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Award Number: DAMD17-98-1-8550

TITLE: In Vivo Testing of Chemopreventive Agents Using the Dog Model of Spontaneous Prostate Carcinogenesis

PRINCIPAL INVESTIGATOR: David J. Waters, DVM, Ph.D.

CONTRACTING ORGANIZATION: Purdue Research Foundation
West Lafayette, IN 47907-1021

REPORT DATE: March 2005

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-03-2005	Final	1 Sep 1998 – 28 Feb 2005

4. TITLE AND SUBTITLE

In Vivo Testing of Chemopreventive Agents Using the Dog Model of Spontaneous Prostate Carcinogenesis

5a. CONTRACT NUMBER

5b. GRANT NUMBER
DAMD17-98-1-8550

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

David J. Waters, DVM, Ph.D.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Purdue Research Foundation
West Lafayette, IN 47907-1021

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
Original contains color plates: ALL DTIC reproductions will be in black and white

14. ABSTRACT – SEE ATTACHED PAGE

15. SUBJECT TERMS
Prostate cancer, chemoprevention, dog, selenium, finasteride, DNA damage, animal model

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	77	

Abstract

The goal of this research was to demonstrate the feasibility of the dog model of spontaneous prostate carcinogenesis as a valuable model system to evaluate chemopreventive agents. Our work has led to significant progress in elucidating the complex relationship between the essential trace mineral selenium and DNA damage, apoptosis, and androgens in the prostate. Our work has generated the first evidence of a non-linear, U-shaped dose : response relationship between selenium status and DNA damage within the prostate. Importantly, the dose : response curve from elderly beagle dogs accurately predicts the relationship between selenium status and prostate cancer risk in men. Moreover, we have demonstrated for the first time that supranutritional selenium intake is associated with increased apoptosis of prostatic epithelial cells in vivo. Our experimental paradigm has also yielded the first information on the influence of dietary selenium intake on intraprostatic levels of androgens. Interestingly, in the dog model, six months treatment with the 5-alpha reductase inhibitor finasteride (with or without selenium) induces prostatic atrophy without significantly reducing the extent of prostatic DNA damage. Our experience indicates the dog model provides a useful model system to study the effects of cancer preventive agents on prostate cells in an appropriate context – in vivo within an aging prostate.

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INTRODUCTION

Dogs and humans share a vulnerability for the spontaneous development of prostate cancer. Prevention rather than treatment may be the best approach to reduce the morbidity and mortality associated with prostate cancer. Our previous work documented the high prevalence of high-grade prostatic intraepithelial neoplasia in elderly pet dogs and its close association with invasive carcinoma. *In vivo* screening of promising chemopreventive agents using the dog model of spontaneous prostate carcinogenesis represents a novel approach to the prevention of prostate cancer. The goal of this Phase II Idea Development Award is to utilize the dog model to define further the anticancer effects of the trace mineral selenium. The scope of this final report includes: (1) continued evaluation of data collected from our Phase I studies on dogs receiving daily supplementation with selenium; (2) dog experiments testing the extent to which manipulation of the androgen milieu within the prostate (using the 5α-reductase inhibitor finasteride) significantly influences the response of the aging prostate to selenium supplementation; and (3) evaluation of data from Phase I and Phase II experiments to address specific questions relevant to the complex relationship between DNA damage, androgens, and apoptosis within the prostate and the reliability of non-invasive biomarkers of prostatic homeostasis. The long-term objective of this research is to utilize the dog as a pre-clinical model to test innovative ideas in cancer prevention and treatment, as well as to further understand the factors that regulate the response of the aging prostate to chemopreventive agents.

BODY

I. Continued Evaluation of Data Collected from Phase I Experiments

1A. What is the Relationship Between Selenium Status and the Level of Genotoxic Stress within the Aging Prostate and Brain?

Using the dog model, we have explored the dose : response relationship between selenium status and DNA damage within the prostate. We studied 49 (8.5 – 10.5 year old) sexually intact male, retired breeder dogs that were randomly assigned to either a control group or to receive daily supplementation with selenomethionine or high selenium yeast at 3 or 6 μg/kg body weight. After 7 months, toenail and prostate tissue specimens were collected immediately after euthanasia and analyzed for total selenium concentration using neutron activation analysis. Dogs from control and selenium-treated groups were combined and subdivided into quartiles based on their toenail selenium level to evaluate the relationship between selenium status and prostatic DNA damage. The extent of DNA damage within the prostate was measured by alkaline Comet assay. We found a non-linear, U-shaped relationship with a relatively narrow range of selenium that optimizes homeostasis within the prostate in terms of DNA damage reduction **(Figure 1A)**. This U-shaped relationship between micronutrient status and biological response was predicted more than 20 years ago by Mertz [1] **(Figure 1B)**. According to the Mertz model, a region of optimal nutrient status lies between two suboptimal (low and high) regions and the extreme values of deficiency and toxicity. Our data provide the first *in vivo* confirmation that Mertz's model is operational for an essential nutrient within the prostate. Importantly, this non-linear U-shaped relationship between selenium status and genotoxic stress within the prostate predicts that not all men will benefit from increasing their selenium status.

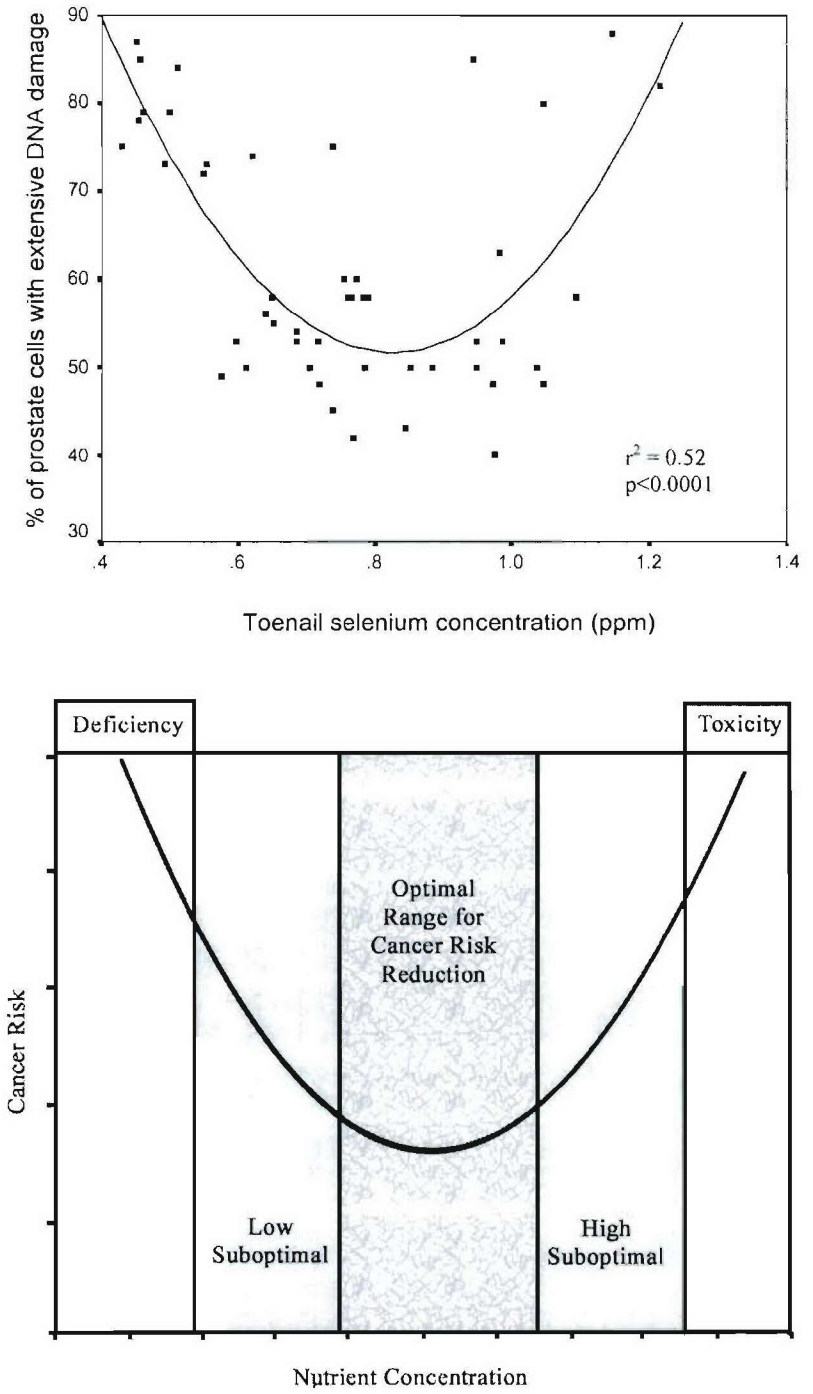


Figure 1. A U-Shaped Dose-Response Curve Defines the Relationship Between Selenium and Genotoxic Stress in Prostate. **(A)** U-shaped dose-response relationship between toenail selenium concentration and prostatic DNA damage in 49 elderly dogs that were physiologically equivalent to 65-year old men. **(B)** Model adapted from Mertz [1] predicting the biological response to an essential nutrient. The data from dogs provides the first *in vivo* confirmation that the Mertz model fits for selenium and procarcinogenic events within the prostate.

To determine whether the U-shaped relationship between selenium status and DNA damage in dogs was unique to the aging prostate, we measured DNA damage in the brain. Similar to our findings in the prostate, there was a U-shaped dose-response relationship between toenail selenium concentration and DNA damage within the aging brain. Importantly, we found the toenail selenium concentration that optimized DNA damage reduction in the prostate also minimized the extent of DNA damage with in the aging brain **(Figure 2)**.

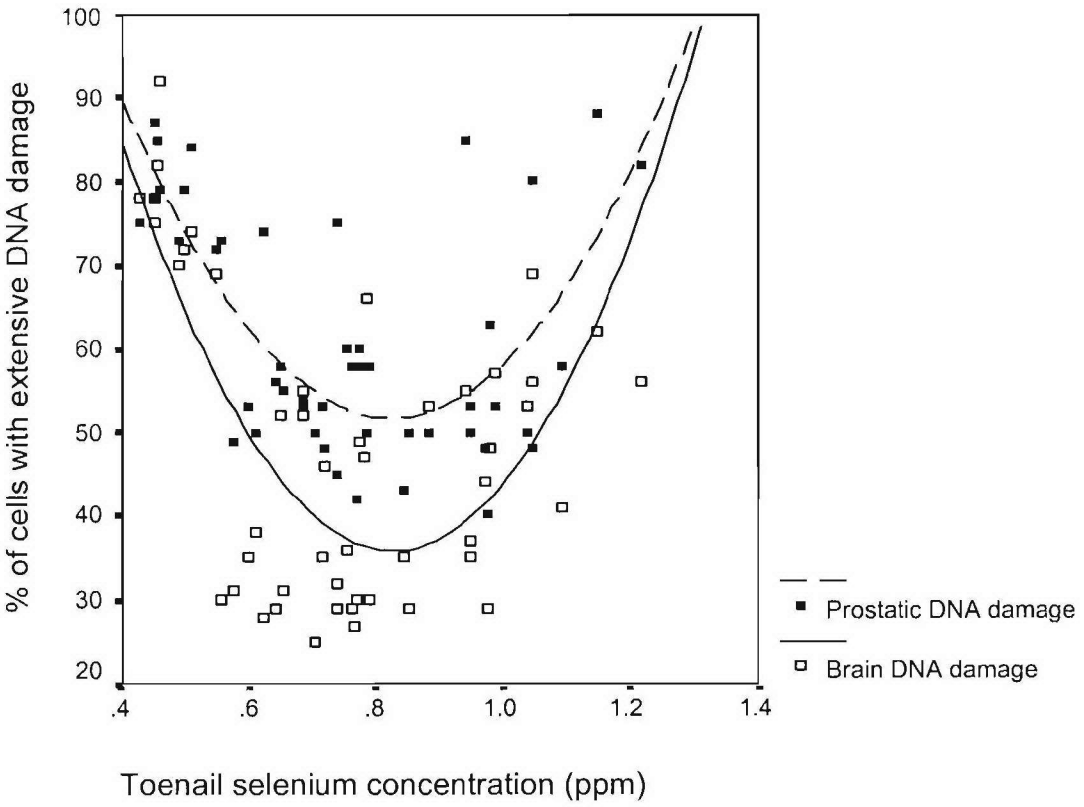


Figure 2. U-Shaped Dose-Response Defines the Relationship Between Toenail Selenium Concentration and Genotoxic Stress in the Brain and Prostate of Elderly Dogs.

1B. Does the U-Shaped Relationship Between Toenail Selenium Concentration and Extent of Prostatic DNA Damage in Elderly Beagle Dogs have Relevance to Selenium and Human Prostate Cancer Risk?

Using data from the Health Professionals Follow-Up Study (HPFS), Yoshizawa et al. [2] found a strong inverse association between toenail selenium concentration and risk for advanced prostate cancer. Interestingly, multivariate analysis demonstrated an *apparent threshold effect*, with no additional prostate cancer protective effect at toenail concentrations exceeding 0.82 ppm. In another study, Brooks *et al.* [3] found a similar threshold effect.

We found that toenail concentrations in the lowest and highest quartiles of elderly beagle dogs in our study (mean of 0.50 ppm and 1.03 ppm, respectively) were quite similar to toenail concentrations seen in the HPFS (median of 0.66 ppm in lowest quintile; median of 1.14 ppm in highest quintile). *Fitting the human data from the HPFS to the dog curve produced an intriguing result – the same level of selenium status that minimizes prostatic DNA damage in dogs also minimizes prostate cancer risk in men.* In the HPFS, the highest risk for prostate cancer was in men in the lowest quintile of toenail selenium (median 0.66 ppm) – a value well outside the optimal range predicted by our model. Lowest prostate cancer risk was in men with a median value of 0.82 ppm, which falls within the optimal range of our model. *Thus, movement along our dog curve from low suboptimal to optimal selenium status* ***(bold arrow in Figure 3)*** *was associated with a 65% reduction in human prostate cancer risk.*

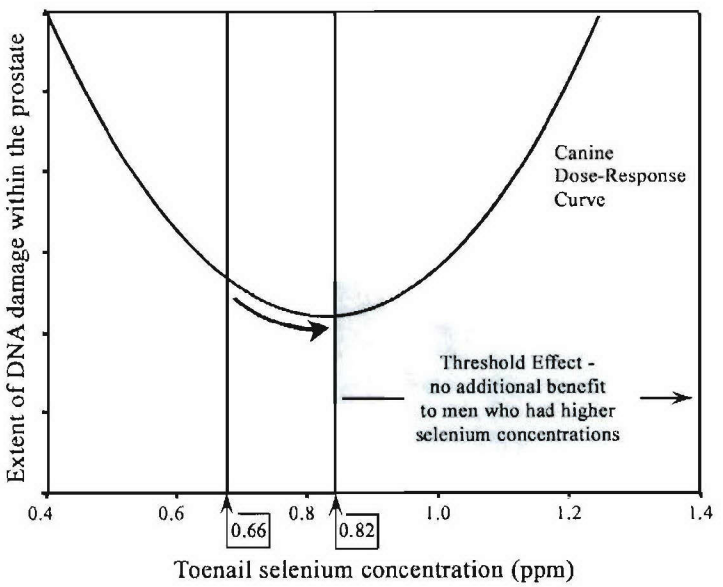


Figure 3. Canine Dose-Response Curve Explains the Effect of Selenium Status on Human Prostate Cancer Risk Reduction in the Health Professionals Follow-Up Study. Men with median selenium status of 0.66 ppm had the highest risk for advanced prostate cancer. Men with median selenium status of 0.82 ppm, a value equivalent to the optimal selenium concentration in the dog model, had the lowest prostate cancer risk. Movement along the dog curve from 0.66 to 0.82 ppm (bold arrow) parallels the 65% reduction in prostate cancer risk for men in the Health Professionals Follow-Up Study.

In addition, we analyzed data from the Nutritional Cancer Prevention Trial of Clark et al. [4, 5], converting plasma selenium to an equivalent toenail selenium concentration.[1] Again, the dog curve correctly predicted that men in the lowest tertile of baseline selenium status (<0.71 ppm) would benefit from selenium supplementation (**Figure 4**). Men in the highest tertile in Clark's study had baseline selenium status (>0.81 ppm) already within the optimum range prior to selenium supplementation; these men did not benefit from selenium supplementation and their post-selenium supplementation selenium status was very high (median, 1.27 ppm).

Taken together, these findings provide strong rationale for using the aging dog prostate to mimic the aging human prostate to further understand the response of prostate cells to selenium. Our results support the hypothesis that toenails are a readily accessible surrogate tissue for monitoring the effects of dietary selenium supplementation on carcinogenic events within the aging prostate. The possibility of a threshold for the prostate cancer protective effects of selenium that can be assayed non-invasively warrants further investigation.

A manuscript reporting these results has been published (Waters et al, Carcinogenesis 2005; 26:1256-1262) and appears in the appendix of this report.

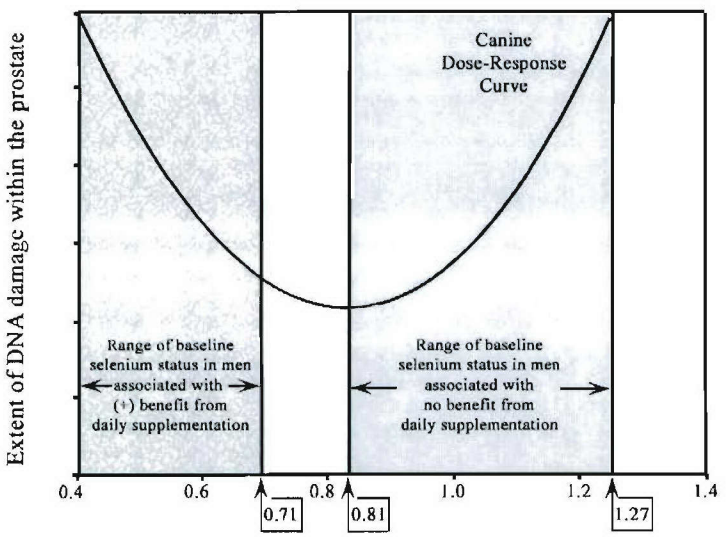


Figure 4. Canine Dose-Response Curve Explains the Effect of Baseline Selenium Status on Human Prostate Cancer Risk Reduction in the Nutritional Prevention of Cancer Trial. Men with baseline selenium status <0.71 ppm had lower than the optimal selenium concentration predicted by the dog model; these men had a 92% reduction in prostate cancer risk after selenium supplementation. Men with baseline selenium status >0.81 ppm were already within the optimal or high suboptimal range predicted by the dog model prior to supplementation; these men had no significant reduction in prostate cancer after selenium supplementation. Following selenium supplementation, men in the highest baseline selenium tertile had a median selenium level of 1.27 ppm, a value clearly exceeding the selenium concentration that minimized DNA damage within the dog prostate. These men had an 88% increased total cancer incidence.

[1] We simultaneously measured toenail and plasma selenium concentration in 12 healthy human volunteers to generate a ratio (6.7 ± 0.7) to convert plasma selenium concentration to predicted toenail values. This technique appears valid because using our conversion, the average plasma selenium concentration in U.S. men (123 ng/ml) is equivalent to a concentration of 0.82 ppm in toenails, which is identical to the median selenium concentration measured in the toenails of men in the HPFS.

II. Evaluation of Data from Phase II Experiments

We conducted a 6 month intervention study in elderly sexually intact male dogs to determine the effect of selenium and antiandrogen on biomarkers of carcinogenesis within the prostate. After prostatic biopsy, 57 dogs were randomized to one of 6 treatment groups: (1) no treatment; (2) selenium supplementation (3μg/kg SelenoExcell yeast); (3) selenium plus low dose (0.25 mg/kg/day) finasteride; (4) selenium plus high dose (1mg/kg/day) finasteride; (5) low dose finasteride without selenium; and (6) high dose finasteride without selenium. After euthanasia, prostate tissues were collected for biomarker analysis. Urine, serum, and toenails were also collected for subsequent measurement of biomarkers. Selenium and finasteride supplementation was well tolerated by all dogs. No technical problems were encountered.

2A. Does Selenium Supplementation Influence the Anti-trophic Effect of Finasteride on the Aging Prostate?

As an initial step in analyzing our experimental results, we focused on the effects of treatment on prostate volume. For each dog, prostate size in 3 dimensions was measured with calipers prior to treatment and after 6-7 months treatment. Prostate weight was calculated using the formula: weight (g) = volume (cm^3) x 0.602 + 1.16. The anti-trophic effect of finasteride on the prostate was assessed by calculating the percent change in prostate volume over the treatment period. Actual prostate weight recorded at the end of the study was strongly correlated with prostate weight calculated from prostate volume ($r = 0.963$; $p < 0.0001$), validating prostate volume as a robust and reliable index of prostate growth. Dogs in the control group had a median change in prostate volume of +15% over the treatment period. Similarly, dogs receiving supranutritional selenium supplementation had a 16% median increase in prostate volume. In contrast, finasteride-treated dogs had a 42% median reduction in prostate volume after 6 months of treatment ($p<0.0001$ vs. control group). Finasteride-treated dogs that received supranutritional selenium had a 38% median reduction in prostate volume, which did not differ from dogs treated with finasteride alone ($p=0.52$).

These data suggest that selenium status does not significantly influence the anti-trophic effects of finasteride on the aging prostate. The dog model enables us to study *in vivo* how differences in selenium status (i.e., nutritionally adequate versus supranutritional dietary intake) influence prostate cell response to other potential cancer preventive agents.

2B. Does Treatment with Finasteride or Finasteride + Selenium Decrease DNA Damage within the Aging Prostate?

Compared with control dogs, finasteride-treated dogs had a non-significant 10% reduction in prostatic damage; no difference in DNA damage was seen between low dose and high dose finasteride groups **(Table 1)**. Dogs that received a combination of finasteride plus selenium did not experience a significant reduction in prostatic DNA damage compared to the other groups **(Table 1)**.

Table 1. The Effect of Finasteride or Finasteride + Selenium on DNA Damage, Apoptosis, and Proliferative Index within the Prostate of Elderly Dogs.

Treatment Group	Prostatic DNA Damage*	Apoptosis **	Proliferative Index ***
	Mean (SD)	Mean (SD)	Mean (SD)
Control (N=10)	84.1 (12.07)	.611 (.781)	1.50 (.663)
Finasteride Treatment			
Low Dose (N=10)	76.6 (10.09)	1.00 (1.32)	2.15 (1.09)
High Dose (N=10)	77.6 (14.29)	.500 (.755)	1.22 (.696)
All Dogs (N=20)	77.1 (12.15)	.764 (1.09)	1.66 (1.00)
Finasteride+Selenium Treatment			
Low Dose +Selenium (N=10)	77.3 (19.44)	.777 (.666)	1.87 (.751)
High Dose +Selenium (N=10)	80.8 (14.41)	.555 (.527)	1.34 (.580)
All Finasteride +Selenium (N=20)	79.1 (16.70)	.666 (.594)	1.60 (.706)

*Prostatic DNA Damage: For each dog, the percentage of prostatic cells that had extensive DNA damage (alkaline Comet assay) was determined.
**Apoptosis: For each dog, the median number of prostatic epithelial cells per 200X microscopic field with positive nuclear staining (TUNEL assay) was determined.
***Proliferative Index: For each dog, the percentage of immunopositive prostatic epithelial cells (MIB-1 immunohistochemistry) was determined.

2C. Does Treatment with Finasteride or Finasteride + Selenium have Significant Effects on Apoptosis or the Proliferative Index of Prostatic Epithelial Cells?

Finasteride treatment of elderly dogs for 6 months resulted in a significant reduction in intraprostatic DHT concentration (mean 1.80 pg/mg tissue versus 6.60 pg/mg tissue in control dogs). Not unexpectedly, 5α-reductase inhibition by finasteride was accompanied by an increase in the concentration of testosterone within the prostate (mean = 7.8 pg/mg tissue in finasteride-treated dogs versus 3.2 pg/mg tissue in control dogs). Despite these significant changes in intraprostatic level of androgens, finasteride treament was not associated with significant changes in apoptosis or proliferative index of prostatic epithelial cells **(Table 1)**.

III. Evaluation of Data from Phase I and Phase II Experiments

3A. Does Toenail Selenium Concentration Reflect the Concentration of Selenium in the Prostate and Brain?

Previous studies in humans and animals did not evaluate whether differences in the tissue concentration of selenium within the prostate or brain were strongly predicted by the non-invasive measurement of selenium in toenails. In 67 elderly dogs, we found a strong positive association between selenium concentration in toenails versus prostate ($r = .709$; $p<0.0001$, Pearson correlation) **(Figure 5A)**. This strong association was observed over the range of selenium status of healthy adults in the United States, including the men who are likely to participate in the SELECT prostate cancer prevention trial.

In contrast, these was a weaker correlation between toenail selenium concentration and selenium concentration in the brain ($r=.273$; $p=0.25$, Pearson correlation) **(Figure 5B)**.

In summary, these results provide the first data on steady state tissue concentration of selenium in prostate and brain after long-term (6 months) dietary selenium supplementation.

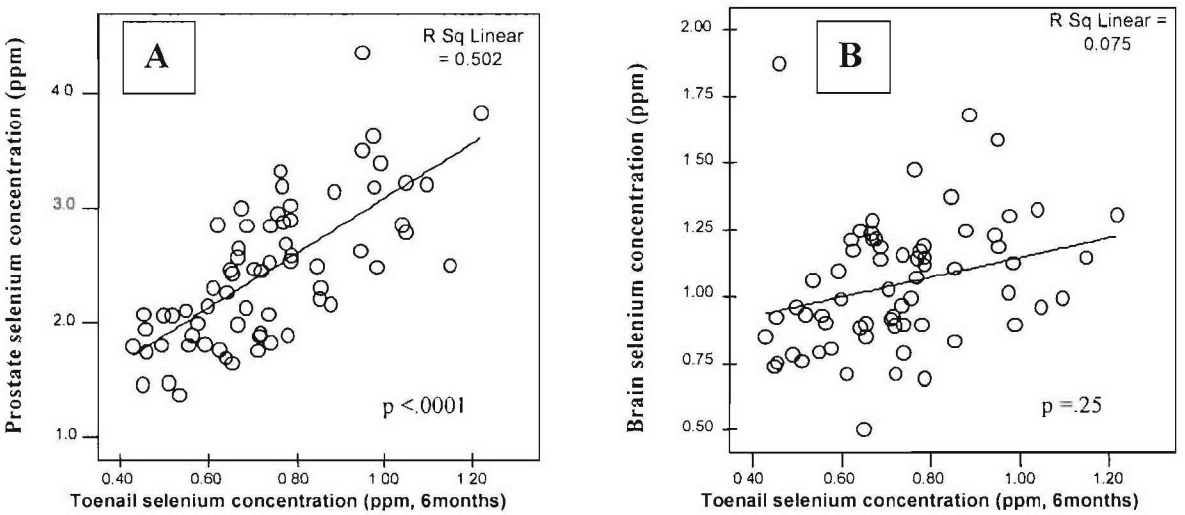


Figure 5. Correlation Between Selenium Concentration in Toenails (ppm) and Selenium Concentration in Prostate (A) and Brain (B).

3B. Can Peripheral Blood Lymphocytes Provide a Window to Assess Genotoxicity within the Prostate?

Previously, we showed that the extent of DNA damage within the aging prostate could be predicted by measuring the concentration of the trace mineral selenium in toenail clippings (see Section 1A of this report). Moreover, this relationship discovered by studying elderly dogs remarkably paralleled the relationship between dietary selenium and prostate cancer risk in men. We sought to extend these findings by testing the hypothesis that the amount of DNA damage measured in circulating blood lymphocytes can be used to non-invasively assess the extent of DNA damage within the prostate. Sixty-seven elderly beagle dogs in the Phase I and Phase II experiments received nutritionally adequate or supranutritional levels of selenium for 7 months, thereby mimicking the range of dietary intake of men in the U.S. At the end of the treatment period, alkaline Comet assay was used to measure DNA damage in peripheral blood lymphocytes (PBLs) and prostate. Pearson and Spearman correlation coefficients were calculated to determine if the extent of prostatic DNA damage was significantly correlated with three different measures of PBL DNA damage: 1) basal damage; 2) total damage after *ex vivo* H_2O_2 challenge; and 3) % inducible damage (total damage after H_2O_2 – basal damage / 100 – basal damage).

In dogs receiving a nutritionally adequate level of selenium, there was no significant association between basal DNA damage in PBLs and damage within the prostate **(Table 2)**.

Table 2. Correlation Between DNA Damage in Peripheral Blood Lymphocytes (PBL) and Genotoxicity within the Prostate.

	PBL Basal DNA Damage		PBL Inducible DNA Damage		PBL Total DNA Damage after H_20_2	
Controls						
Phase I and Phase II (n=18)	r=-.241	p=.34	r=.46	p=.04	r=.417	p=.085
Phase I (n=9)	r=-.150	p=.70	r=.168	p=.67	r=.-081	p=.83
Phase II (n=9)	r=-.273	p=.47	r=.65	p=.058	r=.600	p=.088
Se treated dogs						
Phase I and Phase II (n=49)	r=-.347	p=.01	r=.290	p=.04	r=.197	p=.17
Phase I (n=39)	r=-.100	p=.55	r=.171	p=.297	r=.116	p=.48
Phase II (n=10)	r=-.086	p=.81	r=.03	p=.93	r=-.085	p=.81
Control +Se treated dogs						
Phase I and Phase II (n=67)	r=-.211	p=.09	r=.373	p=.002	r=.319	p=.001
Phase I (n=48)	r=.171	p=.30	r=.171	p=.25	r=.18	p=.23
Phase II (n=19)	r=-.211	p=.71	r=.349	p=.14	r=.305	p=.20

There was a weak negative association between basal DNA damage in PBLs and damage in the prostate of dogs receiving supranutritional levels of selenium ($r = -.347$, $p = .01$). In both treatment groups, the extent of DNA damage in PBLs measured after *ex vivo* H_2O_2 challenge was positively correlated with prostatic DNA damage, indicating that dogs with the most prostatic DNA damage had PBLs that were more susceptible to oxidative stress. Although statistically significant, these associations were relatively weak, accounting for only 4 to 22% of the interindividual variation in prostatic DNA damage **(Table 2, Figure 6)**.

In summary, we conclude that measurement of DNA damage in PBLs using alkaline Comet assay does not provide a reliable method to non-invasively predict genotoxicity within the prostate.

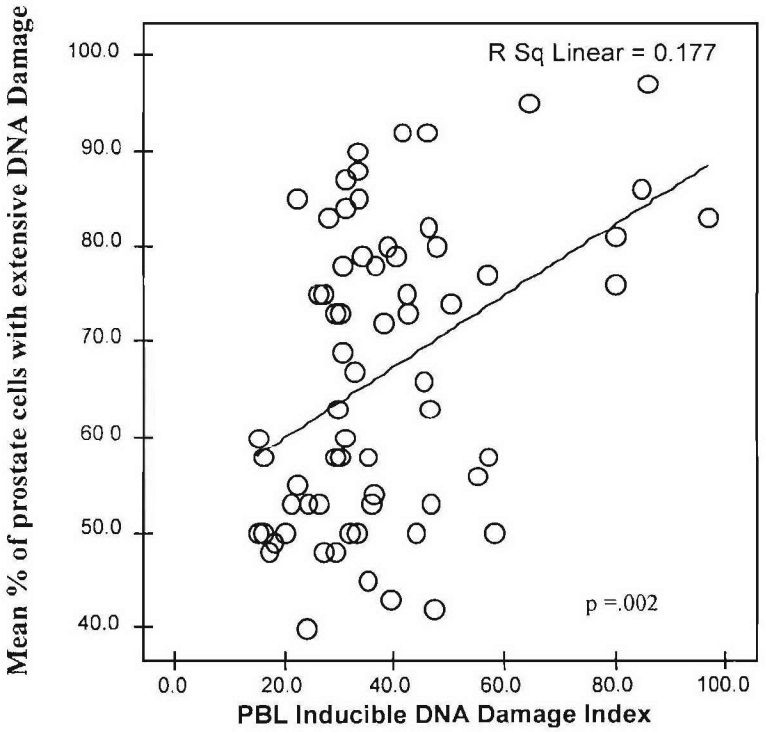
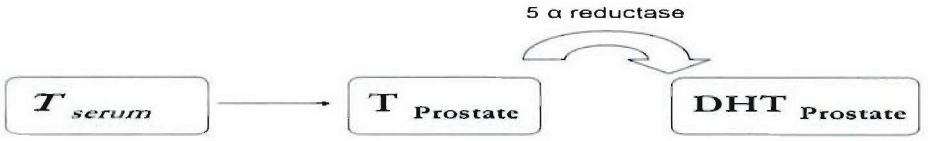


Figure 6. Correlation between H_2O_2 - inducible DNA Damage in Peripheral Blood Lymphocytes (PBL) and Extent of DNA Damage in the Prostate of 67 Dogs.

3C. Is Serum Testosterone Concentration a Useful Predictor of Intraprostatic Concentration of Testosterone (T) or Dihydrotestosterone (DHT)?



DHT levels in the prostate result from the enzymatic conversion of testosterone to DHT by 5α-reductase. As expected, we found a significant positive correlation between testosterone in the prostate and DHT in the prostate **(Table 3, Figure 7A)**. In control dogs, there was a relatively weak but significant correlation between serum testosterone and DHT concentration in the prostate **(Table 3, Figure 7B)**. In contrast, in selenium treated dogs, serum testosterone poorly reflected both intraprostatic concentration of testosterone and DHT (r=0.06 and r=0.05, respectively) **(Figure 7C and 7D)**.

Table 3. Correlation Between Serum Testosterone and Intraprostatic Androgen Concentration.

	Serum Testosterone v/s Testosterone in the Prostate		Serum Testosterone v/s Dihydrotestosterone in the Prostate		Testosterone in the Prostate v/s Dihydrotestosterone in the Prostate	
Controls Phase I and Phase II (n=20)	r=.280	p=.23	r=.484*	p=.03	r=.582*	p=.007
Se treated dogs Phase I and Phase II (n=48)	r=.057	p=.70	r=.051	p=.73	r=.344*	p=.02
Control + Se treated dogs Phase I and Phase II (n=68)	r=.169	p=.17	r=.214	p=.08	r=.456*	p=.000

* Coefficients that are statistically significant (p<0.05)

With selenium treatment, the relationship between testosterone and DHT in the prostate was weakened but remained statistically significant. Taken together these data are consistent with the hypothesis that selenium alters the androgen milieu within the prostate, and that this perturbation likely has a non-linear relationship with the biological dose of selenium.

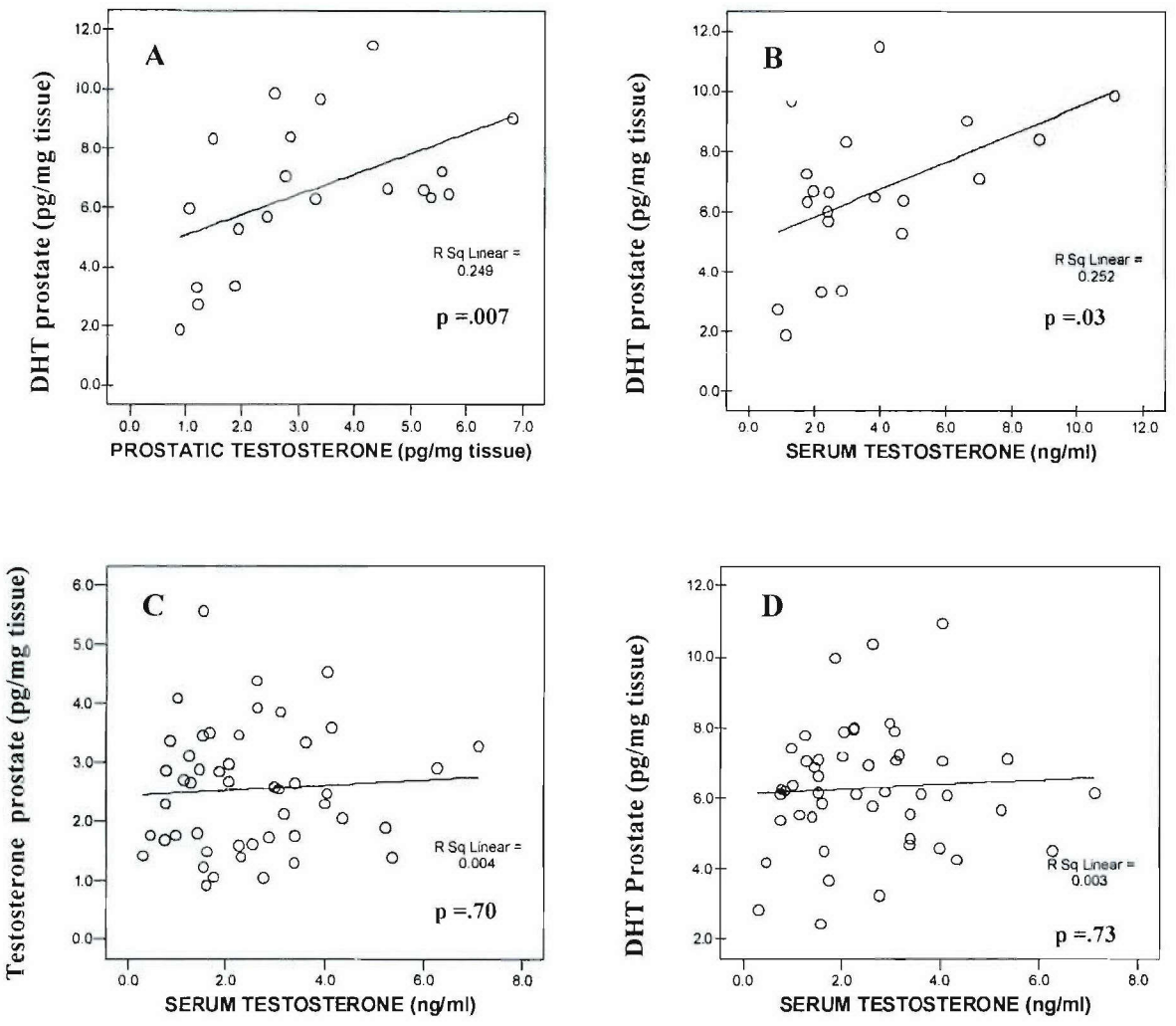


Figure 7.

A. Correlation Between Serum Testosterone and DHT in Prostate of Control Dogs (n=20).

B. Correlation Between Prostatic Testosterone and DHT in Prostate of Control Dogs (n=20).

C. Correlation Between Serum Testosterone and Prostatic Testosterone in Selenium-treated Dogs (n=48).

D. Correlation Between Serum Testosterone and DHT in Prostate in Selenium-treated Dogs (n=48).

To explore further this notion, we combined dogs in the control group and selenium treated groups (n=68) and subdivided these dogs into tertiles on the basis of biological exposure of selenium i.e. low, medium and high levels of selenium within the prostate. We used selenium concentration in the prostate as a measure of biological dose because it integrates: dietary selenium intake; selenium absorption and biodistribution; and prostatic uptake, incorporation, and excretion of selenium.

Based upon a comparison of the toenail selenium concentration in these dogs and in men, we concluded that this population of dogs had a range of selenium status that mimicked the range seen in US men. It should be noted that the range of *intraprostatic* selenium concentration in U.S men is not known, because data on selenium concentration in human prostate tissue are limited to three small studies [6][7][8]. Our analysis showed that over a broad range of intraprostatic selenium concentration there were non-linear, U-shaped relationships between: prostatic selenium concentration and testosterone concentration within the prostate (p=.07, ANOVA) **(Figure 8A);** and prostatic selenium concentration versus DHT : testosterone ratio within the prostate (p=.18, ANOVA) **(Figure 8B)**.

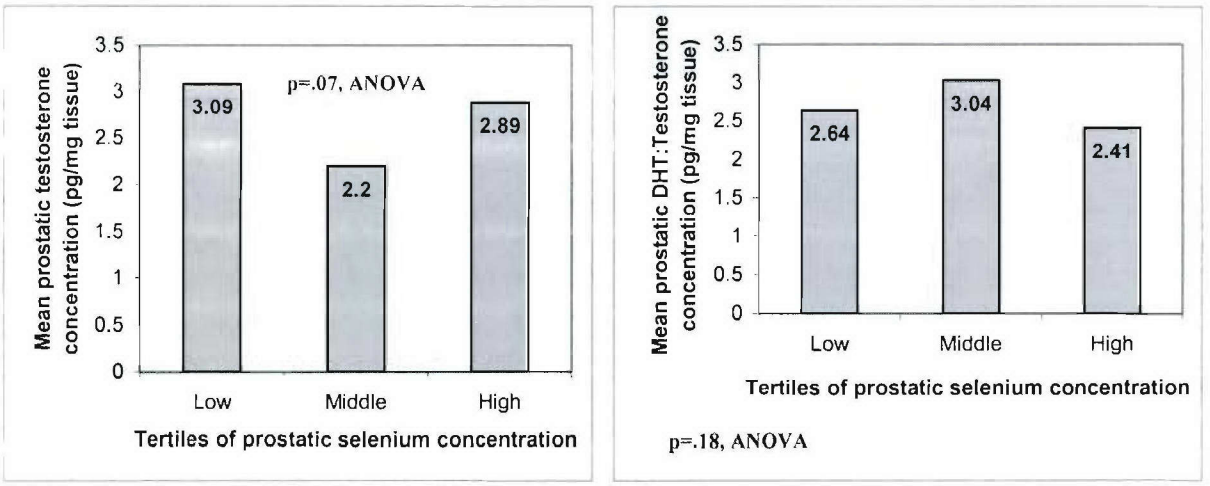


Figure 8. (A) Mean Prostatic Testosterone Levels According to Tertiles of Selenium Concentration in the Prostate (n=67 dogs). (B) Mean Prostatic DHT : Testosterone Levels According to Tertiles of Selenium Concentration in the Prostate (n=67 dogs).

3D. Does Selenium Status Significantly Influence the Androgen Milieu within the Prostate?

There is a strong impression that lifelong androgenic activity within the prostate significantly contributes to prostate cancer progression. However, little is known about the extent to which specific dietary factors can modify or influence the absolute or relative concentration of intraprostatic androgens. Studies in human subjects are ill-suited to directly test these hypotheses because of the difficulty in obtaining prostate tissue for hormone assay. The essential trace mineral selenium is currently being evaluated as a prostate cancer preventive agent, so we studied the effects of selenium supplementation on the androgen milieu within the aging prostate.

Using the dog model, we tested the hypothesis that selenium exerts its anticancer effects by significantly reducing intraprostatic concentrations of androgens. Using radioimmunoassay, we measured the concentration of testosterone (T) and dihydrotestosterone (DHT) in snap-frozen prostate tissue samples obtained at necropsy from dogs after 6 months treatment. We determined the effect of dietary selenium on the following measures of androgen production and metabolism: serum T; intraprostatic T and DHT concentration; intraprostatic DHT : T ratio; prostate T : serum T ratio.

First, we compared these androgen parameters in control dogs (n=20) versus selenium treated dogs (n=48) **(Table 4)**. There was a trend toward lower serum and intraprostatic testosterone levels in selenium-treated dogs (p=.07 and p=.14, respectively). Mean serum testosterone concentration in selenium-treated dogs was 35% lower than in controls; mean intraprostatic levels of testosterone in selenium treated dogs was 21% lower than control dogs. No other differences between treatment groups were noted; mean DHT concentration was similar in both groups.

Table 4. The Influence of Selenium Treatment on Measures of Androgen Status in Elderly Dogs.

Androgen Parameters	Control Dogs (N=20) Mean (SD)	Selenium Treated Dogs (N=48) Mean (SD)	T-test p-value
Serum Testosterone	3.71 (2.73)	2.49 (1.51)	.07
Testosterone in Prostate	3.20 (1.80)	2.55 (1.04)	.14
DHT in Prostate	6.60 (2.49)	6.28 (1.75)	.54
Testosterone in Prostate: Serum Testosterone	1.18 (0.85)	1.51 (1.18)	.25
DHT in Prostate: Testosterone in Prostate	2.51 (1.33)	2.75 (1.04)	.42
Difference in Serum Testosterone Levels Between 0 and 6 Months	-.39 (3.90)	-.80 (2.20)	.67

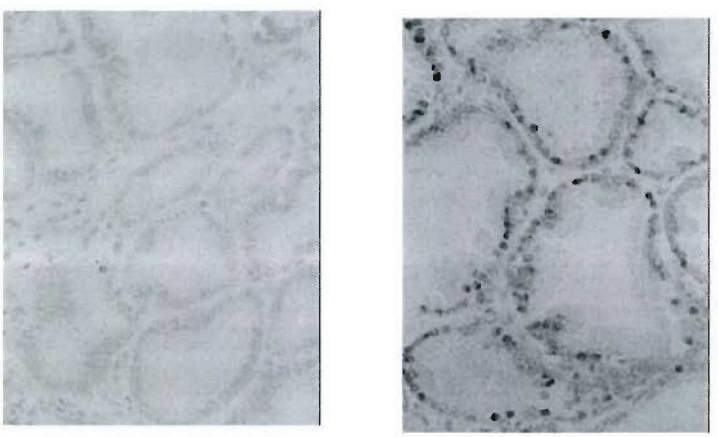
To further evaluate the influence of selenium on androgens, we subdivided the 68 dogs into tertiles on the basis of their prostatic selenium levels and then analyzed for differences in androgen status in the low, middle and high prostatic selenium tertiles. Dogs in the low selenium tertile had the highest serum and intraprostatic testosterone levels **(Figure 8)**. Interestingly, dogs in the middle selenium tertile had the lowest serum and prostatic testosterone levels and the highest $DHT_{Prostate}$: $T_{Prostate}$ **(Figure 8A and 8B)**. The ratio of $DHT_{Prostate}$: $T_{Prostate}$, a surrogate of 5α-reductase activity in the prostate, was lowest in the high selenium tertile (p=.18 ANOVA). Our data suggest that across a range of intraprostatic selenium concentration achievable through dietary selenium supplementation, intraprostatic DHT concentration appears to be quite stable, despite intriguing non-linear trends in serum and prostatic concentration of testosterone and the ratio of $DHT_{Prostate}$: $T_{Prostate}$ ratio.

In summary, changes in dietary selenium intake may influence certain aspects of prostatic intracrinology. However, supranutritional selenium supplementation does not significantly increase or decrease overall androgenic activity within the aging prostate. We conclude that the cancer suppressive effects of selenium are not likely mediated by changing androgen levels within the prostate.

3E. Is Selenium Supplementation Associated with an Upregulation of Prostatic Epithelial Cell Apoptosis In Vivo?

Cell culture studies have shown that selenium can induce apoptosis in a wide variety of cell types, including prostate cancer cells [9]. However, there is no evidence confirming that dietary selenium supplementation can actually increase apoptosis in prostate cells *in vivo*.

In our dog studies, we could directly address this knowledge gap by comparing the amount of epithelial cell apoptosis of control dogs versus dogs supplemented for 6 months with supranutritional levels of dietary selenium. In control dogs receiving a nutritionally adequate intake of dietary selenium, there was a low level of prostatic epithelial cell apoptosis **(Figure 9A)**. Selenium treated dogs had increased apoptosis compared to control dogs (mean number of apoptotic cells per 200 X microscopic field was 3.26 versus 0.76 in control dogs; $p = .10$, t-test). In some dogs, we observed foci of intense apoptosis ≥ 30 times higher than the level seen in typical microscopic fields **(Figure 9B)**. Interestingly, the frequency of these apoptotic "hot spots" was much higher in dogs receiving supranutritional selenium intake. Apoptotic hot spots were seen in 18 of 46 (39%) selenium treated dogs, whereas hot spots occurred in only 2 of 19 (11%) control dogs ($p=.02$, chi-square).



A. Prostate from Control Dog (no selenium treatment) (200X)

B. Prostate from Selenium-Treated Dog (200X)

Figure 9. Daily Selenium Supplementation of Elderly Beagle Dogs for 7 Months is Associated with Upregulation of Apoptosis in Prostate Epithelial Cells.

What is the biological importance of these apoptotic hot spots? To investigate this, dogs were subdivided into tertiles on the basis of DNA damage within the prostate. Dogs with the highest DNA damage had the lowest frequency of apoptotic hot spots **(Figure 10)**. This provides further support for the hypothesis that high levels of apoptosis are associated with a reduction in the extent of DNA damage within the prostate. A more complete understanding of the biological significance of these foci of intense apoptosis within the prostate will be pursued in future investigations.

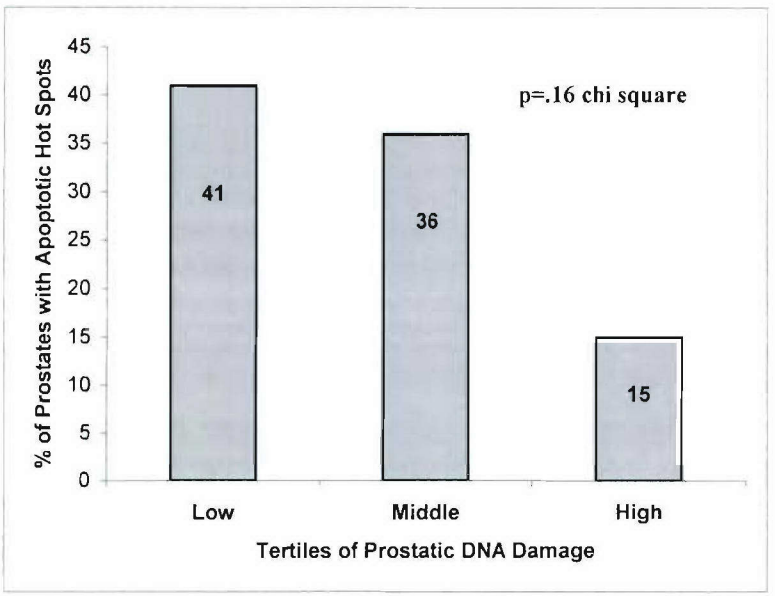


Figure 10. Frequency of Apoptotic Hotspots in Dogs Subdivided According to Low, Middle and High Level of Prostatic DNA Damage.

3F. Does the Level of Androgens within the Prostate Contribute to the Extent of DNA Damage in the Prostate?

Evidence from cell culture experiments suggests that androgens may contribute to the genotoxic stress within the prostate through the generation of reactive oxygen species [10]. However, this hypothesis has not been previously tested *in vivo*.

To test this hypothesis, we compared the extent of prostatic DNA damage (assessed by alkaline Comet assay) in selenium-treated and control dogs that were subdivided into tertiles on the basis of testosterone concentration (low, medium, high) or DHT concentration (low, medium, high) within the prostate. We found that prostatic DNA damage was not significantly affected by intraprostatic testosterone concentration (p=.82, ANOVA) **(Figure 11A)**. In contrast, dogs with the highest DHT concentration within the prostate had significantly greater prostatic DNA damage than dogs with lowest level of intraprostatic DHT (p=.05, ANOVA) **(Figure 11B)**.

To our knowledge, this represents the first *in vivo* evidence that androgens contribute to DNA damage in prostate cells.

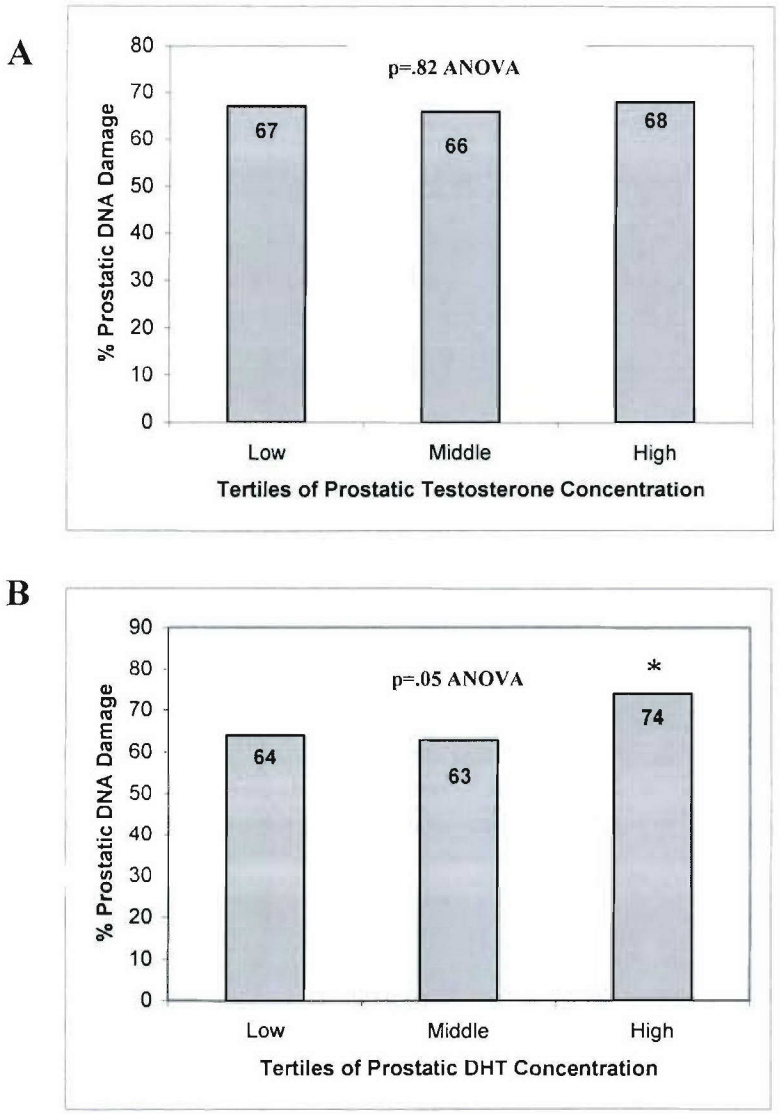


Figure 11. The Association Between Intraprostatic Levels of Testosterone (A) or DHT (B) and the Extent of Prostatic DNA Damage.

3G. Does Selenium Supplementation Induce Prostatic Epithelial Cell Replicative Senescence?

Cells that undergo replicative senescence irreversibly lose their ability to undergo cell division. An intervention that induces replicative senescence in prostatic epithelial cells would be expected to exert an anti-cancer effect on the prostate. It is not known whether dietary supplementation with selenium significantly influences the extent of replicative senescence within the aging prostate.

We sought to test this hypothesis by determining the extent of replicative senescence in prostate tissue by using the senescence-associated βeta-galactosidase (SAβ gal) staining technique of Dimri et al [11]. In this assay, senescent cells stain positive for the enzyme SAβ gal at pH=6. Unfortunately, we were not able to generate meaningful data from this analysis, since SAβ gal deteriorates over time in tissue specimens — even in snap-frozen tissue stored in liquid nitrogen.

3H. *Are Low Circulating Levels of Testosterone Associated With Lower Level of DNA Damage within the Brain?*

To answer this question, we subdivided 67 dogs (control and selenium-treated dogs from Phase I and Phase II) into tertiles on the basis of serum testosterone concentration. Then we compared DNA damage (alkaline Comet assay) in cerebrum of dogs in low, medium and high serum testosterone groups. Our analysis showed that dogs with lowest serum testosterone had the lowest brain DNA damage (p=.01, Kruskal-Wallis test) **(Figure 12)**. These data from brain cells further support the hypothesis that androgens significantly contribute to DNA damage *in vivo*.

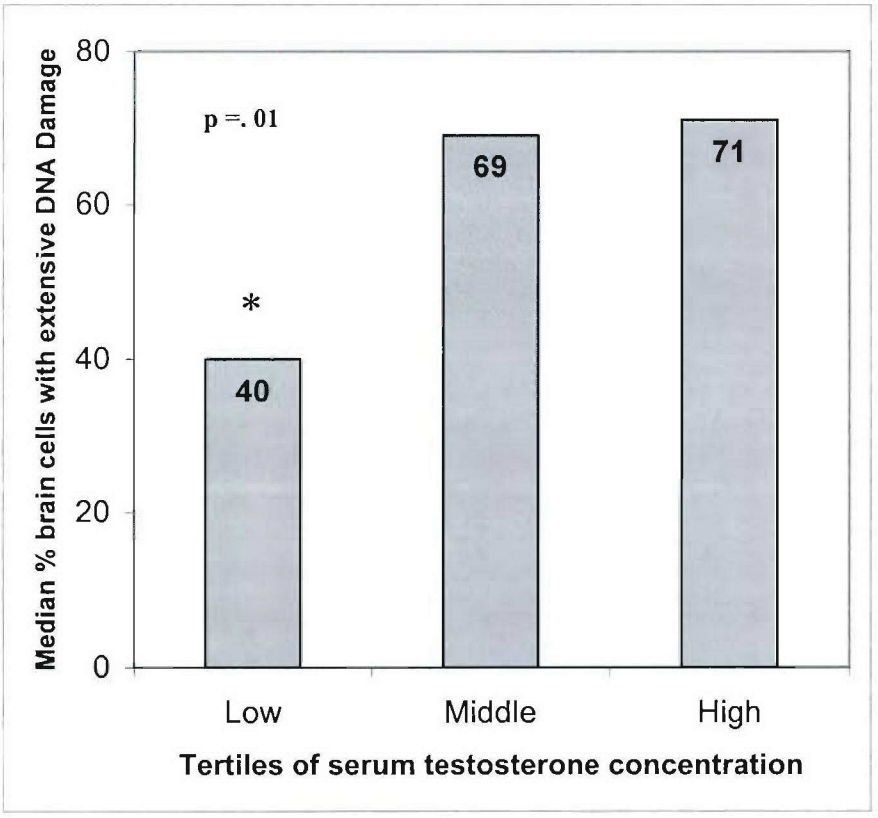


Figure 12. Brain DNA Damage According to Tertiles of Serum Testosterone Concentration. The range (median) for Low, Middle and High Tertiles were 0.30-1.73 (1.25), 1.75-3.09 (2.41), 3.09-11.10 (4.22) ng/ml, respectively.

KEY RESEARCH ACCOMPLISHMENTS

- In elderly beagle dogs, there is a non-linear, U-shaped relationship between selenium status and accumulation of DNA damage within the prostate.

- This dose : response curve indicates a relatively narrow optimal range of selenium that maintains prostatic homeostasis, i.e. more selenium is not necessarily better.

- The optimal selenium status predicted by the dog model appears to have implications for human health, because men with the lowest risk of prostate cancer in the Health Professionals Follow-Up Study had a median toenail selenium concentration of 0.82 ppm, a value that falls within the optimal range predicted by the dog model.

- Furthermore, the dog dose : response curve provides a biological explanation for the findings from Dr. Larry Clark's Nutritional Cancer Prevention Trial — baseline selenium status prior to supplementation predicts whether or not selenium supplementation leads to a reduction in prostate cancer risk.

- The U-shaped dose : response between selenium status and DNA damage also holds true for the brain, not just for the prostate.

- Measuring DNA damage in peripheral blood lymphocytes does not appear to be a reliable method to predict the extent of DNA damage within the aging prostate gland. Our data suggests that selenium concentration in toenails provides a more useful predictor of genotoxicity within the prostate.

- Dietary selenium supplementation has minimal effects on intraprostatic concentration of dihydrotestosterone (DHT). Interestingly, dogs with medium range selenium levels in their prostate have the lowest intraprostatic testosterone levels.

- Serum testosterone is a weak but significant predictor of intraprostatic DHT levels in dogs receiving nutritionally adequate levels of selenium. However, serum testosterone does not predict DHT level in the prostate of dogs receiving supranutritional selenium supplementation. This suggests that the utility of serum testosterone as an indicator of prostatic androgen activity may be dependent upon dietary selenium status.

- Selenium supplementation is associated with an increased frequency of intense apoptosis, so-called apoptotic "hot spots". The significance of these apoptotic hot spots is unknown, but our data provide the strongest evidence to date that dietary selenium supplementation can upregulate prostatic epithelial cell apoptosis *in vivo*.

- Selenium status does not significantly influence the anti-trophic effect of the 5α-reductase inhibitor finasteride on the aging prostate.

- There is a strong correlation between selenium concentration in toenails and prostate tissue. The correlation between selenium content of toenails and brain tissue is weaker.

- The extent of DNA damage in the brain of elderly dogs is lowest in dogs with the lowest serum testosterone levels. This finding supports the notion that androgens may contribute to DNA damage *in vivo*. This has important clinical implications because minimizing DNA damage is a goal common to developing effective anti-aging and cancer prevention strategies.

REPORTABLE OUTCOMES

Manuscripts

Bostwick DG, Adolfsson J, Burke HB, Damber JE, Huland H, Kattan MW, Pavone-Macaluso M, Waters DJ. Report of the committee on epidemiology and statistical methods: World Health Organization's 2004 international consultation on prostate cancer. Epidemiology and statistical methods in prediction of patient outcome. *Scand J Urol Nephrol* 2005; 216: 94-110.

Waters DJ, Shen S, Glickman LT, Cooley DM, Bostwick DG, Qian J, Combs GF Jr, Morris JS. Prostate cancer risk and DNA damage: translational significance of selenium supplementation in a canine model. *Carcinogenesis* 2005; 26(7):1256-1262.

Waters DJ, Chiang EC, Cooley DM, Morris JS. Making sense of sex and supplements: differences in the anticarcinogenic effects of selenium in men and women. *Mutat Res* 2004; 551:91-107.

Bostwick DG, Burke HB, Djakiew D, Euling S, Ho SM, Landolph J, Morrison H, Sonawane B, Shifflett T, Waters DJ, Timms B. Human prostate cancer risk factors. *Cancer* 2004;101(10 Suppl):2371-490.

Waters DJ, Shen S, Cooley DM, Bostwick DG, Qian J, Combs GF Jr, Glickman LT, Oteham C, Schlittler DL, Morris JS. Effects of dietary selenium supplementation on DNA damage and apoptosis in canine prostate. *J Natl Cancer Inst* 2003; 95:237-41.

Five additional manuscripts originating directly from this research are currently being prepared for publication. It is anticipated that preparation of these manuscripts will be completed in 2005.

Published Scientific Abstracts

Shen S, Cooley DM, Schlittler D, Oteham C, Chen Y, Chiang EC, Morris JS, Glickman LT, Bostwick DG, Waters DJ. Effect of dietary selenium intake on androgen levels within the aging prostate. *Cancer Epidemiology Biomarkers & Prevention* 2004; 13: 1836s.

Press Releases

Does Selenium Prevent Prostate Cancer? By Kathleen Wildasin. About.cancer.com, July 10, 2003.

Good News for Men (and Dogs) by Susan Edmiston. Alternative Medicine, October 2003, pp. 45-46.

Does Selenium Reduce the Risk of Developing Prostate Cancer? By Kathleen Wildasin, US TOO International Hot Sheet, October 2003.

Patents

None

Poster and Oral Presentations

Invited Lectures

Relationship Between Selenium Status and the Extent of Genotoxic Stress within the Aging Prostate. International Conference on Antimutagenesis and Anticarcinogenesis, Pisa, ITALY, November 2003.

Prostate Cancer Risk and DNA Damage: Translational Significance of Selenium Supplementation in a Canine Model. Keynote Speaker, University of Missouri Research Reactor (MURR) Science Day, Columbia, MO, May 2004.

Comparative Oncology: A New Path to Progress. Animal Models Initiative, National Cancer Institute, Bethesda, MD, August 2004.

Modeling the Aging Prostate: Implications for Prostate Cancer Prevention. Aging and Cancer Symposium of the 7th International Conference of Anticancer Research, Corfu, GREECE, October 2004.

Comparative Aspects of Prostate Cancer. Wake Forest University Medical School, Winston-Salem, NC, April 2005.

Selenium, DNA Damage and Prostate Cancer. Oklahoma Medical Research Foundation, Oklahoma City, OK, June 2005.

The Public Health Significance of Selenium and Cancer Prevention. University of Modena, Modena, ITALY, June 2005.

Posters Presented at National and International Meetings

Is the Anti-Trophic Effect of the 5α-Reductase Inhibitor Finasteride on the Aging Prostate Influenced by Selenium Status? American Association for Cancer Research Frontiers of Cancer Prevention, Phoenix, AZ, October 2003

Effect of Dietary Selenium Intake on Intraprostatic Androgen Levels Within the Aging Prostate. American Association for Cancer Research Frontiers of Cancer Prevention, Seattle, WA, October 2004

Other: Graduate Education

Based upon the significant research progress made by our group and others in better understanding the anticancer effects of the trace mineral selenium, Dr. Waters developed a graduate level course at Purdue University "Selenium in Health and Disease". The course focused on the relationship between selenium status and cancer risk, particularly the risk of prostate cancer. Discussions addressed the issues of measurement and epidemiology, mechanisms studied using *in vitro* and *in vivo* animal models, and interventional studies with human subjects. Students were enrolled in this 3 credit hour course for the first time in the Spring 2004 semester.

CONCLUSIONS

During the next 10 years, the National Cancer Institute sponsored SELECT trial will study more than 32,000 men to evaluate whether selenium +/- vitamin E will decrease the incidence of human prostate cancer. However, the mechanisms by which selenium modulates key events in multi-step prostatic carcinogenesis are unknown. Our work using the dog model has yielded the first evidence that daily selenium supplementation can significantly decrease DNA damage within the aging prostate [12]. Furthermore, we showed for the first time that selenium can upregulate apoptosis of prostatic epithelial cells *in vivo* [12]. Moreover, our discovery of a non-linear, U-shaped dose : response relationship between selenium and genotoxic damage suggests that not all men will benefit from selenium supplementation. It follows from this new understanding that more selenium is not necessarily better and measurement of baseline nutrient status should be required for all individuals in prevention trials to avoid oversupplementation. Our research addresses a key underexplored area – the further development of an animal model system to study the effects of potential chemopreventive agents on cellular processes that regulate human prostatic carcinogenesis. By studying the response of prostate cells in an appropriate context – *in vivo* within an aging prostate gland – our work has provided new insights into the complex dose : response relationship between selenium, genotoxic stress, and carcinogenesis within the aging prostate. Importantly, we have shown that the response of the human prostate to the anticarcinogenic effects of selenium can be correctly predicted using cost effective, short-term studies in dogs, the non-human species most prone to prostate cancer development [13]. Our experimental paradigm represents a new approach to bridge the gap between laboratory and human studies that can be used to find the appropriate dose of cancer-fighting nutrients for large-scale human cancer prevention trials to reduce prostate cancer mortality. In addition to studying the prostate, our research also addressed the effects of anti-cancer interventions on essential organs, such as the brain. As a result, we have generated tantalizing evidence that supports the notion that androgens contribute significantly to DNA damage within the aging brain and prostate. Capitalizing on the insights gained from our studies funded by the Department of Defense Prostate Cancer Research Program, we are now uniquely positioned to extend these investigations using the dog model to further develop selenium and other agents as practical prostate cancer prevention strategies.

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APPENDIX

Manuscripts

Waters DJ, Shen S, Glickman LT, Cooley DM, Bostwick DG, Qian J, Combs GF Jr, Morris JS. Prostate cancer risk and DNA damage: translational significance of selenium supplementation in a canine model. *Carcinogenesis* 2005; 26:1256-1262.

Waters DJ, Chiang EC, Cooley DM, Morris JS. Making sense of sex and supplements: differences in the anticarcinogenic effects of selenium in men and women. *Mutat Res* 2004; 551:91-107.

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Scientific Abstracts

Is the Anti-Trophic Effect of the 5α-Reductase Inhibitor Finasteride on the Aging Prostate Influenced by Selenium Status? Cooley DM, Shen S, Oteham C, Schlittler D, Glickman LT, Bostwick DG, Morris JS, Combs Jr GF, Waters DJ. American Association for Cancer Research Frontiers of Cancer Prevention, Phoenix, AZ, October 2003

Relationship Between Selenium Status and the Extent of Genotoxic Stress within the Aging Prostate. Waters DJ, Shen S, Cooley DM, Bostwick DG, Qian J, Glickman LT, Morris JS. International Conference on Antimutagenesis and Anticarcinogenesis, Pisa, ITALY, November 2003

Prostate Cancer Risk and DNA Damage: Translational Significance of Selenium Supplementation in a Canine Model. Waters DJ. University of Missouri Research Reactor (MURR) Science Day, Columbia, MO, May 2004

Effect of Dietary Selenium Intake on Intraprostatic Androgen Levels Within the Aging Prostate. Shen S, Cooley DM, Schlittler D, Oteham C, Chen Y, Chiang EC , Morris JS, Glickman LT, Bostwick DG, Waters DJ. American Association for Cancer Research Frontiers of Cancer Prevention, Seattle, WA, October 2004

Can Peripheral Blood Lymphocytes Provide A Window To Assess The Extent Of Genotoxicity Within The Prostate? Shen S, Cooley DM, Schlittler D, Chen Y, Chiang E, Bostwick DG, Morris JS, Glickman LT, Waters DJ. Environmental Mutagen Society Meeting, San Francisco, CA, September 2005

Carcinogenesis vol.26 no.7 pp.1256–1262, 2005
doi:10.1093/carcin/bgi077
Advance Access publication April 7, 2005

Prostate cancer risk and DNA damage: translational significance of selenium supplementation in a canine model

David J.Waters[1,6,*], Shuren Shen[1,6], Lawrence T.Glickman[2], Dawn M.Cooley[1,6], David G.Bostwick[3], Junqi Qian[3], Gerald F.Combs Jr[4] and J.Steven Morris[5]

[1]Department of Veterinary Clinical Sciences and [2]Department of Veterinary Pathobiology, Purdue University, West Lafayette, IN 47907, USA, [3]Bostwick Laboratories, Richmond, VA 23294, USA, [4]Division of Nutritional Sciences, Cornell University, Ithaca, NY 14853, USA, [5]University of Missouri-Columbia Research Reactor Center, Columbia, MO 65211, USA and [6]Gerald P.Murphy Cancer Foundation, West Lafayette, IN 47906, USA

*To whom correspondence should be addressed at: Gerald P.Murphy Cancer Foundation, 3000 Kent Avenue, Suite E2100, West Lafayette, IN 47906, USA. Tel: +1 765 494 9271; Fax: +1 765 775 1006; Email: dwaters@gpmcf.org

Daily supplementation with the essential trace mineral selenium significantly reduced prostate cancer risk in men in the Nutritional Prevention of Cancer Trial. However, the optimal intake of selenium for prostate cancer prevention is unknown. We hypothesized that selenium significantly regulates the extent of genotoxic damage within the aging prostate and that the relationship between dietary selenium intake and DNA damage is non-linear, i.e. more selenium is not necessarily better. To test this hypothesis, we conducted a randomized feeding trial in which 49 elderly beagle dogs (physiologically equivalent to 62–69-year-old men) received nutritionally adequate or supranutritional levels of selenium for 7 months, in order to mimic the range of dietary selenium intake of men in the United States. Our results demonstrate an intriguing U-shaped dose–response relationship between selenium status (toenail selenium concentration) and the extent of DNA damage (alkaline Comet assay) within the prostate. Further, we demonstrate that the concentration of selenium that minimizes DNA damage in the aging dog prostate remarkably parallels the selenium concentration in men that minimizes prostate cancer risk. By studying elderly dogs, the only non-human animal model of spontaneous prostate cancer, we have established a new approach to bridge the gap between laboratory and human studies that can be used to select the appropriate dose of anticancer agents for large-scale human cancer prevention trials. From the U-shaped dose–response, it follows that not all men will necessarily benefit from increasing their selenium intake and that measurement of baseline nutrient status should be required for all individuals in prevention trials to avoid oversupplementation.

Introduction

Epidemiologic data suggest that many people could substantially reduce their cancer risk through relatively simple dietary changes, including supplementation with non-toxic doses of cancer-fighting nutrients (1,2). Prostate cancer is the second leading cause of male cancer-related mortality in the United States (3) and the identification of safe, non-toxic compounds for the prevention of prostate cancer is considered a high research priority. Selenium, an essential nutrient required for a number of metabolically important enzymes, inhibits cancer development in a variety of experimental animal models (4–6). In 1996, Clark *et al.* (7) reported the results of the Nutritional Prevention of Cancer Trial, a 13-year, randomized, placebo-controlled study of older Americans. In this study, daily supplementation of 200 μg of selenium in the form of selenium-enriched yeast was associated with a significant reduction in the risk of several cancers, most notably cancer of the prostate (63% risk reduction) (7). These results suggested that a significant reduction in cancer risk could be achieved using dietary supplementation with low, non-toxic doses of selenium and/or selenium fortification of foods.

The use of selenium supplements in the USA has grown steadily over the last 20 years, both in the number of adults who use supplements and in the amount consumed daily. But, health professionals seldom recommend that supplement users test their nutrient status prior to or after taking supplements. Growing interest in selenium as a prostate cancer preventive agent has led to a large intervention trial, selenium and vitamin E cancer prevention trial (SELECT), that is currently enrolling >32 000 men and will require 12 years to complete (8). However, it is not known what form or dose of selenium offers the most potent prostate cancer protective effects, or whether too much selenium supplementation might be harmful. Observational data from men in the Health Professionals Follow-up Study showed a strong inverse association between selenium status, as measured by toenail selenium concentration, and the risk for developing advanced prostate cancer (9). However, multivariate analysis demonstrated an apparent threshold effect, with no additional prostate cancer protective effect at toenail concentrations exceeding 0.82 p.p.m. In another study, Brooks *et al.* (10) found a similar threshold effect. Taken together, these data suggest that not all men will necessarily benefit from increasing their dietary intake of selenium.

It has been previously hypothesized that the cancer-protective effects of selenium are related to its ability to limit the accumulation of genotoxic damage within the aging prostate (11,12). However, the optimal intake of selenium or other nutrients necessary to protect the prostate from cancer is unknown because previous human and animal studies have not adequately defined the relationship between nutrient dose and genotoxic damage within the prostate. In this study, we tested the hypothesis that the relationship between selenium intake and DNA damage within the prostate and brain is non-linear, i.e. that more selenium is not necessarily better. We studied elderly beagle dogs, that were physiologically equivalent to 62–69-year-old men and free of prostate cancer, to simulate the aging human prostate and to define the dose of

Abbreviations: DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; BSS, Hanks' balanced salt solution; SELECT, selenium and vitamin E cancer prevention trial.

selenium that minimizes genotoxic damage within the prostate. This animal model was used because the influence of aging on prostatic carcinogenesis appears similar in dogs and men, the only two species in which prostate cancer occurs spontaneously with appreciable frequency (13,14). Finally, to determine whether the dose–response data from this animal model were relevant to human health, we compared our results with published data on selenium status and human prostate cancer risk from the Health Professionals Follow-up Study and the Nutritional Prevention of Cancer Trial.

Materials and methods

Experimental animals and study design

In a randomized controlled feeding trial, elderly beagle dogs, physiologically equivalent to 62–69-year-old men (15), received diets containing nutritionally adequate or supranutritional levels of selenium for 7 months to produce a range of dietary selenium exposures similar to that of healthy adult men in USA. Forty-nine elderly (8.5–10.5-year-old) sexually intact male, retired breeder dogs weighing 9–18 kg were purchased from a local supplier. After 4 weeks acclimation, dogs were randomly assigned to a control group ($n = 10$ dogs) or four daily treatment groups: 3 μg/kg/day selenomethionine (L-selenomethionine, Solgar Vitamin and Herb, Leonia, NJ) ($n = 10$ dogs), 6 μg/kg/day selenomethionine ($n = 10$ dogs), 3 μg/kg/day high selenium yeast (SelenoExcell®, Cypress Systems, Fresno, CA) ($n = 10$ dogs) and 6 μg/kg/day high selenium yeast ($n = 9$ dogs). The selenium in the high-selenium yeast product is mostly (~85%) selenomethionine. However, unlike the free L-selenomethionine product, the yeast form is protein-bound. All dogs had nutritionally adequate selenium status confirmed by plasma selenium concentration prior to the start of the experiment. Throughout the experiment, all dogs were fed a selenium-adequate maintenance diet (0.3 p.p.m as fed basis; Science Diet® Canine Maintenance, Hills Pet Nutrition, Topeka, KS). In the control group, daily selenium intake was ~6 μg/kg body weight. After 7 months of treatment, all dogs were euthanized in accordance with guidelines set forth by the American Veterinary Medical Association Panel on Euthanasia (16). All aspects of this experimental protocol were approved by the Purdue University Animal Care and Use Committee.

Measurement of genotoxic damage within the prostate

Within 15 min of euthanasia, the prostate was collected at necropsy and 50–80 mg of prostate tissue was placed in 1 ml of cold Hanks' balanced salt solution (HBSS) containing 20 mM EDTA and 10% dimethyl sulfoxide (DMSO) (17). The tissue was then minced with fine scissors and 50 μl of cell suspension was mixed with 1 ml of RPMI 1640 media containing 10% fetal bovine serum (FBS) for subsequent electrophoresis. Cytospin preparations indicated that >90% of cells had epithelial cell morphology; mean percentage cell viability estimated by trypan blue exclusion was 80%. Histopathologic evaluation of formalin-fixed, step-sectioned prostate tissue sections revealed no foci of carcinoma.

The extent of DNA damage in prostate cells, which is an index of oxidative stress and other genotoxic influences within the prostate, was measured by single cell gel electrophoresis (alkaline Comet assay) using a method previously described (17). Under the conditions of this experiment, the comet tail reflects the electrophoretic migration of DNA fragments resulting from strand breaks, alkali labile sites, crosslinks or base excision repair sites (18). The extent of DNA damage was scored in 100 randomly selected cells from each sample (50 cells from several different fields from each of two replicate slides) by an examiner who was blinded to treatment group. Analysis was performed by one slide reader (SS), thus minimizing variability attributable to intersubject scoring. SYBR green I-stained nucleoids were examined at 200× magnification with an Olympus epifluorescent microscope. Each cell was visually scored on a 0–4 scale according to its appearance using a method described by Collins (19,20) as follows: no damage (type 0), mild to moderate damage (types 1 and 2) and extensive DNA damage (types 3 and 4) (Figure 1). Using this scoring method, the extent of DNA damage within the prostate was expressed in terms of a Comet score (range 0–400) (19) and as the percentage of cells with extensive damage (sum of types 3 and 4 cells).

Measurement of genotoxic damage in brain

Immediately after euthanasia, brain tissue from the cerebral cortex was collected via craniotomy. In all cases, interval from euthanasia to brain tissue harvest was <30 min. For each dog, 50–80 mg of brain tissue was placed in 1 ml of cold HBSS containing 20 mM EDTA and 10% DMSO. The tissue was then minced with fine scissors and 50 μl of cell suspension was mixed with 1 ml of RPMI 1640 media containing 10% FBS for the alkaline Comet assay. Pyramidal neurons were the most prevalent cell type in the tissue harvested from the cerebral cortex.

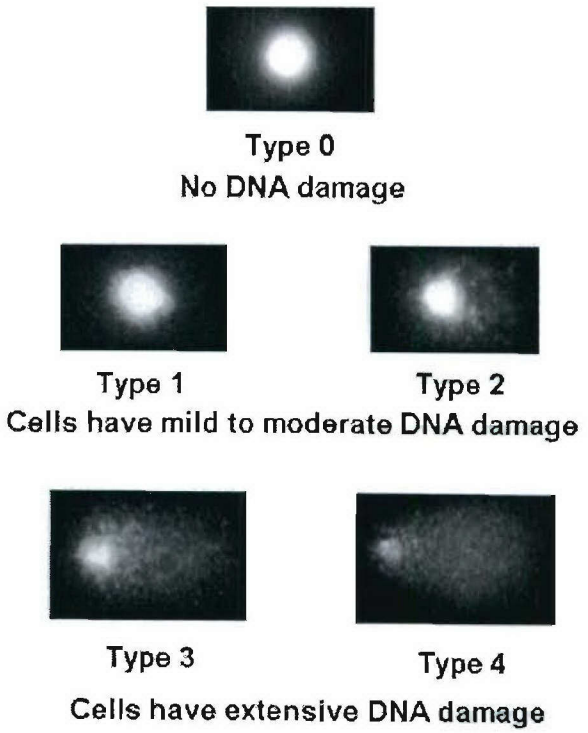

Fig. 1. Alkaline Comet assay: visual scoring method to measure the extent of DNA damage in cells.

Measurement of selenium concentration in prostate, brain and toenails

After 7 months of treatment, toenail clippings and snap frozen prostate and brain tissues were collected from dogs immediately after euthanasia. Specimens from control and selenium supplemented dogs were analyzed together, but in random order, with the supplementation status unknown to laboratory personnel. Nails (49 dogs) and tissues (25 dogs) were analyzed for selenium by instrumental neutron activation analysis at the University of Missouri-Columbia Research Reactor Center (MURR), Columbia, MO using a modification of methods previously described (21–23). Total selenium content in toenail clippings provides a reliable non-invasive measure of selenium status (24–27).

Statistical analysis

The data from selenium-treated and control dogs were combined and Pearson correlation coefficients were calculated to determine if selenium concentration within prostate or brain tissue was significantly ($P < 0.05$) correlated with toenail selenium concentration. Since the relationship between the percentage of prostate cells with extensive DNA damage and toenail selenium concentration (p.p.m) was non-linear, a polynomial regression including a quadratic term was used. To control for multicolinearity in the polynomial regression, the mean toenail selenium concentration was first subtracted from each toenail selenium concentration and then squared (28). All data analyses were done using standard statistical software [SPSS (Version 10.0, Chicago, IL) and SAS System (Version 8.1, SAS Institute, Cary, NC, 1999)].

Results

U-shaped dose–response relationship between selenium status and DNA damage in prostate and brain

There was a non-linear, U-shaped dose–response relationship between toenail selenium concentration and DNA damage within the prostate ($r^2 = 0.52$, $P < 0.0001$), with a relatively narrow range of selenium that minimized the extent of DNA damage in prostate cells (Figure 2A). When dogs with low,

moderate and high selenium status were compared, the relationship between selenium status and prostatic DNA damage could not be explained by selenium dose-dependent differences in prostatic epithelial cell proliferation or apoptosis indices or in the extent of prostatic inflammation (data not shown).

To determine whether the U-shaped relationship between selenium status and DNA damage in dogs was unique to the aging prostate, we conducted a similar analysis of DNA damage in the brain. Similar to our findings in the prostate, there was a U-shaped dose-response relationship between toenail selenium concentration and DNA damage within the aging brain. Importantly, we found the toenail selenium concentration that optimized DNA damage reduction in the prostate also minimized the extent of DNA damage within the aging brain (Figure 3A).

Toenail selenium concentration reflects selenium concentration in prostate and brain tissue

Previous studies in humans and animals did not evaluate whether differences in the tissue concentration of selenium within the prostate or brain were strongly predicted by the non-invasive measurement of selenium in toenails. In elderly dogs, we found a strong positive association between selenium concentration in toenails versus prostate ($r^2 = 0.52$; $P < 0.0001$) and brain ($r^2 = 0.53$; $P < 0.0001$); these strong associations were observed over the range of toenail selenium concentration seen in healthy adults in the USA, including the men who were likely to participate in the SELECT prostate cancer prevention trial (Figure 3B and 3C).

Dog dose-response curve parallels results from human studies

To determine whether the U-shaped dose-response in beagle dogs was relevant to human health, we compared our results with published data on selenium and human prostate cancer risk from the Health Professionals Follow-up Study (9). Toenail selenium concentrations in the lowest and highest quartiles of dogs (mean of 0.50 and 1.03 p.p.m., respectively) were similar to toenail concentrations in men (median of 0.66 p.p.m in lowest quintile; median of 1.14 p.p.m in highest quintile). Fitting the human data from the Health Professionals Follow-up Study to the dose-response curve from dogs produced an intriguing result—the same concentration of selenium that minimized prostatic DNA damage in dogs also minimized advanced prostate cancer risk in men (Figure 4). The highest risk for prostate cancer was observed in men with the lowest toenail selenium (median of 0.66 p.p.m.), which was less than the optimal concentration predicted by the dog model. The lowest risk for prostate cancer occurred in men with a median selenium level of 0.82 p.p.m., which was equivalent to the optimal concentration in the dog model. Thus, movement along the dog dose-response curve from low suboptimal to optimal selenium status (bold arrow in Figure 4) was associated with a 65% reduction in human prostate cancer risk. The canine dose-response curve also accurately predicted a cancer protection threshold, i.e. no additional reduction in prostate cancer risk with selenium >0.82 p.p.m.

We then used the canine dose-response curve to reconcile the results of the Nutritional Prevention of Cancer Trial of Clark *et al.* (7,29). In this large intervention trial, baseline selenium status prior to supplementation was strongly

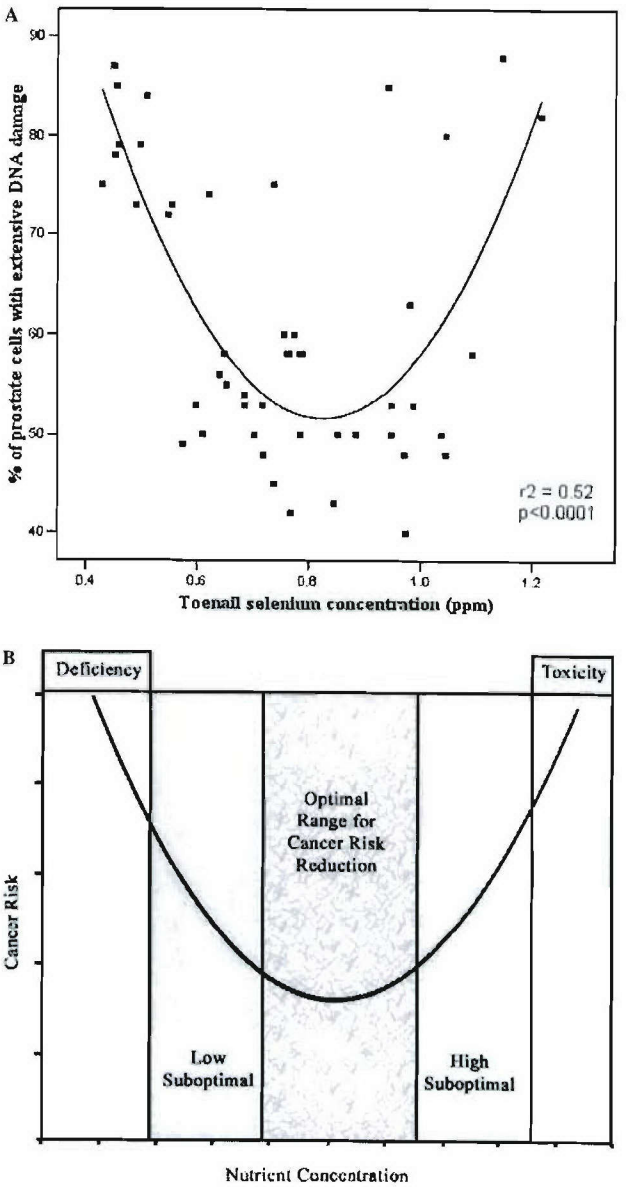

Fig. 2. A U-shaped dose–response curve defines the relationship between selenium and genotoxic stress in prostate. (**A**) U-shaped dose–response relationship between toenail selenium concentration and prostatic DNA damage in 49 elderly dogs that were physiologically equivalent to 62 to 69-year-old men. (**B**) Model adapted from Mertz (31) predicting the biological response to an essential nutrient. The data from dogs provides the first *in vivo* confirmation that the Mertz model fits for selenium and procarcinogenic events within the prostate.

predictive of prostate cancer protection associated with selenium supplementation. Men with the lowest plasma selenium prior to supplementation had a significant 92% reduction in prostate cancer risk in response to daily selenium supplementation. In contrast, men with the highest plasma selenium prior to supplementation did not exhibit a significant reduction in prostate cancer risk. Instead, these men had an alarming and statistically significant 88% increase in overall cancer incidence (30). We simultaneously measured toenail and plasma selenium concentration in 12 healthy human volunteers to

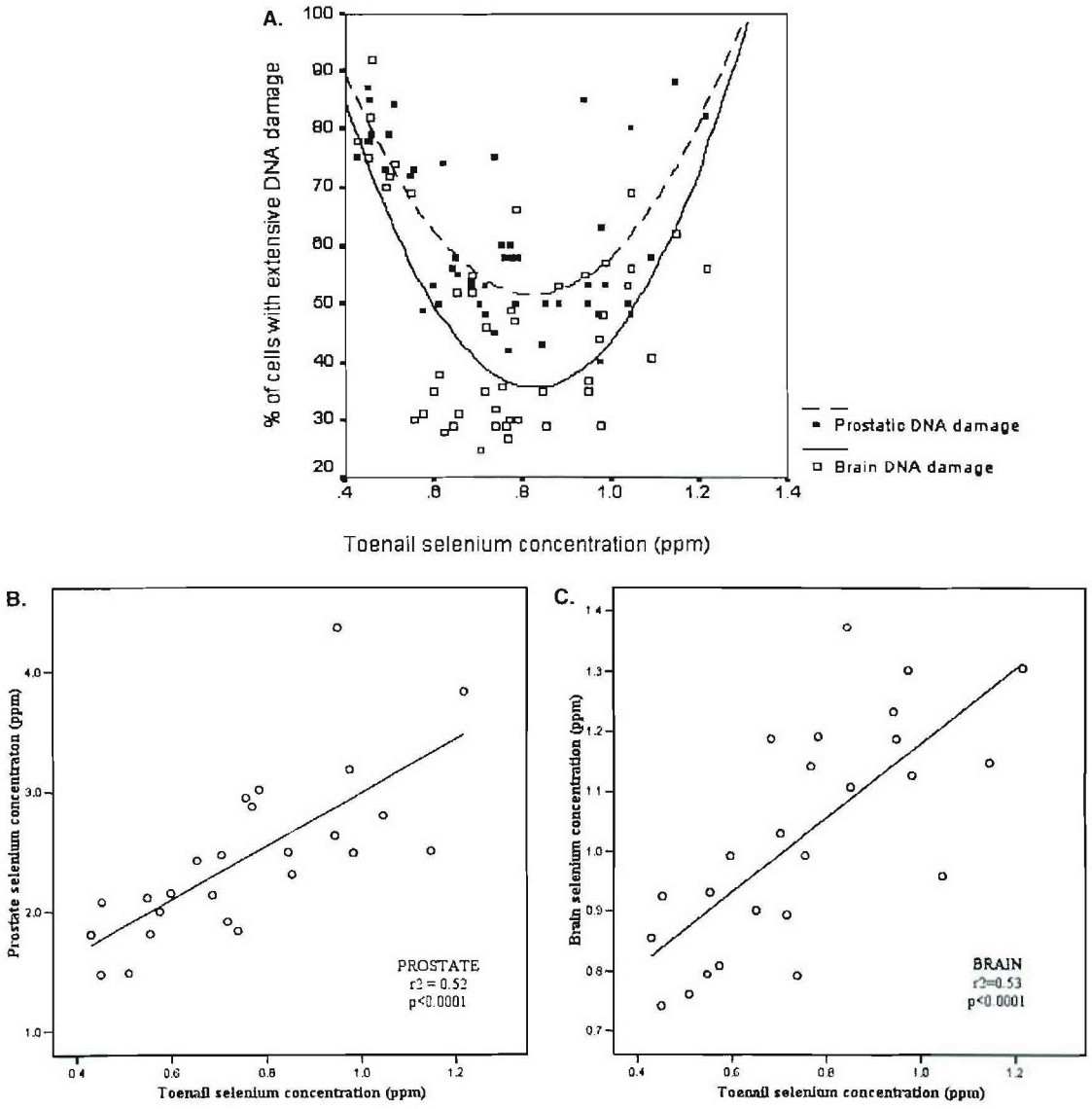

Fig. 3. (A) U-shaped dose–response defines the relationship between toenail selenium concentration and genotoxic stress in the brain of elderly dogs. The same concentration of selenium minimizes the extent of DNA damage within brain and prostate. (B and C) Non-invasive assessment of selenium status using toenails strongly predicts the concentration of selenium in prostate and brain tissues. Correlation between selenium concentration in prostate versus toenails (B) and brain versus toenails (C) in elderly dogs.

generate a ratio (6.7 ± 0.7) that could be used to convert plasma selenium concentration to predicted toenail values. After converting the plasma selenium levels of men in the Nutritional Prevention of Cancer Trial to an equivalent toenail selenium concentration, we found that the dog dose–response curve correctly predicted that men with the lowest baseline selenium status (<0.71 p.p.m.) would benefit from selenium supplementation (Figure 5). Men with the highest baseline selenium status (>0.81 p.p.m.) had a selenium concentration that was equivalent to or exceeded the optimal selenium concentration prior to supplementation; they did not benefit from selenium supplementation. Following supplementation, selenium concentration in these men was further elevated (median, 1.27 p.p.m.) and they experienced an increased total cancer incidence.

Discussion

The results of this study demonstrate the utility of a new approach to bridge the gap between laboratory and human studies that can be used to select the appropriate dose of anticancer nutrients for large-scale human cancer prevention trials. By studying the only non-human species that develops spontaneous prostate cancer, we documented an intriguing U-shaped dose–response relationship between the dietary intake of the essential trace mineral selenium and the extent of DNA damage within the aging prostate gland. Moreover, we found the results of two important human studies that examined selenium and prostate cancer risk—those used to justify the evaluation of selenium in the SELECT trial—were explained using this simple, cost-effective approach. More

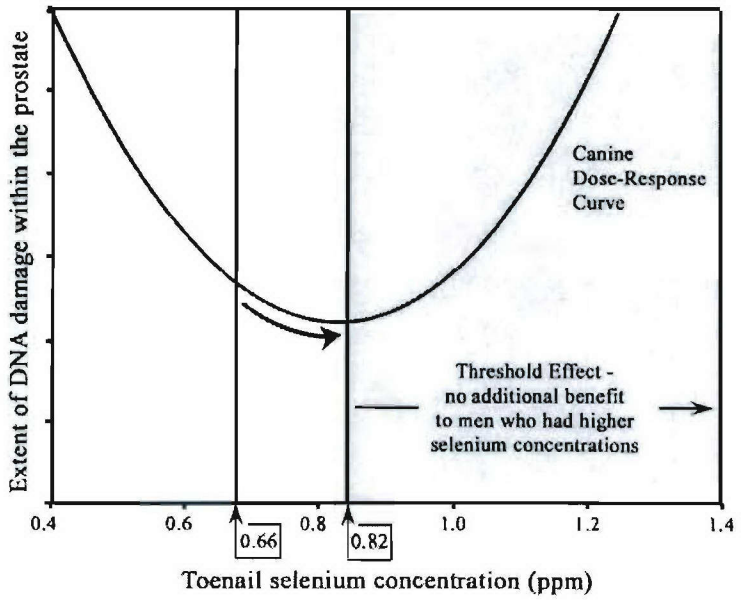

Fig. 4. Canine dose–response curve explains the effect of selenium status on human prostate cancer risk reduction in the Health Professionals Follow-up Study (9). Men with the lowest selenium status (median 0.66 p.p.m.) had lower than optimal selenium concentration predicted by the dog model; these men had the highest risk for advanced prostate cancer. Men with median selenium status of 0.82 p.p.m., a value equivalent to the optimal selenium concentration in the dog model, had the lowest prostate cancer risk. There was no additional prostate cancer risk reduction seen in men with selenium status >0.82 p.p.m., a finding predicted by the dog model. Movement along the dog curve from 0.66 to 0.82 p.p.m. (bold arrow) parallels a 65% reduction in prostate cancer risk for the men in the Health Professionals Follow-up Study.

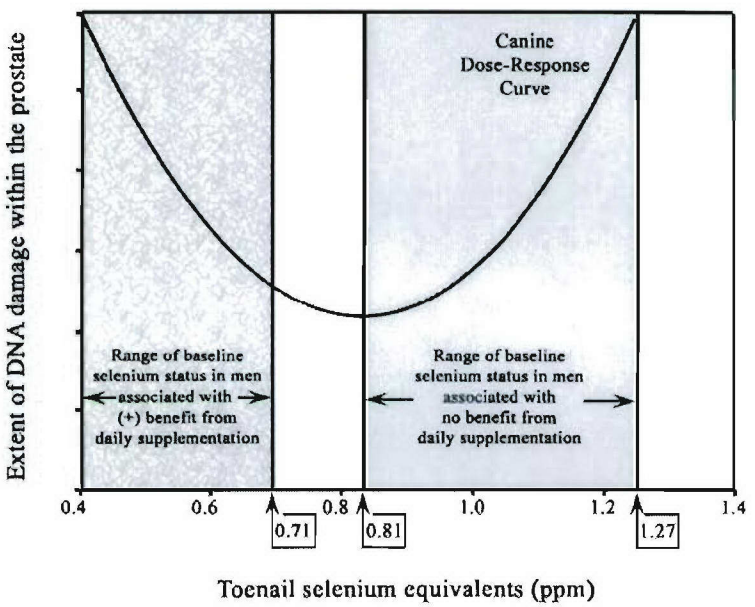

Fig. 5. Canine dose–response curve explains the effect of baseline selenium status on human prostate cancer risk reduction in the Nutritional Prevention of Cancer Trial (7,29,30). Men with baseline selenium status <0.71 p.p.m. had lower than the optimal selenium concentration predicted by the dog model; these men had a statistically significant 92% reduction in prostate cancer risk after selenium supplementation. Men with baseline selenium status >0.81 p.p.m. were already within the optimal or high suboptimal range predicted by the dog model prior to supplementation; these men had no significant reduction in prostate cancer after selenium supplementation. Following selenium supplementation, men in the highest baseline selenium tertile had a median selenium level of 1.27 p.p.m., a value clearly exceeding the selenium concentration that minimized DNA damage within the dog prostate. These men had an 88% increase in total cancer incidence compared with men with the lowest baseline selenium.

than 20 years ago, Mertz (31) proposed that the dose–response relationship between essential nutrients and biological processes was U-shaped. According to the Mertz model, a region of optimal nutrient status lies between two suboptimal (low and high) regions and the extreme values of deficiency and toxicity (Figure 2B). Our data provide further evidence that the Mertz model may indeed be correct—at least for selenium and the prostate. It follows from this new understanding that not all

men will necessarily benefit by increasing their daily selenium intake.

A similar U-shaped dose–response may also hold true for the anticancer effects of other trace minerals and carotenoids. For example, zinc is essential for prostate function (32) and it has been shown that zinc deficiency results in increased oxidative DNA damage and disruption of the p53 tumor suppressor (33). However, men with the highest intake of supplemental zinc had a significant two-fold increased risk of prostate cancer (34). Recently, Nyberg *et al.* (35) found a U-shaped dose–response between the dietary intake of β-carotene and spontaneous mutation frequency in the peripheral blood lymphocytes of humans. The aged dog model correctly predicted the human prostate's response to the anticarcinogenic effects of selenium and may also be applicable to other cancer-preventing nutrients and other anatomic cancer sites.

An important challenge facing scientists in the field of cancer prevention is to identify experimental approaches that can expeditiously define the dose-dependent effects of dietary supplements on health outcomes. Failure to recognize the U-shaped dose-dependent effects of nutritional supplements on carcinogenesis adversely impacted the design of previous cancer prevention trials. For example, in two randomized lung cancer prevention trials, subjects who received high doses of beta-carotene had an unexpected increase in lung cancer incidence compared with placebo-treated controls (36,37). Measurement of baseline nutrient status was not included as a required entry criterion in these beta-carotene trials or in any of the large selenium intervention trials. This is of particular relevance to the ongoing SELECT trial, since the average selenium status of men in the USA is roughly equivalent to a toenail concentration of 0.82 p.p.m., a value that already falls within the optimal range for prostate cancer risk reduction. Our demonstration of a U-shaped dose–response for cancer-fighting nutrients emphasizes that baseline nutrient status in the suboptimal range should be a required entry criterion for prevention trials to avoid the potential deleterious effects of oversupplementation.

Our study introduces to the field of cancer prevention research a powerful new paradigm that reflects the synthesis of three concepts: (i) the importance of using an *in vivo* model system (aging dog prostate) to mimic the aging human prostate prior to the onset of cancer; (ii) the importance of studying a broad dose range that is sufficient to define the U-shaped dose–response relationship between an essential nutrient and pro-carcinogenic processes; (iii) the use of Comet score as a measure of DNA damage that integrates prostatic exposure to genotoxic stress, the susceptibility of prostate cells to DNA damage, and prostatic DNA repair capacity (38,39). Using this approach, it is feasible to select a selenium dose that optimizes DNA damage reduction within a cancer target, such as the prostate and other organs, such as the brain. Future cancer prevention trials with humans could benefit significantly from adopting this paradigm to define the effects of nutrient dose on markers of genotoxic damage and cancer risk.

Finally, our analysis of the complex relationship between selenium, genotoxic damage and cancer risk within the prostate raises important questions regarding the currently recommended intake of this trace mineral. The current recommended daily allowance (RDA) for selenium in men is 70 μg/day, which reflects the selenium intake required to achieve maximal plasma glutathione peroxidase actvity. However, there is growing consensus that nutritionally adequate selenium intake may be suboptimal with respect to reducing disease risk (2,40). Indeed, our analysis showed that selenium status sufficient to saturate the activity of plasma glutathione peroxidase (equivalent to 0.6 p.p.m. selenium concentration in toenails) will not necessarily minimize prostatic DNA damage in the dog model or prostate cancer risk in men. Researchers are aggressively pursuing new functional markers of selenium status that can accurately reflect the biologically effective concentration of selenium that optimizes human health. Since selenium has diverse health-promoting roles, it is likely that a range of markers assessing particular biochemical functions, disease states and tissue specificity will be required. We have presented here the first evidence that prostatic DNA damage measured by Comet assay may serve as a functional marker of selenium's anticarcinogenic effect on the prostate. Importantly, our results suggest that measurement of toenail selenium concentration can provide a non-invasive method for titrating and individualizing optimal selenium intake required for prostate cancer protection.

Acknowledgements

We thank N.Glickman and R.Thorpe for the statistical advice, C.Oteham and D.Schlittler for the technical assistance, R.Borgens for the critical reading of the manuscript and K.Harrington for the preparation of figures. This work was supported by grant PC-970492 from the US Army Medical and Material Command Prostate Cancer Research Program.

Conflict of Interest Statement: None declared.

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Received November 18, 2004; revised March 15, 2005; accepted March 22, 2005

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Review

Making sense of sex and supplements: differences in the anticarcinogenic effects of selenium in men and women

David J. Waters [a,b,*], Emily C. Chiang [b], Dawn M. Cooley [a,b], J. Steven Morris [c]

[a] *Gerald P. Murphy Cancer Foundation, 1291 Cumberland Ave, West Lafayette, IN 47906, USA*
[b] *Department of Veterinary Clinical Sciences, Purdue University, 625 Harrison Street, West Lafayette, IN 47907, USA*
[c] *University of Missouri-Columbia Research Reactor Center, Columbia, MO, USA*

Received 15 December 2003; received in revised form 9 February 2004; accepted 12 February 2004

Available online 7 June 2004

Abstract

The role of the essential trace mineral selenium in human health and disease is currently a subject of intense interest. In particular, the possible cancer preventive effects of dietary selenium supplementation are now being investigated in several large, randomized trials. The association between selenium status, genotoxic damage, and cancer risk remains enigmatic because epidemiologic studies have failed to consistently link low selenium status with increased cancer risk in men and women. In this paper, we considered the evidence that there are sex-based differences in the anticarcinogenic effects of selenium in humans. We focused our review on prospective human studies in which the relationship between selenium status and cancer risk in men and women was directly compared. Results from cohort studies conducted in seven countries (Belgium, China, Finland, Japan, Netherlands, Norway, and United States) were used to assess the strength of association between low selenium status and the incidence of all cancers, sex-specific cancers, and cancers at particular anatomic sites. In general, the available data support the hypothesis that cancer risk in men is more profoundly influenced by selenium status than cancer risk in women. Factors contributing to the apparent difference in the effects of selenium on cancer incidence in men and women may include sex-based differences in the metabolism and/or tissue distribution of selenium, as well as sex- or gender-related factors that influence tumor biology. Studies are needed to further define the dose–response relationship between selenium and cancer risk in men and women. A more complete understanding of the mechanisms by which selenium modulates cancer initiation and progression is needed to optimize dietary selenium supplementation as a practical cancer preventive strategy. Ultimately, achieving the ambitious goal of cancer prevention may require sex- and gender-specific approaches.

Keywords: Cancer incidence; Epidemiology; Cancer prevention; Dietary supplements; Micronutrients; Gender-specific risk; Cohort studies; Sex-based differences

1. Introduction

The trace mineral selenium is an essential component of several metabolically important enzymes, including the antioxidant glutathione peroxidases and thioredoxin reductases [1–3]. Because dietary selenium supplementation inhibits cancer development in

* Corresponding author. Tel.: +1 765 494 9271; fax: +1 765 775 1006.
E-mail address: dwaters@gpmcf.org (D.J. Waters).

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doi:10.1016/j.mrfmmm.2004.02.019

a variety of experimental animal models [4–6], there is growing interest in the prospect that selenium status significantly influences human cancer risk.

To date, the epidemiologic evidence from prospective human studies is inconsistent—some investigations show an increased risk of cancer in individuals with lowest selenium status, whereas other studies report null results [7,8]. In a randomized, placebo-controlled study of older Americans, daily use of an oral selenium supplement substantially reduced the risk of several cancers, most notably cancer of the prostate [9]. These results suggested the exciting possibility that significant reductions in cancer risk may be realized with low, non-toxic doses of selenium that could readily be achieved by dietary supplementation. The cancer protective effects of selenium may be mediated by selenoproteins operating within enzymatic systems which are saturated at relatively low levels of selenium, or by selenium metabolites that increase substantially under conditions of supranutritional selenium intake [10].

In 1987, Kok et al. [11] in the Netherlands reported that low selenium status was associated with increased cancer risk in men, but not in women. They proposed that serum selenium may only be a useful predictor for cancer risk in men. This hypothesized sex-based difference was consistent with earlier data reported from Finland [12] and the United States [13].

In this article, we consider the evidence that there are sex-based differences[1] in the anticarcinogenic effects of selenium in humans. To accomplish this, we critically review data from prospective human studies in which the association between selenium status and subsequent cancer risk in men and women was directly compared. We also review prospective studies that were restricted to males or females as well as studies with both men and women in which sex-specific cancer risk was not reported; our discussion of these studies is limited. The purpose of this review is to provide a conceptual framework for future investigations on the underlying mechanisms and public health implications of the apparent sex-based differences in selenium anticarcinogenesis.

2. Sex-based differences in the association between selenium status and total cancer incidence

Prospective cohort studies provide an opportunity to evaluate the association between nutrient status and the subsequent risk for cancer. In these studies, pre-diagnostic biological samples are collected from a cohort of healthy individuals who are free of cancer. After the cohort is followed longitudinally over time, samples are analyzed from all cancer cases and a matched group of controls who did not develop cancer during the observational period.

Table 1 summarizes data from six prospective cohort studies [11–13,16–18] conducted in five countries (Finland, Japan, Netherlands, Norway, and United States) in which the effect of dietary selenium intake on total cancer incidence in men and women was measured by comparing the serum selenium concentration of cancer cases versus controls without cancer. Among men, cancer cases had significantly lower selenium concentration than controls ($P < 0.05$) in four of six studies. On average, males that subsequently developed cancer at any site had an 8% (range, 5–23%) lower selenium concentration than men who did not develop cancer. In contrast, there was no significant difference between selenium concentration in female cases versus controls in any of the studies. Women with cancer had higher selenium concentration than men with cancer in four of the studies. This is consistent with the findings of Criqui et al. [19] in which mean serum selenium concentration in 74 men that subsequently had cancer mortality was 4 μg/L lower than controls ($P < 0.40$); in contrast, 62 women with cancer mortality had serum selenium concentration 11 μg/L *higher* than controls ($P = 0.03$).

In three studies [11,16,20], the relative risk of cancer in individuals with the lowest serum selenium was compared with the incidence of cancer in individuals with the highest selenium status. In Belgium, Finland, and the Netherlands, men with low selenium status had a significantly higher relative risk (2.2–2.7-fold increase) of cancer at all sites than men with high selenium. In contrast, women with low serum selenium

[1] In this article, we use the terms sex and gender to discuss the differences between men and women. These terms are used in accordance with definitions proposed by the 2001 Institute of Medicine Report, "Exploring the Biological Contributions to Human Health: Does Sex Matter?" [14]. The term sex is used when differences are primarily biological in origin and may be genetic or phenotypic; gender is used when referring to social and cultural influences based on sex [15].

Table 1
Mean pre-diagnostic serum selenium concentration in cancer cases and matched controls from six prospective cohort studies

Cohort	Cases	Mean ± S.D. serum selenium concentration (μg/L)		*P*-value
		Case	Control	
Finland				
Salonen et al. [12]	16 male smokers	49.3	63.5	<0.05
	14 male non-smokers	49.9	58.4	>0.05
	21 female (all non-smokers)	59.5	60.5	>0.05
Knekt et al. [16]	597 male	59.1 ± 17.5	62.5 ± 15.4	<0.001
	499 female	63.6 ± 17.4	63.9 ± 14.3	>0.05
Japan				
Ujiie and Kikuchi [18]	35 male	105.2	112.8	0.18
	38 female	97.4	102.7	0.25
Netherlands				
Kok et al. [11]	40 male	116.7 ± 4.0	126.4 ± 3.1	0.04
	29 female	130.6 ± 6.0	129.3 ± 4.3	0.83
Norway				
Ringstad et al. [17]	26 male	124.0	130.3	0.08
	34 female	123.2	127.9	0.36
USA				
Willett et al. [13]	60 male	127.0	137.0	0.008
	51 female	132.0	134.0	0.57

had a relative risk to develop cancer that did not differ significantly from unity (Fig. 1).

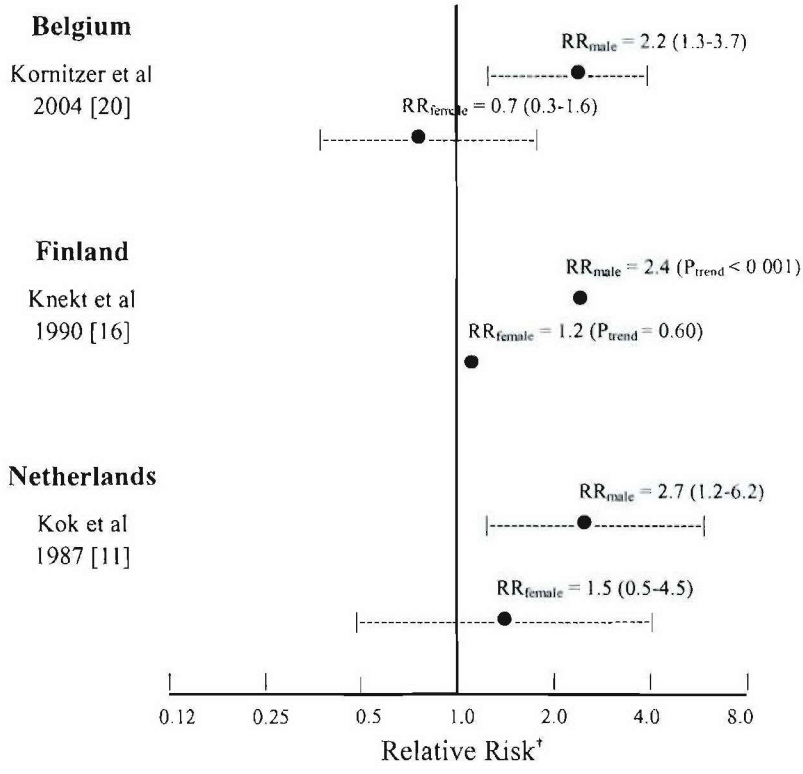
Garland et al. [21] analyzed the association between selenium and cancer risk in women (503 cancer cases and matched controls) in the Nurses Health Study. Toenail selenium concentration was not inversely associated with overall cancer risk or cancer risk at any site. The authors concluded that higher selenium intake within the range typical for US women was not protective against cancer development in women.

Taken together, these studies suggest that overall cancer incidence in men is more profoundly affected by low selenium status than is cancer incidence in women.

3. The association between selenium status and risk of breast cancer and prostate cancer

To further analyze the influence of sex or gender-related factors on the anticarcinogenic effects of selenium, we explored the association between selenium status and risk of two sex-specific cancers—breast cancer and prostate cancer. Fig. 2 summarizes data collected from the largest prospective cohort studies conducted in Finland, Netherlands, and United States. An inverse association between serum selenium concentration and prostate cancer risk was not seen in the Finnish study ($n = 61$ cases) [16]. However, two large studies from the Netherlands ($n = 540$ cases) and United States ($n = 181$ cases) showed that men with low selenium status had a significantly increased risk (RR = 1.5 and 2.9, respectively) of prostate cancer compared to men with high selenium status [22,23].

In a secondary cohort analysis of the Alpha Tocopherol Beta Carotene (ATBC) Cancer Prevention Study, no significant association between low selenium intake and prostate cancer risk was found in the placebo treated or α-tocopherol treated groups [24]. Similarly, low baseline selenium status was not a significant risk factor for subsequent prostate cancer in the Carotene and Retinol Efficacy Trial (CARET) [25]. Among Japanese American men in Hawaii, low selenium status was associated with a significant increased risk of prostate cancer in current smokers



† Represents the relative cancer risk for individuals with low selenium status compared to cancer risk in individuals with high selenium status. For each sex, cancer risk in individuals with high selenium status equals 1.0.

Fig. 1 Relative risk of cancer (all sites) associated with low selenium status in men and women from three prospective cohort studies.

[RR = 5.0 (1.3–10.0)] and past smokers [RR = 2.0 (0.9–5.0)], but not in never smokers [RR = 1.25 (0.5–2.5)] [26]. There was a non-significant trend toward increased prostate cancer risk with low selenium in a Washington County, MD cohort study [27]. More recently, low selenium status was associated with a four-fold increase in prostate cancer risk among participants of the Baltimore Longitudinal Study on Aging [28].

In contrast to prostate cancer, cohort studies lend little support for the hypothesis that low selenium status confers an increased risk of breast cancer [29–34]. Two large studies from the Netherlands (n = 202 cases; RR = 1.1) and United States (n = 434 cases; RR = 0.9) showed a null association between breast cancer risk and selenium status [33,34].

4. Sex-based differences in the association between selenium status and risk of particular cancer types

Next, we sought to determine whether there were sex-based differences in the association between selenium status and cancer incidence at particular anatomic sites. Figs. 3–5 summarize the data from prospective studies in which the risk of specific cancers in men and women was compared. Data from Finland (lung, colorectal, stomach, pancreatic, urinary tract, and non-melanoma skin cancer), Netherlands (lung, colorectal, and stomach cancer), and United States (lung, pancreatic cancer) are summarized below for each cancer site. With two exceptions [30,35], the cutoffs used to define low versus high selenium status in these cohorts are shown in Fig. 6. Table 2

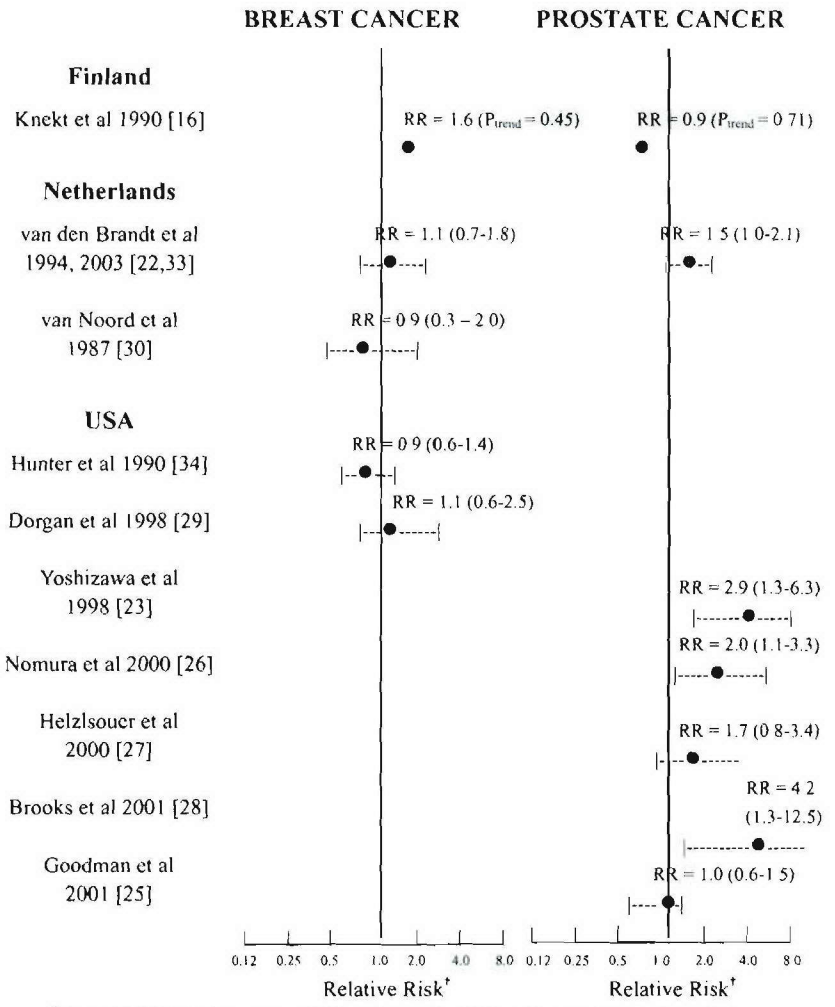


Fig. 2. Relative risk of prostate cancer and breast cancer associated with low selenium status in 11 prospective cohort studies.

shows the factors used in these prospective studies to match cases with controls and to conduct multivariate analysis of cancer risk.

4.1. Lung cancer

The risk of lung cancer in Finnish men was 3.3 times greater in men with low selenium status than in those with high selenium status (P for trend <0.001) [16] (Fig. 3). In that study, there were only nine evaluable cases of lung cancer in women, and therefore no valid conclusions could be posited regarding the association between selenium status and female lung cancer risk.

In a Netherlands cohort study [36], men with low selenium status had a statistically significant two-fold increased risk of lung cancer. Women with low selenium had a 2.5-fold increased risk of lung cancer compared to women with high selenium status, but this

4.2. Colorectal cancer

The association between colorectal cancer risk and selenium status was not profoundly different in men and women in two evaluable studies [16,37] (Fig. 3). Rectal cancer risk was *lowest* in Dutch women with low selenium status, but this did not reach statistical significance [36].

4.3. Stomach cancer

Data from two evaluable studies showed that risk of stomach cancer in men with low selenium status was significantly increased (RR = 2.5 Netherlands; RR = 11.1 Finland) [16,37] (Fig. 4). In contrast, low selenium status in women did not confer an increased risk of stomach cancer. In fact, the relative risk of

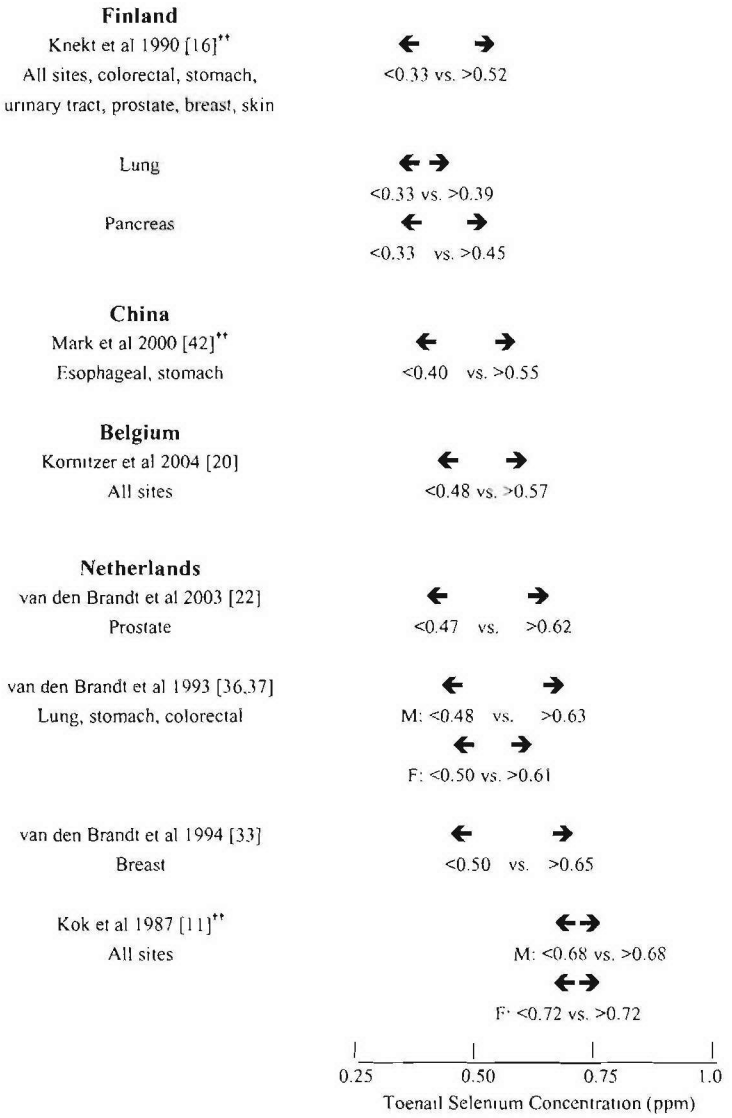
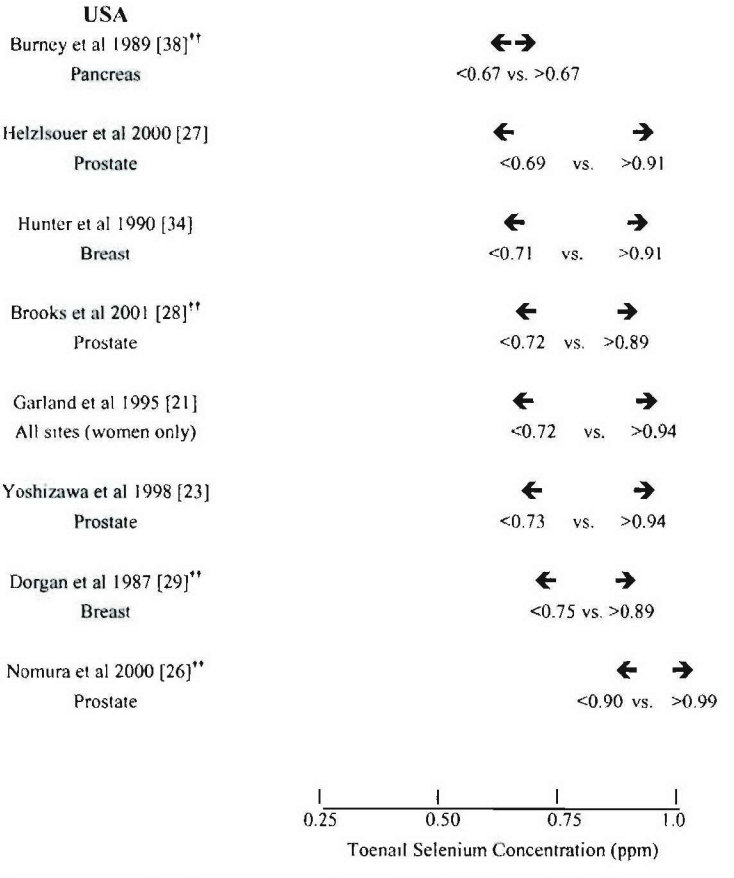


Fig. 6. Cutoffs used to define low vs. high selenium groups[†] within study cohorts from Finland, China, Belgium, Netherlands, and United States.



†Arrows represent the selenium concentration cutoffs for the low and high selenium groups that were used to estimate the relative risk of cancer associated with low selenium status. Relative risks are shown in Figures 1-5.

†† For the purpose of comparing studies in which selenium status was measured by either serum or toenail selenium levels, the serum selenium concentration reported in these six studies are expressed as toenail selenium equivalents here. The toenail and plasma selenium concentration in 12 healthy human volunteers were simultaneously measured to generate a ratio (6.7 ± 0.7) that could be used to convert plasma selenium concentration to predicted toenail values [J.S. Morris, unpublished data]. In this figure, toenail selenium equivalents (ppm) = serum selenium concentration (μg/L) x 0.0067.

Fig. 6. (*Continued*)

stomach cancer was *lower* in Dutch women with low selenium compared to those with high selenium status [37].

4.4. Pancreatic cancer

Data from both Finland [16] and the United States [38] showed statistically significant sex-based differences in the association between selenium status and risk of pancreatic cancer (Fig. 4).

4.5. Urinary tract cancer

The relationship between selenium and risk of urinary tract cancer in Finnish men and women supported the hypothesis that there are sex-based differences in

Table 2
Factors used in prospective studies for matching cases with controls and for multivariate analysis of the association between cancer risk and selenium status

Cohort	Factors				Other
	Sex	Age	Smoking status	Sample collection	
Belgium					
Kornitzer et al. [20]	✓	✓			Body mass index; intake of alcohol, total energy, total fat, saturated fat, dietary fiber, retinol, and Vitamin C
China					
Mark et al. [42]	✓	✓			
Finland					
Salonen et al. [12]	✓	✓	✓		
Knekt et al. [16]	✓	✓	✓		Residence
Japan					
Ujiie and Kikuchi [18]	✓	✓			Residence
Netherlands					
Kok et al. [11]	✓	✓	✓		
van Noord et al. [30]	✓	✓		✓	Residence, premenopausal status
van den Brandt et al. [22,33,36,37]	✓	✓	✓	✓	Education level; intake of alcohol and energy [33], beta-carotene and Vitamin C [37]
Norway					
Ringstad et al. [17]	✓	✓	✓	✓	Residence
USA					
Willett et al. [13]	✓	✓	✓	✓	Initial blood pressure, antihypertensive medication, randomization, parity and menopausal status (women)
Burney et al. [38]	✓			✓	Race
Hunter et al. [34]	✓	✓		✓	Intake of alcohol
Garland et al. [21]	✓	✓	✓		Toenail weight, laboratory batch
Comstock et al. [35]	✓	✓	✓	✓	
Yoshizawa et al. [23]	✓	✓	✓	✓	Body mass index, residence; intake of lycopene, saturated fat and calcium
Dorgan et al. [29]	✓	✓	✓	✓	Body mass index, time of diagnosis, total serum cholesterol
Nomura et al. [26]	✓	✓	✓	✓	Subgroups
Helzlsouer et al. [27]	✓	✓		✓	Race
Brooks et al. [28]	✓	✓	✓	✓	Body mass index, intake of alcohol
Goodman et al. [25]	✓	✓	✓	✓	Year of randomization, intervention arm, exposure population

the anticarcinogenic effects of selenium [16] (Fig. 5). Males with low serum selenium had a non-significant *increased* relative risk of 1.2 compared to males with high selenium status. However, females with low serum selenium had an 80% *decreased* urinary tract cancer risk ($P = 0.06$) compared to females with high selenium status.

4.6. Non-melanoma skin cancer

Men in the Finnish cohort [16] who had the lowest serum selenium had a non-significant two-fold *increased* skin cancer risk. In contrast, females with low serum selenium had a non-significant 40% *decreased* risk of skin cancer (Fig. 5).

5. Results of the Nutritional Cancer Prevention Trial

In 1983, Clark et al. [9] launched the Nutritional Cancer Prevention Trial (NCPT) to determine whether daily selenium supplementation with selenium would significantly decrease the incidence of cancer in patients with non-melanoma skin cancer. In the NCPT, 1312 participants (980 men, 332 women) were randomized to treatment groups that received placebo or 200 μg selenium daily in the form of high selenium yeast.[2] When data from the entire blinded treatment period were analyzed [39], men receiving selenium supplementation had a 33% reduction in overall cancer incidence [hazard ratio (95% CI) = 0.67 (0.50–0.89; $P = 0.005$]. In contrast, women who received supplementation had a non-significant *increase* in total cancer incidence [hazard ratio = 1.20 (0.66–2.20; $P = 0.55$]. The apparent cancer protective effect of selenium supplementation was limited to males, even after adjusting for age and smoking status. Selenium supplementation was associated with a 26% reduction in risk of lung cancer and a 54% reduction in risk of colorectal cancer, but these results were not statistically significant after a mean of 7.4 years follow-up. However, the significant reduction in prostate cancer that was originally reported remained highly significant (52% reduction; $P = 0.005$) [40]. Low baseline selenium status prior to supplementation was an important predictor of the prostate cancer protective effects of dietary selenium supplementation [39,40]. It is interesting to note that selenium supplementation was associated with a non-significant *increase* in the incidence of five cancer types: melanoma, bladder, breast, head and neck, and lymphoma/leukemia [39].

Taken together, the results of this interventional trial support the hypothesis that overall cancer incidence in men may be more responsive to changes in selenium status than in women. However, a balanced interpretation of the NCPT results must consider that neither overall cancer mortality nor site-specific cancer incidence (with the exception of non-melanoma skin cancer) were primary endpoints of the study. Moreover, this trial was not adequately powered to detect a cancer protective effect in women because 75% of the participants were men.

6. Results of the General Population Trial (Linxian, China)

From 1986 to 1991, the General Population Trial was conducted in Linxian, China to determine if nutritional supplementation could significantly reduce cancer incidence, cancer mortality, or overall mortality [41]. Twenty-nine thousand five hundred and eighty-four adults were randomized to receive placebo or one of seven different combinations of nutrient supplements. Compared with the placebo group, a significant 13% reduction in overall cancer mortality was observed in the group receiving Factor D, a supplement containing selenium (50 μg) in the form of selenized yeast, β-carotene (15 mg), and α-tocopherol (30 mg). However, no information was provided on whether men and women receiving this selenium-containing supplement experienced equivalent cancer protective effects.

Recently, Mark et al. [42] analyzed the relationship between pre-trial (baseline) serum selenium concentration and subsequent risk of developing esophageal and gastric cancer in the participants of the General Population Trial. Low baseline selenium status was associated with a significant increase in risk of esophageal cancer [RR = 1.8 (1.4–2.3)] and cancer of the gastric cardia [RR = 2.1 (1.5–3.0)], but not cancers affecting the non-cardiac region of the stomach [RR = 0.9 (0.5–1.8)]. Relative risk estimates for cancers at these three sites were nearly identical in men and women. Interestingly, among individuals with low baseline selenium status, the high risk of esophageal and gastric cardia cancers was not significantly influenced by selenium treatment, i.e. the development of incident cancers was similar in the selenium supplemented and non-supplemented groups. Apparently, the high risk of cancer associated with low selenium status could not be reduced by daily supplementation with 50 μg of selenium.[3]

[2] High selenium yeast contains a cocktail of different organic selenium compounds; selenomethionine is the most abundant form of selenium in this supplement.

[3] This level of supplementation resulted in a more than two-fold increase in total daily selenium intake because the estimated selenium intake in residents of Linxian was 36–42 μg selenium/day [43].

The results of the General Population Trial do not support the hypothesis that there are differences between men and women in the association between low selenium status and subsequent cancer risk. However, the epidemic rate of esophageal and gastric cancer (these sites accounted for 87% of all cancer deaths) and consistently low concentration of several micronutrients in the inhabitants of Linxian make it difficult to generalize these findings to Western populations [44].

7. Other studies

To determine whether the overarching hypothesis—that low selenium status has a stronger association with cancer risk in men than in women—was refuted by other prospective studies, we also reviewed studies that were restricted to males or females as well as those that included both men and women in which analysis of sex-specific cancer risk was not reported. These included 24 reports relating pre-diagnostic selenium concentration in blood or toenails to subsequent cancer incidence at the following anatomic sites: stomach, colon, rectum, or gastrointestinal; lung or respiratory; lymphoma, leukemia, or hematologic; urinary bladder and other urogenital; skin (squamous cell or basal cell carcinoma, melanoma); oropharyngeal; hepatocellular carcinoma; cervical and ovarian; all sites [19,45–66]. Nine of these studies had exclusively or predominately males [45–53] and three studies had exclusively females [54–56]. In the remaining studies, sex-based analysis was not reported [57–64] or was incomplete [19,65–67].

Although the results of these studies varied, none persuasively argued against the hypothesis. However, conclusions based upon a survey of the literature may overestimate real differences in the relationship between selenium and cancer risk in men and women. There may have been no significant differences in the association between selenium status and cancer risk in men and women in studies in which results of sex-based analysis were not reported. We also recognize that there is inherent bias which favors publication of significant rather than non-significant results.

8. Potential underlying explanations for the apparent sex-based differences in the anticarcinogenic effects of selenium

8.1. Sex-specific cancers affecting men and women may differ in their response to selenium

Differences in the association between selenium status and total cancer incidence in men and women may reflect that certain sex-specific cancers of men (e.g. prostate cancer) are selenium responsive, whereas those affecting women (e.g. breast cancer) are insensitive to changes in selenium status. However, Garland et al. [21] showed a null association between selenium status and the incidence of 503 non-breast cancers in women of the Nurses Health Study. This suggests that breast cancer cannot solely account for the weak association observed between selenium status and overall cancer incidence in women.

8.2. Sex-based differences in tumor biology

Growing evidence suggests there are sex-based differences in the biology of particular types of cancer that affect both men and women [68]. For example, the frequency of G to T transversions in the p53 tumor suppressor gene are higher in the lung cancers of female smokers than in male smokers [69]. After adjusting for smoking exposure, non-tumorous lung tissue of women had higher levels of DNA adducts than lung tissue from men [70]. It has been speculated that some of the sex-based differences in tumor biology might reflect a diminished DNA repair capacity in women [71]. It is plausible that sex-based differences in selenium's effects on cancer incidence are the consequence of differences in certain tumor cell or host factors that favor cancer progression in men and women. A more complete understanding of the molecular and cellular biological differences between the cancers of men and women could help to elucidate the specific mechanisms by which selenium exerts its anticancer effects.

8.3. Sex-based differences in the dose–response relationship between selenium and cancer prevention

The dose–response for the anticarcinogenic effect of selenium may be significantly modified by sex or

gender-related factors. If this hypothesis is correct, the level of selenium that minimizes cancer risk would be different in men and women. Indeed, in some studies [16,37], women with the lowest selenium levels had the *lowest* risk for colorectal, stomach, urinary tract, and non-melanoma skin cancers. Analysis of data collected from four Canadian provinces suggested that men and women have non-linear dose–response relationships that are not superimposable [72]. In the Canadian study, the slope of the regression between estimated age-adjusted cancer death rates (all sites) and toenail selenium concentration was steeper for males, indicating that estimated cancer mortality in men was more strongly influenced by incremental changes in selenium status [72]. Sizeable international differences in selenium status (i.e. toenail selenium levels in the low selenium status group within cohorts from Finland, Netherlands, and United States were <0.33, <0.50, and <0.91 ppm, respectively; Fig. 6) provide an opportunity to determine whether selenium's influence on cancer incidence is strengthened or abrogated within populations that have relatively low selenium intake.

8.4. Sex-based differences in metabolism or tissue distribution of selenium

From animal studies, it is apparent that there are sex-based differences in the metabolism and tissue concentrations of selenium [73]. The vulnerability of dogs and rodents to the toxicity of selenium compounds is influenced by sex; males were more sensitive than females to the toxic effects of intragastric or oral doses of L-selenomethionine [74]. Interestingly, in some rat studies, sex-based differences in toxicity were observed despite equivalent plasma concentrations in males and females [74].

Population-based studies reveal differences in the toenail selenium concentration of men and women, suggesting that the biodistribution of dietary selenium in humans is influenced by sex-based factors. Mean toenail selenium level in men was lower than in women in the Netherlands [75], Canada [72], and United States [76]. It is unclear whether high concentrations of selenium harbored within "priority tissues" of the male reproductive tract contribute to the lower toenail selenium concentrations seen in men. It is unlikely that the sex-based differences in selenium status can be explained by higher dietary selenium intake in women. Whole body residence time of selenium has been estimated by Patterson et al. [77] to be greater in men than in women. Also, urinary excretion of selenium per kilogram of body weight in females may be higher than in males [78]. However, in contradiction to the aforementioned studies, analysis of 7102 male and 7517 female participants in NHANES III showed that mean serum selenium concentration was slightly higher in men (men = 124 μg/L versus women = 122 μg/L; $P < 0.0001$) [79].

Although unproven, men and women may differ in the rate of formation or tissue distribution of certain anticarcinogenic metabolites [6] of selenium. This raises an important methodologic issue because measurement of total selenium concentration within nails or blood may be an insensitive means of detecting individual differences in the concentration of cancer fighting selenium metabolites.

8.5. Sex-based differences in the interaction between selenium and other factors

There may be differences between men and women in the extent to which selenium status is influenced by confounding variables, such as health-related behaviors or dietary intake of other nutrients. For example, alcohol consumption was positively associated with serum selenium in women, but not in the men of NHANES III [79]. Also, the inverse association between toenail selenium concentration and smoking was reported to be stronger in men than in women [75].

9. Knowledge gaps and summary

In a recent review of the epidemiology of selenium and human cancer, Vinceti et al. [7] stated that "the relationship between the trace element selenium and the etiology of human cancer in humans remains elusive and intriguing". In order to understand the role that selenium plays in cancer protection, the biological factors and methodological issues contributing to the inconsistency of the epidemiological evidence linking low selenium status and increased cancer risk must be identified. In this survey, we evaluated the strength of evidence supporting the hypothesis that there are sex-based differences in the anticarcinogenic effects of

selenium. We conclude that, in general, the data support the hypothesis that cancer risk in men is more profoundly influenced by selenium status than in women. However, our analysis revealed relatively few informative prospective studies that directly compared the association between selenium and cancer risk in men and women. This was particularly true for men and women living in the United States. The most consistent sex-based difference within Western populations was the association between low selenium status and cancer incidence at all sites, and in particular, the cancers of the stomach and pancreas. Data supporting a difference in men and women was weakest for colorectal cancer. The influence of sex on the anticancer effects of selenium has not been extensively evaluated in animal tumor models. Relevant hypotheses could be formally tested using the most appropriate animal models and selenium doses relevant to human populations [80].

In several published studies [57–64], the results from sex-specific analysis of cancer incidence were not reported. Future studies should report the results of these analyses, even if no differences between men and women are found. All analyses should appropriately consider potential confounding variables, such as age and smoking status. Clearly, a more complete understanding of the extent to which sex modifies the influence of nutritional status (and other factors) on cancer risk is needed to establish sound health recommendations.

Finally, the anticarcinogenic dose–response of most cancer-fighting nutrients is unknown. It is doubtful that observational data from cohort studies can reliably predict the cancer risk reduction achievable with high doses of nutrient supplements, because the expected nutrient levels in supplement users are likely to exceed the range seen in the general population [25]. As scientists and clinicians seek to identify the dietary intake of selenium that minimizes cancer risk, it will be important to determine whether the dose–response relationship between selenium and anticarcinogenesis is non-linear [81,82]. A non-linear dose–response predicts that not all persons will benefit from increasing their selenium intake through daily supplementation. The possibility that the anticarcinogenic effects of selenium may differ significantly between men and women contributes further to the complexity of this already challenging area of inquiry.

Acknowledgements

The research of DJW on selenium and cancer prevention is supported by the Department of Defense Prostate Cancer Research Program. The authors also thank the Purdue University Center on Aging and the Life Course for the award of a Lynn Research Fellowship to ECC.

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BRIEF COMMUNICATION

Effects of Dietary Selenium Supplementation on DNA Damage and Apoptosis in Canine Prostate

David J. Waters, Shuren Shen, Dawn M. Cooley, David G. Bostwick, Junqi Qian, Gerald F. Combs, Jr., Lawrence T. Glickman, Carol Oteham, Deborah Schlittler, J. Steven Morris

The trace mineral selenium inhibits cancer development in a variety of experimental animal models. We used an *in vivo* canine model to evaluate the effects of dietary selenium supplementation on DNA damage in prostate tissue and on apoptosis in prostate epithelial cells. Sexually intact elderly male beagle dogs were randomly assigned to receive an unsupplemented diet (control group) or diets that were supplemented with selenium (treatment group), either as selenomethionine or as high-selenium yeast at 3 μg/kg or 6 μg/kg body weight per day for 7 months. The extent of DNA damage in prostate cells and in peripheral blood lymphocytes, as determined by the alkaline comet assay, was lower among the selenium-supplemented dogs than among the control dogs (prostate $P<.001$; peripheral blood lymphocytes $P = .003$; analysis of variance) but was not associated with the activity of the antioxidant enzyme glutathione peroxidase in plasma. The median number of terminal deoxynucleotidyl transferase–mediated dUTP nick end-labeling-positive (i.e., apoptotic) prostate epithelial cells was 3.7 (interquartile range = 1.1–7.6) for the selenium-supplemented dogs and 1.7 (interquartile range = 0.2–2.8) for the control dogs ($P = .04$, Mann–Whitney U test). These data suggest that dietary selenium supplementation decreases DNA damage and increases epithelial cell apoptosis within the aging canine prostate. [J Natl Cancer Inst 2003;95: 237–41]

Prostate cancer is the second leading cause of cancer-related mortality among men in the United States *(1)*. Selenium, an essential nutrient required for the activities of a number of metabolically important enzymes, including the antioxidant glutathione peroxidase, inhibits cancer development in a variety of experimental animal models *(2–4)*. In 2001, the National Cancer Institute initiated the Selenium and Vitamin E Prostate Cancer Prevention Trial (SELECT) to evaluate whether daily dietary supplementation with selenium and/or vitamin E decreases the incidence of prostate cancer. However, it is not known what dietary form or dose of selenium might offer the most potent cancer-protective effects.

Selenium-dependent glutathione peroxidase and thioredoxin reductase protect the body from the endogenous products of cellular metabolism that have been implicated in DNA damage, mutagenesis, and carcinogenesis *(5–7)*. A shift in the pro-oxidant–antioxidant balance within the prostate has been proposed as a factor that contributes to prostate carcinogenesis *(8–11)*. We hypothesized that selenium supplementation exerts its anticarcinogenic effect by reducing the naturally occurring genotoxic stress within the aging prostate. Because the influence of aging on prostate cancer development is similar in dogs and humans, the only two species in which prostate cancer occurs spontaneously with appreciable frequency *(12,13)*, we examined the effects of dietary selenium supplementation on DNA damage and apoptosis in elderly beagle dogs that were physiologically equivalent to 62- to 69-year-old men and free of prostate cancer.

Forty-nine elderly (i.e., 8.5- to 10.5-year-old) sexually intact male, retired breeder dogs weighing 9–18 kg were purchased from a local supplier. After 4 weeks of acclimation, the dogs were randomly assigned to the control group (n = 10 dogs), which was fed a maintenance diet that contained 0.3 ppm selenium (Science Diet® Canine Maintenance; Hills Pet Nutrition, Inc., Topeka, KS), or to one of the four daily treatment groups, which received either the maintenance diet plus 3 μg/kg/day selenomethionine (Solgar Vitamin and Herb, Leonia, NJ) (n = 10 dogs), 6 μg/kg/day selenomethionine (n = 10 dogs), 3 μg/kg/day high-selenium yeast (SelenoExcell®; Cypress Systems, Fresno, CA) (n = 10 dogs), or 6 μg/kg/day high-selenium yeast (n = 9 dogs). The daily selenium intake for the dogs in the control group was approximately 6 μg/kg body weight. All dogs had nutritionally adequate selenium status prior to the start of the experiment [mean pretreatment plasma selenium concentration *(14)* was 275 ng/mL (range = 228–339 ng/mL)]. The dogs were fed their respective diets for 7 months. At the end of that period, peripheral blood lymphocytes were harvested from whole blood *(15–17)* that was obtained from each dog, and the dogs were then euthanized in accordance with guidelines set forth by the American Veterinary Medical Association Panel on Euthanasia *(18)*. The prostate was collected *in toto* from each dog within 15 minutes after euthanasia. Prostate tissue (50–80 mg) was harvested fresh to prepare prostate cell suspensions for alkaline comet assay. The remaining prostate was fixed in formalin, embedded in paraffin, and step-sectioned at 4-mm intervals.

The extent of DNA damage in prostate cells and in peripheral blood lymphocytes was measured by single-cell gel electrophoresis (alkaline comet assay) *(19)*. The extent of DNA damage was visually scored in 100 randomly selected cells from each sample using previously described criteria *(20,21)* (Fig. 1, A). The ApopTag™ peroxidase *in situ* apoptosis detection kit (Intergen, Inc., Purchase, NY) and a modification of the terminal deoxynucleotidyl trans-

Affiliations of authors: D. J. Waters, S. Shen, D. M. Cooley, Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN, and Gerald P. Murphy Cancer Foundation, Seattle, WA; D.G. Bostwick, J. Qian, Bostwick Laboratories, Richmond, VA; G. F. Combs, Jr., Division of Nutritional Sciences, Cornell University, Ithaca, NY; L. T Glickman (Department of Veterinary Pathobiology), C. Oteham, D. Schlittler (Department of Veterinary Clinical Sciences), Purdue University; J. S. Morris; University of Missouri–Columbia Research Reactor Center, Columbia.

Correspondence to: David J. Waters, D.V.M., Ph.D., Purdue University, School of Veterinary Medicine, 625 Harrison St., West Lafayette, IN 47907–2026 (e-mail: dwaters@gpmcf.org).

See "Notes" following "References."

Journal of the National Cancer Institute, Vol. 95, No. 3,

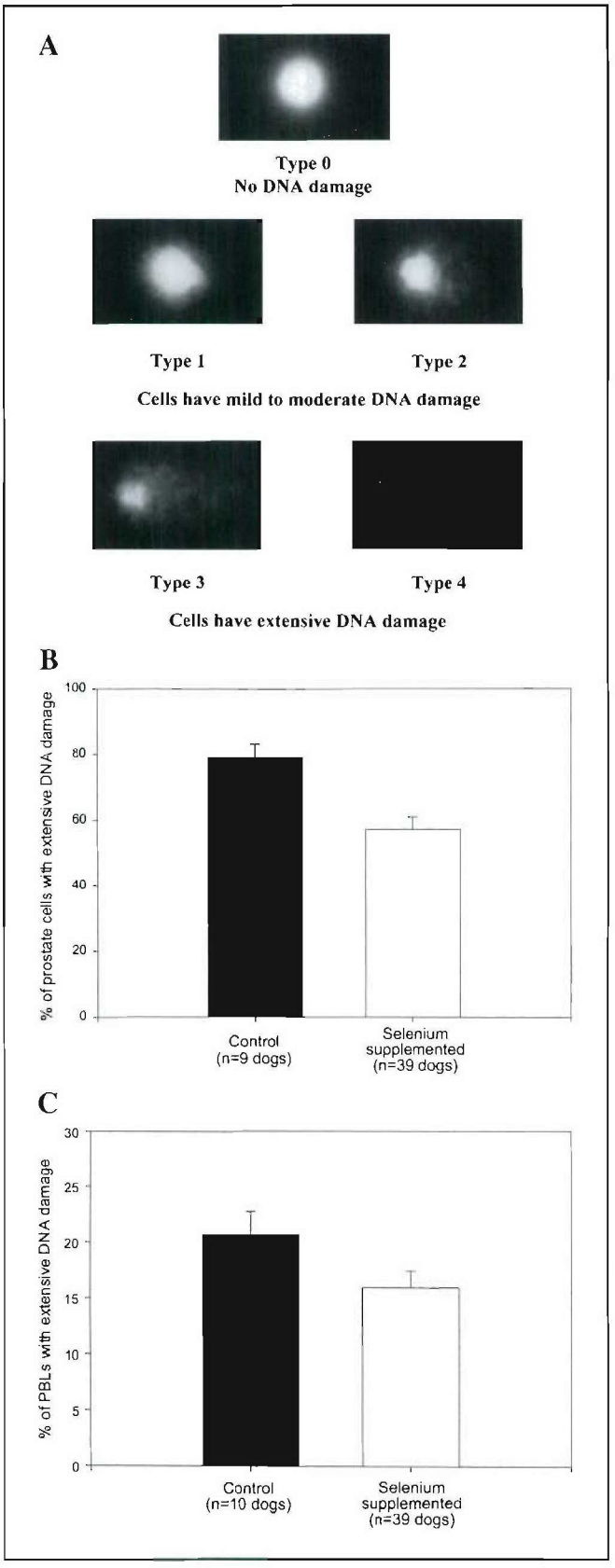

Fig. 1. DNA damage in prostate cells and peripheral blood lymphocytes (PBLs) from control dogs and dogs that received daily selenium supplementation. **A**) Extent of DNA damage in prostate cells and PBLs was measured by single-cell gel electrophoresis (alkaline comet assay) as described by Singh et al. *(19)*. Under the assay conditions used in this experiment, comet tails reflect the electrophoretic migration of DNA fragments that result from strand breaks, alkali-labile sites, crosslinks, or base excision repair sites *(19)*. Extent of DNA damage was scored in 100 randomly selected cells from each sample (50 cells from several different fields from each of two replicate slides) by an examiner who was blinded to treatment group. Each cell was visually scored as previously described *(20, 21)* according to the following criteria: no damage (type 0), mild to moderate damage (type 1 and type 2), and extensive DNA damage (type 3 and type 4). Extent of DNA damage within prostate cells or PBLs was expressed as the percentage of cells with extensive DNA damage (the total number of cells that displayed type 3 or type 4 DNA damage). **B**) DNA damage in prostate cells. Within 15 minutes of euthanasia, the prostate was collected from each dog at necropsy, and 50–80 mg of prostate tissue was placed in 1 mL of cold Hanks' balanced salt solution containing 20 m*M* EDTA and 10% dimethyl sulfoxide (DMSO) *(24)*. One dog in the control group had a tissue sample that was insufficient for further analysis. Tissue was then minced with fine scissors, and 50 μL of the resulting cell suspension was mixed with 1 mL of RPMI-1640 medium containing 10% fetal bovine serum for subsequent electrophoresis. Cytospin preparations of the cell suspensions indicated that greater than 90% of the cells had an epithelial morphology; the mean percentage of viable cells, as estimated by the trypan blue exclusion assay, was 80%. **Bars** = mean percentage (and the upper 95% confidence interval) of prostate cells that displayed type 3 or type 4 DNA damage. **C**) DNA damage in PBLs. PBLs were freshly harvested from whole blood *(15–17)* that was obtained from each dog after 7 months of treatment and prior to euthanasia. Cytospin preparations confirmed that more than 90% of the cells in this enriched cell population were lymphocytes; mean percentage of viable cells, as estimated by the trypan blue exclusion assay, was 91%.

ferase–mediated dUTP nick end-labeling (TUNEL) method *(22)* were used to determine the frequency of apoptosis within sections of dog prostatic tissue. Histopathologic evaluation of formalin-fixed, step-sectioned prostate tissue sections stained with hematoxylin and eosin revealed no foci of carcinoma in any of the dogs. The activity of selenium-dependent glutathione peroxidase in plasma collected immediately prior to euthanasia was assayed by the method of Lawrence and Burk *(23)* using 0.25 m*M* H_2O_2 as the acceptor substrate. All aspects of this experimental protocol were approved by the Purdue University Animal Care and Use Committee.

Analysis of variance was used to determine the statistical significance of differences between the control dogs and the selenium-supplemented dogs in the extent of DNA damage in prostate cells or peripheral blood lymphocytes after 7 months on the respective diets. Because no consistent differences in effects were observed with respect to the different forms or doses of selenium the dogs received, in all analyses control dogs were compared with all selenium-supplemented dogs. The median number of apoptotic epithelial cells within prostate tissue sections from control and selenium-supplemented dogs per ×200 microscope field were compared with the use of the Mann–Whitney *U* test. Fisher's exact test was used to compare the percentage of dogs in each treatment group that had more than 30 apoptotic cells per ×200 microscope field. This cutoff point represented a level of apoptosis that exceeded the mean number plus three standard deviations of apoptotic cells in prostate samples from dogs fed the control diet. A *P* value of less than .05 was considered statistically significant, and all tests of statistical significance were two-sided.

After 7 months of treatment, the percentage of prostate epithelial cells and peripheral blood lymphocytes with extensive (i.e., types 3 and 4; Fig. 1) DNA damage was statistically significantly lower in the selenium-supplemented dogs than in the control dogs (mean percentage of prostate cells with extensive DNA damage was 79.1% for the control group and 57.2% for the selenium-treated group [difference = 21.9%, 95% confidence interval [CI] = 13.6% to 30.1%, $P<.001$]; mean percentage of peripheral blood lymphocytes with ex-

tensive DNA damage was 20.7% for the control group and 15.9% for the selenium-treated group [difference = 4.8%, 95% CI = 1.7% to 7.9%, P = .003]) (Fig. 1, B and C). The mean percentage of prostate cells with extensive DNA damage in dogs in each of the four selenium treatment groups was statistically significantly lower than it was in dogs in the control group (mean percentage of prostate cells with extensive DNA damage was 79.1% for control dogs and 49.1% for dogs receiving 6 μg/kg/day high-selenium yeast [difference = 30.0%, 95% CI = 23.8% to 36.2%, P<.001]; 56.9% for dogs receiving 3 μg/kg/day high-selenium yeast [difference = 22.2%, 95% CI = 13.5% to 30.9%, P = .003]; 63.9% for dogs receiving 6 μg/kg/day selenomethionine [difference = 15.2%, 95% CI = 4.0% to 26.4%, P = .01]; and 58.1% for dogs receiving 3 μg/kg/day selenomethionine [difference = 21.0%, 95% CI = 13.5% to 28.5%, P<.001]). After 7 months of treatment, the mean (± standard deviation) glutathione peroxidase activity in plasma of control dogs that received a selenium-adequate diet was 25.5 ± 6.1 nm/mg protein, which was not statistically significantly different from the mean glutathione peroxidase activity in plasma of selenium-treated dogs (P>.05).

A very low level of apoptosis was observed within prostate cells from the

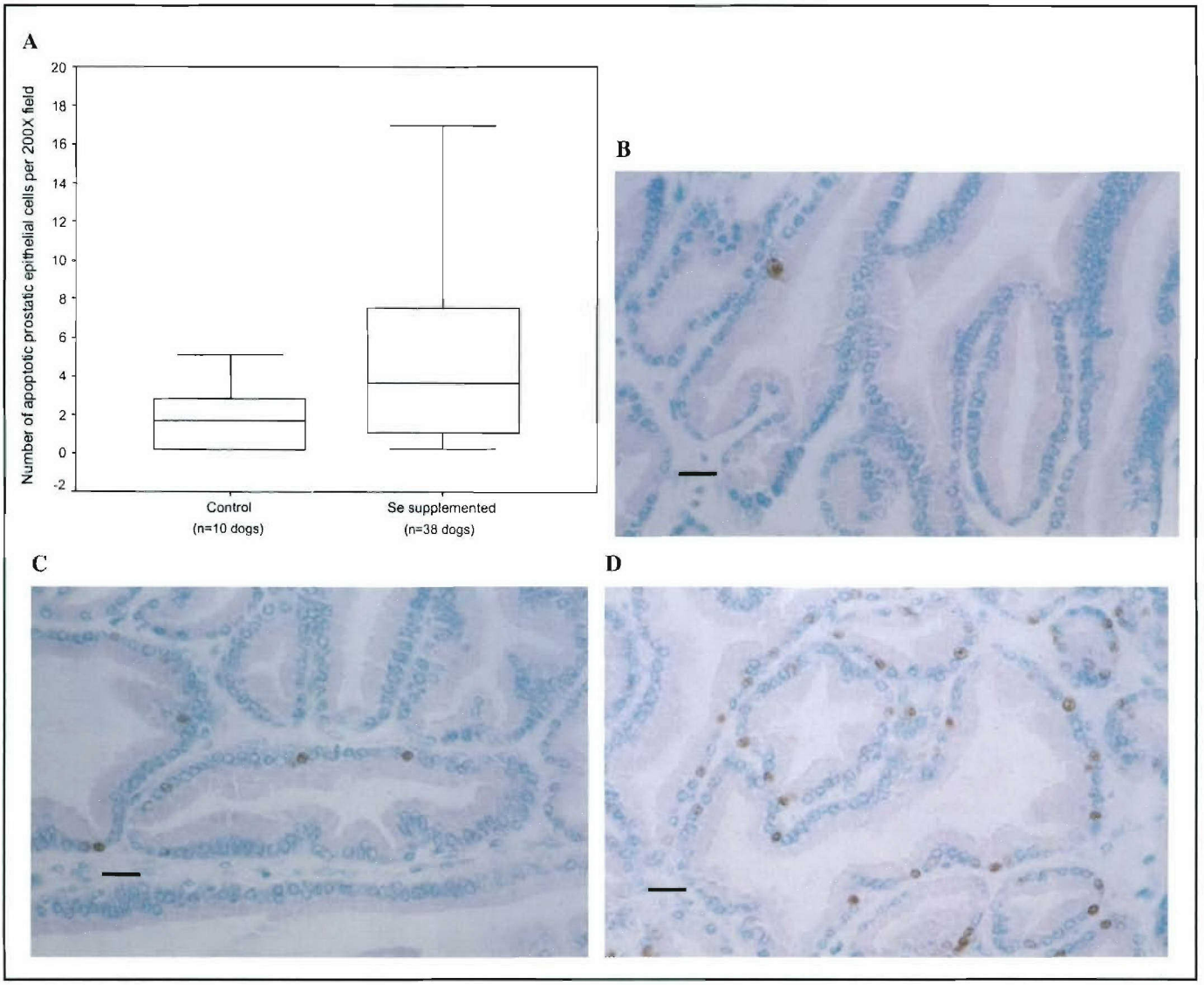

Fig. 2. Prostatic epithelial cell apoptosis in control dogs and dogs receiving daily selenium supplementation. A modified terminal deoxynucleotidyl transferase–mediated dUTP nick end-labeling (TUNEL) method was used to measure prostatic epithelial cell apoptosis *in situ* in formalin-fixed tissue specimens *(22)*. For each dog, the number of prostate epithelial cells with positive nuclear staining was counted in randomly selected, noncontiguous, ×200 microscopic fields. An average of 23 fields in one tissue section was evaluated for each dog. Immunopositive stromal cells, inflammatory cells, or epithelial cells that were shed into the acinar lumen were not counted. Microscopic fields that contained areas that displayed intense inflammation were not scored. **A**) Data are displayed in a box and whisker plot (prostate tissue from one selenium-supplemented dog did not react to staining). The **center horizontal line** indicates the median value for each group. The length of each box (interquartile range) indicates the range of the central 50% of values, with the box edges placed at the first and third quartiles. **Whiskers** (the lines extending beyond the box) show the range of observed values that are within 1.5 times the interquartile range. **Panels B, C,** and **D**) Representative photomicrographs of TUNEL-stained prostate tissue from a control dog (**B**) and a selenium-treated dog (**C**) demonstrate the increased number of epithelial cells with TUNEL-positive nuclear staining (brown) associated with selenium treatment. **Panel D** shows a region of markedly increased apoptosis ("hot spot") within the prostate of a selenium-treated dog. In each of these ×200 photomicrographs, the scale bar = 50 μm.

10 control dogs (median number of TUNEL-positive epithelial cells/×200 field = 1.7 cells; interquartile range = 0.2–2.8 cells) (Fig. 2, B). By contrast, 38 dogs treated with selenium for 7 months had an approximately twofold increase in the median number of apoptotic cells per field compared with control dogs (median = 3.7 cells; range = 1.1–7.6 cells) (P = .04) (Fig. 2, A and C). Foci of increased apoptosis (i.e., apoptotic hot spots), which were defined as those microscopic fields in which there were more than 30 apoptotic cells, were present in prostate tissue sections from 16 (42%) of 38 selenium-supplemented dogs (Fig. 2, D) but in prostate tissue sections from only one (10%) of 10 control dogs (P = .07). There were also no statistically significant differences between the two groups of dogs when the cutoff point for apoptotic hotspots was 20, 40, or 50 apoptotic cells per ×200 microscope field (P = .07 for each cutoff point).

Our results show that daily supplementation with nontoxic doses of selenium is associated with a decrease in the steady-state level of DNA damage and an increase in epithelial cell apoptosis within the aging canine prostate. Importantly, these effects of selenium supplementation were observed in dogs that had no histologic evidence of prostate cancer and that were of a comparable physiologic age to that of men enrolled in SELECT. We used the alkaline comet assay as a simple, robust method to assess DNA integrity in prostate cells to measure the effect of nutritional intervention on the level of genotoxic stress within the prostate. Two different forms and doses of selenium were consistently associated with a reduction in the steady-state level of DNA damage within the prostate of elderly dogs to levels lower than those measured in the prostate of young adult dogs (data not shown). These biologic responses within the canine prostate were accompanied by statistically significant increases in plasma and toenail selenium concentrations over the treatment period (data not shown). At the end of the study, mean concentration of selenium in toenails collected from selenium-treated dogs was roughly equivalent to the average selenium level found in toenails of men in the Health Professionals Study (data not shown) *(25)*.

The specific mechanism by which selenium supplementation exerts its anticarcinogenic effect on the prostate is unknown *(26,27)*. A reduction in the steady-state level of DNA damage within prostatic epithelial cells could result from a decrease in the rate of DNA damage formation, an increase in the rate or efficiency of DNA damage repair *(28)*, or the preferential elimination of epithelial cells that have the most extensive DNA damage. With regard to the latter possibility, selenium has been shown to induce apoptosis in several *in vitro* models of cancer *(27,29–32)*. Our data support the hypothesis that selenium sensitizes prostatic epithelial cells with extensive DNA damage to apoptosis *in vivo*. Our data also suggest that the effects of selenium on the level of DNA damage are independent of the effects of selenium supplementation on glutathione peroxidase activity. This observation in dogs is consistent with data from a randomized clinical trial of selenium supplementation in humans *(14)*, in which a 63% reduction in prostate cancer incidence was observed in selenium-supplemented men who already had maximal expression of plasma glutathione peroxidase prior to intervention (Combs GF Jr, Clark LC: unpublished data).

In summary, daily supplementation with nontoxic doses of selenomethionine or high-selenium yeast given prior to the development of carcinoma is associated with a reduction in the accumulation of genotoxic damage within the aging canine prostate. Therefore, selenium may benefit the aging prostate by decreasing the accumulation of DNA damage in epithelial cells even before these cells show cytologic changes suggestive of malignancy. We believe that DNA damage and apoptosis are selenium-responsive events that may be important regulatory points in multistep prostatic carcinogenesis. Further study of the process of carcinogenesis within the prostate of animal species vulnerable to spontaneous cancer development may provide important insights into the putative anticancer mechanisms of selenium and identify biomarkers that predict the prostate's response to selenium.

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NOTES

Supported in part by grant PC-970492 from the U.S. Army Medical Research and Materiel Command Prostate Cancer Research Program (to D. J. Waters).

We thank Karen Coffman, Maxine Nichols, and Patty Bonney for technical assistance. We also acknowledge Nancy Martin and Sam Royer for their expert assistance with histotechnology and medical photography.

Manuscript received March 21, 2002; revised November 13, 2002; accepted November 20, 2002.

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with Erin Breitenbach

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In The Spotlight

Does Selenium Prevent Prostate Cancer?

Learn about new research on how selenium inhibits the development of prostate cancer.

Thursday July 10, 2003 #

Men: Easy on the Zinc

Men who take too much zinc may have an increased risk for prostate cancer. Results of a National Cancer Institute (NCI) study reported last week showed that men who took more than 100 mg a day of zinc - far above the recommended level of 11 mg a day - had twice the risk of developing prostate cancer. Prostate cancer is the second most common cause of cancer death for men in the U.S. but studies have shown various dietary factors may reduce prostate cancer risk.

Sunday July 06, 2003 #

Do You Know Enough About Skin Cancer?

You may already know that a sun-safe lifestyle helps prevent skin cancer, but it's a good idea to review some of those frequently asked questions just to make sure. Also available online is a tool for testing your skin cancer FAQ.

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Pancreatic Cancer
Prostate Cancer

Does Selenium Prevent Prostate Cancer?

By: Kathleen Wildasin

David J. Waters, DVM, PhD, Director of the Gerald P. Murphy Cancer Foundation and Professor of Comparative Oncology at Purdue University, is leading a research team in the investigation of how selenium, a nutrient essential to the functioning of several metabolically important enzymes, inhibits the development of prostate cancer.

"Using elderly beagles to mimic 65-year-old men, we evaluated the effect of selenium on prostate cells in an appropriate context ... in vivo in an aging prostate gland," Waters said.

Although most information on the mechanisms of anticancer agents has been gleaned from studies using animal tumor models, studying prostate cancer in the laboratory has been hampered by the fact that only one non-human species, the dog, develops this cancer spontaneously and with appreciable frequency.

The research of Waters and colleagues complements the Selenium and Vitamin E Cancer Prevention Trial (SELECT), a study initiated in 2001 by the National Cancer Institute to evaluate whether selenium and/or vitamin E decreases the incidence of human prostate cancer. The largest prostate cancer prevention study ever undertaken, SELECT will evaluate more than 32,000 men during a 12-year period. The Murphy Foundation is one of more than 400 sites in North America that will enroll men into the SELECT Trial.

"In this study supported by the Department of Defense Prostate Cancer Research Program, we found that 7 months of daily oral supplementation, using the same form and dose of selenium currently being used in SELECT, significantly reduced the accumulation of DNA damage within prostate cells," Waters said.

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In the February 5, 2003 issue of the Journal of the National Cancer Institute, the group
also reported that daily selenium supplementation was accompanied by a two-fold increase in prostate cell apoptosis. Apoptosis, an orderly process of cell death, can remove damaged cells from the prostate, which may lower the risk of cancer.

"Although several previous studies have shown that selenium can induce apoptosis in the cell culture laboratory, our results represent the most convincing evidence to date that DNA damage and apoptosis are selenium-responsive events within the prostate," Waters said.

The long-term research goal of Dr. Waters' comparative oncology team is to accelerate the development and application of effective cancer prevention strategies that will benefit both people and pet animals who are at high risk of developing cancer.

For more information regarding this article, contact Kathleen Wildasin at kwildasin@insightbb.com.
For more information regarding research on selenium and prostate cancer, visit the Murphy Foundation website (www.gpmcf.org), under the section "About Selenium."

Bio: Kathleen Wildasin is a full-time freelance medical/science writer and editor. She holds B.A. degrees in biology and music theory/composition from Indiana University and the University of Minnesota, respectively, and an M.A. degree in music theory from the University of Iowa. Her articles have been published in magazines, education manuals, newsletters, and online, and her medical thriller and short stories have received recognition in national writing competitions. She lives in Lexington, Kentucky.

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Good News for **Men (and Dogs)**

With the help of some elderly beagles, experts discover an easy way to prevent prostate cancer.

BY SUSAN EDMISTON

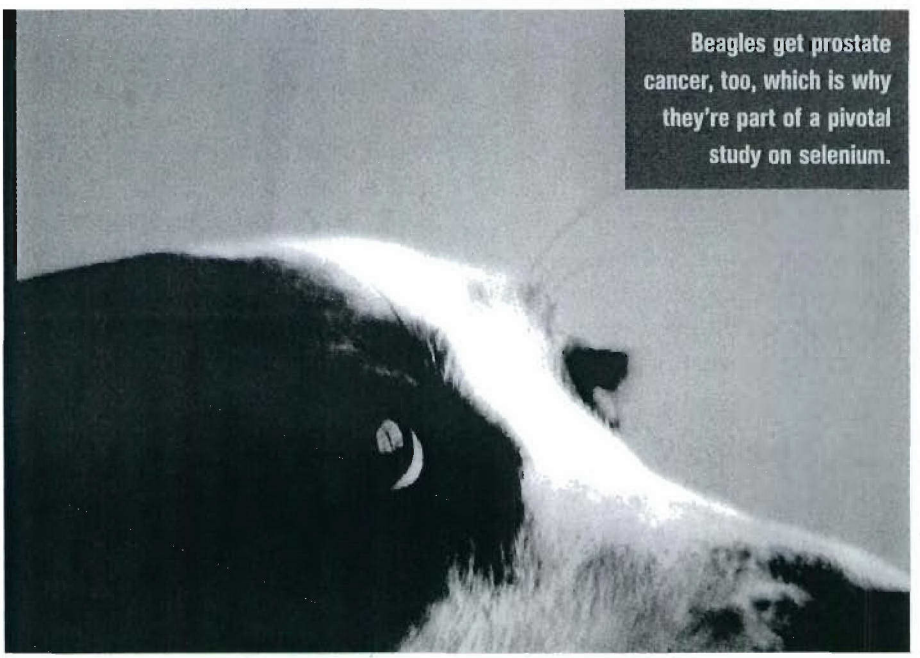
Men, take note: The evidence for an easy, inexpensive way to prevent your number-one malignancy—prostate cancer—has reached critical mass. Prostate cancer strikes nearly 200,000 men each year and kills more than 30,000, and it can devastate a man's sex life. But you may be able to avoid that fate by taking a simple daily supplement of the mineral selenium.

In fact, the evidence for selenium has swelled into a tide even the FDA couldn't ignore. Last February the agency, notoriously reluctant to give any supplement its imprimatur, allowed health claims to be made for selenium, stating that the mineral may reduce the risk of certain cancers. Although it permitted only a qualified claim—research has yet to determine exact dosages and other factors that may affect the supplement's effectiveness—the agency's action put selenium on the map as one of the most powerful weapons in our anticancer arsenal.

Research first linked higher levels of selenium to reduced cancer risk in the 1960s. But the results of a ten-year study, published in 1996, thrust the mineral into the spotlight. The late Larry Clark, then associate professor of epidemiology at the University of Arizona Cancer Center, had done a series of studies linking skin cancer to low selenium levels and decided to put his theory to the ultimate test: a double-blind, placebo-controlled study. For an average of four and a half years, 1,312 volunteers took either brewer's yeast tablets containing 200 micrograms of selenium or placebos.

Clark was surprised to find that the selenium had no effect on the skin cancers he was studying. But, as another selenium expert put it, "Then serendipity stepped in." Poring over his data, Clark noticed that the three leading cancers in men—lung, prostate, and colon—were significantly lower in the people taking selenium. He redesigned the study to collect more complete information and ultimately found a moderate decrease in cancer overall, but a whopping 63 percent lower risk of prostate cancer among the selenium-takers. (The study found no decrease in cancers for women, but since it focuses primarily on men—as does most subsequent selenium research—the jury's still out on whether women can benefit from supplements, too.)

Other researchers rushed to follow Clark's trail. In 1987, at Harvard, 33,737 male health professionals were asked to send in their toenail clippings, a measure of long-term selenium intake. Four years later, when the researchers matched the men to their clippings, they found that the rate of prostate cancer had decreased by one-half to two-thirds in those with the highest selenium levels.



Beagles get prostate cancer, too, which is why they're part of a pivotal study on selenium.

But perhaps the most exciting evidence of selenium's powers comes from a bunch of elderly beagles. As a

comparative oncologist (an expert in cancers affecting both humans and animals), David Waters, of Purdue University, knew that beagles also tend to develop prostate cancer with age, and that selenium had been shown to lower the risk in men. But he wanted to know *how* selenium worked its magic. So he assembled 49 dogs that were roughly equivalent in age to 65-year-old men and gave 39 of them 200 micrograms of selenium (twice what most Americans get from their daily diet). They also nibbled a brand of dog food that contains trace levels of selenium. The other ten pooches ate only the dog food.

The results, published last year in *Journal of the National Cancer Institute*, were impressive. After seven months, dogs who chewed selenium supplements along with their daily chow fared much better than those who didn't. Among the untreated dogs, nearly 80 percent of their prostate cells had extensive DNA damage, compared with 57 percent of cells from animals who got the extra selenium. When scientists examined the prostate tissue of all the dogs, they didn't find greater antioxidant activity in the selenium-takers—the mechanism they expected to be responsible for curbing cell damage—but they did find a much higher level of something called apoptosis.

Apoptosis is a normal biological process that, in effect, helps damaged cells commit suicide. When cells deteriorate or go haywire because of radiation, viral infection, aging, or the kind of aberrant growth that occurs with cancer, this process shuts them down, limiting the damage they can do. Waters' group found twice the level of apoptosis in the prostate tissue of the selenium-supplemented dogs as in the untreated beagles.

Does this mean men should all immediately begin taking 200 micrograms of selenium a day?

Many experts say yes, among them pioneering physician Dean Ornish, in Sausalito, California. "If a drug company came out with a medication that could reduce the risk of cancer to this degree, just about every doctor in the country would prescribe it," he says. "The potential benefit is great, the cost is very low, and so are the side effects and risks." Ornish's soon-to-be-reported Prostate Cancer Lifestyle Trial includes selenium supplements along with a low-fat plant-based diet and other cancer-reducing strategies.

"If a drug company came out with a medication that could reduce the risk of cancer to this degree, just about every doctor in the country would prescribe it."

John Finley, a scientist at the Human Nutrition Research Center in Grand Forks, North Dakota, also thinks men should be taking a daily supplement with 100 to 200 micrograms of selenium. (Until more research is done, it can't hurt for women to hedge their bets against cancer with a supplement, too.) Most people have blood levels of selenium of about 120 mcg just from diet alone, says Finley, but it takes around 300 mcg to get the benefits.

Some experts recommend even more. Stephen Strum, former medical director of the Prostate Cancer Research Institute in Los Angeles, thinks it's perfectly safe to take daily doses in the 400 to 800 mcg range—the amount physicians routinely recommend in England. But don't overdo it. In doses above 1,000 micrograms, selenium can lead to a disease called selenosis, which may cause neurological problems, hair loss, and deformed nails. Anyone taking large amounts should watch for side effects—oddly enough, the first sign that you may be headed toward selenosis is a garlicky smell on your breath and skin—and work with a physician to find the right dosage.

Some experts think selenium might work best when taken along with vitamin E. A vast ten-year study, called SELECT (Selenium and Vitamin E Cancer Prevention Trial), sponsored by the National Cancer Institute, was launched in 2001 to find out. In the meantime, you can't go wrong with a daily dose of both supplements.

Tell your friends about them, too—including any beagles you know.

Susan Edmiston is a contributing writer.

>>> Selenium User's Guide

What is it? A trace mineral that's been shown to prevent prostate cancer, and possibly other cancers as well.

Dosage: Many experts recommend a multivitamin with 100 to 200 micrograms of selenium. But daily doses in the 400 to 800 mcg range are considered safe, too.

Risks: In amounts above 1,000 mcg, the mineral can lead to a disease called selenosis, which causes neurological problems, hair loss, and deformed nails. —S.E.

Arthur S. Aubry/Photodisc

Us Too! Prostate Cancer Education & Support — Know Your PSA

Prostate Cancer Hot Sheet

Us Too! International — October 2003

Mortality From Four Leading Cancers Continues To Fall In US

Mortality from the four most common cancers in the US — lung, breast, prostate, and colorectal — continued to drop in the late 1990s, according to a report released Tuesday in the Journal of the National Cancer Institute.

Mortality for all cancer sites combined started to drop in 1994 and stabilized from 1998 through 2000, indicate the findings from the "Annual Report to the Nation on the Status of Cancer, 1975-2000." The report is a joint effort of many US health groups including the Centers for Disease Control and Prevention (CDC) and the American Cancer Society.

"This report shows that we have made some progress in reducing the burden of cancer in the US, but much still needs to be done to reach the Healthy People 2010 goals — including wider application of what science has shown to be effective in preventing, screening, and treating cancer," CDC director Julie Gerberding said in a statement.

Analysis of data from state and metropolitan area cancer registries revealed that incidence rates for all cancer sites increased between 1975 and 1992 and then dropped between 1992 and 1995, lead author Dr. Hannah K. Weir, from the Atlanta-based CDC, and colleagues note. Rates stabilized between 1995 and 2000.

The apparent stability in this most recent period was actually the result of two divergent trends, the researchers note. Although the incidence of lung cancer among men continued to fall, this trend was offset by a rise in new cases of breast and prostate cancer.

Death rates from all cancer sites started

(continued on page 5)

The Outdoor Channel Partners With *Us Too!* To Fight Prostate Cancer

The Outdoor Channel, a subsidiary of Outdoor Channel Holdings, Inc., and *Us Too!* International announced today a commitment to join forces in the war against prostate cancer. Executives from both organizations met to lock in the agreement at The National Conference on Prostate Cancer held in September in Burbank, California and release the news on the first day of National Prostate Cancer Awareness Week.

Based in Temecula, CA, The Outdoor Channel is a national cable network

dedicated to providing the best in traditional outdoor programming to America's sixty million anglers and hunters. The Outdoor Channel is available to nearly sixty million homes in the U.S. through a combination of cable networks and satellite providers. The network recently announced its launch to an international audience, reaching nearly eight million homes in Latin America. For more information, visit The Outdoor Channel's web site at www.outdoorchannel.com.

The partnership calls on The Outdoor Channel to lend its resources in an

(continued on page 8)

Brothers, not Father, Have More Affect on Prostate Cancer

Men are more at risk for prostate cancer if their brother — rather than their father — has the disease, according to new research. Researchers say this new finding may suggest that the risk is related to shared environmental factors like diet.

Led by Deborah Watkins Bruner, Ph.D., at Fox Chase Cancer Center in Philadelphia, researchers analyzed 23 published studies. They found an increased risk of prostate cancer for men with a family history, but if the affected family member was a brother, the risk increased nearly threefold. Links between first-degree relatives (father, son or brother) and second-degree relatives (a grandfather or uncle) were examined to see which relationship posed the greatest risk. Researchers found the risk increased 1.8 fold when the affected relative was a second-degree family member and 2.1 fold when the relative with prostate cancer was a father.

Bruner says, "Unlike the maternal-child pattern we see with inherited breast cancers, a brother with prostate cancer was associated with a significantly increased risk of the disease compared to a father or any other relative with the disease." In addition to the environmental factors, she theorizes the age of onset of the disease may reveal a stronger genetic risk. Although more research is needed, she says a combination of genetic and environmental factors likely contribute to increased risk.

Bruner also says, "We need to assess the risk of disease associated with younger age [less than 65 or 70 years] of onset, dietary habits and lifestyle behaviors that may interact with inherited genes to increase prostate cancer risk."

Source: International Journal of Cancer

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CRAIG KUREY, DIRECTOR OF OPERATIONS
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DOROTHY WIENCEK, PATIENT INFORMATION ASSISTANT
5003 FAIRVIEW AVENUE - DOWNERS GROVE, IL 60515
PHONE:(630) 795-1002 / FAX: (630) 795-1602
WEBSITE: WWW.USTOO.ORG
SUPPORT HOTLINE (OUTSIDE CHICAGO) 1-800-80-USTOO!

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RESEARCHERS ORGANIZE PROSTATE CANCER INTO GENETICALLY DISTINCT CATEGORIES

Daniel J. George, MD
Veritas Medicine

With over 180,000 new cases of diagnosed each year, there is an enormous number of men with prostate cancer. But is it all the same disease?

The natural history of prostate cancer has taught us that a subset of men - roughly 30,000 each year - will die from this disease, despite our best treatment efforts. Another subset - perhaps as many as 90,000 cases each year - may be incidental and pose minimal risk to the patient. Why then do we classify all these cancers by the same name?

The Gleason score, or grade of prostate cancer, has been the most successful method to date for sub-classifying the disease. The Gleason score, however, is not based upon any molecular or genetic markers in prostate cancer. In this month's issue of the journal Cancer Research, investigators at the Dana-Farber Cancer Institute published an attempt to sub-classify prostate cancers by their genetic makeup. A technique known as single nucleotide polymorphisms, or SNP mapping, allows researchers to create a genetic fingerprint of tumors. Cancers with similar fingerprints are clustered together to create an overall genetic map.

The efforts of the Dana-Farber team represent one of the first to genetically sub-classify prostate cancer, but more work still needs to be done to get a complete picture of the different types of prostate cancer. The SNP mapping technique is likely to improve with greater technological breakthroughs, and the general principle of classifying tumors according to their genetic profile has been validated in other diseases such as lymphoma. Ultimately, a more accurate classification of prostate cancers should lead to treatments customized to certain types pf prostate cancer, and perhaps target therapy more effectively.

Reference:
ME Lieberfarb et al. Genome-wide Loss of Heterozygosity Analysis from Laser Capture Microdissected Prostate Cancer Using Single Nucleotide Polymorphic Allele (SNP) Arrays and a Novel Bioinformatics Platform ChipSNP Cancer Research 63, 4781-4785 (2003).

TREATMENT OF ORGAN CONFINED PROSTATE CANCER WITH THIRD GENERATION CRYOSURGERY: PRELIMINARY MULTICENTER EXPERIENCE

Han KR, Cohen JK, Miller RJ, Pantuck AJ, Freitas DG, Cuevas CA, Kim HL, Lugg J, Childs SJ, Shuman B, Jayson MA, Shore ND, Moore Y, Zisman A, Lee JY, Ugarte R, Mynderse LA, Wilson TM, Sweat SD, Zincke H, Belldegrun AS.

J Urol. 2003 Oct;170(4):1126-1130

SUMMARY:

PURPOSE
Cryosurgical ablation of the prostate is 1 approach to the treatment of localized prostate cancer. Third generation cryosurgery uses gas driven probes that allow for a decrease in probe diameter to 17 gauge (1.5 mm). The safety, morbidity and preliminary prostate specific antigen (PSA) results of 122 cases are reported.

MATERIALS AND METHODS
A total of 106 patients have undergone percutaneous cryosurgery using a brachytherapy template with at least 12 months of PSA followup. Immediate and delayed morbidities were evaluated. PSA results at 3 and 12 months were recorded, and failure was defined as the inability to reach a nadir of 0.4 ng/ml or less.

RESULTS
Complications in patients undergoing primary cryosurgery included tissue sloughing (5%), incontinence (pads, 3%), urge incontinence/no pads (5%), transient urinary retention (3.3%) and rectal discomfort (2.6%). There were no cases of fistulas or infections. Postoperative impotence was 87% in previously potent patients. For patients who underwent salvage cryosurgery there were no fistulas reported and 2 (11%) patients required pads after salvage cryosurgery. A total of 96 (81%) patients achieved a PSA nadir of 0.4 ng/ml or less at 3 months of followup,

(continued on page 8)

WHAT EVERY DOCTOR WHO TREATS MALE PATIENTS SHOULD KNOW

Stephen B. Strum, MD

Prostate cancer diagnosis clinical practice guidelines:

Every man should have an annual PSA and DRE starting at forty years of age. Men at risk due to family history of prostate cancer (brothers, fathers, uncles), men with family history of breast cancer (mothers, sisters, aunts) and black men should begin annual screening at age 35.

A PSA of 2.0 and over at any age should be investigated to rule out prostate cancer (PC).

A first step in investigation of PSA's elevated at 2.0 and above should be a free PSA percentage test.

- A free PSA percentage of over 25% is associated with a low risk of prostate cancer.
- A free PSA percentage of under 15% is associated with a higher risk of prostate cancer
- A benign cause of an elevated PSA and a correspondingly low free PSA percentage would be prostatitis. Four to six weeks of Cipro or similar antibiotic should be prescribed prior to recommending a biopsy if prostatitis symptoms are noted or if expressed prostatic secretions (EPS) are consistent with prostatitis.
- BPH (benign prostate hyperplasia) does not cause a low free PSA percentage. It may cause an elevated PSA, however. So in the case of an elevated PSA but a high free PSA percentage, an estimate of gland volume by DRE or a transrectal ultrasound of the prostate may reveal findings consistent with a diagnosis of BPH.

Blood sampling for PSA determinations, done at least three months apart, and by the same laboratory using the same testing procedure, are necessary to establish PSA velocity (PSAV) and PSA doubling time (PSADT).

- A PSAV that exceeds 0.75 ng/ml/yr is associated with a higher probability of PC.
- A PSADT of less than 12 years is associated with a higher probability of PC.

PSA's that bounce up and down are more indicative of benign processes than malignant processes.

PSA's that show a persistent rise over time, particularly three consecutive rises, three months apart are suspicious for prostate cancer regardless of the level of the PSA.
Gland volume in cubic centimeters (cc) multiplied by 0.066 yields the amount of PSA produced by a normal, non-malignant gland. Any amount of PSA in excess of this should be considered to be produced by a malignant process until proven otherwise.

Pussycats vs. tigers:

Pussycats in general, have low PSA values (under 10) and long doubling times, as well as low PSA velocities. If a biopsy is done on a patient with a PSA that is under 10, the Gleason score often turns out to be (3,3). Depending on the calculated tumor volume, T-stage and other factors, many of these patients may be candidates for objectified observation as well as for any of the currently FDA approved local therapies. Patients who choose to monitor their disease status rather than seek immediate local treatment need to be vigilant and need to be aware that if disease progression is evident, they may need to consider a form of local treatment before the window of opportunity for successful local treatment slams shut.

Tigers in general, have high PSA's (over 10) OR very low PSA's associated with very aggressive, high Gleason score cancers. These are very dangerous because they often escape investigation for long periods of time since the PSA's appear to be in the so-called normal range. Investigating all PSA's 2.0 and over will help to catch these prostate cancers while they are still organ-confined and treatable with local therapies. The probability of spotting these low PSA/high Gleason score cancers is enhanced if patients and doctors monitor PSA levels over time to note any persistent increases even if the PSA is very low. High Gleason score cancers often have reverted to such a primitive state that they no longer secrete PSA into the blood. Therefore, in cases such as this, the normal guidelines for PSA velocity and doubling time may not be applicable.

SELECTED RESOURCES FOR PHYSICIANS AND PATIENTS:

On the Web:

The Prostate Cancer Research Institute (PCRI) web site at www.pcri.org. This site has a wealth of information including the Prostate Cancer Address Book listing expert prostate cancer physicians, software tools, and articles and the newsletter INSIGHTS.

The Phoenix5 web site at www.phoenix5.org This is a vast resource for the prostate cancer student, with information on nearly every aspect of the disease as well as an excellent glossary, many first person stories and the prostate cancer journal of the webmaster who died of prostate cancer in June, 2003.

Us Too! INTERNATIONAL – Prostate Cancer Education and Support website at www.ustoo.org The world's largest independent, charitable network of education and support groups for men with prostate cancer and their families.

In print:

"A Primer on Prostate Cancer, The Empowered Patient's Guide" by Stephen B. Strum, MD and Donna Pogliano, copyright 2002. Available through ***Us Too!*** for $20 (plus $5 s&h) through the ***Us Too!*** website or by calling (317) 558-4858 and at web booksellers and fine bookstores everywhere Everything you ever wanted to know about prostate cancer

AFRAID I HAVE BAD NEWS . . . TWELVE STEPS TO HANDLE A DISTURBING DIAGNOSIS

By Elizabeth Austin

AARP Magazine - May-June 2003

It's the bombshell everyone dreads. The doctor calls and asks you to visit so you can discuss your test results. Your biopsy has come back positive. Or your EKG is abnormal. Or your blood test revealed something questionable. Without warning and without preparation, you're suddenly battling a serious health problem.

What happens next? That depends partly on your individual situation. A cancer scare will bring one set of challenges and choices, and a life-threatening heart ailment will bring others. But experts say there are basic steps that all patients should take, no matter what illness they're facing. This 12-step plan will help you get the best possible care—and the greatest chance for a quick, successful recovery.

1. Start building your team. Don't try to get through this battle alone. Ask at least one trusted person to be your full-time advocate who can accompany you to doctor appointments, says Joni Rodgers, author of Bald in the Land of Big Hair, a memoir of her battle with lymphatic cancer. "You need someone who is objective and isn't going to hear just what they want to hear," she explains.

"Your best choice is someone who is not excitable or confrontational and who is good at taking notes," adds Marsha Hurst, Ph.D., director of the health advocacy program at Sarah Lawrence College in Bronxville, New York. If your sister gets hysterical, or your husband's ears hear only good news, ask a friend to step in.

You'll also need to designate an information manager, someone to return the 20 daily phone calls you'll soon be getting from concerned friends and relatives—or those half-forgotten acquaintances who want to hear all the gory details. Don't be timid about ducking out of distressing conversations. A good escape speech: "Although it means so much to me that you're interested, I'm not always able to talk about this. But I'll promise to keep you updated." Then give the friend's number to your information manager.

Call in outstanding favors when asking people to help; if you fed someone's goldfish for a week in 1982, that counts. "The definite skill that every survivor cultivates is the ability to assemble a team they can rely on," says Rodgers.

2. Don't let a gung-ho doctor rush you. Sometimes speed saves lives. When Rodgers was diagnosed with advanced cancer, her life depended on getting immediate treatment (starting the next day). "I had to depend on doctors to make a good decision in that moment," she explains. But whenever possible, take a few days, or even a couple of weeks, to ponder all your options—including the ones your physician may not know about. This is especially hard after you get hit with a diagnosis, and you're anxious. "I felt like I had a roach on me—get it off, get it off!" admits Rodgers. But jumping in to treatment too quickly—and without taking all of the steps outlined in this article—can lead to regret.

3. Take a hard look at your primary care doctor. If you've got a rare disease, the internist you've seen for years may be intrigued—but he's probably not the best physician to monitor your treatment. Make sure your doctor is up to speed on your particular condition. You can get the lowdown on him by calling your state board of medicine and checking his history and training at www.healthgrades.com. Also, directly ask your doctor if he feels qualified to treat you, and if he regularly performs the surgical procedure you may need done. If he's not the expert you need, he should be happy to refer you to a specialist who's better able to handle your case, says Richard A. Wherry, M.D., a family physician in Dahlonega, Georgia. "I never worry about losing control, because that's not what this is about." If he can't admit his limitations, consider changing doctors—if your insurance plan is flexible enough to allow this on short notice.

4. Invest 40 bucks in a microcassette tape recorder. This will allow you to record your talks with your doctor. "You can listen to it when you're not so upset and also let your family or other doctors listen to it," says journalist Curtis Pesmen, who wrote about his battle with colon cancer in Esquire. (Having a verbatim record can also help bring another doctor up to speed when you're looking for a second opinion.) Also, buy a heavy-duty, hard-to-lose notebook, and hand it over to your advocate during appointments. Don't even think about trying to write while you're listening to a doctor talk about your life. "It's like trying to take notes while you're being attacked by a dog," Rodgers says.

5. Tap two brains. Don't hesitate to get a second opinion—and don't feel uneasy about telling your doctor you want one. "When one of my patients gets a second opinion, only two things can happen, and they're both good," says Wherry, who is also on the Board of Directors of the American Academy of Family Physicians. "Either I'm right, or the other doctor finds something I didn't diagnose and the patient comes out ahead." Let statistics encourage you: In about one in five cases, the second opinion yields a different diagnosis, says Charles Inlander, president of the People's Medical Society, a consumer health advocacy group in Allentown, Pennsylvania. And even if the second doctor agrees with the diagnosis, she may have different ideas for the best treatment.

A political tip: Don't ask for a second opinion from another physician in your own doctor's practice; they're not likely to contradict each other. A doctor who works with a different hospital, preferably outside your insurance network, is usually the most unbiased choice. (Many insurance plans will pay part of the cost of consulting a specialist outside your network.)

When you're investigating treatment options with each doctor, make sure you're getting the whole story. "Ask 'What is the most aggressive treatment, what's more conservative, and what are the points in between?' " Inlander advises. Then ask the specialist what he or she thinks is the smartest strategy and why. Follow up by asking whether your insurance company covers the other options. If it doesn't, ask why.

You're likely to wind up with some conflicting opinions, which isn't necessarily bad. Tell your primary doctor the options you're considering and ask for help in determining the risks and benefits of each. "I try to take it from the patient's perspective and ask, 'If you had a preference and the outcomes were similar, what would you like to do?' " says Wherry. "Ultimately, you're the one who has to make this decision."

If there's major disagreement, seek a tiebreaker. Some health insurance companies will pay for a third specialist, Inlander says.

6. Make hurried doctors listen. You'll likely encounter several doctors of different skills and temperaments during this journey. Remember that some of the best physicians are the worst communicators; prescription pads never talk back. To make her doctor listen, Rodgers practiced this line: "I need to say something, and if you promise to listen without interrupting, I promise to speak for 90 seconds or less." It's a surefire way to get silence. It sounds far more reasonable than "just two minutes"—which doctors hear as patient-speak for "a half-hour or so." And, if you're well-prepared, 90 seconds is enough time to say everything you need to say (the "Gettysburg Address" took scarcely longer than that).

7. Get educated, not distraught. Finding health news and research about your condition on the Internet can be helpful, but it can also be a source of misinformation and needless worry. To ensure you're getting reliable information, stick with websites backed by known organizations. A prominent one is MedlinePlus (medlineplus.gov), a site jointly provided by the U.S. National Library of Medicine and the National Institutes of Health. Also, the site at healthfinder.gov has links to more than 1,800 health-related organizations.

Offline, some hospitals and university medical centers offer well-stocked medical libraries, with librarians and research assistants to help patients wade through them. For example, the Stanford University Medical Center's Health Library offers free research help to anyone seeking information on an illness or treatment. "We walk through every patient's case individually and provide scientifically based medical information to help them make informed decisions about their health care," says the director of special patient services, Barbara Ralston. To reach the library, call 800-295-5177 or visit healthlibrary.stanford.edu.

Don't hand your doctor a thick sheaf of medical journal articles and expect him or her to read them on the spot. Instead, Inlander suggests, use your research to create a list of half a dozen "talking points," and offer your doctor copies of your research.

8. Choose your hospital wisely. The closest hospital may be convenient, but it's probably a poor choice unless its staff has a great deal of experience in treating patients in your situation. You can get a quick read on this by checking www.healthgrades.com, and by calling the hospital and asking the medical director how often its doctors treat your condition. If you find that the closest qualified hospital is 500 miles away, ask your doctor if he can consult with the specialists there.

9. After checking in, shake some hands. "When you get into your hospital room, the first thing you should do is call and ask the hospital's patient representative to come up so you can introduce yourself," Inlander says. "If you encounter problems, that person is responsible for making it right." (Ask for the patient representative's number when you check in, or ask a nurse.) Your friendliness will pay off if you have a problem; the advocate knows how to intervene if the night staff keeps waking you up to take your sleeping pill, for instance.

10. Chat up the nurses. It could yield more than extra pillows. "They have terrific insider information," says Dr. Hurst. Not only can they make your stay more comfortable, they can give you important treatment advice, too. You may need to listen for code words; a nurse could lose her job for telling you she wouldn't let your surgeon cut her hair. But if you hear a hint that she thinks you'd be better off with another doctor, take it seriously.

11. Stay sane. The emotional stress of battling a serious illness can take a large toll on your mental health—and the stability of your relationships. Joining a support group and venting to others who have been in your shoes can help; just make sure they're an optimistic bunch. "You can learn from other people who have gone through this situation," says Inlander, "but avoid groups that don't give you positive vibes."

12. Be blissfully self-indulgent. When you're recuperating, forget about being the "perfect patient"—cheerful, brave, and attuned to everyone else's needs. Take all the slack that friends and family readily give you during this furlough, and don't feel guilty. Karma will come around. "The good news, if you can call it that, is that everything you go through will help you be part of someone else's support system six months or a year from now," says Pesmen, who—knock on wood—has been cancer-free for two years. "It's a small bonus at the end of a long, hard ride."

Elizabeth Austin is an award-winning health writer in Chicago.

CANCER MORTALITY CONTINUES TO FALL

(continued from page 1)

to decline in 1994, the investigators state. Although men continued to experience a slight fall in death rates throughout the 1990s, women's rates essentially stabilized between 1998 and 2000.

Mortality due to lung cancer continues to drop among black and white men, while the rate of increase in death rates has slowed among women, the findings indicate. Death rates for cancer of the breast, prostate, and colon all continue to fall.

In a related editorial, Dr. M. J. Quinn, from the National Cancer Intelligence Centre in London, comments that "a principal strength of the report is that it provides a wealth of information on the cancer trends in terms of both incidence and mortality."

"The establishment in the US of state cancer registries, in addition to the high quality Surveillance, Epidemiology, and End Result (SEER) registries, is a major step forward in cancer control," Dr. Quinn adds.

EARLY DETECTION REDUCES PROSTATE CANCER DEATHS

New research shows earlier detection and wide use of hormone treatment have driven down prostate cancer's death rates.

A British researcher said death rates dropped by one-third in North America and by 20 percent in Europe since 1990 among men aged 65 to 74.

Previous studies have demonstrated hormone treatment delays the progression of prostate cancer and makes patients feel better. The new study offers evidence that the approach can save lives.

Prostate cancer is most often driven by the male sex hormone testosterone. Therapy blunts the ability of the hormone to stimulate cancer cells.

The findings were presented September 22 at a European conference on cancer.

Make YOUR Best Treatment Choice for Early Prostate Cancer

Rachel Snyder - CancerSource

"In the late 80s, we found it was men with prostate cancer who wanted the least involvement in making their treatment decision—" says Lesley Degner, RN, PhD, "how things have changed!"

Today most men newly diagnosed with early stage (localized) prostate cancer—cancer that has not spread outside of the prostate gland—will not be content with saying, "Yes doctor, whatever you think is best." The number of treatment choices has gone up, and the side effects can change a man's life. This will cause many men to get more involved and to seek more information in order to make the "best choice," as described by Donna Berry, RN, AOCN, PhD.

In interviews with CancerSource, Degner and Berry provided treatment decision-making advice for men with early stage prostate cancer. They each have more than 25 years of experience studying decision-making and the human response to cancer, and new research recently published.[1,2] Read what they have to say for help in making the best treatment choice for you.

The Diagnosis

If a man has some of the symptoms of prostate cancer, a doctor or nurse will first ask questions about these symptoms. A physical exam and other tests will be done. If any of these test results suggest that cancer may be present, the doctor will order a biopsy of the prostate. A biopsy is the only sure way of knowing whether a problem is because of cancer

It takes about a week for the biopsy results to come back. "Physicians have their own preferences and styles," says Berry. "Men should ask their physician before the biopsy how they will hear about the results, for example, 'Will you call me or will you let me know at my next appointment?'"

Men should start learning about treatment options soon after their diagnosis. Many men find themselves shocked, "I have cancer?!" This can make it hard to take in all of the information. Many people feel like they need to make a treatment decision quickly so the cancer doesn't spread. "Men shouldn't feel rushed to make a treatment decision even if they or their family are feeling anxious about it," says Degner. "A lot of people, when they're diagnosed with cancer, think it's growing like a mushroom. While there are some tumors that are extremely aggressive and very rapidly growing, the majority of prostate cancer tumors have been there for a long time, it was just that they were undetectable."

If you can tolerate waiting and the doctor says it is ok (the tumor is not growing fast), try to slow the treatment decision-making pace down. "Think very carefully about what you're doing and look at all of the options," says Degner.

"With prostate cancer it's never a bad idea to get a second opinion, it's a good idea!" says Berry. "The treatments for prostate cancer are very diverse, so many men feel comfortable when they talk to different specialist." A man should consider a second opinion as soon as they hear the biopsy results.

Your Treatment Information and Discussion

Before hearing about the different treatment options, the man should tell the doctor about himself. "What happens too often is we load the person up with information and we don't listen," says Berry. "The conversation should focus on what the doctor needs to know about the man so decisions can be framed around who the man is and how the treatment fits into his life."

Both Degner's and Berry's new studies showed how personalizing the treatment discussion and information could help a man with his decision. Degner's study1 looked at 74 couples in which a partner was newly diagnosed with early stage prostate cancer. In counseling sessions, these men and their partners talked about what was most important to them at the time of diagnosis. For example, for some men sexuality after treatment was the most important to them. So, these men were given information about treatment based on how it would affect their sexuality. After receiving individualized information, four months after diagnosis most men reported that they took on a more active role in making the decision and their partners took on less of a role than they thought they would, and everyone involved had lower levels of stress.

Berry's study[2] looked at 44 men who were within 6 months of a diagnosis of early stage (localized) prostate cancer. The study was to see how men came to "making the best choice" for treatment. The researchers concluded that men make "the best choice for me" based on the medical information they received from all sources (the first doctor, second opinions, Internet, friends, etc.), plus personal factors (their job, past experiences with cancer, etc.). "The health care team has to customize the education they give men based on who they are and what they do," says Berry, "it's not enough to just provide medical information."

You may have to start the conversation about yourself, don't depend on your doctor to do so. "At a minimum men should be talking about what they do for a living, for recreation, who they know that's had cancer and what are the stories they've heard about men with prostate cancer or other people who have had cancer," says Berry. "Men can make a decision based on misinformation if they haven't talked to their doctor about what they've heard and what their priorities are."

Berry recommends that you lead your doctor towards this discussion by saying, "Well, before I hear about the treatment options and outcomes, I would like to tell you more about myself because it has a lot to do with my decision." For example, "I have a job where I walk a lot in my work. It's really important that you know that I can't get to a bathroom on the job, and I can't afford to take too much time off after surgery." Knowing this information, when the doctor talks about incontinence (unable to control urine) he can personalize the information. Rather than saying, "Your chances of incontinence are 15 percent," he can say, "Your chances of incontinence are 15 percent, and if you had surgery you may have to be

prepared to take six months off of work to resolve this side effect."

The Choices

"Since there is no one best treatment for localized prostate cancer, most men are given the choice in treatment," says Berry, "the doctor and the man must work together to decide." Here is basic treatment information and questions that will help you successfully work with your health care team.

Common treatment options for early stage (localized) prostate cancer:

- Surgery (called prostatectomy). Surgical removal of the prostate and any remaining tumor.
- Radiation Therapy. X-rays used to kill cancer cells. External beam radiation is given outside of the body. Brachetherapy is done inside the body by placing radiation seeds into the prostate.
- Watchful Waiting. Monitoring or checking cancer that is growing slowly and will not do any harm for a long time, if ever.
- Hormonal Therapy. Lowers or blocks the male hormone, testosterone, to slow the growth of prostate cancer. This can be done by removal of the testicles, by giving an injection, or taking a pill.
- Cryosurgery. Kills cancer cells by freezing the prostate gland.

Most men are given the major treatment options of surgery and radiation, and they are usually told about watchful waiting. Whether the other options, such as cryosurgery or hormonal therapy, are discussed depends on the doctor "It's hard to image that someone who has spent 10 to 20 years learning to do surgery would say that surgery is not a good option," says Berry. It is good to talk to doctors from various specialties and try to gather more information.

"In our research we have found that thousands and thousands of men, even if they're not able to say it, want to know their chances of a cure and how far the disease has spread," says Degner. "Write down your top questions before your discussion, and don't leave without having them answered. If you can't say them, give the doctor the piece of paper."

Men should ask about the doctor's record. For example, how much experience does the doctor have doing the treatment? Do they perform two or 100 a year? This will be a bigger issue in a small town or more rural setting. Men should also hear what the doctor's outcomes are. For example, what percent of the men cannot control their urine after the surgery and what percent are able to be totally dry. "Physicians will often quote the literature," says Berry, "but you don't want a quotation of an unknown expert, you want to base your decision on the record of the physician whose office you're sitting in."

Men should ask about the treatment scheduling. How often do they have treatment, how much time does it take, how much follow-up will it require? They should ask who will work with them on making the treatment decision and after it is made. "These questions are important just so the man knows what the routine is going to be," says Berry. "It's not just the short term stuff that you have to think about," says Degner, "make sure you also ask about the long term side effects." For example, with prostate cancer, the most common are erectile dysfunction (cannot get a penile erection) and urinary incontinence.

"Often times you're just focused on getting through the treatment, which is important," says Degner. "But, most people go on and survive their cancer and live to die of something else. You don't want to be living with the serious side effects of your cancer treatment for the rest of your life. But if you have to, it would be nice to know about it before you're treated, so you can at least make the choice."

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2. Berry DL, Ellis WJ, Woods NF, Schwien C, Mullen KH, Yang C. (2003). Treatment Decision Making by Men with Localized Prostate Cancer: The Influence of Personal Factors. Urologic Oncology, 2003, MarApr;21(2):93100.

Questions to ask about prostate cancer treatments

If you are having treatment

- What are my treatment choices?
- What are the expected benefits of each kind of treatment?
- What are the risks of each treatment?
- What are the side effects of each treatment?
- Are there new treatments or clinical trials that I should consider?
- What are my chances of being cured?
- How will we know if this is working?
- How will each treatment affect my daily life?
- What are the chances of the tumor coming back again?

Surgery

If considering surgery

- What kinds of surgery can I consider? Which operation do you recommend for me?
- Will I need radiation after surgery?
- How will I feel after surgery?
- Where will the scars be? What will they look like?
- Will I have to do special exercises after surgery?
- When can I get back to my normal activities?

Radiotherapy

If you are having radiotherapy

- What is the goal of this treatment?
- How will the radiation be given?
- How many treatments will I get? Over what period of time?
- When will the treatment begin? When will it end?
- How will I feel during radiation therapy?
- What can I do to take care of myself during therapy?

Hormonal Therapy

If you are having hormonal therapy

- Why do I need this treatment?
- What drugs will I be taking? How often? For how long? What will they do?
- What can I do about side effects?
- If I need hormonal treatment, which would be better for me, drugs or an operation?
- How long will I be on this

DOES SELENIUM REDUCE THE RISK OF DEVELOPING PROSTATE CANCER?

By Kathleen A. Wildasin

Results of a recent experimental study offer new insights into how dietary supplementation with a trace mineral might reduce the risk of prostate cancer.

David J. Waters, DVM, PhD, Director of the Gerald P. Murphy Cancer Foundation and Professor of Comparative Oncology at Purdue University, is leading a research team in the investigation of how selenium, a nutrient essential to the functioning of several metabolically important enzymes, inhibits the development of prostate cancer.

"Using elderly beagles to mimic 65-year-old men, we evaluated the effect of selenium on prostate cells in an appropriate context ... *in vivo* in an aging prostate gland," Waters said.

Although most information on the mechanisms of anticancer agents has been gleaned from studies using animal tumor models, studying prostate cancer in the laboratory has been hampered by the fact that only one non-human species, the dog, develops this cancer spontaneously and with appreciable frequency.

The research of Waters and colleagues complements the Selenium and Vitamin E Cancer Prevention Trial (SELECT), a study initiated in 2001 by the National Cancer Institute to evaluate whether selenium and/or vitamin E decreases the incidence of human prostate cancer. The largest prostate cancer prevention study ever undertaken, SELECT will evaluate more than 32,000 men during a 12-year period. The Gerald P. Murphy Cancer Foundation, a not-for-profit cancer research organization in West Lafayette, IN and Seattle, WA, is one of more than 400 sites in North America enrolling men into the SELECT Trial.

"In this study supported by the Department of Defense Prostate Cancer Research Program, we found that 7 months of daily oral supplementation, using the same form and dose of selenium currently being used in SELECT, significantly reduced the accumulation of DNA damage within prostate cells," Waters said.

In the February 5, 2003 issue of the *Journal of the National Cancer Institute*, the group also reported that daily selenium supplementation was accompanied by a two-fold increase in prostate cell apoptosis. Apoptosis, an orderly process of cell death, can remove damaged cells from the prostate, which may lower the risk of cancer.

"Although several previous studies have shown that selenium can induce apoptosis in the cell culture laboratory, our results represent the most convincing evidence to date that DNA damage and apoptosis are selenium-responsive events within the prostate," Waters said.

Does this study provide evidence that selenium supplementation can be used to effectively treat prostate cancer?

"In our experiments, we studied the effects of selenium on the aging prostate gland prior to the development of prostate cancer," Waters said. "One should always use caution before concluding that an intervention that is beneficial in a prevention setting will also be beneficial for treatment."

Several scientists, including Waters, are actively investigating the effect of selenium on cancer cells in the laboratory. "There is still a lot about selenium's effect on the prostate that remains unknown to us," Waters conceded.

The long-term research goal of Dr. Waters' comparative oncology team is to accelerate the development and application of effective cancer prevention and treatment strategies that will benefit both people and pet animals who are at high risk of developing cancer.

For more information regarding this article, contact Kathleen Wildasin at kwildasin@insightbb.com

For more information regarding research on selenium and prostate cancer, visit the Murphy Foundation website (www.gpmcf.org), under the section "About Selenium."

SOURCES:

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Us Too! AND OUTDOOR CHANNEL PARTNERSHIP

(continued from page 1)

effort to promote awareness, education, prevention and treatment of the disease that will be newly diagnosed in more than 220,000 men in the USA this year. To this end, the International cable network will draw upon its reach to more than 60 million homes in the United States and Latin America.

"Every sixteen minutes someone dies of prostate cancer in this country alone," said John Page, President and CEO of *Us Too!* INTERNATIONAL. "This simply does not have to be. There are more effective treatment options today than ever before, and death from Prostate Cancer can be practically 100% avoidable if men simply take responsibility for their health and get tested annually to detect the disease early."

"Our viewers are mostly male, and men of an age ideal for early awareness and education of prostate cancer prevention," said Amy Hendrickson, Senior Vice President of Affiliate Sales and Marketing for The Outdoor Channel. "We believe this is one of the most effective and meaningful ways we can fulfill our responsibility to help better the community that we serve."

POSITIVE MULTICENTER CRYO RESULTS

(continued from page 2)

while 79 of 106 (75%) remained free from biochemical recurrence at 12 months. A total of 42 (78%) low risk patients (Gleason score 7 or less and PSA 10 or less) remained with a PSA of 0.4 ng/ml or less at 12 months of followup, compared to 37 (71%) high risk patients. All patients were discharged within 24 hours.

CONCLUSIONS

After a followup of 1 year 3rd generation cryosurgery appears to be well tolerated and minimally invasive. The use of ultrathin needles through a brachytherapy template allows for a simple percutaneous procedure and a relatively short learning curve. A prospective multicenter trial is ongoing to determine the long-term efficacy of this technique.

American Association for Cancer Research Frontiers of Cancer Prevention, Phoenix, AZ, October 2003

Is the Anti-Trophic Effect of the 5α-Reductase Inhibitor Finasteride on the Aging Prostate Influenced by Selenium Status?

Dawn M. Cooley[5], Shuren Shen[5] , Carol Oteham[1], Deborah Schlittler[1], Lawrence T. Glickman[1], David G. Bostwick[2], J. Steven Morris[3], Gerald F. Combs Jr[4], David J. Waters[1]

Purdue University[1], West Lafayette, IN; Bostwick Laboratories[2], Richmond, VA; University of Missouri-Columbia Research Reactor Center[3], Columbia, MO, Cornell University[4], Ithaca, NY; Gerald P. Murphy Cancer Foundation[5], Seattle, WA.

In previous work, we showed that daily selenium supplementation reduced DNA damage and increased epithelial cell apoptosis within the aged dog prostate. These results add to a growing body of evidence that trace minerals, such as selenium or zinc, play an important role in genomic protection and growth control within the prostate. Daily treatment with finasteride, a 5α-reductase inhibitor that exerts potent anti-trophic effects on the prostate, is currently under extensive evaluation as an approach to prostate cancer prevention. The purpose of this study was to test the hypothesis that selenium status significantly influences the prostate's response to daily treatment with finasteride. We studied elderly (8.5-10.5 year-old) sexually intact male beagle dogs with nutritionally adequate selenium status. Fifty-seven dogs were randomly assigned to: a control group (n=13 dogs); or to receive daily supplementation with finasteride (0.5 mg/kg) (n=9 dogs); supranutritional dietary selenium (3 μg/kg high selenium yeast, SelenoExcell, Cypress Systems, Fresno, CA) (n=15 dogs); or high selenium yeast plus finasteride (n=10 dogs). As the first step in analyzing our experimental results, we focused on the effects of treatment on prostate volume. For each dog, prostate size in 3 dimensions was measured with calipers prior to treatment and after 6 to 7 months treatment. Prostate weight was calculated using the formula: weight (g) = volume (cm^3) x 0.602 + 1.16. The anti-trophic effect of finasteride on the prostate was assessed by calculating the percent change in prostate volume over the treatment period. Actual prostate weight recorded at the end of the study was strongly correlated with prostate weight calculated from prostate volume ($r = 0.963$; $p < 0.0001$), validating prostate volume as a robust and reliable index of prostate growth. Dogs in the control group had a median change in prostate volume of +15% over the treatment period. Similarly, dogs receiving supranutritional selenium supplementation had a 16% median increase in prostate volume. In contrast, finasteride-treated dogs had a 42% median reduction in prostate volume after 6 months of treatment ($p<0.0001$ vs. control group). Finasteride-treated dogs that received supranutritional selenium had a 38% median reduction in prostate volume, which did not differ from dogs treated with finasteride alone ($p=0.52$). These preliminary data suggest that selenium status does not significantly influence the anti-trophic effects of finasteride on the aging prostate. The dog model enables us to study *in vivo* how differences in selenium status (i.e., nutritionally adequate versus supranutritional) influence prostate cell response to other potential cancer preventive agents. Further analysis of these dogs will determine to what extent the combination of selenium and finasteride affect biomarkers of growth regulation and carcinogenesis within the aging prostate.

International Conference on Antimutagenesis and Anticarcinogenesis, Pisa, ITALY, November 2003

RELATIONSHIP BETWEEN SELENIUM STATUS AND THE EXTENT OF GENOTOXIC STRESS WITHIN THE AGING PROSTATE

David J. Waters[1,5] , Shuren Shen[1,5] , Dawn M. Cooley[1,5], David G. Bostwick[3], Junqi Qian[3], Lawrence T. Glickman[2], J. Steven Morris[4]

Departments of Veterinary Clinical Sciences[1] and Veterinary Pathobiology[2] (ltg@purdue.edu); Bostwick Laboratories[3] (bostwick@bostwicklaboratories.com, jqian@bostwicklaboratories.com); University of Missouri-Columbia Research Reactor Center[4] (morrisj@missouri.edu); Gerald P. Murphy Cancer Foundation[5] (dwaters@gpmcf.org, sshen@gpmcf.org, dmc@gpmcf.org).

Analysis of toenails from men in the Health Professionals Follow-up Study showed an inverse association between selenium status and risk for advanced prostate cancer, with no substantial reduction in prostate cancer risk in men with toenail selenium concentration exceeding 0.85 ppm. In a previous study, we found that daily supplementation with selenomethionine or high selenium yeast significantly reduced DNA damage within the prostate of elderly dogs of a comparable physiologic age to men enrolled in the Selenium and Vitamin E Prostate Cancer Prevention Trial (SELECT). The objective of this study was to determine if toenail selenium concentration provides a readily accessible surrogate biomarker predictive of the extent of genotoxic damage within the prostate. We studied 49 (8.5 – 10.5 year old) sexually intact male, retired breeder dogs that were randomly assigned to either a control group or to receive daily supplementation with selenomethionine or high selenium yeast at 3 or 6 μg/kg body weight. After 7 months, toenail and prostate tissue specimens were collected immediately after euthanasia and analyzed for total selenium concentration using neutron activation analysis. Dogs from control and selenium treated groups were combined and subdivided into quartiles based on their toenail selenium concentration to evaluate the relationship between toenail selenium level and extent of DNA damage within the prostate as measured by alkaline comet assay. Dogs with the lowest toenail selenium concentration had the highest extent of genotoxic damage within the prostate (ANOVA, $p < 0.0001$). The relationship between the percentage of cells with extensively damaged DNA and toenail selenium concentration was non-linear and U-shaped. In dogs that had toenail concentrations in the two lowest quartiles, there was a significant inverse correlation between DNA damage within the prostate and selenium status ($r = -0.78$, $p < 0.0001$). There was no additional decrease in DNA damage within the prostate of dogs that had toenail selenium concentration in the two highest quartiles (i.e. >0.75 ppm). In fact, the extent of DNA damage increased with increasing concentrations of toenail selenium in dogs in the two highest quartiles ($r = 0.44$, $p = 0.03$). A strong positive correlation was found between intraprostatic and toenail concentrations of selenium ($r = 0.72$, $p < 0.0001$). These findings support the hypothesis that toenails are a readily accessible surrogate tissue for monitoring the effects of dietary selenium supplementation on total selenium levels and carcinogenic events within the aging prostate. The possibility of a threshold for the prostate cancer protective effects of selenium that can be assayed non-invasively, warrants further investigation.

University of Missouri Research Reactor (MURR) Science Day, Columbia, MO, May 2004

Prostate Cancer Risk and DNA Damage: Translational Significance of Selenium Supplementation in a Canine Model

Keynote Speaker: David J. Waters, DVM, PhD
Professor of Comparative Oncology, Purdue University
Director, Gerald P. Murphy Cancer Foundation

Daily supplementation with the essential trace mineral selenium significantly reduced prostate cancer risk in men in the Nutritional Prevention of Cancer Trial. However, the optimal intake of selenium for prostate cancer prevention is unknown. We are conducting randomized feeding trials in dogs to study the consequence of nutritionally adequate or supranutritional selenium status at concentrations that mimic the range of selenium intake of healthy men in the United States. By studying elderly dogs, the only non-human animal model of spontaneous prostate cancer, we are defining the dose-response relationship between dietary selenium and prostatic DNA damage. Our results have important implications for the design of human cancer prevention trials and for optimizing selenium supplementation as a practical cancer prevention strategy.

American Association for Cancer Research Frontiers of Cancer Prevention, Seattle, WA, October 2004

Effect of Dietary Selenium Intake on Intraprostatic Androgen Levels Within the Aging Prostate.

Shen S[1,4], Cooley DM[1,2], Schlittler DL[2], Oteham C[2], Chen Y[2], Chiang EC[2], Morris JS[3], Glickman LT[2], Bostwick DG[4], Waters DJ[1,2]

Gerald P. Murphy Cancer Foundation[1], West Lafayette, IN; Purdue University[2], West Lafayette, IN; University of Missouri-Columbia Research Reactor Center[3], Columbia, MO, Bostwick Laboratories[4], Richmond, VA

There is a strong impression that lifelong androgenic activity within the prostate significantly contributes to prostate cancer progression. However, little is known about the relationship between serum and tissue hormone levels or whether specific dietary factors significantly modify the absolute or relative concentration of intraprostatic androgens. Studies in human subjects are ill-suited to directly test these hypotheses because of the difficulty in collecting prostate tissue for hormone assay. The essential trace mineral selenium (Se) is currently being evaluated as a prostate cancer preventive agent, so we tested the hypothesis that Se supplementation modulates the androgen milieu within the aging prostate. In a randomized feeding trial, elderly beagle dogs (age equivalent of 65 year-old men) received nutritionally adequate or supranutritional levels of Se, thereby mimicking the range of dietary Se intake of U.S. men. Twenty-five dogs were randomly assigned to daily supplementation with 0, 3μg/kg, or 6 μg/kg Se as selenomethionine or high Se yeast. After 6 months treatment, samples were collected for Se measurement by neutron activation analysis (toenails, prostate) and hormone levels by RIA (serum, prostate). Mann-Whitney U Test and Spearman correlation coefficients were used to determine the relationship between measures of Se status (toenails, prostate) and androgen production and metabolism (serum testosterone (T), intraprostatic T and dihydrotestosterone (DHT) concentration, intraprostatic DHT:T ratio, prostate T : serum T ratio). For some analyses, dogs were divided into tertiles on the basis of Se concentration within the prostate. Se supplemented dogs had significantly higher mean intraprostatic Se level than control dogs that received nutritionally adequate Se intake (2.61 ppm vs. 1.79 ppm; $p=0.01$). There was a strong positive association between Se concentration in prostate tissue and in toenails, a non-invasive marker of Se status ($r=0.82$, $p<0.0001$). Overall, serum T concentration was not strongly correlated with intraprostatic levels of T ($r=0.11$, $p=0.61$) or DHT ($r=0.17$, $p=0.41$). Dogs with higher intraprostatic Se had higher T in their prostate tissue (mean T concentration was 2.3 vs. 3.9 pg T / ng tissue in lowest vs. highest tertiles, $p=0.006$) and a non-significant trend toward increased circulating T levels in serum over the experimental period (mean percent change in serum T was −19.8% vs. 48.5% in lowest vs. highest tertiles, $p=0.41$). The ratio of T in prostate : serum, which integrates the T uptake and metabolism by the prostate, was unchanged over the range of intraprostatic Se concentration ($r=0.16$, $p=0.43$). The DHT:T ratio, a surrogate for 5α-reductase activity, was significantly lower in dogs with the highest intraprostatic level of Se (mean DHT:T of 2.75 vs. 1.70 in lowest vs. highest tertiles, $p=0.02$). Consequently, there was no difference between the mean DHT concentration in prostates with low or high Se concentration (6.2 pg DHT / mg tissue in lowest and highest tertiles). Although changes in dietary Se intake may influence certain aspects of prostatic intracrinology, supranutritional Se supplementation does not significantly increase or decrease overall androgenic activity within the aging prostate. We conclude that the cancer suppressive effects of Se are not likely mediated by changing androgen levels within the prostate.

Environmental Mutagen Society Meeting, San Francisco, CA, September 2005

Can Peripheral Blood Lymphocytes Provide A Window To Assess The Extent Of Genotoxicity Within The Prostate?

Shen S, Cooley DM, Schlittler D, Chen Y, Chiang E, Bostwick DG, Morris JS, Glickman LT, Waters DJ

Previously we showed that the extent of DNA damage within the aging prostate could be predicted by measuring the concentration of the trace mineral selenium in toenail clippings. Moreover, this relationship discovered by studying elderly dogs remarkably paralleled the relationship between dietary selenium and prostate cancer risk in men. Herein, we extend this work to test the hypothesis that the amount of DNA damage measured in circulating blood lymphocytes can be used to non-invasively assess the extent of DNA damage within the prostate. In a randomized feeding trial, 67 elderly beagle dogs (physiologically equivalent to 65 year-old men) received nutritionally adequate or supranutritional levels of selenium for 7 months, in order to mimic the range of dietary intake of men in the U.S. At the end of the treatment period, alkaline Comet assay was used to measure DNA damage in peripheral blood lymphocytes (PBLs) and prostate. Pearson and Spearman correlation coefficients were calculated to determine if the extent of prostatic DNA damage was significantly correlated with 3 different measures of PBL DNA damage: 1) basal damage; 2) total damage after *ex vivo* H_2O_2 challenge; and 3) % inducible damage (total damage after H_2O_2 – basal damage / 100 – basal damage). In dogs receiving a nutritionally adequate level of selenium, there was no significant association between basal DNA damage in PBLs and damage within the prostate. There was a weak negative association between basal DNA damage in PBLs and damage in the prostate of dogs receiving supranutritional levels of selenium ($r = -.347$, $p = .01$). In both treatment groups, the extent of DNA damage in PBLs measured after *ex vivo* H_2O_2 challenge was positively correlated with prostatic DNA damage, indicating that dogs with the most prostatic DNA damage had PBLs that were more susceptible to oxidative stress. Although statistically significant, these associations were relatively weak, accounting for only 4-22% of the interindividual variation in prostatic DNA damage. We conclude that measurement of DNA damage in PBLs using alkaline Comet assay does not provide a reliable method to non-invasively predict genotoxicity within the prostate. Supported by PC-970492 from the US Army Prostate Cancer Research Program.