Quality control in the neutron activation analysis of biological markers for selenium in epidemiological investigations

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Instrumental neutron activation analysis is routinely used at the MURR to quantify selenium in prospectively-collected biologic markers including blood serum and toenails. These specimens are typically collected from well-defined cohort populations participating in investigations assessing selenium intake and incidence of chronic disease endpoints. These epidemiological investigations, whether observational (case-control) or clinical (intervention), typically generate thousands of samples. The purpose of this paper is to assess, through evaluation of quality control results, if the achievable accuracy and precision in the measurement of selenium using NAA is adequate to determine a relative risk of 1.2 at high confidence in epidemiological studies.

Introduction

Selenium is a required micronutrient and its deficiency has been associated with a broad spectrum of chronic pathologies as recently reviewed by HATFIELD.1 Selenocysteine (Sec), a cysteine analog with selenium replacing sulfur, is now recognized as the 21st amino acid. Understanding the molecular biology of Sec is widely accepted as the key to explaining the selenium-responsive pathologies. The mechanism for the insertion of Sec in eukaryotes has been reported2,3 and the potential misannotation of selenoprotein, due to the dual function of the UGA codon as both a stop codon and Sec insertion signal, has been described,4 and recognized as a major challenge in accurately identifying new selenoproteins.5 Nevertheless, careful application of independent bioinformatic approaches has now led to the identification of 26 selenoproteins in higher eukaryotes; however, the functions of many of these remain unknown.6

Advancing the selenoproteome is the foundation for understanding selenium-responsive chronic disease, regardless of the endpoint. However, substantial differences exist among health professionals regarding the dietary intake of selenium needed for optimal status and maximum protection against chronic disease. The RDA, or so-called nutritional requirement for selenium, as established by the National Research Council, is 55 to 75 micrograms per day for adults with the higher value recommended for lactating females.7 BURK8 has reported that most estimates of selenium intake in the U.S. are in the range of 80 to 120 micrograms per day concluding that: “routine supplementation with selenium cannot be recommended”. A comprehensive discussion of the rationale for the nutritional requirement for selenium can be found in Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids.9 While the approach used to establish dietary recommendations for selenium, based on saturation of a family of selenoproteins, is rational and consistent with that used for other nutrients, a new paradigm, with growing support, suggests protective effects against chronic diseases such as cancer and cardiovascular disease can be realized from intakes perhaps 2 to 3 times the RDA for selenium. The evidence for this so-called supranutritional requirement has come from a growing body of carefully executed epidemiological studies employing both case-control and intervention designs and using prospectively collected biologic monitors, such as blood plasma and sera and toenails, to assess selenium status. Examples of such studies are discussed in the context of assessing the current knowledge of the selenium requirement in an excellent review by THOMSON.10

We have used neutron activation analysis in our laboratory to assess selenium status in numerous epidemiological studies. For example, WILLETT et al.11 were the first to report a case-control difference in serum selenium in a cancer study using prediagnostic specimens. In the case of prostate cancer using prospectively collected samples in the Health Professionals Follow-Up Study (HPFS), YOSHIZAWA has reported case-control differences using toenails12 and LI using plasma.13 From this same cohort, using the toenail biologic monitor, YOSHIZAWA reported differences in coronary heart disease in men,14 and, using toenails from both the HPFS and the Nurses’ Health Study, RAJPATHAK et al.15 reported differences
in selenium status in men and women with diabetes and second endpoint cardiovascular disease. JORDAN et al.\textsuperscript{16} using toenails prospectively collected in the Johnson County Osteoarthritis Project (North Carolina) reported increased risk of knee and hip osteoarthritis associated with a low selenium status in both Caucasians and African Americans.

All of these studies indirectly support the new selenium paradigm suggesting that intakes in excess of the nutritional requirement, based on saturation of selenoproteins, are protective against chronic disease. Nevertheless, restraint in recommending indiscriminant supplementation of the U.S. population is prudent. Selenium is toxic at intakes not greatly in excess of those achieved by the increasing fraction of the population that routinely takes a selenium supplement. In a reevaluation of the Nutritional Prevention of Cancer paradigm suggesting that intakes in excess of the 200 microgram per day supplement. Others on the selenium treatment arms may actually have experienced an increased risk. This is consistent with a report by WATERS et al.\textsuperscript{18} who showed a “U-shaped” response to selenium status, measured using toenails, with DNA damage in prostatic tissue in a canine PC model.

There are several analytical challenges in today’s epidemiological studies of selenium and chronic disease. There is a relatively narrow therapeutic range of selenium intake, consequently differences in selenium concentrations having biological significance may be relatively small in the biologic monitors. Prospectively collected specimens are irreplaceable, consequently the desire to conserve sample by limiting the size of test aliquots is frequently encountered. To have sufficient statistical power to detect the relative risks at the level desired requires the analysis of large numbers of specimens, frequently in the thousands. Determination of lower relative risk at higher confidence is desired by the analytic epidemiology community.

The major concerns in epidemiological studies are misclassification and control of confounding. Misclassification is generally a random event arising when a variable, or even the endpoint, is inaccurately assigned in the data set. Confounding occurs when the effect of interest is mixed with one or more other effects. Control of confounding can be achieved by stratification, isolating the study effect if possible; or through multivariant modeling when stratification is not feasible. A power estimation, generally done as part of the experimental design, takes into account the known sources of variation and error. Epidemiological power estimates are complex algorithms that compute the relative risk (RR) along a continuum. The gold standard might be defined as the capability to detect a RR of 1.2 with a 95\% confidence. To accomplish this in a study of sub-optimal selenium status as a hypothetical risk factor for a chronic disease, requires the selenium determinations be done with an accuracy of 10\% or better in an epidemiological context minimizing misclassification and confounding. In this study we report on the quality control results from two ongoing epidemiological studies in which the assessment of selenium status is derived from the analysis of prospectively collected serum and toenail samples. We also report on the quality control results in a pilot study assessing the use of toenails as a monitor of trace-element intake.

Experimental

Description of the Epidemiological Studies

\textit{The Harvard Prostate Cancer Study:} The subjects in this study are participants in the Health Professionals Follow-Up Study from which pre-diagnostic blood serum samples were collected and stored at \textasciitilde 80 °C until cases and controls were subsequently identified. Aliquots (100 microliters) were prepared and transferred frozen to the MURR Center for selenium determinations. This sample set, 4th in a continuing series, consisted of 518 samples including 60 aliquots of a quality control serum added at Harvard and indistinguishable from the test samples. All samples were blinded to the MURR Center regarding all demographic descriptors as well as the case-control status. To the 60 QC serum samples imbedded by Harvard we added 71 additional serum samples, 35 and 36 in each at two different selenium concentrations. These QC samples were also distributed throughout the test samples and blinded to the NAA analyst.

\textit{The One Source Multivitamin Study:} The objective of this pilot study was to determine what effect, if any, the use of the over-the-counter One Source Multivitamins (Perrigo Co., Allegan, MI) has on toenail concentrations for those trace-element nutrients in One Source, including selenium. The study included 74 female and 52 male subjects subdivided into groups with 37 and 26 treatment/control pairs, respectively. In addition to sex, the subjects were matched on age, BMI and cigarette smoking (never, past, current). The results for the quality control samples are reported in this paper. The QC samples consisted of twenty replicate samples from the same non-participant, obtained on 4 collection dates spanning 9 months, and included as 5-replicate sub-samples. Of the 126 test samples, duplicate pairs were prepared for 62 and these were separated and randomly inserted in the analysis batch. The identity of the QC samples was blinded to the NAA analyst. Ten of the participant replicate pairs, ranging in selenium concentration from 0.8 to 1.6 µg/g, were reanalyzed a second time unblinded.