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

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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 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

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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.

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 Walter Willett chose more than 30 years ago when he reported the results of
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 toenails1) 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.

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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.
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,
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 conditions.

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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].
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 usefully place into 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 lowest quintile 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...
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].

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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.

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

† 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.