After selenium cessation, PSA levels at 12 months remained low but were not significantly different from those measured in the placebo arm (74). These results suggest that Se-enriched yeast has specific effects on the prostate; however, the specific mechanisms remain unknown. The function of PSA in healthy individuals is unclear; thus, the effects of reducing PSA levels as in this study are unknown. Because Se-enriched yeast had no effect on the ratio of DHT:T, as shown in this study, its inhibitory effect on PSA levels in healthy men appears to be independent of androgen metabolism. Clearly, additional studies are urgently needed to understand the mechanisms that may account for the effect of Se-enriched yeast on PSA levels.

In summary we demonstrated that supplementation of Se-enriched yeast (247 μg/day for 9 months) to healthy adult men clearly enhanced blood GSH levels and decreased bound:free GSH ratio, suggesting a decrease in oxidative stress. Although it reduced serum PSA levels, it had no effect on the conversion of T to DHT. The results clearly show that continuous selenium supplementation is essential because its effect vanished after cessation. The same hypothesis tested here is currently being examined in prostate cancer patients and in individuals with elevated PSA.

References

1. Willett, W. C., Polk, B. F., Morris, J. S., Stampfer, M. J., Pressel, S., Rosner, B., Taylor, J. O., Schneider, K., and Hames, C. G. Prediagnostic serum selenium and risk of cancer. Lancet, 2: 130–134, 1983.

2. Clark, L. C., and Alberts, D. S. Selenium and cancer: risk or protection? J. Natl. Cancer Inst. (Bethesda), 87: 473–475, 1995.

3. van den Brandt, P. A., Goldbohm, R. A., van't Veer, P., Bode, P., Dorant, E., Hermus, R. J., and Sturmans, F. A prospective cohort study on selenium status and the risk of lung cancer. Cancer Res., 53: 4860–4865, 1993.

4. Kabuto, M., Imai, H., Yonezawa, C., Neriishi, K., Akiba, S., Kato, H., Suzuki, T., Land, C. E., and Blot, W. J. Prediagnostic serum selenium and zinc levels and subsequent risk of lung and stomach cancer in Japan. Cancer Epidemiol. Biomark. Prev., 3: 465–469, 1994.

5. El-Bayoumy, K. The role of selenium in cancer prevention. In: V. T. DeVita, S. Hellman, and S. A. Rosenberg (eds.), Cancer Prevention, pp. 1–15. Philadelphia: J. B. Lippincott Co., 1991.

6. Webber, M. M., Perez-Ripoll, E. A., and James, G. T. Inhibitory effects of selenium on the growth of DU-145 human prostate carcinoma cells in vitro. Biochem. Biophys. Res. Commun., 130: 603–609, 1985.

7. Blot, W. J., Li, J. Y., Taylor, P. R., Guo, W., Dawsey, S., Wang, G. Q., Yang, C. S., Zheng, S. F., Gail, M., Li, G. Y., Yu, V., Liu, J., Tangrea, J., Sun, Y., Liu, F., Jr., Fraumeni, J. F., Zhang, Y. H., and Li, B. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. J. Natl. Cancer Inst. (Bethesda), 85: 1483–1492, 1993.

8. Brooks, J. D., Metter, E. J., Chan, D. W., Sokoll, L. J., Landis, P., Nelson, W. G., Muller, D., Andres, R., and Carter, H. B. Plasma selenium level before diagnosis and the risk of prostate cancer development. J. Urol., 166: 2034–2038, 2001.

9. El-Bayoumy, K. The protective role of selenium on genetic damage and on cancer. Mutat. Res., 475: 123–139, 2001.

10. Greenwald, P., and Lieberman, R. Chemoprevention trials for prostate cancer. In: L. W. K. Chung, W. B. Isaacs, and J. W. Simons (eds.), Prostate Cancer: Biology, Genetics, and the New Therapeutics, pp. 499–518. Totowa, NJ: Humana Press Inc., 2000.

11. Brooks, J. D., and Nelson, W. G. Chemoprevention of prostate cancer. In: L. W. K. Chung, W. B. Isaacs, and J. W. Simons (eds.), Prostate Cancer: Biology, Genetics, and the New Therapeutics, pp. 365–375. Totowa, NJ: Human Press Inc., 2000.

12. Platz, E. A., and Helzlsouer, K. J. Selenium, zinc, and prostate cancer. Epidemiol. Rev., 23: 93–101, 2001.

13. Clark, L. C., Dalkin, B., Krongrad, A., Combs, G. F., Jr., Turnbull, B. W., Slate, E. H., Witherington, R., Herlong, J. H., Janosko, E., Carpenter, D., Borosso, C., Falk, S., and Rounder, J. Decreased incidence of prostate cancer with selenium supplementation: results of a double-blind cancer prevention trial. Br. J. Urol., 81: 730–734, 1998.

14. Clark, L. C., Combs, G. F., Jr., Turnbull, B. W., Slate, E. H., Chalker, D. K., Chow, J., Davis, L. S., Glover, R. A., Graham, G. F., Gross, E. G., Krongrad, A., Lesher, J. L., Jr., Park, H. K., Sanders, B. B., Jr., Smith, C. L., and Taylor, J. R. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. J. Am. Med. Assoc., 276: 1957–1963, 1996.

15. Duffield-Lillico, A. J., Reid, M. E., Turnbull, B. W., Combs, G. F., Jr., Slate, E. H., Fischbach, L. A., Marshall, J. R., and Clark, L. C. Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: a summary report of the Nutritional Prevention Cancer Trial. Cancer Epidemiol. Biomark. Prev., 11: 630–639, 2002.

16. Rayman, M. P. The importance of selenium to human health. Lancet, 356: 233–241, 2000.

17. Klein, E. A., Thompson, I. M., Lippman, S. M., Goodman, P. J., Albanes, D., Taylor, P. R., and Coltman, C. SELECT: the Selenium and Vitamin E Cancer Prevention Trial: rationale and design. Prostate Cancer Prostatic Dis., 3: 145–151, 2000.

18. Hoque, A., Albanes, D., Lippman, S. M., Spitz, M. R., Taylor, P. R., Klein, E. A., Thompson, I. M., Goodman, P., Stanford, J. L., Crowley, J. J., Coltman, C. A., and Santella, R. M. Molecular epidemiologic studies within the Selenium and Vitamin E Cancer Prevention Trial (SELECT). Cancer Causes Control, 12: 627–633, 2001.

19. Jemal, A., Thomas, A., Murray, T., and Thun, M. Cancer statistics, 2002. CA Cancer J. Clin., 52: 23–47, 2002.

20. Garnick, M. B. Prostate cancer screening, diagnosis, and management. Ann. Int. Med., 118: 804–818, 1993.

21. Kolonel, L. N., Hinds, M. W., and Hankin, J. H. Cancer patterns among migrant and native-born Japanese in Hawaii in relation to smoking, drinking and dietary habits. In: H. V. Gelboin, B. MacMahon, T. Matsushima, T. Sugimura, S. Takayama, and H. Takebe (eds.), Genetics and Environmental Factors in Exper-

imental and Human Cancer, pp. 327–340. Tokyo: Japan Scientific Society Press, 1980.

22. Breslow, N., Chan, C. W., Dhom, G., Drury, R. A. B., Franks, L. M., Gellei, B., Lee, Y. S., Lundberg, S., Spanke, B., Sternby, N. H., and Tulinium, H. Latent carcinoma of the prostate at autopsy in seven areas. Int. J. Cancer, *20:* 680–688, 1977.

23. Yantani, R., Kusano, I., Shiraishi, T., Hyashi, T., and Stemmermann, G. N. Latent prostatic carcinoma: pathological and epidemiological aspects. Jpn. J. Clin. Oncol., *19:* 319–326, 1989.

24. Wynder, E. L., and Fair, W. R. Prostate cancer: nutrition adjunct therapy. J. Urol., *156:* 1364–1365, 1996.

25. Greenwald, P. Principles of cancer prevention: diet and nutrition. *In:* V. T. DeVita, Jr., S. Hellman, and S. A. Rosenberg (eds.), Cancer: Principles and Practice of Oncology, pp. 167–180. Philadelphia: J. B. Lippincott, 1989.

26. Rohan, T. E., Howe, G. R., Burch, J. D., and Jain, M. Dietary factors and risk of prostate cancer: a case control study in Ontario, Canada. Cancer Causes Control, *6:* 145–154, 1995.

27. Anderson, S. O., Wolk, A., Bergstrom, R., Giovannucci, E., Lindgren, C., Baron, J., and Adami, H. O. Energy, nutrient intake and prostate cancer risk: a population based case-control study in Sweden. Int. J. Cancer, *68:* 716–722, 1996.

28. Mettlin, C., Selenskas, S., Natarajan, N., and Huben, R. β-Carotene and animal fats and their relationship to prostate cancer risk. A case-control study. Cancer (Phila.), *64:* 85–94, 1989.

29. Block, G., Patterson, B., and Subar, A. Fruit, vegetables and cancer prevention: a review of the epidemiological evidence. Nutr. Cancer, *18:* 1–29, 1992.

30. Jacobs, D. R., Jr., Marquart, L., Slavin, J., and Kushi, L. H. Whole-grain intake and cancer: an expanded review and meta-analysis. Nutr. Cancer, *30:* 85–96, 1998.

31. Hebert, J. R., Hurley, T. G., Olendzki, B. C., Teas, J., Ma, Y., and Hampl, J. S. Nutritional and socioeconomic factors in relation to prostate cancer mortality: a cross-national study. J. Natl. Cancer Inst. (Bethesda), *90:* 1637–1647, 1998.

32. Steinberg, G. D., Carter, B. S., Beaty, T. H., Childs, B., and Walsh, P. C. Family history and the risk of prostate cancer. Prostate, *17:* 337–347, 1990.

33. Woolf, C. M. An investigation of the familial aspects of carcinoma of the prostate. Cancer (Phila.), *3:* 739–744, 1960.

34. Cannon, L., Bishop, D. T., Skolnick, M., Hunt, S., Lyon, J. L., and Smart, C. R. Genetic epidemiology of prostate cancer in the Utah Mormon genealogy. Cancer Surv., *1:* 47–69, 1982.

35. Whittemore, A. S., Wu, A. H., Kolonel, L. N., John, E. M., Gallagher, R. P., Howe, G. R., West, D. W., Teh, C. Z., and Stamey, T. Family history and prostate cancer risk in black, white, and Asian men in the United States and Canada. Am. J. Epidemiol., *141:* 732–740, 1995.

36. Coetzee, G. A., and Ross, R. K. Re: prostate cancer and the androgen receptor. J. Natl. Cancer Inst. (Bethesda), *86:* 872–873, 1994.

37. Imperato-McGinley, J., Guerrero, L., Gautier, T., and Peterson, R. E. Steroid 5α-reductase deficiency in man: an inherited form of male pseudohermaphroditism. Science (Wash. DC), *186:* 1212–1215, 1974.

38. Walsh, P. C., Madden, J. D., Marrod, M. J., Goldstein, J. L., MacDonald, P. C., and Wilson, J. P. Familial incomplete male pseudohermaphroditism, type 2. Decreased dihydrotestosterone formation in pseudovaginal perineoscrotal hypospadias. N. Engl. J. Med., *291:* 944–949, 1974.

39. Bruun, E., Frandsen, H., Nielsen, K., Rasmussen, L. D., Vinnergaard, T., and Frimodt-Moller, C. Dihydrotestosterone measured in core biopsies from prostatic tissues. Am. J. Clin. Oncol., *11* (Suppl. 2)*:* S27−S29, 1988.

40. Habib, F. K., Bissas, A., Neill, W. A., Busuttil, A., and Chisholm, G. D. Flow cytometric analysis of cellular DNA in human prostate cancer: relationship to 5α-reductase activity of the tissue. Urol. Res., *17:* 239–243, 1989.

41. Ross, R., Bernstein, L., Judd, H., Hanisch, R., Pike, M., and Henderson, B. Serum testosterone levels in healthy young black and white men. J. Natl. Cancer Inst. (Bethesda), *76:* 45–48, 1986.

42. Prehn, R. T. On the prevention and therapy of prostate cancer by androgen administration. Cancer Res., *59:* 4161–4164, 1999.

43. Brawley, O. W., Ford, L. G., Thompson, I., Perlman, J. A., and Kramer, B. S. 5-α-Reductase inhibition and prostate cancer prevention. Cancer Epidemiol. Biomark. Prev., *3:* 177–182, 1994.

44. Burks, D. A., and Littleton, R. H. The epidemiology of prostate cancer in black men. Henry Ford Hosp. Med. J., *40:* 89–92, 1992.

45. Ross, R. K., and Henderson, B. E. Do diet and androgens alter prostate cancer risk via a common etiologic pathway? J. Natl. Cancer Inst. (Bethesda), *86:* 252–254, 1994.

46. Pienta, K. J., and Esper, P. S. Risk factors for prostate cancer. Ann. Int. Med., *118:* 793–803, 1993.

47. Gormley, G. J. Chemoprevention strategies for prostate cancer: the role of 5-α-reductase inhibitors. J. Cell. Biochem., *16H:* 113–117, 1992.

48. Coffey, D. S., and Pienta, K. J. New concepts in studying the control of normal and cancer growth of the prostate. *In:* A. R. Liss (ed.), Current Concepts and Approaches to the Study of Prostate Cancer, pp. 1–73. New York: Alan R. Liss Inc., 1987.

49. Pienta, K. J., Partin, A. W., and Coffey, D. S. Cancer as a disease of DNA organization and dynamic cell structure. Cancer Res., *49:* 2525–2532, 1989.

50. Grino, P. B., Griffin, J. E., and Wilson, J. D. Testosterone at high concentrations interacts with the human androgen receptor similarly to dihydrotestosterone. Endocrinology, *126:* 1165–1172, 1990.

51. Cohen, S. A., and Michaud, D. P. Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino acids via high performance liquid chromatography. Anal. Biochem., *211:* 279–287, 1993.

52. Cohen, S. A., De Antonis, K., and Michaud, D. P. Compositional protein analysis using 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, a novel derivatization reagent. *In:* R. H. Angeletti (ed.), Techniques in Protein Chemistry IV, pp. 289–298. San Diego, CA., Academic Press, 1993.

53. Richie, J. P., Jr., Skowronski, L., Abraham, P., and Leutzinger, Y. Blood glutathione concentrations in a large-scale human study. Clin. Chem., *42:* 64–70, 1996.

54. Tietze, F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione. Anal. Biochem., *27:* 502–522, 1969.

55. Owens, C. W. I., and Belcher, R. V. A colorimetric micro-method for the determination of glutathione. J. Biochem., *94:* 705–711, 1965.

56. Sohn, O. S., Blackwell, L., Mathis, J., Assad, W. W., Reddy, B. S., and El-Bayoumy, K. Excretion and tissue distribution of selenium following treatment of male F344 rats with benzylselenocyanate or sodium selenite. Drug Metab. Dispos., *19:* 865–870, 1991.

57. Voth-Beach, L. M., and Schrader, D. E. Graphite furnace atomic absorption spectroscopy: new approaches to matrix modification. Spectroscopy, *1:* 49–59, 1986.

58. Fleiss, J. L. The Design and Analysis of Clinical Experiments, Chapter 8. Repeated measurements studies, pp. 220–240. New York: John Wiley & Sons, 1986.

59. Richie, J. P., Jr., Abraham, P., and Leutzinger, Y. Long-term stability of blood glutathione and cysteine levels in human subjects. Clin. Chem., *42:* 1100–1105, 1996.

60. Pryor, W. A. Cigarette smoke radicals and the role of free radicals in chemical carcinogenicity. Environ. Health Perspect., *105:* 875–882, 1997.

61. Hickey, K., Do, K-A., and Green, A. Smoking and prostate cancer. Epidemiol. Rev., *23:* 115–125, 2001.

62. Schafer, F. Q., and Buettner, G. R. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic. Biol Med., *30:* 1191–1212, 2001.

63. Richie, J. P., Jr. The role of glutathione in aging and cancer. Exp. Gerontol., *27:* 615–626, 1992.

64. Locigno, R., and Castronovo, V. Reduced glutathione system: role in cancer development, prevention and treatment (review). Int. J. Oncol., *19:* 221–236, 2001.

65. Ketterer, B. Protective role of glutathione and glutathione transferases in mutagenesis and carcinogenesis. Mutat. Res., *202:* 343–361, 1988.

66. Hall, A. G. The role of glutathione in the regulation of apoptosis. Eur. Clin. Invest., *29:* 238–245, 1999.

67. Klatt, P., and Lamas, S. Regulation of protein function by *S*-glutathiolation in response to oxidative and nitrosative stress. Eur. J. Biochem., *267:* 4928–4944, 2000.

68. Cotgreave, I. A., and Gerdes, R. G. Recent trends in glutathione biochemistry: glutathione-protein interactions: a molecular link between oxidative stress and cell proliferation? Biochem. Biophys. Res. Commun., *242:* 1–9, 1998.

69. Delmas-Beauvieux, M-C., Peuchant, E., Couchouron, A., Constans, J., Sergeant, C., Simonoff, M., Pellegrin, J-L., Leng, B., Conri, C., and Clerc, M. The enzymatic antioxidant system in blood and glutathione status in human immunodeficiency virus (HIV)-infected patients: effects of supplementation with selenium of β-carotene. Am. J. Clin. Nutr., *64:* 101–107, 1996.

70. Habib, F. K., Ross, M., Tate, R., and Chisholm, G. D. Differential effect of finasteride on the tissue androgen concentrations in benign prostatic hyperplasia. Clin. Endocrinol., *46:* 137–144, 1997.

71. Geller, J. Effect of finasteride, a 5 α-reductase inhibitor on prostate tissue androgens and prostate-specific antigen. J. Clin. Endocrinol. Metab., *71:* 1552–1555, 1990.

72. Presti, J. C., Jr., Fair, W. R., Andriole, G., Sogani, P. C., Seidmon, E. J., Ferguson, D., Ng, J., and Gormley, G. J. Multicenter, randomized, double-blind, placebo controlled study to investigate the effect of finasteride (MK-906) on stage D prostate cancer. J. Urol., *148:* 1201–1204, 1992.

73. Abdalla, I., Ray, P., Vaida, F., and Vijayakumar, S. Racial differences in prostate-specific antigen levels and prostate-specific antigen densities in patients with prostate cancer. Am. J. Clin. Oncol., *22:* 537–541, 1999.

74. Diamandis, I. P. Prostate-specific antigen: a cancer fighter and a valuable messenger? Clin. Chem., *46:* 896–900, 2000.