Free Radical Biology and Medicine 127 (2018) 36–45

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Review Article

Five threads: How U-shaped thinking weaves together dogs, men, selenium, and prostate cancer risk☆

David J. Waters*, Emily C. Chiang

Center for Exceptional Longevity Studies, Gerald P. Murphy Cancer Foundation, 3000 Kent Avenue, Suite D1-104, West Lafayette, IN, USA

ARTICLE INFO

Keywords:
Translational research
Comparative oncology
Prostate cancer prevention
Non-linear dose response
Personalized medicine
Precision nutrition

ABSTRACT

Prostate cancer is one of the leading causes of cancer-related mortality among men living in developed countries, making the development of safe, practical approaches to prostate cancer risk reduction a high research priority. The relationship between prostate cancer risk and selenium, an essential nutrient required for a number of metabolically important enzymes including glutathione peroxidases, has been investigated, but a satisfactory integration of results has proven elusive. Dogs, like men, naturally develop prostate cancer during aging, providing an appropriate context to study the effects of selenium supplementation on the dysregulation of homeostasis that drives cancer development within the aging prostate. In this paper, we summarize the translational significance of research results gained from dog studies on selenium and prostate cancer risk. Our discovery of a U-shaped dose-response between toenail selenium concentration and prostatic DNA damage in dogs remarkably parallels data on the relationship between selenium status and prostate cancer risk in men. Notably, the dog U-curve provides a plausible explanation for the unanticipated increase in prostate cancer incidence among men with highest baseline selenium who received selenium supplementation in the largest-ever prostate cancer prevention trial (SELECT). Moreover, the dog U-curve guided the discovery of a non-antioxidant, anti-carcinogenic mechanism of organic selenium — the preferential triggering of apoptosis in DNA damaged cells, which we have termed “homeostatic housecleaning”. Taken together, the data from dogs and men indicate that increasing selenium status will not necessarily be associated with prostate cancer risk reduction. Landing in the trough of the U — achieving mid-range selenium status — is better than being too low or too high. Personalizing health promotion in a more-is-not-necessarily-better world poses distinctive challenges. Dog studies can be relied upon to contribute important insights into dose-dependent and form-dependent effects — two critical aspects of selenium biology that will have to be disentangled if the burgeoning science of selenium is to be translated into effective strategies for human disease prevention. Beyond contributing to understanding the role of selenium in biology, our work situates the concept of U-shaped thinking at the core of personalized medicine and precision nutrition.

1. Introduction

In August 2017, a joint meeting of the 11th International Symposium on Selenium in Biology and Medicine and the 5th International Conference on Selenium in the Environment and Human Health was convened in celebration of the 200th anniversary of the discovery of selenium. The aim of this paper, which was delivered at the conference, is to review the translational impact of dog studies on selenium and prostate cancer risk in men. The work directly addresses the challenge of optimizing selenium status for prostate cancer risk reduction, while provoking fresh thinking about mechanisms of selenium anti-carcinogenesis that may extend beyond the redox activity of this essential nutrient.

Since the seminal studies of Charles Huggins in the 1940s [1,2], scientists have been in search of the genetic and non-genetic factors that dictate who will develop prostate cancer. The notion that selenium status may be an important determinant of prostate cancer risk in men first gained traction in 1996 when Clark and co-workers published the landmark results of the Nutritional Prevention of Cancer (NPC) Trial [3]. NPC was a selenium-yeast supplementation trial (200 µg of selenium-yeast daily; average supplementation duration, 4.5 years) with skin cancer recurrence as the primary endpoint. Selenium

☆ A talk delivered at the Se 2017 Conference in Stockholm, Sweden August 16, 2017 titled “Of Dogs and Men: A Review of the Translational Impact of Dog Studies on Selenium and Prostate Cancer Risk”.

* Corresponding author.
E-mail address: dwaters@gpmcf.org (D.J. Waters).

https://doi.org/10.1016/j.freeradbiomed.2017.12.039
Received 5 November 2017; Received in revised form 21 December 2017; Accepted 31 December 2017
Available online 02 January 2018

supplementation did not suppress the development of skin cancer. However, as serendipity would have it, the investigators found that daily supplementation with selenium-yeast significantly reduced prostate cancer incidence by 63% (RR, 95% CI = 0.37, 0.18–0.71) [3]. This exciting finding fueled the design and launch of the largest-ever prostate cancer prevention trial in men called SELECT — Selenium and Vitamin E Cancer Prevention Trial. In SELECT, more than 32,000 men were randomized to receive either vitamin E, selenium in the form of selenomethionine, both vitamin E and selenium, or placebo. The trial began in 2001 with an expected completion date of 2012. Prostate cancer incidence was the primary endpoint.

2. A Story Unfolds

At the time that SELECT was launched, there were still many important unanswered questions about selenium and cancer prevention, including the nutrient's anticancer mechanism and the most effective dose. Our research team perceived these gaps in understanding as a research opportunity because of our expert knowledge of the prostate cancers that occur naturally in pet dogs [4–7]. Dogs and humans are the only two species that develop spontaneous prostate cancer with appreciable frequency — not rats or mice, moose or zebras. We posited that the aging dog prostate could provide a unique opportunity to study the effect of selenium on prostate cells *in an appropriate context*. We would study the response of prostatic epithelial cells to selenium in vivo within the complex milieu of the aging prostate gland, consisting of epithelial cell-stroma cell interactions, oxidative stress, inflammation, declining androgen levels, and stromal senescence. It is difficult to imagine how such a complex context could be readily duplicated in the cell culture laboratory.

Our research method hinged upon conducting a randomized feeding trial in elderly beagle dogs physiologically equivalent to the men who were enrolled in SELECT. The design enabled us to assess the impact of *supranutritional selenium status versus selenium adequacy* in an appropriate in vivo context. Second, we could evaluate outcome measures over a wide range of selenium status that would mirror the selenium status achievable in human populations. Moreover, we could directly compare the prostatic response to selenomethionine (used in SELECT) versus selenium-yeast (used in the NPC Trial).

Our first major result was to show that dietary selenium supplementation could significantly reduce DNA damage in the aging dog prostate [8]. In our randomized feeding trial, 49 elderly male beagle dogs were studied. The dogs were free of prostate cancer and physiologically equivalent to 62–69-year-old men [9]. All dogs were fed a selenium-adequate maintenance diet and were randomized to either a control group or selenium-treated groups. Selenium-treated dogs received daily supplementation with either selenomethionine or selenium-yeast (SelenoExcell, Cypress Systems) at either a low dose (3 μg/kg body weight) or a higher dose (6 μg/kg body weight) for seven months. After seven months, dogs were euthanized and prostatic DNA damage was measured by alkaline Comet assay in cell preparations from fresh prostatic tissue. Dogs supplemented with either form of selenium — selenomethionine or selenium-yeast — had a significant 28% reduction in the percentage of prostatic epithelial cells with extensive DNA damage. We also found a 2-fold higher apoptosis in prostatic epithelial cells in selenium-supplemented dogs compared to dogs in the control group. These experimental results challenged us to consider the relation between these two observations: How does one reconcile *decreased* DNA damage coinciding with *increased* apoptosis? This concurrence, which initially seemed counterintuitive, would become the subject of closer examination.

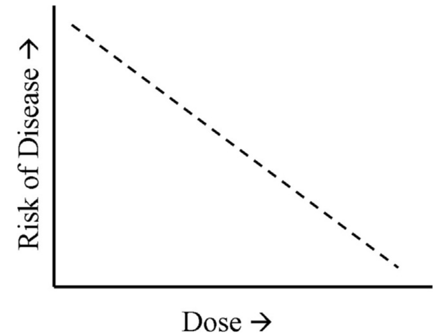

Fig. 1. Finding the optimal dose for disease risk reduction. A hypothetical linear relationship is shown between the risk of a target disease and the dose of a disease preventive agent, consistent with the notion that "more is better".

Next, our work moved on to pursue an important question: *Could dogs help to pinpoint the optimal selenium status for prostate cancer risk reduction*? When investigators conduct dose-response studies, they attempt to decipher the relationship between dose and risk of disease. Fig. 1 illustrates a linear dose-response relationship, in which more of an agent results in further decrease in disease risk. But many years ago, Walter Mertz working at the United States Department of Agriculture (USDA) proposed that the dose-response between any essential nutrient and biological response is not linear, but U-shaped — progressing through states of deficiency, low suboptimal, optimal, high suboptimal, and toxicity [10] (Fig. 2). Mertz did not have much data, but he had an idea.

With the tension of these two possibilities in mind, we probed the dose-response relationship between selenium status and DNA damage within the aging prostate. Selenium status was determined by measuring toenail selenium concentration using neutron activation analysis [12] — the same technique used to analyze toenail selenium concentration of men in the Health Professionals Follow-Up Study and SELECT. Prostatic DNA damage was measured by alkaline Comet assay and expressed as the percentage of extensively damaged prostatic epithelial cells. The results are shown in Fig. 3. We found that the relationship between selenium status and prostatic DNA damage was U-shaped. This result, published in *Carcinogenesis*, was the first demonstration of a U-shaped dose-response between a cancer-fighting nutrient and a cancer-relevant read-out within the prostate [11]. We went on to demonstrate that this U-shaped relationship was not peculiar to the prostate. Fig. 4 shows dose-response data from prostate and brain. Taken together, our results suggest that, if selenium is a 'good thing', more of a 'good thing' is not necessarily better. The anthropologist and systems thinker Gregory Bateson expressed this idea more eloquently: "There are no monotone values in biology." [13]

It is altogether natural for scientists to be skeptical about new findings, asking themselves: Are the results of these *animal* studies relevant to the relationship between selenium status and *human* prostate cancer risk? After all, our studies were of dogs not men, and we chose DNA damage, not cancer, as our endpoint. Consider the following statement:

> These findings suggest that additional selenium could potentially benefit only the subgroup of the population with low selenium levels and that it would not reduce disease in subjects with moderate to high selenium levels.

This seems to be a reasonable interpretation of our dog studies. But these are not our words. These are the words that epidemiologist Walt Willett chose more than 30 years ago when he reported the results of

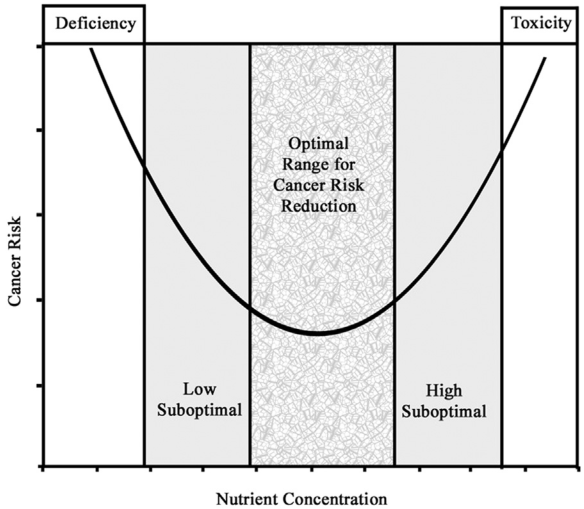

Fig. 2. Dose-response model adapted from Mertz [10]. The U-curve predicts that the biological response to an essential nutrient is characterized by an optimal middle range, consistent with the notion that "more is not necessarily better" [11] (with permission).

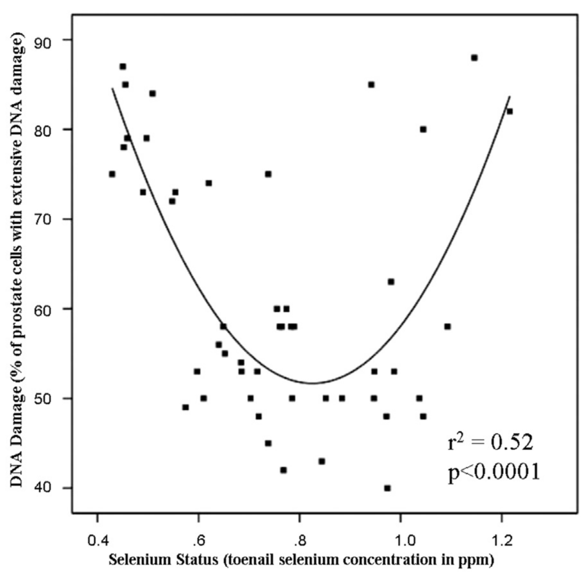

Fig. 3. The dog U-curve. The dog U-curve was discovered by studying the dose-response relationship between selenium status and prostatic DNA damage in elderly dogs over a range of selenium concentration achievable in human populations [11] (with permission). Each data point represents the result from one dog.

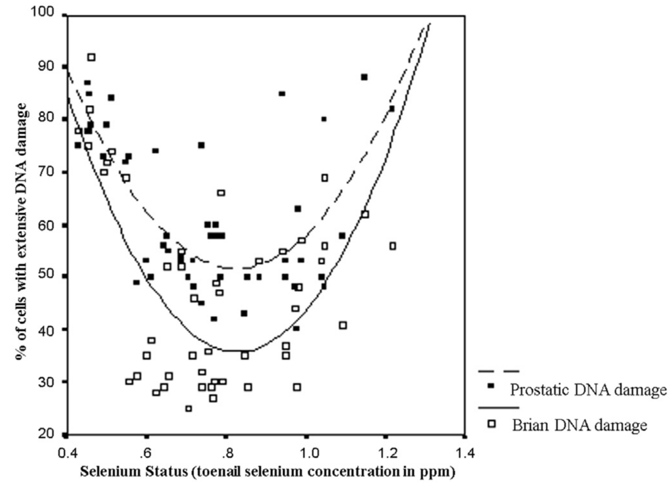

Fig. 4. Two U-shaped curves. A U-shaped dose-response between selenium status and DNA damage was observed within the brain as well as the prostate of elderly dogs [11] (with permission).

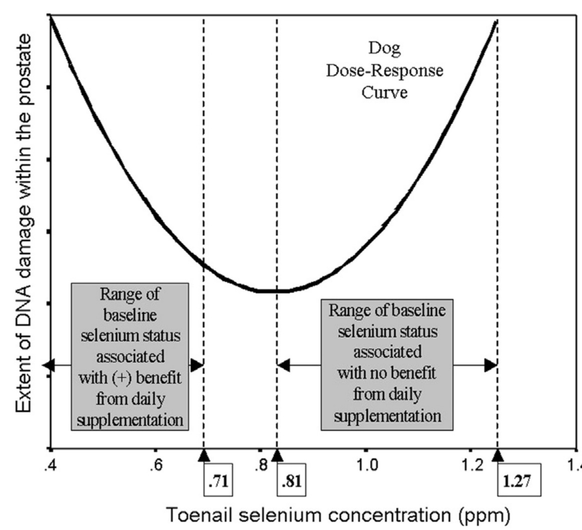

Fig. 5. Dog U-curve explains the impact of baseline selenium status on human prostate cancer risk reduction achieved by selenium supplementation in the Nutritional Prevention Cancer Trial. Men in the lowest tertile of selenium status (left grey box) benefited from selenium supplementation, whereas men in the highest tertile (right grey box) did not benefit from selenium supplementation [11] (with permission).

the first prospective cohort study on selenium and cancer risk in humans [14]. Apparently, this mode of thinking was forgotten somewhere along the way.

Now let us consider what translational significance does the dog U-curve hold for us by re-examining the results from Clark's NPC Trial, the study in which the idea of selenium and prostate cancer prevention first gained eminence. Not all men in the NPC Trial benefited from selenium supplementation [15]. Men in the lowest tertile of selenium status prior to supplementation (equivalent to < .71 ppm Se in toenails[1]) experienced an impressive 92% prostate cancer risk reduction in response to selenium supplementation. In contrast, men in the highest tertile of baseline selenium (equivalent to > .81 ppm Se in toenails) experienced no prostate cancer risk reduction. In fact, these men had an 88% *increase* in overall cancer incidence. Fig. 5 shows that the dog U-curve predicts the results of the NPC Trial — both the benefit observed in men with the lowest baseline selenium, and the undesired effect in men with the highest baseline selenium status.

We reasoned that if the dog U-curve could predict the results of human studies, such as the NPC Trial, the dog U-curve could also provoke a meaningful re-evaluation of the mechanisms of selenium anti-carcinogenesis. Conventional wisdom holds that selenium prevents oxidative damage, and the resultant protection from damage is fundamental to this nutrient's cancer-protective effect [16–18]. Indeed, at its inception, the SELECT cancer prevention trial was named "Antioxidant Chemoprevention". But maximum expression of glutathione peroxidases and other selenoenzymes occurs at a selenium status that is less than optimal for prostate cancer risk reduction

[1] Toenail and plasma selenium concentration in healthy human volunteers (n = 12) were simultaneously measured to generate a ratio (6.7 ± 0.7) that could be used to convert plasma selenium values to predicted toenail values [11]. Toenail Se (ppm) = plasma Se (μg/L) x 0.0067 is utilized here and elsewhere in this manuscript where data on selenium status provided by studies were limited to plasma Se.

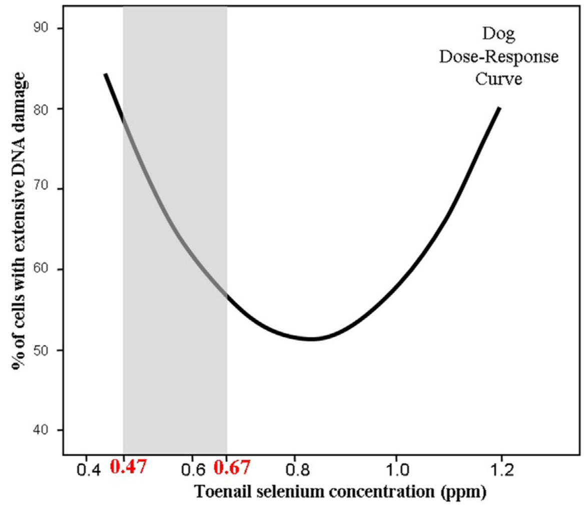

Fig. 6. Search for a mechanism of selenium anti-carcinogenesis is guided by the dog U-curve. Maximum expression of plasma glutathione peroxidase occurs at a selenium status of 70–100 µg/L plasma Se [20–23], equivalent to 0.47 − 0.67 ppm Se in toenails, which is less than the level needed for optimal prostate cancer risk reduction.

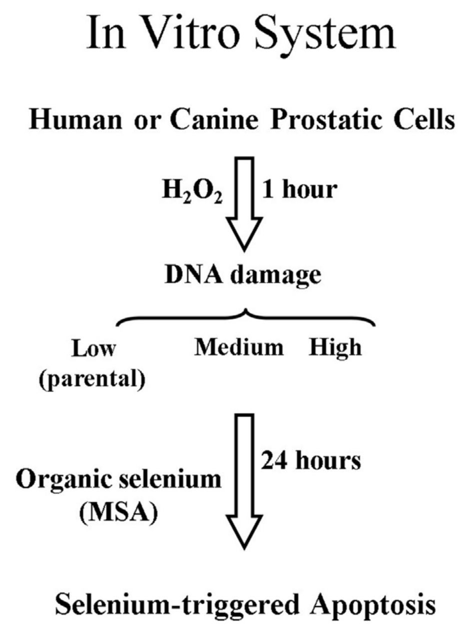

Fig. 9. Testing the homeostatic housecleaning hypothesis. Diagram outlines the cell culture experiments used to evaluate the extent to which DNA damage sensitizes human and canine prostatic cells to methylseleninic acid (MSA)-triggered apoptosis [24].

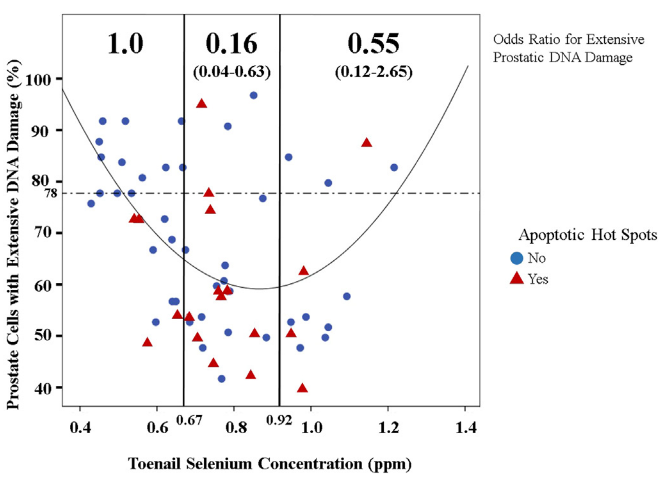

Fig. 7. Critical nexus of selenium, DNA damage, and apoptosis leads to mechanistic insight. Dog U-curve reveals selenium status associated with lowest prostatic DNA damage (trough of U) is also associated with highest intensity of prostatic epithelial cell apoptosis [19] (with permission).

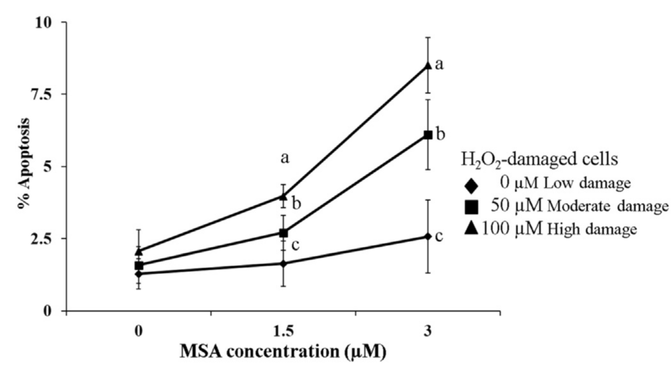

Fig. 10. Evaluation of selenium-triggered apoptosis. Hydrogen peroxide (H_2O_2)-induced DNA damage sensitizes DU-145 human prostate cancer cells to methylseleninic acid (MSA)-triggered apoptosis. ^{abc}Statistically different, $p < 0.05$, compared within each MSA exposure [24] (with permission).

(Fig. 6). Therefore, we reasoned that any robust explanation of how to achieve maximum cancer risk reduction must require further mechanistic explanation. Guided by the dog U-curve, our thinking turned to examine more carefully the relationship between selenium status, DNA damage, and apoptosis.

Our reassessment of this critical nexus was published in *Dose Response* in 2009 [19]. Fig. 7 shows a plot of prostatic DNA damage versus selenium status from dogs in our randomized selenium supplementation trial. Each dot represents a dog after seven months of study. When we divided selenium status into three zones — low

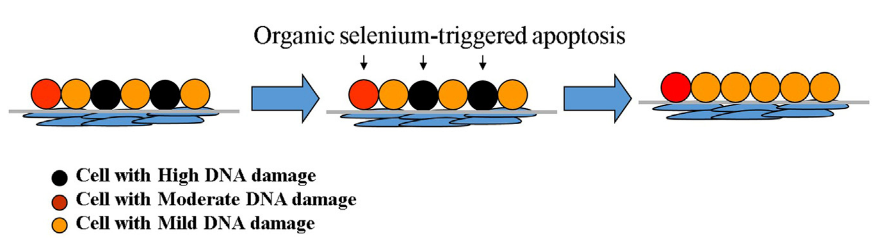

Fig. 8. Hypothesis: Organic selenium preferentially triggers apoptosis in cells with the highest DNA damage [19]. The hypothesis predicts that dietary selenium supplementation can selectively eliminate prostatic epithelial cells with highest DNA damage (black circles) resulting in a reduction in the steady-state level of DNA damage within the aging prostate.

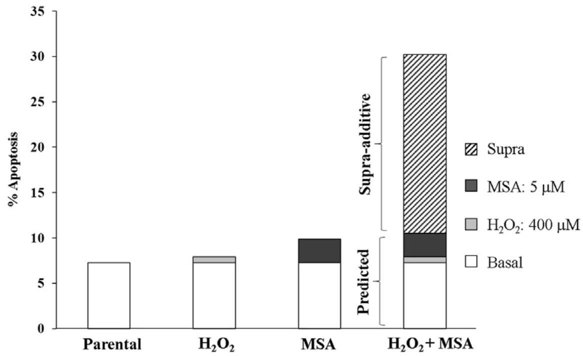

Fig. 11. Evaluation of selenium-triggered apoptosis. Non-cytotoxic DNA damage induced by hydrogen peroxide (H_2O_2) sensitizes TR5P canine prostate cancer cells to methylseleninic acid (MSA)-triggered apoptosis, resulting in a supra-additive effect [24] (with permission).

vs. middle vs. high — dogs with selenium status in the optimal middle range (exceeding a level equivalent to .67 ppm Se in toenails, the selenium status at which antioxidant selenoenzymes in blood are reportedly maximally expressed [20–23]) were 84% less likely to have extensive prostatic DNA damage compared to dogs with low selenium status. But here is where the data pattern gets very interesting: *The optimal middle range of selenium status where DNA damage was minimized is precisely where apoptosis was maximized*. Compared to dogs in the low selenium zone, dogs with middle selenium status had a two-fold increase in the median number of prostatic cells undergoing apoptosis. Moreover, dogs in the optimal middle range of selenium had a four-fold increase in the frequency of "apoptotic hotspots", defined as foci of intense apoptosis more than 15-fold higher than the level of apoptosis observed in tissue sections from unsupplemented control dogs [8]. Of considerable consequence, there was no significant difference in apoptosis between dogs in the low versus high selenium zones.

Earlier in this paper, our attention was drawn to the question: How can we reconcile *decreased* DNA damage and *increased* apoptosis in the prostate of selenium-supplemented dogs? Apoptosis is usually considered a DNA damage response. Then why would *higher* apoptosis accompany *lower* DNA damage? To address this difficulty, we put forward a new hypothesis: *Selenium preferentially triggers apoptosis in prostatic cells with the highest DNA damage* (Fig. 8). According to this hypothesis, steady-state level of DNA damage would decrease with supranutritional selenium supplementation because the remaining epithelial cells have less damage, not because of increased protection. Based upon this best-fit explanation of the observations from our in vivo dog studies, we proposed that selenium can selectively sweep away the most DNA damaged cells, a process we termed "homeostatic housecleaning" [19].

To test this new idea, we extended our in vivo work to utilize an in vitro cell culture system in which DNA damage could be more precisely controlled. Fig. 9 shows a schematic outline of our in vitro experimental paradigm [24]. Brief non-cytotoxic exposures to hydrogen peroxide or other DNA damaging agents were used to create populations of human and canine prostatic cells with low, medium, or high levels of DNA damage. Then, these cell populations were exposed to organic selenium in the form of methylseleninic acid (MSA) and the extent of selenium-triggered apoptosis was measured.

Fig. 10 shows representative results of experiments using the DU-145 human prostate cancer cell line. For two different doses of MSA, apoptosis triggered by selenium was significantly higher in cells with the highest DNA damage, compared to cells with low damage [24]. Confirmation that non-cytotoxic DNA damage sensitizes cell populations to selenium-triggered apoptosis is evidenced by a clear supra-additive effect (Fig. 11). The Figure shows that, in the TR5P canine prostate cancer cell line developed in our laboratory, the intensity of apoptosis triggered by selenium in cells with the highest DNA damage far exceeded the sum of apoptosis expected under basal

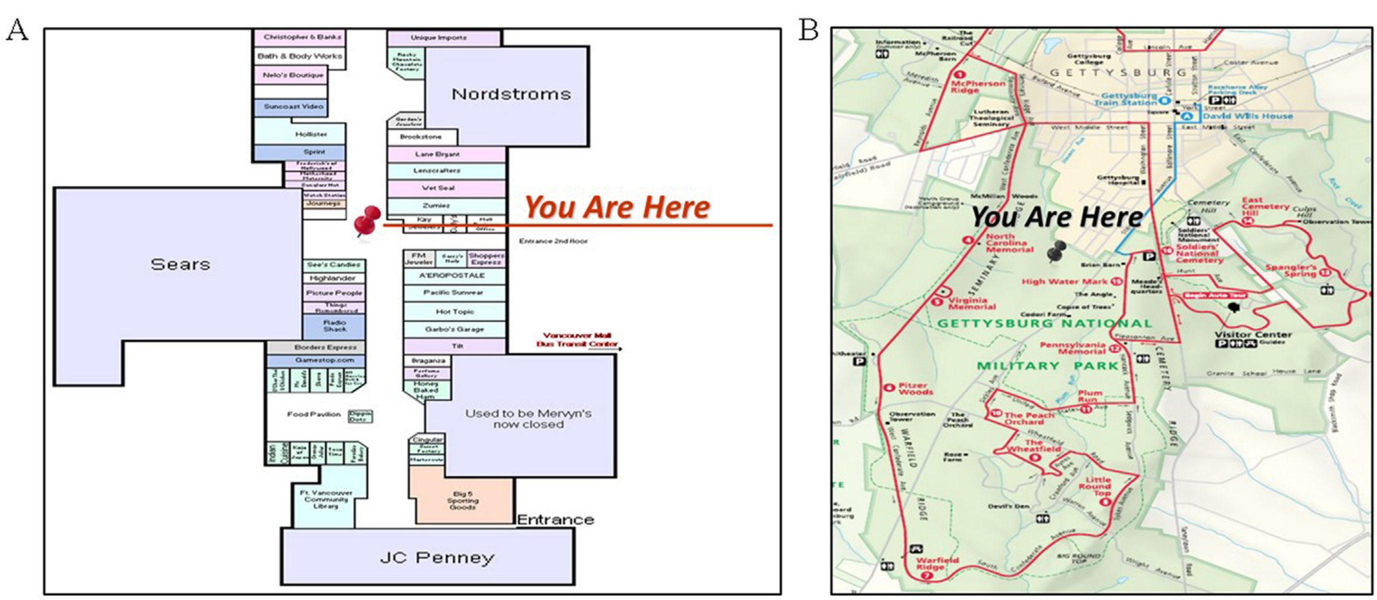

Fig. 12. Maps serve as useful tools for navigating uncertainty. These maps of a shopping mall in Vancouver (A) and of the Gettysburg National Military Park (B) provide valuable information on "You are Here", analogous to the information that the dog U-curve contributes to explaining the disappointing results of the Selenium and Vitamin E Cancer Prevention Trial (SELECT) (see Figs. 13 and 14).

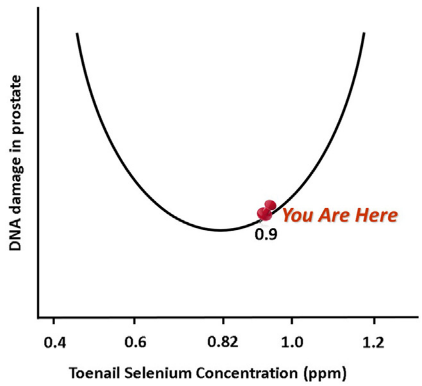

Fig. 13. The dog U-curve: A map to interpret the disappointing results of the Selenium and Vitamin E Cancer Prevention Trial (SELECT). The dog U-curve predicts that the selenium status of the average man in SELECT prior to selenium supplementation ('You are Here') is already in the optimal range for minimizing prostate cancer risk.

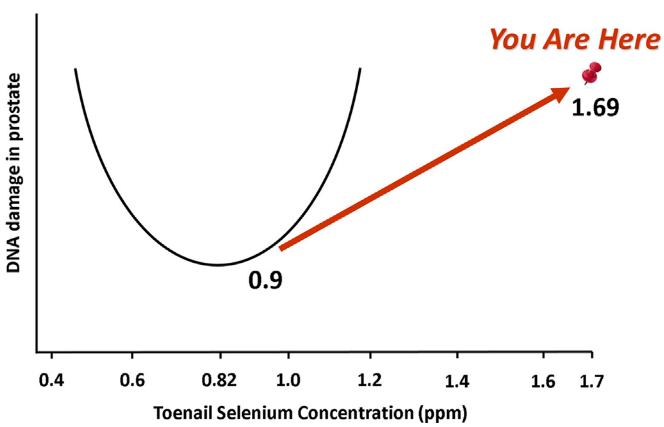

Fig. 14. The dog U-curve: A map to interpret the disappointing results of the Selenium and Vitamin E Cancer Prevention Trial (SELECT). The dog U-curve predicts that the selenium status of the average man in SELECT following selenium supplementation ('You are Here') clearly exceeds the optimal range for minimizing prostate cancer risk.

conditions, hydrogen peroxide alone, and MSA alone. Taken together, our in vitro experiments demonstrated the homeostatic housecleaning effect of organic selenium on human and canine prostatic cells. The work marked a satisfying progression that differed from the more typical sequence in which hypothesis-building in vitro experiments are followed by in vivo testing. The homeostatic housecleaning hypothesis — a proposition conceived in an in vivo setting by carefully observing the response of epithelial cells in the aging prostate to supranutritional selenium — had been carried back into the laboratory to undergo validation [24].

At this point, let us return to the SELECT study. Seven years after its inception, SELECT was stopped early. From highly publicized news releases, the lay public received notice of its failure: "Prostate cancer prevention study halted. Vitamin E, selenium no help in preventing prostate cancer." [25] "Vitamins get 'F' in cancer prevention." [26] SELECT was stopped because interim analysis showed no prostate cancer protection and a statistically non-significant increase in type II diabetes mellitus in men receiving selenium supplementation [27]. What happened? Is it possible that when it comes to the "unexpected" results of SELECT, there is a chance that we are just lost? In instances of uncertain navigation, maps can serve as useful tools. If you are in a shopping mall in Vancouver and you are lost, you look for a map like the one in Fig. 12A and you find 'You Are Here'. If you are lost at the battlefield where Abraham Lincoln delivered his famous Gettysburg Address, you look for a map (Fig. 12B), you find out 'You Are Here', and you are no longer lost. If you are the average man enrolled in SELECT *before* selenium supplementation, 'You Are Here' (Fig. 13) — in the trough of the dog U-curve, already in the optimal range. And if you are the average man in SELECT *after* selenium supplementation, 'You Are Here' (Fig. 14) — you have been supplemented into a potentially dangerous place, equivalent to a plasma selenium concentration of more than 250 μg/L [27]. After SELECT was stopped, a letter was sent to each participant stating: "We now know that selenium and vitamin E do not prevent prostate cancer." Is that what we really know? Here is what we believe we know: *There are no monotone values in biology*. The problem of high baseline selenium and risk for oversupplementation — which we noted earlier in our analysis of the NPC Trial results (Fig. 5) — was again operational in SELECT and became the focus of a subsequent report by Kristal et al. [28]. The report revealed that, in the SELECT study, daily selenium supplementation was associated with a two-fold *increase* in risk for prostate cancer in men with the highest baseline selenium status (plasma Se > 149 μg/L, equivalent to > 1.0 ppm Se in toenails).

How then should we situate the disappointing results of SELECT? We contend that the critical hypothesis has yet to be tested: *Can men with low, suboptimal selenium status achieve cancer risk reduction through daily selenium supplementation?* Interestingly, Fig. 15 shows that this hypothesis could be tested by enrolling the average man living in many countries in the world, because the average selenium status in those countries is in the low suboptimal range of the dog U-curve — equivalent to less than .8 ppm Se in toenails (plasma Se < 119 μg/L). The figure shows clearly that this situation does not hold true for men living in Canada or the United States, where SELECT was performed.

In an editorial published in the *American Journal of Clinical Nutrition,* the state-of-the-understanding in a post-SELECT world was framed as follows: "Selenium and prostate cancer: The puzzle isn't finished yet." [29] Today, as attempts to situate the null results of SELECT continue, investigators wonder whether the failure could have been the consequence of supplementation with the wrong form of selenium. It is impossible to know whether selenium-yeast would have been more active than selenomethionine had it been used in SELECT. No inferences could be made because SELECT did not test different forms of selenium. And though the dog U-curve provided a comfortable conceptual landing point to adequately explain the disappointing results of SELECT, we extended our studies in dogs to address what was becoming a question of accelerating interest: *When it comes to prostate cancer risk reduction, are selenomethionine and selenium-yeast equipotent?* To shed further light on the "wrong form" hypothesis, we utilized dog studies to obtain a direct, head-to-head comparison of the target tissue potency of selenomethionine versus selenium-yeast on a suite of readouts in the aging prostate that reflect flux through multiple gene networks — cell proliferation, apoptosis, DNA damage, and androgen levels [30]. And though gaps in understanding remain and disagreement regarding the "wrong form" hypothesis persist [31,32], analysis of our dog data did not support the notion that the null results of SELECT were attributable to differences in prostatic consequences achievable through daily supplementation with these two forms of selenium [30].

Free Radical Biology and Medicine 127 (2018) 36–45

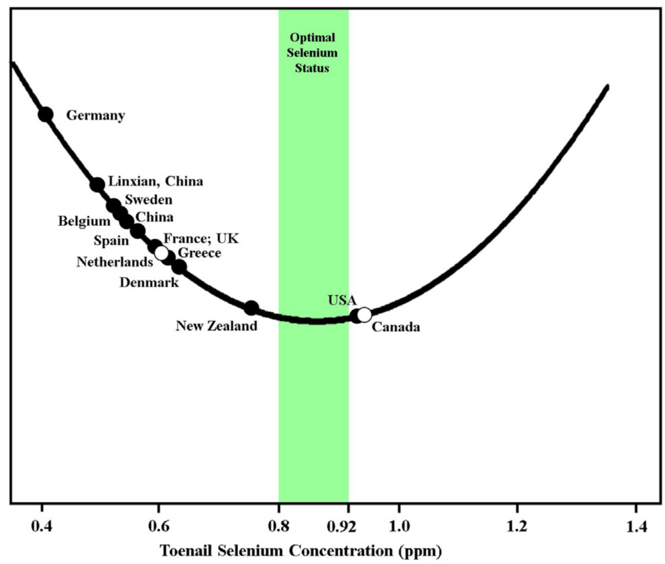

Fig. 15. Average selenium status of men living in 13 countries: Implications for cancer prevention trial design. The average man living in many countries (USA and Canada are exceptions), has a selenium status that falls well below the optimal level for prostate cancer risk reduction predicted by the dog U-curve (shown in green), suggesting they might benefit from selenium supplementation [modified from 19]. For each country, studies are cited that provide information on the selenium status of 40–65 year-old men (see Appendix for more details). Selenium status represents selenium concentration measured in toenails (open circles) or derived from serum selenium values[1] (closed circles). Toenail selenium concentration of 0.8 – 0.92 ppm corresponds to plasma selenium concentration of 119 – 137 μg/L [11].

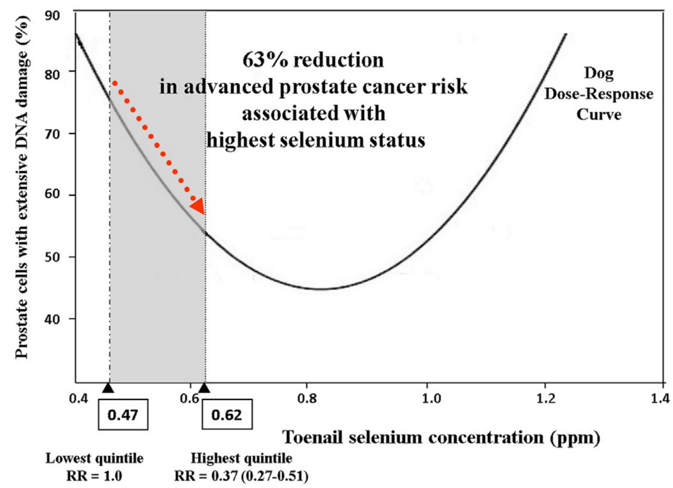

Fig. 16. The relationship between selenium and prostate cancer risk: The Netherlands experience. The dog U-curve is useful for placing into context the linear dose-response reported among men living in the Netherlands [34].

3. Synthesis

Our work points to the need for U-shaped thinking — seeing selenium biology through the lens of U-shaped dose responses. The concept of U-shaped thinking can serve as a valuable navigational tool, steering our thinking away from a consideration of whether selenium is ‘good’ or ‘bad’, pointing us toward a more nuanced view of the shifting contexts of health and disease. This line of thinking aligns with a comprehensive review of the scientific literature on selenium and human health [33]. In her *Lancet* article, professor Rayman states: “The crucial factor that needs to be emphasized with regard to the health effects of selenium is the inextricable U-shaped link with status; whereas additional selenium intake may benefit people with low status, those with adequate to high status might be affected adversely and should not take selenium supplements.” [33]

With this apparent clarity, it is expected that U-shaped thinking will significantly shape the dynamic discourse about selenium, health, and disease that no doubt lies ahead. That is not to say, however, that we will not encounter reports claiming linear dose-responses between selenium and disease outcomes. For example, in a paper reporting the Netherlands experience with selenium and prostate cancer risk, investigators showed an inverse (linear) association between toenail selenium levels and prostate cancer risk [34]. In this observational study, there was a 63% reduction in advanced prostate cancer risk in men in the highest quintile of selenium status compared to men in the lowest quintile. Based upon these results, more selenium would appear to be better for prostate cancer risk reduction. But Fig. 16 shows that the dog U-curve can comfortably place in context the linear dose-response of men living in the Netherlands — *all of the men in the Netherlands fit precisely in the downswing of the dog U-curve.*

As scientists and health professionals, the challenge ahead will be to apply our understanding of disease risk and health promotion, which will always be fragmented and incomplete. Here, looking through the lens of U-shaped thinking, we have considered the relationship between selenium and prostate cancer risk, attempting a fruitful integration using four angles of vision (Fig. 17), beginning with the dog U-curve generated by studying prostatic DNA damage over a broad range of selenium status. Second, in the NPC Trial, the U-shaped relationship became apparent when we considered how baseline selenium status influenced the response to further selenium supplementation. In the Netherlands study, men could only inform us of the possible benefit that might be attainable by moving along the downswing of the U-curve. Finally, the selenium replete men in SELECT could only tell us what they could — a significant *increase* in prostate cancer among men with the highest baseline selenium status as supplementation moved them along the upswing of the U-curve.

In a U-shaped world, more of an essential nutrient, such as selenium, is not necessarily better for disease risk reduction. It follows from this understanding that baseline nutrient status should be required for all individuals enrolled in prevention trials to avoid oversupplementation. The work presented here also stimulates an increased appreciation for the power of *comparative oncology* — the opportunity to capitalize on the similarities and differences between the naturally-occurring cancers of pets and people [35]. When it comes to selenium, we believe that dog studies can contribute important insights into dose-dependent and form-dependent effects — aspects of selenium biology that will have to be further elucidated if the steadily expanding science of selenium is to be translated into effective strategies for human disease prevention. Perhaps it is not surprising that the dog U-curve — generated through careful study of prostatic cell response to selenium *in an appropriate context* — can usefully place into context the results of human studies and can even contribute to the search for anti-cancer mechanisms. Not surprisingly, we would contend. For it is the very nature of scientific inquiry that the amount of attention we devote to context calibrates our scholarly advance.

Although the reader may find the observations woven together here to be intellectually satisfying, this construction should mainly be considered a solid starting point for further inquiry. For example, does U-shaped thinking help to describe the relationship between selenium

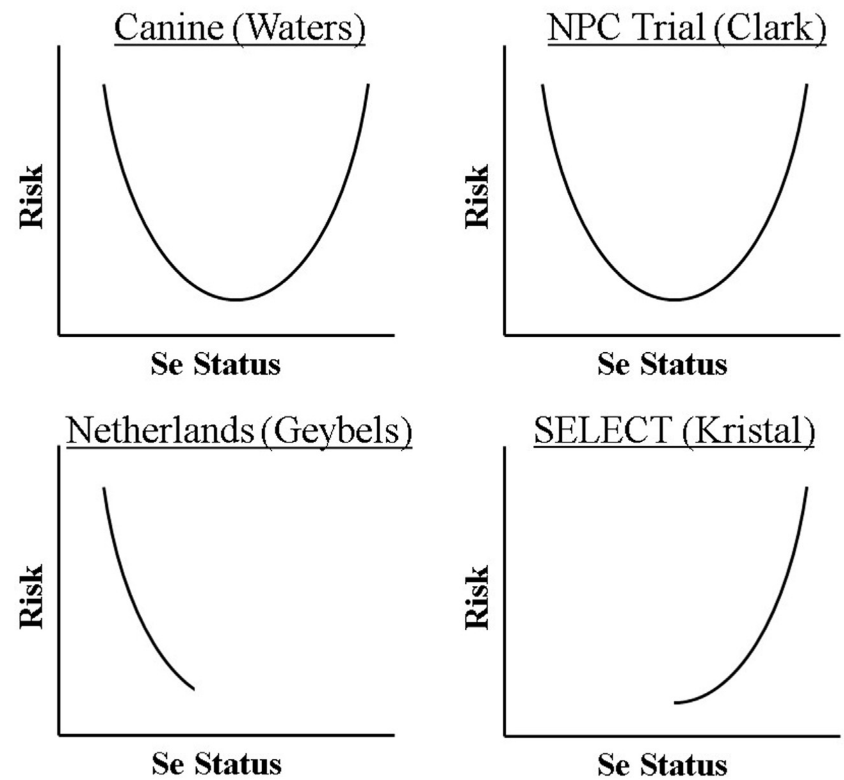

Fig. 17. Selenium and prostate cancer risk: Four angles of vision. An integration of results from four studies [3,8,28,34] is seen through the lens of U-shaped thinking (see text for explanation).

status and cancers affecting women? And though breast cancer and other cancers affecting women seem to be relatively insensitive to changes in selenium status [36], the question provokes broader consideration of whether there are significant sex-specific differences in the dose-response of selenium with other non-cancer health outcomes, such as diabetes mellitus, cardiovascular disease, or dementia [37]. Also, it is expected that an increased awareness of the importance of non-linear dose-responses catalyzed by U-shaped thinking will lead to the discovery of more complex dose-response relationships between selenium status and health outcomes, such as the bimodal dose-response for selenium and type II diabetes mellitus reported by Wang and colleagues [38]. Finally, this paper has focused on cancer-related consequences of supplementation with organic forms of selenium. It is interesting to speculate on the extent to which inorganic forms of selenium (e.g., selenite, selenate) may produce similar dose responses. Here it is notable that, in our hands, selenite failed to provoke the preferential apoptosis of DNA-damaged cells induced by organic selenium that we have termed homeostatic housecleaning [39].

Finally, just as scientists interested in the biology of aging study 100-year-old humans (centenarians), our research team is currently conducting the first systematic study of the oldest-old pet dogs — “canine centenarians” — living in North America. This trailblazing work seeks to gain clues to better understanding the biology of exceptional longevity in pets and people. A possible connection between selenium status, healthspan, and longevity has been recently reported in a study of mice carrying humanized telomeres [40]. Among mammals, dogs are endowed with key aspects of telomere biology — relatively short telomere length, absence of telomerase in somatic cells — that naturally place them in close proximity to humans [41]. Researching long-lived dogs that display cancer resistance offers great opportunity to utilize U-shaped thinking to weave together key exposures and windows of vulnerability, including the role that selenoproteins, selenium metabolites, and other regulators of redox networks play in achieving highly successful aging and side-stepping cancer mortality [42,43].

Acknowledgment

The authors acknowledge the critical contributions made by our scientific collaborators, particularly David G. Bostwick, Gerald F. Combs, Jr., Lawrence T. Glickman, and J. Steven Morris, and for expert assistance by members of our research team, especially Shuren Shen, Dawn Cooley, Seema Kengeri, Deborah Schlittler, and Carol Oteham. This work was supported by the US Army Medical and Materiel Command Prostate Cancer Program (PC-970492), an American Cancer Society Institutional Grant (IRG-17), and funding from Bostwick Laboratories and The Brookdale Foundation.

Appendix A

See Table A1

Table A1
Supporting information on the selenium status of men living in 13 countries used to position those countries on the dog U-curve in Fig. 15. For each country, studies are cited that provide an estimate of selenium status for men within the age range of 40–65 years.

Country	Study cohort [ref]	Participant characteristics	Number of men	Selenium status (mean ± SD)	Equivalent to toenail selenium† (ppm)
Germany	The Lipid Analytic Cologne Study [44]	Never-smokers, not diagnosed with atherosclerotic cardiovascular disease or diabetes, aged 20–92 years, average 51.0 years	362	Serum: 60.4 ± 28.7 µg/L	0.40
China (Linxian)	The General Population Trial of Linxian, China [45]	Healthy adults aged 40–69 years, average 57.7 years, living in 4 Linxian communes	608	Serum: 0.9 µM	0.49
Sweden	Uppsala Longitudinal Study of Adult Men [46]	50 year-old male residents of Uppsala county	1005	Serum: 77.4 µg/L	0.52
Belgium	Belgian Interuniversity Study on Nutrition and Health [47]	Control subjects, average age 62.4 years	430	Serum: 79.6 ± 14.2 µg/L	0.53
China	A cross-sectional study in Shaanxi Province [48]	Adults aged 18–70 years, average 48.5 years, living in the study area for at least 5 years	1932	Serum: 80.5 µg/L	0.54
Spain	Hortega Survey [49]	Adults aged 15–85 years, living in Valladolid	721	Plasma: 83.4 µg/L	0.56
France	The SU.VI.M.AX Study [50]	Adults aged 40–60 years	4915	Serum: 1.1 ± 0.2 µM	0.58
UK	The U.K. National Diet and Nutrition Survey [51]	Adults aged 19–64 years, average 40.8 years*	472	Plasma: 1.1 ± 0.2 µM*	0.58
Netherlands	The Netherlands Cohort Study [52]	Cancer-free residents, aged 55–69 years, average 61.3 years	1061	Toenail: 0.6 ± 0.1 ppm	0.60
Greece	The ATTICA Study [53]	Residents of Attica, average age 40 years	296	Serum: 90.5 ± 35.1 µg/L	0.61
Denmark	The Copenhagen Male Study [54]	Caucasian males aged 53–74 years, average 63 years	3179	Serum: 1.2 µM	0.63
New Zealand	[55]	Caucasian males aged 20–81 years, average 52.3 years, living in Auckland; not taking more than 50 µg Se/day	503	Serum: 111.6 ± 1.21 µg/L	0.75
USA	NHANES 2003–2004 [56]	Adults aged over 40 years, average 58.6 years	536	Serum: 139 µg/L	0.93
Canada	The Canadian Study of Diet, Lifestyle and Health [57]	Free-living individuals, average age 55 years	377	Toenail: 0.94 ± 0.1 ppm	0.94

† Toenail and plasma selenium concentration in healthy human volunteers (n = 12) were simultaneously measured to generate a ratio (6.7 ± 0.7) that could be used to convert plasma selenium values to predicted toenail values [11]. Toenail Se (ppm) = plasma Se (µg/L) x 0.0067 is utilized here and elsewhere in this manuscript where data on selenium status provided by studies were limited to plasma Se.

* Information on age of participants and selenium status reported for entire cohort of men and women.

References

[1] C. Huggins, Effect of orchiectomy and irradiation on cancer of the prostate, Ann. Surg. 115 (1942) 1192–1200.
[2] C. Huggins, Prostatic cancer treated by orchiectomy: the five year results, J. Am. Med. Assoc. 131 (1946) 576–581.
[3] L.C. Clark, G.F. Combs Jr, B.W. Turnbull, E.H. Slate, D.K. Chalker, et al., Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group, J. Am. Med. Assoc. 276 (1996) 1957–1963.
[4] D.J. Waters, G.J. Patronek, D.G. Bostwick, L.T. Glickman, Comparing the age at prostate cancer diagnosis in human and dogs, J. Natl. Cancer Inst. 88 (1996) 1686–1687.
[5] D.J. Waters, D.G. Boswtick, Prostatic intraepithelial neoplasia occurs spontaneously in the canine prostate, J. Urol. 157 (1997) 713–716.
[6] D.J. Waters, W.A. Sakr, D.W. Hayden, C.M. Lang, L. McKinney, et al., Workgroup 4: spontaneous prostate carcinoma in dogs and nonhuman primates, Prostate 36 (1998) 64–67.
[7] K.K. Cornell, D.G. Bostwick, D.M. Cooley, G. Hall, H.J. Harvey, et al., Clinical and pathologic aspects of spontaneous canine prostate carcinoma: a retrospective analysis of 76 cases, Prostate 45 (2000) 173–183.
[8] D.J. Waters, S. Shen, D.M. Cooley, D.G. Bostwick, J. Qian, et al., Effects of dietary selenium supplementation on DNA damage and apoptosis in canine prostate, J. Natl. Cancer Inst. 95 (2003) 237–241.
[9] G.J. Patronek, D.J. Waters, L.T. Glickman, Comparative longevity of pet dogs and humans: implications for gerontology research, J. Gerontol. A Biol. Sci. Med. Sci. 52 (1997) B171–B178.
[10] W. Mertz, The essential trace elements, Science 213 (1981) 1332–1338.
[11] D.J. Waters, S. Shen, L.T. Glickman, D.M. Cooley, D.G. Bostwick, et al., Prostate cancer risk and DNA damage: translational significance of selenium supplementation in a canine model, Carcinogenesis 26 (2005) 1256–1562.
[12] D.M. McKown, J.S. Morris, Rapid measurement of selenium in biological samples using instrumental neutron activation analysis, J. Radioanal. Chem. 43 (1978) 411–420.
[13] G. Bateson, Mind and Nature: a Necessary Unity, Wildwood House, London, 1979.
[14] W.C. Willett, B.F. Polk, J.S. Morris, M.J. Stampfer, S. Pressel, et al., Prediagnostic serum selenium and risk of cancer, Lancet 2 (1983) 130–134.
[15] A.J. Duffield-Lillico, B.L. Dalkin, M.E. Reid, B.W. Turnbull, E.H. Slate, E.T. Jacobs, et al., Selenium supplementation, baseline plasma selenium status and incidence of prostate cancer: an analysis of the complete treatment period of the nutritional prevention of cancer trial, BJU Int. 91 (2003) 608–612.
[16] G. Hocman, Chemoprevention of cancer: selenium, Int. J. Biochem. 20 (1998) 123–132.
[17] D. Medina, H. Lane, F. Shepherd, Effects of selenium on mouse mammary tumorigenesis and glutathione peroxidase activity, Anticancer Res. 1 (1981) 377–382.
[18] H. Li, P.W. Kantoff, E. Giovannucci, M.F. Leitzmann, J.M. Gaziano, et al., Manganese superoxide dismutase polymorphism, prediagnostic antioxidant status, and risk of clinical significant prostate cancer, Cancer Res. 65 (2005) 2498–2504.
[19] E.C. Chiang, S. Shen, S.S. Kengeri, H. Xu, G.F. Combs, et al., Defining the optimal selenium dose for prostate cancer risk reduction: insights from the U-shaped relationship between selenium status, DNA damage, and apoptosis, Dose Response 8 (2009) 285–300.
[20] C.D. Thomson, M.F. Robinson, J.A. Butler, P.D. Whanger, Long-term supplementation with selenite and selenomethionine: selenium and glutathione peroxidase in blood components of New Zealand women, Br. J. Nutr. 69 (1993) 577–588.
[21] J. Nève, Human selenium supplementation as assessed by changes in blood selenium concentration and glutathione peroxidase activity, J. Trace Elem. Med. Biol. 9 (1995) 65–73.
[22] G.F. Combs Jr., Selenium in global food systems, Br. J. Nutr. 85 (2001) 517–547.
[23] G.F. Combs Jr., Biomarkers of selenium status, Nutrients 7 (2015) 2209–2236.
[24] E.C. Chiang, D.G. Bostwick, D.J. Waters, Homeostatic housecleaning effect of selenium: evidence that noncytotoxic oxidant-induced damage sensitizes prostate cancer cells to organic selenium-triggered apoptosis, Biofactors 39 (2013) 575–588.
[25] WebMD, Prostate cancer prevention study halted. 〈http://www.webmd.com/prostate-cancer/news/20081028/prostate-cancer-prevention-study-halted#1〉 (Accessed 27 August 2017).
[26] USA Today, Vitamins get ‘F’ in cancer prevention. 〈https://usatoday30.usatoday.com/news/health/2009-01-06-vitamins-heart_N.htm〉 (Accessed 27 August 2017).
[27] S.M. Lippman, E.A. Klein, P.J. Goodman, M.S. Lucia, I.M. Thompson, et al., Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT), J. Am. Med. Assoc. 301 (2009) 39–51.
[28] A.R. Kristal, A.K. Darke, J.S. Morris, C.M. Tangen, P.J. Goodman, et al., Baseline selenium status and effects of selenium and vitamin E supplementation on prostate cancer risk, J. Natl. Cancer Inst. 106 (2014) djt456.
[29] E.L. Richman, J.M. Chan, Selenium and prostate cancer: the puzzle isn't finished yet, Am. J. Clin. Nutr. 96 (2012) 1–2.
[30] D.J. Waters, S. Shen, S.S. Kengeri, E.C. Chiang, G.F. Combs Jret al., Prostatic response to supranutritional selenium supplementation: comparison of the target tissue potency of selenomethionine vs. selenium-yeast on markers of prostatic homeostasis, Nutrients 4 (2012) 1650–1663.
[31] D.L. Hatfield, V.N. Gladyshev, The outcome of Selenium and Vitamin E Cancer Prevention Trial (SELECT) reveals the need for better understanding of selenium biology, Mol. Interv. 9 (2009) 18–21.
[32] J.P. Richie, Jr, A. Das, A.M. Calcagnotto, R. Sinha, W. Neidig, et al., Comparative effects of two different forms of selenium on oxidative stress biomarkers in healthy men: a randomized clinical trial, Cancer Prev. Res. 7 (2014) 796–804.
[33] M.P. Rayman, Selenium and human health, Lancet 379 (2012) 1256–1268.
[34] M.S. Geybels, B.A. Verhage, F.J. van Schooten, R.A. Goldbohm, P.A. van den Brandt, Advanced prostate cancer risk in relation to toenail selenium levels, J. Natl. Cancer Inst. 105 (2013) 1394–1401.
[35] D.J. Waters, K. Wildasin, Cancer clues from pet dogs, Sci. Am. 24 (2015) 54–61.
[36] D.J. Waters, E.C. Chiang, D.M. Cooley, J.S. Morris, Making sense of sex and supplements: differences in the anticarcinogeneic effects of selenium in men and women, Mutat. Res. 551 (2004) 91–107.
[37] L. Schomburg, U. Schweizer, Hierarchical regulation of selenoprotein expression and sex-specific effects of selenium, Biochim. Biophys. Acta 2009 (1790) 1453–1462.
[38] X.L. Wang, T.B. Yang, J. Wei, G.H. Lei, C. Zeng, Association between serum selenium level and type 2 diabetes mellitus: a non-linear dose-response meta-analysis of observational studies, Nutr. J. 15 (2016) 48, http://dx.doi.org/10.1186/s12937-0160169-6.
[39] E.C. Chiang, D.G. Bostwick, D.J. Waters, Selenium form-dependent anti-carcinogenesis: preferential elimination of oxidant-damaged prostate cancer cell populations by methylseleninic acid is not shared by selenite, Vitam. Miner. 4 (2015) 126, http://dx.doi.org/10.4172/2376-1318.1000126.
[40] R.T. Wu, L. Cao, E. Mattson, K.W. Witwer, J. Cao, et al., Opposing impacts on healthspan and longevity by limiting dietary selenium in telomere dysfunctional mice, Aging Cell 16 (2017) 125–135.
[41] N.M. Gomes, O.A. Ryder, M.L. Houck, S.J. Charter, W. Walker, et al., Comparative biology of mammalian telomeres: hypotheses on ancestral states and the roles of telomeres in longevity determination, Aging Cell 10 (2011) 761–768.
[42] Y.-M. Go, D.P. Jones, Redox theory of aging: implications for health and disease, Clin. Sci. 131 (2017) 1669–1688.
[43] D.M. Cooley, D.L. Schlittler, L.T. Glickman, M. Hayek, D.J. Waters, Exceptional longevity in pet dogs is accompanied by cancer resistance and delayed onset of major diseases, J. Gerontol. A Biol. Sci. Med. Sci. 58 (2003) B1078–B1084.
[44] H.K. Berthold, B. Michalke, W. Krone, E. Guallar, I. Gouni-Berthold, Influence of serum selenium concentrations on hypertension: the Lipid analytic Cologne cross-sectional study, J. Hypertens. 30 (2012) 1328–1335.
[45] W.-Q. Wei, C.C. Abnet, Y.-L. Qiao, S.M. Dawsey, Z.-W. Dong, X.-D. Sun, et al., Prospective study of serum selenium concentrations and esophageal and gastric cardia cancer, heart disease, stroke, and total death, Am. J. Clin. Nutr. 79 (2004) 80–85.
[46] B. Grundmark, B. Zethelius, H. Garmo, L. Holmberg, Serum levels of selenium and smoking habits at age 50 influence long term prostate cancer risk: a 34 year ULSAM follow-up, BMC Cancer 11 (2011) 431–441.
[47] M. Kornitzer, F. Valente, D.D. Bacquer, J. Neve, G.D. Backer, Serum selenium and cancer mortality: a nested case-control study within an age- and sex-stratified sample of the Belgian adult population, Eur. J. Clin. Nutr. 58 (2004) 98–104.
[48] Q. Wu, M.P. Rayman, H. Lv, L. Schomburg, B. Cui, et al., Low population selenium status is associated with increased prevalence of thyroid disease, J. Clin. Endocrinol. Metab. 100 (2015) 4037–4047.
[49] I. Galan-Chilet, M. Tellez-Plaza, E. Guallar, G. de Marco, R. Lopez-Izquierdo, et al., Plasma selenium levels and oxidative stress biomarkers: a gene-environment interaction population-based study, Free Radic. Biol. Med. 74 (2014) 229–236.
[50] J. Amaud, S. Bertrais, A.M. Roussel, N. Amault, D. Ruffieux, et al., Serum selenium determinants in French adults: the SU. VI.M.AX study, Br. J. Nutr. 95 (2006) 313–320.
[51] S. Stranges, M. Laclaustra, C. Ji, F.P. Cappuccio, A. Navas-Acien, et al., Higher selenium status is associated with adverse blood lipid profile in British adults, J. Nutr. 140 (2010) 81–87.
[52] D.H.E. Maasland, L.J. Schouten, B. Kremer, P.A. van den Brandt, Toenail selenium status and risk of subtypes of head-neck cancer: the Netherlands Cohort Study, Eur. J. Cancer 60 (2016) 83–92.
[53] S. Letsiou, T. Nomikos, D. Panagiotakos, S.A. Pergantis, E. Fragopoulou, et al., Serum total selenium status in Greek adults and its relation to age. The ATTICA study cohort, Biol. Trace Elem. Res. 128 (2009) 8–17.
[54] P. Suadicani, H.O. Hein, F. Gyntelberg, Serum selenium level and risk of lung cancer mortality: a 16-year follow-up of the Copenhagen Male Study, Eur. Respir. J. 39 (2012) 1443–1448.
[55] N. Karunasinghe, D.Y. Han, S. Zhu, J. Yu, K. Lange, et al., Serum selenium and single-nucleotide polymorphisms in genes for selenoproteins: relationship to markers of oxidative stress in men from Auckland, New Zealand, Genes Nutr. 7 (2012) 179–190.
[56] M. Laclaustra, S. Stranger, A. Navas-Acien, J.M. Ordovas, E. Guallar, Serum selenium and serum lipids in US adults: National Health and Nutrition Examination Survey (NHANES) 2003–2004, Atherosclerosis 210 (2010) 643–648.
[57] J.S. Morris, T. Rohan, C.L. Soskolne, M. Jain, T.L. Horsman, et al., Selenium status and cancer mortality in subjects residing in four Canadian provinces, J. Radioanal. Nucl. Chem. 249 (2001) 421–427.