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Portable X-ray fluorescence of zinc and selenium with nail clippings – visit 3 of the Mother and Infant Nutrition Investigation (MINI)

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ABSTRACT

Introduction: Recent advances in portable X-ray fluorescence (XRF) have opened up the possibility of a rapid and cost-effective method for measuring elemental content in nail clippings.

Two elements of particular interest for intake assessment through nail clipping measurement are zinc and selenium. The New Zealand-based Mother and Infant Nutrition Investigation (MINI) provides an ideal testing ground for the application of portable XRF in the measurement of zinc and selenium. Two aspects requiring further study with portable XRF are the optimal time of measurement and the optimal approach to energy spectrum analysis.

Methods: A subset of nail clippings from 20 mother-infant pairings were initially selected for analysis over three separate MINI study visits. This paper considers measurements from visit 3 (12 months postpartum). For portable XRF, a mono-energetic X-ray beam was used to excite characteristic X-rays from zinc and selenium. Individual XRF measurements were made for 60 s (real time). In each case, analysis of the elemental signal was performed in four different ways: (1) automated system output concentration; (2) element signal to total signal ratio; (3) element signal to Compton scatter signal; (4) element signal to coherent scatter signal. XRF results were compared against zinc and selenium concentrations obtained from a “gold standard” method of inductively coupled plasma mass spectrometry (ICP-MS).

Results: ICP-MS results from the visit 3 nail clippings determined a mean zinc concentration of 104 μg/g and a mean selenium concentration of 0.606 μg/g. Analysis of XRF energy spectra from 60 s measurements showed that zinc was clearly detected in all cases. The selenium signal was more challenging, but was also detected from all spectra. For both zinc and selenium, correlations between XRF results and ICP-MS concentrations were highly significant ($p < 0.01$) in all cases, with the zinc results more strongly correlated than selenium. Of the four approaches to XRF signal analysis, the three approaches involving an external software package provided superior results relative to the automated system output. Correlations of XRF results with ICP-MS concentrations were similar between these three approaches.

Conclusion: ICP-MS concentrations for both zinc and selenium were found to be lower from visit 3 relative to results from visit 1 and visit 2. This may indicate overall dietary changes postpartum. Correlations of XRF results with ICP-MS concentrations suggest that a relatively short 60 s measurement time is adequate for a reliable zinc assessment, but not for selenium. Although correlations of XRF results with ICP-MS concentrations were similar between three different methods of spectra analysis, practical considerations may favor use of the element signal to total signal approach.

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1. Introduction

The Mother and Infant Nutrition Investigation (MINI) is a cohort study that followed a population of women and infants from the North Island of New Zealand [1], over the first year postpartum. The overall goal of MINI is to assess nutrition and related health outcomes, with particular interest directed toward the intake and status of iodine, selenium, and iron. Potential study participants eligible for MINI were breastfeeding women over 16 years of age with a healthy term infant. Visits of participating mothers and infants with the study team were made 3 months (visit 1), 6 months (visit 2), and 12 months (visit 3) postpartum, with a range of assessments at each time point. These included a dietary assessment, the collection of urine, blood, breast milk, and toenail clippings from the mothers, and the collection of urine, toenail, and fingernail clippings from the infants [1].

Using dietary, urine, blood, and breast milk assessments, previously published results from the MINI study indicated iodine deficiency in the group of mothers and suboptimal iodine status for the group of infants [2]. There was a determination of suboptimal selenium intake for both groups, with the potential for insufficient selenium status for some mothers [3]. These findings were largely consistent with historical and ongoing concerns regarding nutrition gaps in New Zealand [1–4]. While the toenail and fingernail clippings from the MINI study have not yet been fully analyzed with respect to elemental concentrations, a subset of nail clippings from visit 1 and visit 2 were examined for zinc and selenium using two different measurement techniques [5]. Nail clipping results from the novel method of portable X-ray fluorescence (XRF) were compared with concentrations obtained from a "gold standard" method of inductively coupled plasma mass spectrometry (ICP-MS).

The investigation of portable XRF as a measurement technique for zinc and selenium in nail clippings is motivated by interest in establishing a new and more convenient approach to monitoring zinc and selenium status in humans [5]. Such an approach could have significant impact in the fields of public health and nutrition in applications such as the large-scale screening of populations or the monitoring of dietary intervention programs. Given the overall nutritional focus of the MINI study, the assessment of selenium was a clear choice as an element of interest in the nail clippings. The measurement of zinc was also an obvious selection since zinc is typically present in nail clippings at concentrations exceeding 100 times that of selenium [6] and therefore more easily detected. Both zinc and selenium are essential minerals for human nutrition, and there is a need for reliable biomarkers of zinc and selenium status [5]. Nail zinc concentration has been described as an emerging biomarker for zinc [7]. Nail selenium concentration has been shown to correlate with the better-established biomarkers of plasma (or serum) and whole-blood selenium [8,9].

Recent advances in portable XRF have raised the possibility of a rapid, cost-effective, non-destructive, mobile, and straightforward approach to zinc and selenium assessment through the analysis of nail clippings [10]. While these advantages of portable XRF are significant, measurements of nail clippings using portable XRF will not be as accurate nor as precise as measurements using a gold standard technique such as ICP-MS. With MINI study samples, it was previously shown that nail clipping results from portable XRF correlate well with ICP-MS concentrations for both zinc and selenium [5]. Not surprisingly, given the relative magnitudes of concentration in human nail, the observed correlation was generally stronger for zinc measurements compared with selenium measurements. To assess the impact of XRF measurement time on the results, clippings from visit 1 were scanned for 300 s, and clippings from visit 2 were scanned for 180 s. In general, while the shorter XRF scan time increased the relative standard deviation of measurement, it did not adversely affect the observed correlation between XRF and ICP-MS results [5].

The focus of the current paper was to examine a subset of nail clippings from visit 3 (12 months postpartum) of the MINI study, using the same two techniques of portable XRF and ICP-MS to measure zinc and selenium [5]. In this study, the XRF measurement time was reduced to an even shorter interval of 60 s to see whether this provided a viable approach for assessing zinc and selenium in nail clippings. While a shorter measurement time increases the potential throughput of samples, this gain in time efficiency must be balanced against an expected loss of XRF measurement quality. This tradeoff was examined in the current study. In addition, a variety of different data normalization approaches were implemented together for the first time with human nail clippings using this particular portable XRF system. In a previous experimental study, these approaches were compared using artificial nail clippings (often referred to as phantom nail clippings) [11]. Another study, using Monte Carlo simulations, suggested that one of these normalization approaches could be preferable over the others for applications to zinc measurement [12]. In the current paper, results from these different normalization strategies were compared against each other following detailed analyses of the XRF energy spectra detected from human nail clippings. Overall, results from this study will inform development of the portable XRF technique for assessing zinc and selenium in nail clippings, and suggest optimal protocols for future studies.

2. Methods

The MINI study protocol was originally approved by the Northern A Health and Disability Ethics Committee (15/NTA/172) and subsequently by the Mount Allison University Research Ethics Board (102846). Each participant or their parent provided informed written consent prior to enrollment in the study. Nail clippings were provided from 87 mother-infant pairings at three different study visits. These nail clippings were self-collected before each visit and consisted of toenail clippings from the mothers, and both toenail and fingernail clippings from the infants [1]. From the initial 87 mother-infant pairings who participated in the full range of MINI study assessments, a subset of 20 pairings were selected randomly for a detailed comparison of zinc and selenium nail clipping results using both portable XRF and ICP-MS. The smaller subset was used to provide representative results while still preserving some clippings for future study. Results from visit 1 and visit 2 with respect to these 20 pairings were reported previously [5]. The current paper considers results from visit 3 (12 months postpartum). Nail clippings from each pairing were divided into three separate groupings prior to analysis: (1) clippings from a big toe of a mother; (2) clippings from the other toes of a mother; (3) clippings from the toes and fingers of an infant. At the beginning of the study, there were 20 sets of clippings from each of these three groupings, for a total of 60 sets of clippings available for analysis. Due to study attrition and incomplete collection of clippings, samples available from visit 3 consisted of 16 sets of clippings from a big toe of a mother, 16 sets from the other toes of a mother, and 15 sets from the toes and fingers of an infant, for a total of 47 sets of clippings. Before XRF analysis, nail clippings were washed through repeated cleanings with both acetone and water [13,14].

The measurement of nail clippings from the MINI study by portable XRF followed a procedure described previously [5]. This XRF measurement method is briefly summarized here, as carried out with nail clipping samples obtained from visit 3. From each of the 47 sets of nail clippings, four relatively flat nail fragments were prepared by mounting them in an open-ended XRF sample cell between two sheets of 4 μm-thick prolene film (SCP Science; Baie-d'Urfé, QC, Canada). Each fragment was measured in turn using an HD Mobile XRF system (X-ray Optical Systems; East Greenbush, NY, USA), operated in benchtop mode. Individual XRF measurements lasted for 60 s (real time). Using X-ray optics, the HD Mobile XRF system provided a mono-energetic X-ray beam at an energy of ~17.5 keV for efficient excitation of characteristic X-rays from zinc and selenium. The X-ray tube operated at a tube current of ~200 μA and a tube voltage ranging up to 50 kV. The radiation detector used was a silicon drift detector having an area of 25 mm^2.

Data analysis of the XRF measurements was performed in four distinct ways to obtain XRF results for zinc and selenium. Two of these approaches have recently been used in other XRF studies with human nail clippings [15,16], including with the analysis of results from visit 1 and visit 2 of the MINI study [5]. The first approach was the use of automated factory-calibrated concentrations output directly from the XRF system. This allowed calculation of a weighted mean concentration from the four independent measurements made with each set of nail clippings. This approach was not expected to yield accurate concentrations since the factory calibration of the system assumed a sample matrix (plastic) which was not the same composition as human nail. Nonetheless, these system output concentrations are useful for comparison purposes, and were anticipated to scale linearly with the measured ICP-MS concentrations [5,15,16]. The second approach was made possible through analysis of zinc and selenium signals in the energy spectrum using PyMca [17,18], an external software package. Fitting of the energy spectrum from each individual XRF measurement was first performed using PyMca to identify contributions from a range of elements including zinc, selenium, nickel, copper, arsenic, lead, and bromine. The zinc and selenium signals were then normalized with respect to the total counts over the entire energy spectrum. This approach provided the total area ratio (TAR), the total area of signal detections from a particular element divided by the total area of signal over the entire energy spectrum [11]. The TAR technique provides a unitless result which has been shown to scale linearly with elemental concentrations as assessed by ICP-MS [5,15,16]. The weighted mean TAR result from the four independent measurements was determined for each set of nail clippings.

The third and fourth approaches to XRF data analysis in the current study were applied with human nail clipping measurements for the first time using this system. Similar approaches were, however, used previously in a study of artificial nail clippings [11]. Both techniques involved analysis of energy spectra using PyMca and were therefore very similar to the TAR method in terms of implementation. The third approach simply normalized the zinc and selenium signals against the counts detected from the broad Compton scatter peak. The fourth approach normalized the zinc and selenium signals against the counts detected from the much smaller (and more narrow) coherent scatter peak. Once again, using both of these Compton and coherent normalization approaches, weighted mean results were calculated based on the four independent measurements from each set of nail clippings.

Following non-destructive analysis by portable XRF, the nail clippings were measured for zinc and selenium concentration using ICP-MS. A more detailed description of the measurement of nail clippings from the MINI study by ICP-MS has been provided previously [5]. For completeness, the ICP-MS method is summarized here. Each set of nail clippings was first prepared for measurement. For the toenail clippings of the mother, the combined masses of the nail fragments in each set analyzed by XRF were sufficient for ICP-MS measurement. For the infant clippings, however, the combined masses in a given set were very low. Therefore, all clippings originally obtained from a given infant were used in the measurement by ICP-MS. The nail clipping samples were weighed to determine a wet mass. Using sonication, samples were then cleaned with acetone and water, dried overnight at 105 °C in a convection oven (Heratherm 60 L gravity oven, Thermo Scientific; Waltham, MA, USA), and reweighed for a dry mass. The samples were dissolved using a microwave digestor with concentrated nitric acid, water, and hydrogen peroxide. Following this digestion, samples were diluted with water to 10 mL.

An iCAP Q ICP-MS system (Thermo Scientific; Waltham, MA, USA) was then employed to assess the total concentrations of zinc and selenium in each sample. An autosampler introduced the samples to the system, and a scandium internal standard was used. The collision gas was a high-purity helium (>99.999 %) and the system used kinetic energy discrimination to remove polyatomic interference. A calibration curve was constructed from various dilutions of a multi-element calibration standard (Inorganic Ventures; Christiansburg, VA, USA). Check standards (1 μg/L and 10 μg/L) were measured after every 20 samples for quality control. The overall data were collected and analyzed with Qtegra Intelligent Scientific Data Solution software (Thermo Scientific; Waltham, MA, USA).

Results from the portable XRF and ICP-MS measurements from the nail clippings of visit 3 of the MINI study were compared for both zinc and selenium. A small number of outliers (two for zinc and one for selenium) were removed based on the standard deviation of the residuals determined from the linear relationships between XRF and ICP-MS results. Since ICP-MS was considered the gold standard, summary statistics for concentrations of zinc and selenium were reviewed from this method. Mean concentrations were compared from visit 3 with the visit 1 and visit 2 results. A paired *t*-test was used to investigate differences between visit 3 and previous results for both zinc and selenium. A difference was defined to be significant for $p < 0.05$ and highly significant for $p < 0.01$. Best fit linear relationships between XRF results and ICP-MS concentrations were established for both zinc and selenium. A correlation was defined to be significant for $p < 0.05$ and highly significant for $p < 0.01$. Correlations between XRF and ICP-MS results were examined when using the automated output from the XRF system, as well as when using the TAR, Compton normalization, and coherent normalization approaches to XRF energy spectrum analysis.

3. Results

From the 47 sets of MINI study visit 3 clippings available for analysis with respect to zinc, two sets were removed as outliers, leaving 45 sets for a full comparison of data. Of the 47 sets of clippings available for analysis with respect to selenium, one set returned an ICP-MS result below the limit of detection and one set was removed as an outlier, leaving 45 sets for a full comparison. Summaries of the ICP-MS concentration results obtained from measurements of zinc and selenium in the clipping sets are provided in Table 1.

A typical energy spectrum from XRF measurement of a single nail fragment for 60 s is given in Fig. 1. The Fig. 1(a) plot illustrates the relative magnitudes of the zinc and selenium signals. The fitting of this spectrum by PyMca shows a clear detection of characteristic X-rays from zinc at 8.6 keV (Kα) and 9.6 keV (Kβ). The detection of characteristic X-rays from selenium is more challenging, but the expanded Fig. 1(b) plot shows a small peak at 11.2 keV (Kα). PyMca also provided a fit for a selenium signal at 12.5 keV (Kβ), but this peak is very small and not discernable by eye. A particular advantage of the mono-energetic excitation used by the XRF system in this study was the very small, flat, background continuum over the energy range of interest.

For comparison between the XRF results and ICP-MS results obtained in this study, four distinct approaches to the analysis of the XRF data were available: (1) the automated output of concentration results directly from the XRF system using factory default calibrations; (2) the calculation of TAR normalized results from PyMca analysis of the energy spectra; (3) the calculation of Compton scatter normalized results from PyMca analysis of the energy spectra; and (4) the calculation of coherent scatter normalized results from PyMca analysis of the energy spectra.

When considering the concentration results output directly from the XRF system, the concentration values were expected in advance to be inaccurate, for the reason noted above in the Methods section. For zinc,

Table 1
Summary statistics from ICP-MS concentration results obtained for zinc and selenium from the MINI study visit 3 participants.

	Zinc	Selenium
N	45	45
Minimum	60.8 μg/g	0.374 μg/g
Maximum	171 μg/g	1.02 μg/g
Mean	104 μg/g	0.606 μg/g
Standard deviation	22.0 μg/g	0.133 μg/g
Median	97.4 μg/g	0.608 μg/g

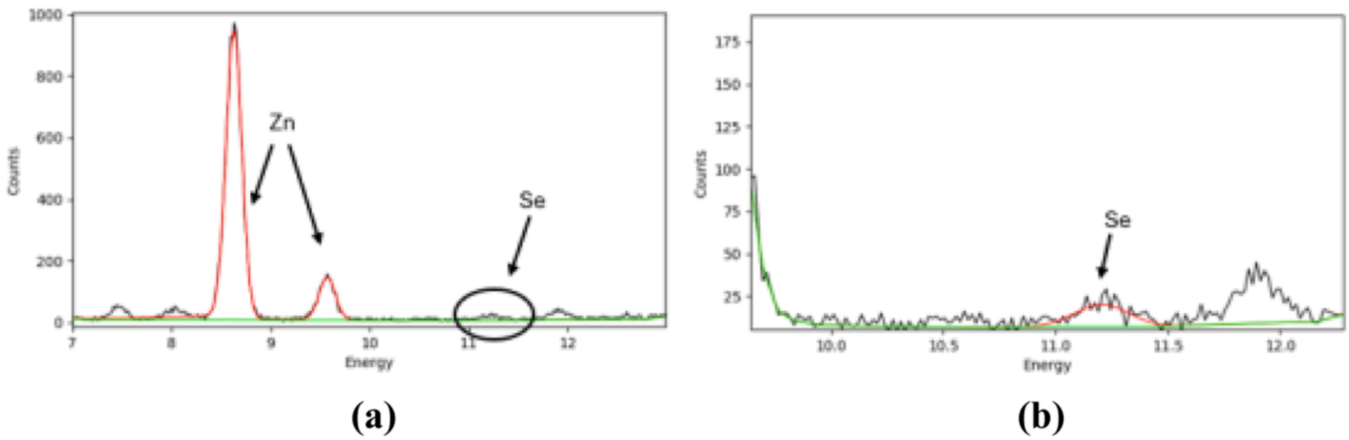

Fig. 1. Energy spectrum obtained from a 60 s XRF measurement of a single nail fragment, with data fit by PyMca software. (a) The plot on the left shows counts as a function of energy (keV). The red peaks represents the fit for zinc (Zn). The signal from selenium (Se) is difficult to discern at this scale. (b) The plot on the right shows the same energy spectrum, with the count scale and energy scale expanded. The red peak represents the fit for selenium (Se).

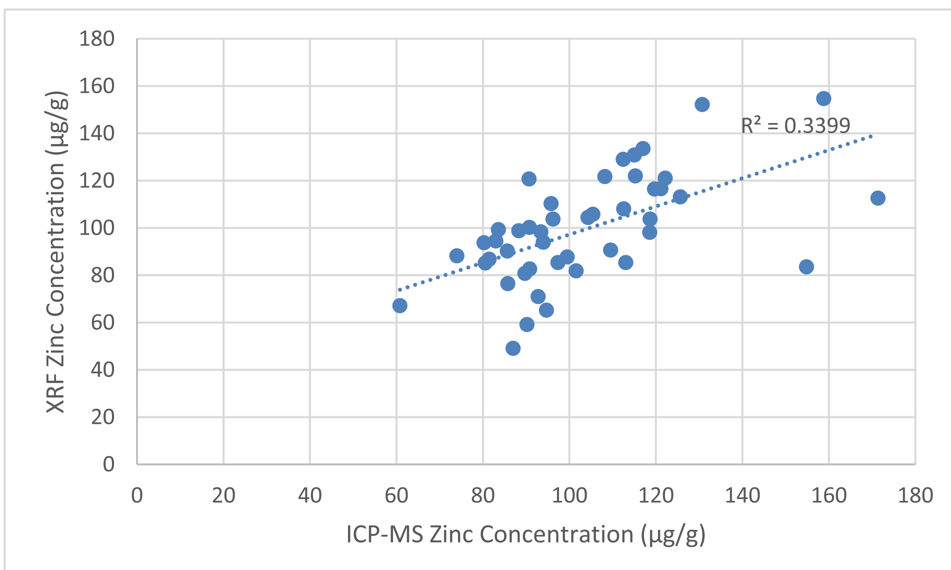

Fig. 2. XRF system output zinc concentration (µg/g) as a function of ICP-MS zinc concentration (µg/g) for nail clippings from visit 3 of the MINI study. The best fit linear relationship between the variables is provided as a dotted line.

Fig. 2 provides the relevant comparison between XRF system output concentrations and ICP-MS concentrations from the visit 3 participants. The correlation was highly significant ($p < 0.0001$, r^2=0.34) and the parameters for the linear equation of best fit from this relationship are given in Table 2. For selenium, the XRF concentrations were below the limit of detection according to the HD Mobile output, and a similar comparison of selenium concentration results was therefore not possible. It should be noted, however, that PyMca analysis of individual XRF energy spectra did provide a selenium signal for comparison with ICP-MS concentrations, and these results are described below.

Using the TAR normalization approach to energy spectrum analysis with the PyMca software, XRF results were obtained for both zinc and selenium from all nail clipping measurements. Fig. 3 presents a comparison between XRF TAR normalized zinc signals and ICP-MS zinc concentrations from the visit 3 participants. The correlation was highly significant ($p < 0.00001$, r^2= 0.54). Likewise, Fig. 4 provides the comparison between XRF TAR normalized selenium signals and ICP-MS selenium concentrations. The correlation was not as strong for selenium, but still highly significant ($p < 0.001$, r^2=0.27). The parameters for the linear equations of best fit from these relationships are given in Table 2 for zinc and in Table 3 for selenium.

Table 2
Linear equation of best fit slopes and y-intercepts for the zinc data presented in Figs. 2, 3, 5, and 7. Parameter values and their uncertainties are provided.

	Slope	y-intercept
Fig. 2	0.60 ± 0.13	38 ± 13
Fig. 3	0.000091 ± 0.000013	0.0050 ± 0.0014
Fig. 5	0.000285 ± 0.000041	0.0162 ± 0.0043
Fig. 7	0.00124 ± 0.00016	0.068 ± 0.017

Introducing instead the Compton scatter normalization approach to energy spectrum analysis using the PyMca software, Fig. 5 provides a comparison between XRF Compton normalized zinc signal and ICP-MS zinc concentration from the visit 3 participants. The correlation was highly significant ($p < 0.00001$, r^2=0.53). Fig. 6, in turn, gives the comparison between XRF Compton normalized selenium signal and ICP-MS selenium concentration from the visit 3 participants. The correlation was not as strong for selenium, but still highly significant ($p < 0.01$, r^2=0.21). The parameters for the linear equations of best fit from these relationships are provided in Table 2 for zinc and in Table 3 for

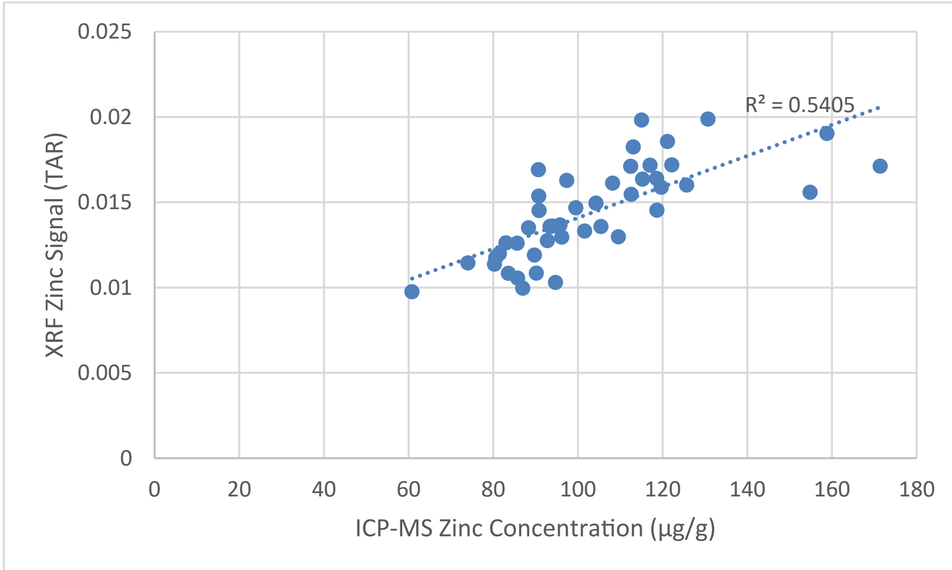

Fig. 3. XRF Total Area Ratio (TAR) zinc signal as a function of ICP-MS zinc concentration (μg/g) for nail clippings from visit 3 of the MINI study. The best fit linear relationship between the variables is provided as a dotted line.

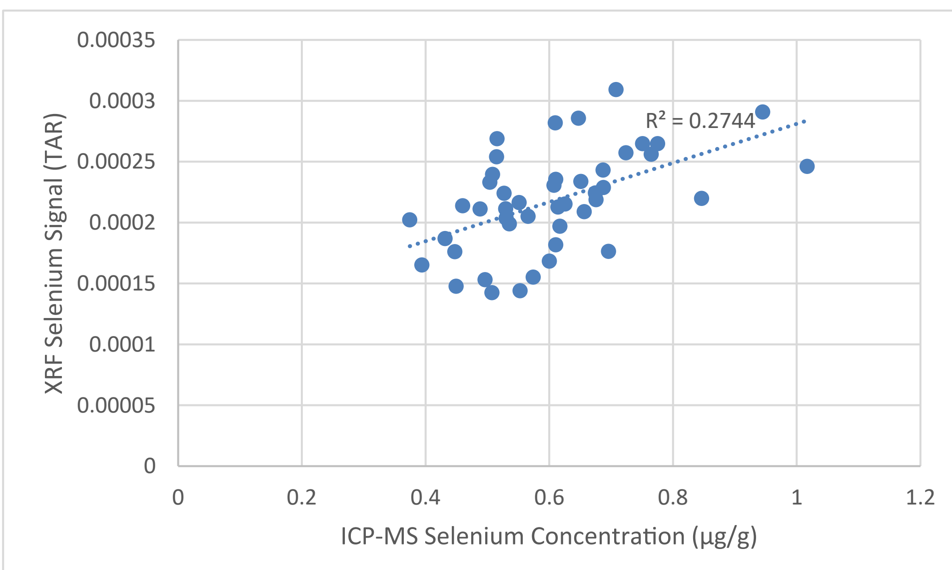

Fig. 4. XRF Total Area Ratio (TAR) selenium signal as a function of ICP-MS selenium concentration (μg/g) for nail clippings from visit 3 of the MINI study. The best fit linear relationship between the variables is provided as a dotted line.

Table 3
Linear equation of best fit slopes and y-intercepts for the selenium data presented in Figs. 4, 6, and 8. Parameter values and their uncertainties are provided.

	Slope	y-intercept
Fig. 4	0.000161 ± 0.000040	0.000120 ± 0.000025
Fig. 6	0.00045 ± 0.00014	0.000342 ± 0.000084
Fig. 8	0.00203 ± 0.00060	0.00143 ± 0.00038

selenium.

Finally, using the coherent scatter normalization approach to energy spectrum analysis with the PyMca software, Fig. 7 presents a comparison between XRF coherent normalized zinc signal and ICP-MS zinc concentration from the visit 3 participants. The correlation was highly significant ($p < 0.00001$, r^2=0.57). Fig. 8 gives the comparison between XRF coherent normalized selenium signal and ICP-MS selenium concentration from the visit 3 participants. The correlation was not as strong for selenium, but still highly significant ($p < 0.01$, r^2=0.21). The parameters for the linear equations of best fit from these relationships are again provided in Table 2 for zinc and in Table 3 for selenium.

4. Discussion

Zinc concentrations and selenium concentrations obtained from ICP-MS measurements of nail clippings from visit 3 of the MINI study may be compared against those observed from visit 1 and visit 2 [5]. Overall, the nail clipping levels from visit 3 decreased relative to the earlier visits. The average zinc concentration from visit 3 was 104 μg/g, compared with 122 μg/g from visit 1 and 127 μg/g from visit 2. A paired *t*-test indicated a highly significant decrease in zinc concentrations from visit 1 to visit 3 ($p < 0.001$) and from visit 2 to visit 3 ($p < 0.001$). Likewise,

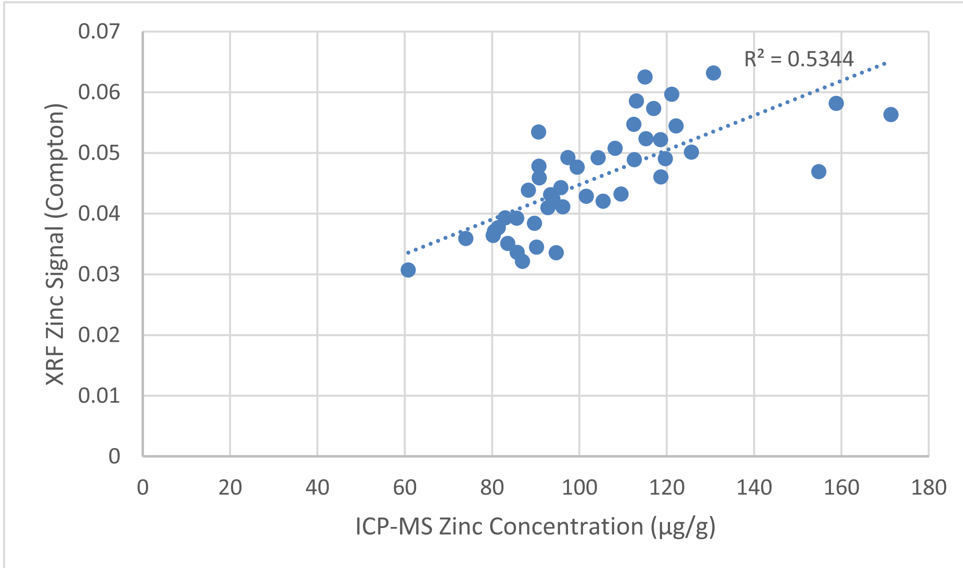

Fig. 5. XRF zinc signal (Compton) as a function of ICP-MS zinc concentration (μg/g) for nail clippings from visit 3 of the MINI study. The best fit linear relationship between the variables is provided as a dotted line.

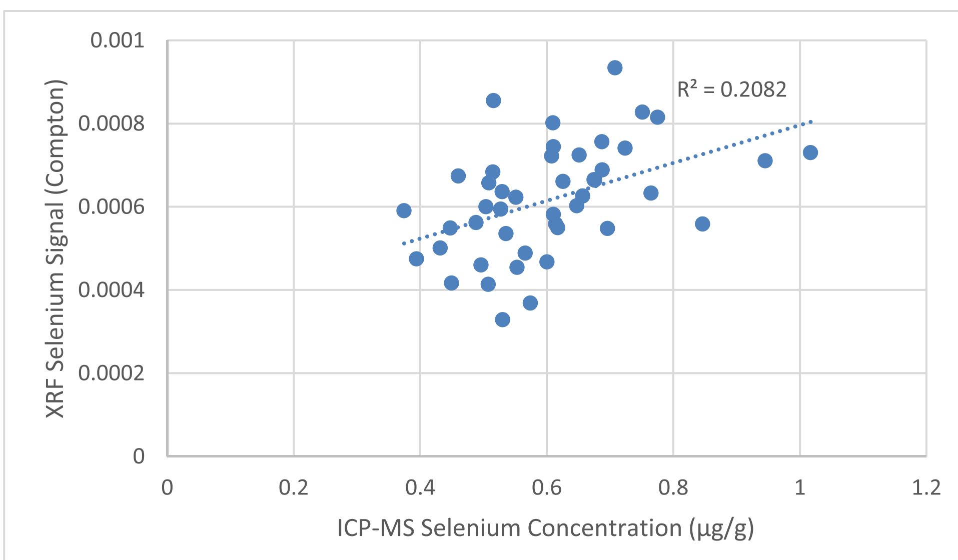

Fig. 6. XRF selenium signal (Compton) as a function of ICP-MS selenium concentration (μg/g) for nail clippings from visit 3 of the MINI study. The best fit linear relationship between the variables is provided as a dotted line.

the average selenium concentration from visit 3 was 0.606 μg/g, relative to 0.659 μg/g from visit 1 and 0.646 μg/g from visit 2. A paired *t*-test indicated a highly significant decrease in selenium concentrations from visit 2 to visit 3 ($p < 0.01$) only. The reason for any decrease in concentrations is unclear. Since the collection of nail clippings from visit 3 took place a full 12 months postpartum, it is possible the observed trends reflect an overall shift in diet following pregnancy and delivery. Any initial changes in intake following pregnancy would not be immediately evident from clippings since nails take a substantial amount of time to grow out [19,20]. For the toes of the mothers in particular, this time lag between dietary change and its evidence in clippings would last months, and perhaps over 10 months in the case of the big toe [20].

The XRF measurement results from visit 3 were compared with the ICP-MS concentration results to see how well the values correlated. In general, a stronger correlation would provide better evidence that the XRF method is a reliable indicator of elemental content in nail clippings. There were two aspects of special interest from this study: how the correlations from visit 3 compared with those from the earlier visits, and how the various different methods of XRF data analysis from visit 3 compared with each other.

4.1. Visit 3 correlations relative to visit 1 and visit 2

The key variable between the three sets of data from the three visits was the XRF measurement time for each nail fragment. Visit 1 made use of a 300 s measurement time, while visit 2 applied a 180 s measurement, and visit 3 used a 60 s measurement. The relatively short time of 60 s was chosen for the current study to see whether a more rapid XRF scan could still produce reasonable results for both zinc and selenium. The associated efficiencies for XRF measurement throughput could potentially provide a significant benefit for future public health applications involving large population groups.

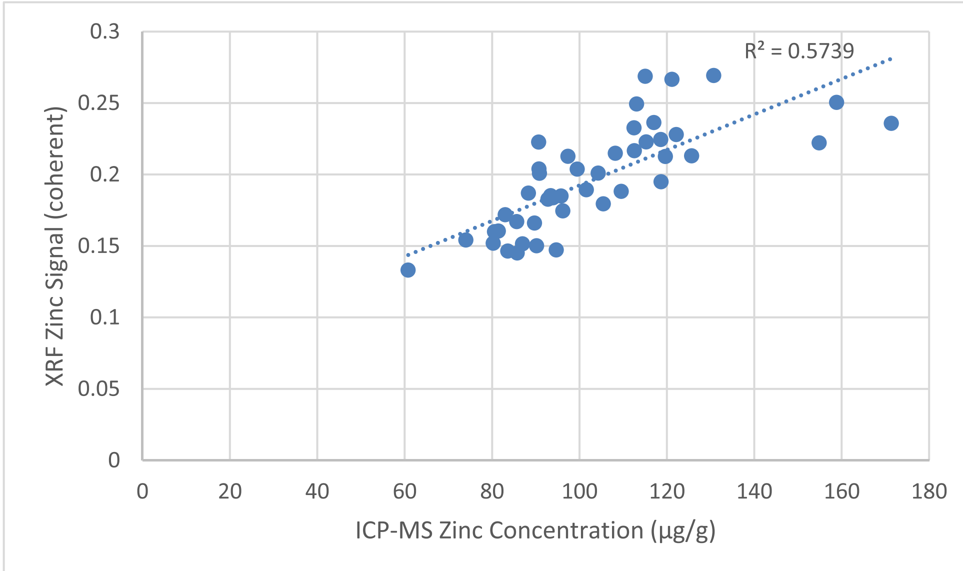

Fig. 7. XRF zinc signal (coherent) as a function of ICP-MS zinc concentration (μg/g) for nail clippings from visit 3 of the MINI study. The best fit linear relationship between the variables is provided as a dotted line.

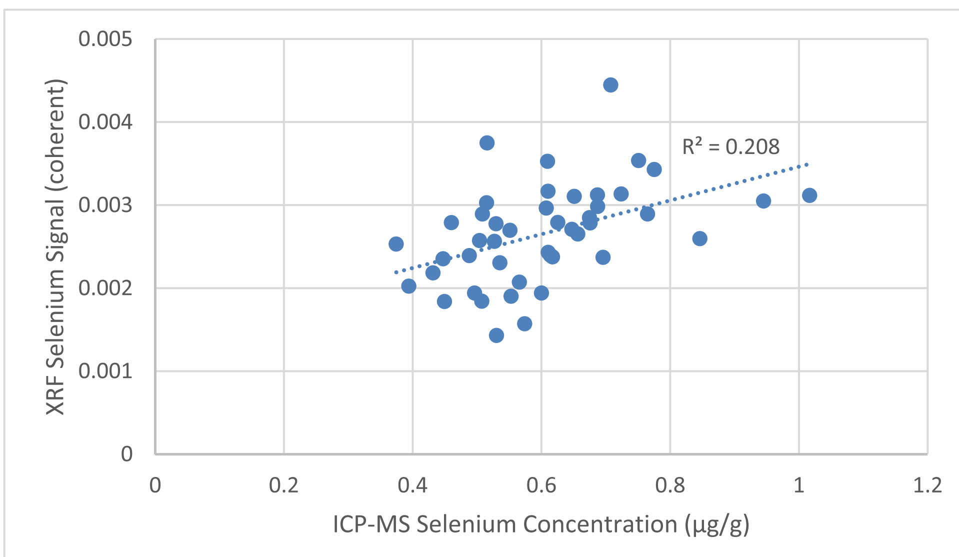

Fig. 8. XRF selenium signal (coherent) as a function of ICP-MS selenium concentration (μg/g) for nail clippings from visit 3 of the MINI study. The best fit linear relationship between the variables is provided as a dotted line.

For the visit 3 zinc results, when using the automated XRF system output zinc concentrations, the correlation with the ICP-MS zinc concentrations proved to be relatively weak. The observed coefficient of determination (r^2) between the two variables was 0.34. This compares with the corresponding results of $r^2 = 0.77$ from visit 1 and $r^2 = 0.83$ from visit 2 [5]. When instead using the XRF energy spectrum analysis with the TAR approach to determine a zinc signal, the visit 3 correlation with ICP-MS zinc concentrations was higher ($r^2 = 0.54$). This result compares with $r^2 = 0.74$ from visit 1 and $r^2 = 0.95$ from visit 2 [5]. The recording of automated XRF system output concentrations was not possible for selenium since the individual readings registered as below the limit of detection. XRF selenium results were, however, obtained from PyMca analysis of the energy spectrum. Using the XRF spectrum analysis with the TAR approach to generate a selenium signal, the visit 3 correlation with ICP-MS selenium concentrations was lower ($r^2 = 0.27$) than was the case from the zinc signal. This compares with selenium results of $r^2 = 0.53$ from visit 1 and $r^2 = 0.70$ from visit 2. Overall, these results suggest that a 60 s measurement time may be adequate for zinc if a rapid assessment is necessary, but a longer scan is preferable. In the case of selenium assessment, a rapid 60 s measurement time is likely not adequate to provide reliable results. The contrast between zinc and selenium results here is a consequence of the much higher concentrations observed for zinc in nail clippings relative to selenium. In general, when considering the visit 3 data, analysis of XRF energy spectra using the TAR approach proved to be superior to the use of automated XRF system output concentrations. This observation is consistent with findings from previous studies [5,15,16].

4.2. Visit 3 correlations using three different approaches to analysis of XRF energy spectra

The first of these different approaches, the TAR method, has been applied with some success through a variety of recent XRF studies [5,11,15,16]. The other two approaches involved assessment of the Compton and coherent scatter peaks, respectively, and were introduced for the first time when using this XRF system with human nail clippings. A recent Monte Carlo simulation study examined all three approaches from the perspective of zinc assessment in nail clippings, using the same XRF system design as employed in the current experimental study [12]. The Monte Carlo study introduced different thicknesses of nail clipping and determined that the coherent scatter method may be preferable for its ability to reduce measurement variabilities resulting from clipping thickness [12]. However, a previous experimental study involving artificial nail clippings found no substantial difference between three similar approaches, with only a slightly better precision shown by the TAR method from repeat measurements [11].

For zinc signal assessment, the three different approaches yielded similar correlations with ICP-MS zinc concentration. Coefficients of determination were $r^2 = 0.54$ for TAR, $r^2 = 0.53$ for the Compton-based approach, and $r^2 = 0.57$ for the coherent. A similar finding was evident from the selenium results: $r^2 = 0.27$ for TAR, $r^2 = 0.21$ for Compton, and $r^2 = 0.21$ for coherent. On balance, these results suggest the three approaches are equally viable, with no clear choice in terms of superior outcome. Practical considerations, however, may favor the TAR approach over the other two methods. The TAR method has the advantage of simplicity: calculation of the denominator in the ratio of values requires only the determination of the total number of detected counts, summed over all energies. There is no user-based interpretation concerning the extent of observed scatter peaks (Compton or coherent) and therefore no judgement as to the boundaries of the region of interest. For the same reason, results from the TAR approach would also be expected to be more consistent, facilitating comparisons between different studies.

Overall, the MINI study visit 3 results relating to the measurement of zinc and selenium in nail clippings have provided new insight into both the dietary intake of the study group and the ongoing development of portable XRF methodology. When performing an energy spectrum analysis using PyMca, the 60 s XRF measurement time applied to individual nail fragments provided zinc results which correlated fairly well with zinc concentrations from ICP-MS. The correlation was not as strong in the case of selenium measurements, indicating a longer XRF measurement time would be needed for a reliable assessment of this element. Three different approaches to analyzing XRF energy spectra, TAR, Compton normalization, and coherent normalization, yielded similar results in terms of their correlations with ICP-MS concentrations. On balance, the TAR approach is recommended for future application due to its simplicity and ease of comparison between different data sets. Equations of best fit between the XRF and ICP-MS results from this study, and those determined from previous data sets, could be used in the future as calibration lines to determine concentration values directly from XRF measurement. This would provide a convenient and practical means of assessing zinc and selenium status. The XRF method as a whole holds significant potential for monitoring of trace elements in nail clippings, especially in situations requiring rapid measurements, portability, or low cost.

CRediT authorship contribution statement

Louise Brough: Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Jane Coad:** Supervision, Project administration, Methodology, Investigation, Conceptualization. **Ying Jin:** Investigation, Formal analysis. **David Fleming:** Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis. **Abir Lefsay:** Validation, Investigation, Formal analysis. **Jong Sung Kim:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis. **Jasmine Ouellette:** Investigation. **Andrianna Scott:** Investigation, Formal analysis.

Declaration of Competing Interest

Concerning the manuscript, “Portable X-ray Fluorescence of Zinc and Selenium with Nail Clippings – Visit 3 of the Mother and Infant Nutrition Investigation (MINI)”, the authors declare that we have no conflicts of interest.

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Author_statement

Please find enclosed a research article submission toward the ISTERH conference special issue of the Journal of Trace Elements in Medicine and Biology. The title of the article is “Portable X-ray Fluorescence of Zinc and Selenium with Nail Clippings – Visit 3 of the Mother and Infant Nutrition Investigation (MINI)”. All authors have reviewed this manuscript.

This manuscript is not being considered for publication elsewhere. The authors have no conflicts of interest to declare.

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