Abstract

Objective and Approach: A study, conducted in Toronto, Canada, between 2009 and 2011, measured the bone lead concentrations of volunteers aged 1–82 years using in vivo x-ray fluorescence (XRF) technology.

Main results: Bone lead levels were lower compared to Ontario in vivo XRF studies from the early 1990s. In adults, the slope of tibia lead content versus age was reduced by 36–56%, i.e. bone lead levels for a given age group were approximately half compared to the same age group 17 years prior. Further, bone lead levels of individuals fell over that time period. In 2010, an average person aged 57 years had a bone lead level approximately 1/3 less than their bone lead level age 40 years in 1993. Using this data, the half-lives of lead in the tibia were estimated as 7–26 years. Tibia lead levels were found to be low in children. The reduction in bone tibia content in children was not significant ($p = 0.07$), but using data from additional north eastern US studies, there is evidence that childhood tibia stores are lower than in the 1990s.

Significance: In vivo XRF analysis shows that there has been a reduction in the level of lead in bone in Canada over the last two decades. Public health measures have been very successful in reducing ongoing exposure to lead and in reducing bone lead stores.

List of abbreviations

XRF X-ray fluorescence
CHMS Canadian Health Measures Survey
TIMS Thermal ionization mass spectrometer
Pb Lead
Cd Cadmium
K Potassium
REB Research ethics board

Introduction

A major knowledge gap in Canada is the current extent of chronic exposure to lead in the Canadian population. Protective measures, such as the removal of lead from gasoline, paint and solder, and regulation of lead in drinking water, have been introduced over the previous three decades. However, the extent of the presumed reduction in chronic exposure to lead (as assessed via levels of stored lead in bone) in the general population was unknown, as most bone lead surveys have been performed on predominantly male Canadian workers (Fleming et al 1997, 1998, 1999; Brito et al 2000, 2001).
An individual’s current blood lead level (BLL) is a complex function of a number of factors which include recent exposure, sex, age, nutritional status and exposure history. Studies in the literature assume that it is a reflection of the person’s recent exposure to lead but, at some level, may reflect their prior exposure history (Erkkila et al 1992, Webber et al 1995, Smith et al 1996, Fleming et al 1997, Gulson et al 1998, Popovic et al 2005). The concentration of lead in bone is considered an indicator of chronic exposure and overall body burden and can be measured non-invasively and painlessly by using x-ray based technologies (Somervaille et al 1985, 1988, et al). If only BLL is measured, it is difficult to ascertain how much of the BLL is a contribution from recent lead exposure and how much is a measure of endogenous lead being released back into the bloodstream from long-term bone stores.

This feasibility study was conducted to measure bone, serum and whole blood lead levels among healthy Canadian volunteers across the lifespan who were not occupationally exposed to lead. The study population was intended to comprise 240 healthy, male and female volunteers from all age groups. The objective of this study was to assess whether we could collect serum and whole blood, measure bone lead and administer a questionnaire in a 1 h appointment time slot. This paper summarises our methods for measurement of lead in bone, the feasibility of bone measurements in the general population across the lifespan, and the bone (tibia and calcaneus) lead measurement results obtained for all age groups, from children as young as 18 months, to adults over the age of 80 years. We believe this is the first report of $^{109}$Cd K XRF measurements of both tibia and calcaneus data for children under age 6. Furthermore, we compare current tibia lead levels in adults, to results from studies we conducted in Ontario in the early 1990s, to assess reductions in chronic exposure to lead in Ontario, Canada and to determine whether historical lead is being released from the tibia of adults (due to bone remodelling) at rates comparable with other reported studies. We also compare childhood and teenager exposure with data from Canada and the northern USA in the 1990s to assess whether childhood accumulation of lead into bone has changed.

**Materials and methods**

**Study location**

Between September 2009 and January 2011, a research clinic was established at St. Joseph’s Health Centre in the city of Toronto, Canada. The hospital is on the shore of Lake Ontario, beside a major highway and is surrounded by older houses. The hospital was chosen because it is a research institution with the necessary administrative infrastructure, relative ease of accessibility to volunteers, and it is situated in a mixed ethnic and socio-economic population. In addition, for individuals with elevated blood lead levels, our referral physician (RB) had privileges at the hospital.

We received Health Canada, St. Joseph’s hospital and McMaster University research ethics board (REB) approval for this study. All amendments to the research protocol were approved by all three REBs.

**Consent forms**

Three consent/assent forms were designed depending on the age of the participant. Informed Consent was obtained from participants (≥16 years of age). For children 16–17 years of age, consent could be obtained from the participant alone or the parent/guardian may also have provided consent.

The Informed Assent Form was completed by children ages 7–15 years of age in order to express their assent in respect to this research. A child’s agreement to participate was sought in addition to parental consent when the minor was sufficiently mature to understand the nature of his/her participation in the research study. An assessment of the following were considered for child participants: (a) what the child wants to know; (b) what the child can understand; (c) what the child’s decision making capacity is; and (d) what the child needs to know in order to exercise his/her decisional capacity.

Informed Parental Consent was obtained for children under 7 years of age, or for those who lacked the maturity to assent. The child’s assent or lack of assent was documented by the research staff.

**Advertising**

Advertising flyers and posters with information on the purpose of the study, what it entailed, who could participate and how to contact the study coordinator were distributed and posted on notice boards in the hospital, doctors’ offices and community centres in the surrounding area and at the University of Toronto.

**Recruitment**

Healthy volunteers from various age/gender categories with the ability to consent, assent or have parental consent and who could communicate in English were eligible for the study. The original goal was to recruit 240 subjects; however, the commercial courier misplaced one shipment of pre-cleaned serum containers required for the testing visit. As a result, there were no serum specimens ($n = 32$) for the first group of participants. Therefore, 32 additional subjects were recruited in order to have a full complement of whole blood, serum and bone lead testing.
Participants were compensated with $50 to cover costs involved in their participation (parking, food, transit, etc).

**Appointments procedure**

Up to 10 back-to-back appointments were scheduled daily in 1 h blocks with 22 min for each bone lead test, and about 16 min each to administer the consent form and to draw blood. Daytime, evening and weekend clinics were scheduled in order to accommodate volunteers.

The clinic was laid out so that subjects could move from ‘station’ to ‘station’ as quickly as possible and thus keep to the 1 h appointment (figure 1). Subjects started with informed consent outside of the room. Adults were then taken into the room where a large comfortable chair was used for blood collection. Then, volunteers moved to the bone lead system where bone lead measurements were taken from the tibia, and calcaneus. Adult volunteers were given a questionnaire to complete while their bone lead levels were being measured.

With parental consent/assent, children had a topical anesthetic cream (MAXILENE®) applied to their arm (for the blood draw) prior to their tibia and calcaneus bone lead measurements. The questionnaire for children under 7 was filled out by their parent or guardian while the child had their bone lead levels measured. Children then had their blood drawn at the end of the appointment by a pediatric nurse.

**Questionnaire**

Three questionnaires were developed depending on the age of participants in the study. Questionnaire A was completed by all participants and the parent/guardians of child participants. It collected information on socioeconomic status, health, possible lead exposures, including hobbies; employment history; etc and for women, questions related to bone turnover (oral contraceptive use, menopausal status). Questionnaire B was developed for minor children over the age of 7 who completed the questionnaire with the assistance of a parent or guardian. The questions were similar to the adult questionnaire but excluded questions pertinent only to adults. Questionnaire C was completed by parents of the youngest children and was designed to collect information about possible sources of lead exposure such as whether the child exhibited behaviors such as pica.

**Bone lead measurement system**

The $^{109}$Cd K XRF bone lead measurement system was re-located from McMaster University in Hamilton to St. Joseph’s Health Centre in Toronto. Details of the system have been previously published (Nie et al 2004). The system delivers a very small radiation dose (Nie et al 2006) and volunteers were asked to sit in a chair and remain fairly still while the instrument is placed near the shin or heel (figure 2).

The $^{109}$Cd radiation source was transferred from the McMaster University to the hospital site license and the radiation source was locked and stored on site each night. The bone lead system was left at St. Joseph’s for a period of 18 months. McMaster University bone lead measurement technologists travelled from Hamilton, Ontario, to the hospital in Toronto to conduct the measurements.

McMaster University had never previously measured bone lead levels of children younger than six years of age, so the XRF technicians were instructed to try to measure children by working with them and their parents to make the measurements as comfortable as possible. Distractions such as DVDs, comic books and games were...
available for use. The technicians were told to stop the measurements immediately if children expressed their wish to discontinue the procedure.

In vivo measurements were interspersed with calibration standard measurements which were conducted in a pseudo-random order: at the beginning and end of each day, and in all empty appointment slots. Log books were updated for each measurement and recorded volunteer or calibration standard identification codes, system measurement variables, and notes of unusual occurrences.

**Blood collection**

A child life specialist and pediatric nurse did all the blood draws. The skin surface was thoroughly wiped twice with clean alcohol wipes. Using a safety butterfly needle, approximately 6 ml of blood were slowly drawn into a standard sterile plastic syringe. When complete and after removing the butterfly, 1 ml of blood (for whole blood analysis) was slowly ejected from the syringe into a vacutainer tube (BD Canada, Tan-top, with K2 EDTA) and capped. The whole blood samples were stored at 4 °C. Periodically, samples were shipped unfrozen to the analytical laboratory at the University of Texas at Dallas, using a commercial courier.

**Lead analysis in blood**

Lead concentrations in whole blood were measured by isotope dilution mass spectrometry, using a 205Pb spike, which allows the isotope ratios (206Pb/207Pb and 208Pb/207Pb) of the sample to be measured at the same time. Ratios were measured on a multicolonlector Finnigan MAT 261 thermal ionization mass spectrometer (TIMS). Accuracy of blood Pb concentrations is 0.1% and ±0.001 in 206Pb/207Pb and ±0.002 in 208Pb/207Pb. A procedural blank of 50 pg Pb is negligible.
Statistical methods
A goal of this work was to compare results obtained between 2009 and 2011 with previously published data obtained between 1991 and 1994. This work therefore followed the previously published statistical methods to allow comparisons to be made. Data were analysed using group averaged data (of the age group categories that were recruited). Student t-tests were used to test differences between groups. Relationships between variables were explored using linear regressions. The Minitab 17 statistical package was used to perform this analysis. Tests were performed to determine if mathematical models other than linear fits were better fits to the data. Models were compared to each other on the basis of Aikake’s Information Criteria and the CurveExpert Professional software package was used to perform the analysis.

Results

Study location
The set up and operation of the bone lead system at a remote site was found to be feasible. There were two occasions when technical problems led to appointment cancellation: (i) the liquid nitrogen dewar attached to the detector was not filled, so the system could not be started on time, and (ii) a ‘glitch’ in the control software meant the system had to be restarted which cancelled one appointment. All appointments were re-scheduled.

Recruitment
There were a total of 263 participants in the study and included men, women and children (aged 1–82), which was 96% of our target (table 1). Girls <11 were very difficult to recruit and additional recruitment strategies were employed to obtain sufficient numbers for this group. One of the primary reasons for low recruitment of girls <11 appears to be related to concerns over venous blood collection. Consequently, the decision was taken to close the study without full recruitment.

Appointments
It was feasible to fit all of the testing into a 1 h slot. As the study progressed, the staff became more experienced and the time constraint was less challenging. The time restriction resulted in less precise bone lead measurements with an uncertainty of 25% higher (thus worse) on average than if the study had permitted a 90 min time slot (Behinaein et al 2014). However, difficulty in sitting still for a longer period of time might offset the benefits of less uncertainty with a longer time slot.

Bone lead measurement feasibility
A measurement was made for the tibia and calcaneus bone of every person in the study. However, a small number (nine out of 526) of the measurements were stopped early as noted in the measurement log book. Measurements that were planned for 22 min were stopped, in some cases, before the completion of the scan when children indicated their desire to discontinue with the procedure. The three youngest children in the study, all <25 months old, had both tibia and calcaneus measurements stopped early. The next youngest child (26 months) managed a full tibia measurement, but the calcaneus measurement was stopped early. One child of 4 years of age asked for the calcaneus measurement to be stopped. An 11-year-old girl halted her tibia measurement because she felt unwell, but returned for a full calcaneus measurement. The log book also reported continual motion in subjects <3 years old.

The uncertainty of a bone lead measurement was derived from the statistical uncertainty of the measured x-ray signals. The bone lead measurement uncertainties have been discussed in an earlier paper (Behinaein et al 2014), so only summary data will be presented here. For children <3 years of age, both tibia and calcaneus have significantly poorer bone lead measurement uncertainties (p < 0.01) than measurements in the rest of the population. This is predominantly because these measurements were stopped early.

Bone lead measurement results
We have previously published some data regarding the relationship between age, sex and bone lead levels for this population (Behinaein et al 2017) so only a summary of group averaged data will be discussed here. Figure 3 shows plots of average tibia Pb content against average age for each of the recruitment groups with data from male and female participants shown separately in figure 3(a) and data from male and female participants combined shown in figure 3(b). Data were initially analysed separately by sex to examine if there are differences in bone lead levels due to sex-specific differences in bone metabolism. In general, the average tibia Pb content was found to be similar between the sexes for the age categories, with the exception of the age category 11–20 years. Consequently, the data were combined and the average tibia Pb content was reported.

Figure 4 shows plots of average calcaneus Pb content against average age for each of the recruitment groups with data from male and female participants shown separately in figure 4(a) and data from male and female
participants combined shown in figure 4(b). The average calcaneus Pb content was not significantly different for males and females in any age category, so once again data were combined.

**Adults**

As can be seen in figure 3, above the age of 20, average tibia lead content increases almost linearly with age. This pattern of increase in environmentally exposed populations has been observed in our previous studies (Gamblin et al 1994, Roy et al 1997, McNeill et al 2000). Data from previous studies are directly comparable to the results from the current study, since the $^{109}$Cd K XRF measurement systems are inter-calibrated. A linear regression of the individual data points (rather than the group averaged data presented in figure 3) of tibia lead content versus age, in adults over the age of 20, suggests that the lead content of the tibia increases by $0.16 \pm 0.03 \mu g \text{Pb g}^{-1} \text{bone mineral}$ per year of age ($p < 0.001$) with a $y$ intercept of $-3.5 \pm 1.6 \mu g \text{Pb g}^{-1} \text{bone mineral}$ ($p = 0.03$). These data suggest an $x$-axis intercept of approximate age 22, similar to that found in other studies (McNeill et al 2000).

As can be seen in figure 4, above the age of 20, average calcaneus lead content increases relatively linearly with age, with perhaps a suggestion of a turnover above age 60. A linear regression of the individual data points (rather than group averaged data shown in figure 4 of calcaneus lead content versus age in adults over the age of 20, suggests that the lead content of the calcaneus increases by $0.27 \pm 0.07 \mu g \text{Pb g}^{-1} \text{bone mineral}$ per year of age ($p < 0.001$) with a (not significant) $y$ intercept of $-5.1 \pm 3.5 \mu g \text{Pb g}^{-1} \text{bone mineral}$ ($p = 0.14$). These data suggest an $x$-axis intercept of approximate age 18. Above age 65, the plot appears to turn over, although the group average value is not, in this case, significantly different from that predicted by the regression.
Comparison of adult results to bone lead levels approximately 17 years earlier

In the 1990s, scientists at McMaster University performed a series of bone lead measurements of environmentally exposed subjects at the McMaster University Medical Centre (MUMC) in Hamilton, Ontario. MUMC is 62 km (38 miles) from St. Joseph’s Health Centre in Toronto. Both medical centres are located in major urban centres (which are part of a larger conurbation known colloquially as the Golden Horseshoe) on the shores of Lake Ontario. They have been subject to the same Federal and Provincial legislation and regulation regarding lead. The bone lead data were published in two papers (Gamblin et al 1994, Roy et al 1997) and our study data were compared to both published sets of results. The $^{109}$Cd K XRF bone Pb measurement system used in those studies was an earlier generation of the system that was used in this study (McNeill et al 2000). As new systems have been developed, they have been continuously inter-calibrated with older systems allowing bone Pb measurements that are traceable to national standards and which are directly comparable between studies. We consider the data from the two earlier studies a good comparison group to determine how cumulative lead exposure has changed in Southern Ontario in the last 15–20 years.

Relationships of tibia lead versus age for subjects aged 6–81 years of age were published in Roy et al (1997). They provided regression data for males only, females only, and males and females combined. Linear regressions were therefore performed using the data from this study for the individual sexes and then both combined for volunteers aged 6–83 and compared to the results in Roy et al (1997). Data are shown in table 2 and figure 5(a). All three relationships between tibia lead content and age had significantly lower slopes in 2009–2011 as compared to 1992–1994.
to 1992–1994. In all instances, the level of tibia lead content for a given age in 2009–2011 is approximately 50% of that found nearly two decades earlier.

Relationships of tibia lead versus age for men aged 23–70 years of age and women aged 19–81 years of age were published in Gamblin et al (1994). Regressions of our data over these matched age ranges are also presented in table 2 and figure 5(b). These comparisons are consistent with the results found when comparing to Roy et al (1997). In both cases, as can be seen in figures 5(a) and (b), the relationship of tibia lead content to age has a significantly lower slope in 2009–2011 as compared to 1991–1994. Table 2 also shows the ratio of the slopes of tibia lead content versus age determined in 2009–2011 to the slopes of tibia lead content versus age observed in 1993. The average ratio of slopes is 0.48 ± 0.04 (±SEM) suggesting that tibia lead content for a given age group in 2009–2011 is approximately 50% of that found nearly two decades earlier.

Roy et al (1997) also published data regarding the relationship of calcaneus lead content versus age in women but their published slope of the relationship of calcaneus content versus age was not statistically significant. We include the calcaneus data in table 2 for completeness, even though the Roy et al (1997) results are not significant.

The five sets of comparison data for the tibia content of adults presents statistically significant results and demonstrates a high degree of consistency in the data. The relationship between tibia lead content and age in southern Ontario, Canada is a factor of approximately 2 less now than 15–20 years ago.

The data also show that tibia lead content has fallen over time regardless of age. Table 3 presents a comparison of the measured group average tibia content for different age groups between 2009 and 2011 and the estimated tibia content of those same groups of people between 1991 and 1994 calculated from the published regression data in Roy et al (1997) and Gamblin et al (1994). As can be seen from the table, tibia lead levels in individuals are predicted to have been significantly higher 17 years ago.

Children

Figure 3 may suggest an apparent higher tibia lead content in younger children as compared to teenagers and young adults. The standard error of the mean on the under 5 years age group average is relatively large and so the age 5 and under group average tibia Pb content is not significantly different from the age 11–19 group (p = 0.15). In addition, a linear regression of individual data points (rather than group averaged data) of tibia versus age in all pre-pubertal children up to and including age 11 (an age range chosen as being representative of pre-pubertal children in Canada (Steingraber 2007)) was not significant. There was no observable relationship between tibia lead content and age through puberty i.e. in children between the ages of 12 and 19.

Figure 4 shows a higher calcaneus Pb content in younger children as compared to teenagers and young adults as assessed from group average data. The mean calcaneus content of the age 5 and under group was found to be significantly higher at the 95% confidence level than both the age 11–19 group (p = 0.03) and the age 6–10 group (p = 0.03). A linear regression of the individual data points (rather than the group averaged data) of calcaneus versus age in all children up to and including age 11 found a significant relationship (p < 0.001) with calcaneus lead content decreasing by 6.6 ± 1.5 µg Pb g⁻¹ bone mineral per year between age 1 and 11. Several non-linear models, including exponential and polynomial models were tested to see if they provided a better model of the relationship of calcaneus lead content versus age than linear. An exponential model was found to provide the best fit overall, and using Akaike’s Information Criterion, had a likelihood of 99.999% of being the better model. The relationship of bone lead to age using this model was determined to be

### Table 2. Comparison of relationship between tibia lead content and age as determined in this study with data published by Roy et al. All group comparisons were age-matched.

| Comparison data                        | Slope of tibia versus age, male and female, age 20 and over | Slope of tibia versus age, age 6–81 years | Slope of tibia versus age, age 6–81 years | Slope of tibia versus age, age 23–70 years | Slope of tibia versus age, age 19–81 years | Slope of calcaneus versus age |
|----------------------------------------|-------------------------------------------------------------|-------------------------------------------|-------------------------------------------|--------------------------------------------|------------------------------------------|-----------------------------|
| Roy et al (µg Pb g⁻¹ bone mineral per year) | —                                                           | 0.29 ± 0.04                               | 0.23 ± 0.04                               | 0.24 ± 0.03                                | —                                         | 0.12 ± 0.11                 |
| Gamblin et al (µg Pb g⁻¹ bone mineral per year) | —                                                           | 0.11 ± 0.02                               | 0.13 ± 0.03                               | 0.11 ± 0.02                                | —                                         | —                           |
| Current study (µg Pb g⁻¹ bone mineral per year) | 0.16 ± 0.03                                                | 0.38 ± 0.09                               | 0.56 ± 0.16                               | 0.46 ± 0.10                                | 0.50 ± 0.10                              | 1.75 ± 1.70 |
| Ratio of current to historical bone-age relationship | —                                                           | 0.38 ± 0.09                               | 0.56 ± 0.16                               | 0.46 ± 0.10                                | 0.50 ± 0.10                              | —                           |

*a Significantly different from unity, p < 0.001.

*b Significantly different from unity, p = 0.05.

*c Not significantly different from unity, p = 0.67.
Calcaneus Pb content = 4.1 * e^{-7.1*age}.

The comparison of the exponential to linear fit of the calcaneus content versus age is shown in figure 6(a). It appears that much of the exponential relationship is driven by one high point (which has a high measurement uncertainty). A number of methods could be tested to determine if this is the case, including fits weighted by the measurement uncertainty. However, we performed the simplest test: this data point was removed and the fits were retested as shown in figure 6(b). The likelihood of the exponential fit being better than the linear fit was reduced to 94%. We interpret this to mean that there is evidence therefore that the ratio of lead to bone mineral content in the calcaneus is significantly higher in children under age 5 than in children over age 5, but the determination of the exact nature of the relationship of calcaneus content with age in early childhood requires more data.

Comparison of childhood results to bone lead levels 17 years earlier
Roy et al (1997) performed measurements of children down to age 6 between 1991 and 1994. Figure 7 shows the comparison of the recruitment group averaged data from 2009–2011 to data from 1991–1994. For the 6–10 age group, tibia lead levels were lower in 2009–2011, but the difference was only suggestive (p = 0.09). For the 11–19 age group, there was no significant difference between 1991–1994 and 2009–2011 (p = 0.55). There was no observed relationship between tibia content and age either between 1991 and 1994 or between 2009 and
2011. Therefore the average tibia content of all children between ages 6–19 were compared. The average 6–19 year old tibia content between 1991 and 1994 was 5.8 ± 2.7 µg Pb g⁻¹ bone mineral. The average 6–19 year old calcaneus content between 2009 and 2011 was 0.63 ± 1.0 µg Pb g⁻¹ bone mineral. While the calculated average tibia lead content is lower now than 17 years earlier, the statistical significance of the difference is only suggestive (p = 0.074). We discuss this further below.

**Discussion**

**Adults**

The data from our study show that lead body burden, as assessed by K XRF measurements of the tibia, for different age groups in Canada is now 50% of the level observed for those age groups between 1991 and 1994.

We are confident that this observed reduction is not an artifact of the comparison groups. Home postal code information showed the volunteers in 2009–2011 were recruited from Toronto and other linked municipalities, including Hamilton, in the large urbanization that extends around the western edge of Lake Ontario, so there was overlap in the recruitment areas in the early 1990s and 2009–2011. Although this study employed a convenience-sampling strategy for recruitment, (a strategy also employed between 1991–1994), the data are representative of the southern Ontario population. Volunteer data broadly matches Statistics Canada’s reporting on a number of factors including, for example, household income, for the Greater Toronto Hamilton Area. Importantly, the blood Pb data from this study are comparable to data from the canadian health measures survey (CHMS) from the same time period. The CHMS collects health and biomonitoring data from a nationally representative sample of the Canadian population. Table 4 shows that the blood Pb data reported by age in this Toronto-based study compare very well to national CHMS blood Pb data (The Canada Health Measures Survey, Government of Canada), suggesting that bone Pb data from this Toronto-based study can be argued to be representative of Canadian exposure. Finally, this observed reduction in bone Pb level is not unexpected given patterns of blood Pb levels in North America over time. Figure 8 compares mean blood Pb levels in this study with the CHMS survey and the US National Health and Nutrition Survey data (Pirkle et al 1994, Tsoi et al 2016). As can be seen, these blood Pb data compare well with CHMS, and this study and CHMS both compare well with NHANES. Assuming Canadian Pb exposure levels have fallen in the same pattern as in the US, it would be expected that bone Pb levels are significantly lower than in the early 1990s, as observed.

Since 1994, more stringent health measures have been put in place by the federal and provincial governments in Canada such as removing lead from gasoline, solder and paint and monitoring of lead in drinking water. Our data show that governmental risk management of lead has been extremely successful with an observable reduction of a factor of 2 in population body burden for adults.

In the past, we and other research centres have used relationships of tibia lead versus age to define ‘usual’ exposure. This allows us to perform ¹⁰⁹Cd K XRF bone lead measurements and identify individuals with higher than usual exposure. The current ‘usual’ bone lead exposure level was based on data from 15–20 years ago; however, based on our results, we and other centres should redefine ‘high’ and ‘usual’ exposure to a lower level. We suggest that a current bone lead content that is statistically significantly greater (at the 99% confidence level) than the bone lead content for a given age predicted from these data collected between 2009 and 2011 should now be considered to be a higher than ‘usual’ exposure in Ontario, Canada.

**Table 3.** Comparison of current measured group average tibia content for different age groups (assessed between 2009 and 2011) and estimated tibia content of those same age groups in 1993 (assessed between 1991 and 1994). The reduction in tibia lead content was used to calculate a half-life of lead in the tibia to determine whether the predicted reduction is reasonable. The method is described in the Discussion section.

| Average age of recruitment groups during current study (2009–2011) (years) | Measured group average tibia content during current study (2009–2011) (µg Pb g⁻¹ bone mineral) | Predicted average age of current study (2009–2011) recruitment groups in 1993 (years) | Predicted 1993 tibia content (µg Pb g⁻¹ bone mineral) of age groups calculated from Roy et al (1997) regression data | Upper and lower bound of estimated half-life in tibia (years) |
|---|---|---|---|---|
| 29.6 | 0.9 ± 0.8 | 12.6 | 2.4 ± 1.2 | 7–9 |
| 43 | 3.4 ± 0.8 | 26 | 5.8 ± 1.2 | 13–19 |
| 56.5 | 6.0 ± 1.0 | 39.5 | 9.0 ± 1.2 | 17–26 |
| 70.4 | 6.5 ± 1.6 | 53.4 | 12.4 ± 1.2 | 13–18 |
The reduction in ongoing exposure to lead has been so significant in the Canadian adult population that endogenous lead is being released from bone faster than it is being incorporated into the bone matrix; consequently, bone lead levels are falling in individuals. The average age of our 50–65 year old age group assessed between 2009 and 2011 was age 57. From these data, an average 57 year old in Ontario in 2010 had a tibia lead content of approximately 6 µg Pb g⁻¹ bone mineral; at age 40 in 1993 we can predict (Gamblin et al 1994, Roy et al 1997) from the Roy et al regression data (table 3) that the average Ontarian had a tibia level of 9 µg Pb g⁻¹ bone mineral. This age group has seen a 1/3 reduction in their bone lead burden in the last two decades. Individuals’ bone lead stores are less because historical lead has been removed from the tibia (because of bone turnover) faster than ongoing exposure has increased tibia lead content.

To assess whether this drop in tibia lead content is reasonable based on our understanding of lead pharmacokinetics, we calculated half-lives for lead in tibia from the data. The change in bone tibia values can be used to calculate half-life estimates for lead in the tibia using the equation:

\[
\frac{(\text{Tibia } 2010 - \Delta \text{Tibia})}{\text{Tibia } 1993} = e^{-\ln(2) t / t_{1/2}}
\]

where \( t \) is the time between bone tibia assessments and \( t_{1/2} \) is the half-life of lead in the tibia. The correction factor, \( \Delta \text{Tibia} \), is applied to take into account the lead accumulated into the tibia from ongoing lead exposure between 1993 and 2010. This correction is necessary because
Figure 7. A plot comparing recruitment group average tibia lead content measured between 2009 and 2011 (noted as 2010 on the figure) and data collected between 1991 and 1994 (noted as 1993 on figure) averaged over the same age groups. Error bars indicate standard error of the mean. Data from 1991–1994 are taken from Roy et al (1997). Children under age 6 were not measured between 1991 and 1994.

Table 4. Comparison of geometric mean blood Pb data obtained in this Toronto-based study with geometric mean blood Pb data from the Canada Health Measures Survey 2009–2011 (The Canada Health Measures Survey, Government of Canada). Toronto data are presented with 95% confidence intervals (95% CI). Toronto data match well to national data.

| Age group (years) | CHMS all (µg dl\(^{-1}\)) (95% CI) | Toronto all (µg dl\(^{-1}\)) (95% CI) | CHMS male (µg dl\(^{-1}\)) (95% CI) | Toronto male (µg dl\(^{-1}\)) (95% CI) | CHMS female (µg dl\(^{-1}\)) (95% CI) | Toronto female (µg dl\(^{-1}\)) (95% CI) |
|------------------|-----------------------------------|----------------------------------------|----------------------------------|----------------------------------------|--------------------------------------|----------------------------------------|
| 3–5              | 0.93 (0.86–1.0)                   | 0.91 (0.7–1.2)                        |                                  |                                        |                                      |                                        |
| 6–11             | 0.79 (0.74–0.84)                  | 0.87 (0.8–1.0)                        | 0.79 (0.73–0.86)                 | 0.80 (0.7–0.9)                        | 0.78 (0.72–0.85)                     | 1.02 (0.8–1.3)                         |
| 12–19            | 0.71 (0.68–0.75)                  | 0.78 (0.7–0.9)                        | 0.84 (0.8–0.87)                  | 0.91 (0.7–1.1)                        | 0.6 (0.56–0.65)                      | 0.67 (0.6–0.8)                         |
| 20–39            | 0.98 (0.88–1.1)                   | 1.01 (0.9–1.1)                        | 1.10 (1.0–1.3)                   | 1.10 (0.9–1.3)                        | 0.85 (0.74–0.98)                     | 0.93 (0.8–1.1)                         |
| 40–59            | 1.40 (1.3–1.5)                    | 1.36 (1.2–1.5)                        | 1.60 (1.5–1.7)                   | 1.29 (1.0–1.6)                        | 1.3 (1.2–1.4)                        | 1.43 (1.2–1.7)                         |
| 60–79            | 1.90 (1.8–1.9)                    | 1.79 (1.5–2.2)                        | 2.00 (1.9–2.2)                   | 1.96 (1.4–2.7)                        | 1.7 (1.6–1.8)                        | 1.69 (1.3–2.1)                         |

Figure 8. A comparison of mean blood Pb levels (for the total population) from this study to NHANES (Pirkle et al 1994, Centers for Disease Control and Prevention (CDC) 1997, 2005, Tsai et al 2016) and CHMS (The Canada Health Measures Survey, Government of Canada, Wilker et al (2011)) data. As can be seen, recent data are comparable between all three studies. The NHANES data shows a significant drop in blood Pb levels over the last four decades in the U.S. and Canadian blood Pb levels have fallen in a similar pattern.
Tibia 2010 = ΔTibia + Tibia 1993 \cdot e^{-\ln(2) \cdot t/18}.  

Between 1993 and 2010, there was both input into bone from blood, and loss from bone with a half-life of \( t_{1/2} \). The correction factor, \( \Delta \text{Tibia} \), i.e. the increased tibia content, was calculated from

\[ \Delta \text{Tibia} = \text{CBLI} \times \text{blood-bone - transfer coefficient}. \]

CBLI, a cumulative blood lead index, was calculated as the integral of blood lead values between 1993 and 2010 and was calculated using NHANES (Centers for Disease Control and Prevention (CDC) 1997, 2005) blood lead data as a surrogate for historical geometric mean BLL in southern Ontario between 1993 and 2010. The blood-bone transfer coefficient is an empirically derived estimate of the relationship between a cumulative blood lead index (CBLI, derived by integrating blood lead levels over time) and tibia lead content. We used upper and lower bound estimates of this coefficient determined in two studies of high (Somervaille et al 1988) and low (Erkkila et al 1992) exposure lead workers. The upper bound estimate for the coefficient was 0.06 (Somervaille et al 1988), the lower bound estimate was 0.03 (Erkkila et al 1992).

Using these data, the estimate of the half-life of lead in the tibia was found to be in the range 7–26 years. Estimates are shown in table 3. The predicted range was wide: from 7–26 years. However, our data and our values for half-life are in line with data obtained from the Normative Aging Study in Boston (Wilker et al 2011). They showed a 1.4% reduction in tibia lead content per year, which would be a 24% reduction in tibia lead content over 17 years which compares well with our estimate of 33 ± 12% reduction for current 57 year olds. Using the Boston data and assuming (unreasonably) no input to bone from blood, this would indicate an extreme upper bound on half-life of 49 years from the Boston data.

Our data are also in line with previous work we published regarding Canadian smelter workers (Brito et al 2001). In the smelter worker cohort, we showed an age effect: half lives in the tibia in people under 40 years of age were 4–8 years and half-lives in the tibia in workers over the age of 40 were 10–24 years. We observed similar results here. In this study (see table 3) in our youngest age group aged 20–35 we calculated half-life upper and lower limits of 7–9 years while for our 50–65 age group we calculated upper and lower half-life bounds of 17–26 years. (Our age group 65 + shows a half-life of 13–18 years.) The half-lives are of the same order as found in smelter workers and the pattern of a variation in half-life with age is similar. Overall, we believe the observed reduction in tibia lead content is reasonable and is as would be predicted from bone turn-over rates and reductions in population blood lead values. Lead levels in the tibia have been reduced in individuals in southern Ontario.

### Childhood

To our knowledge, this is the first report of \(^{109}\text{Cd K XRF both tibia and calcaneus lead content in children under age 6}. One previous North American study using this technology measured a small number of young children, but did not publish or use the data in analyses because individual measurement uncertainties were high (Nie et al 2011). An alternative technology was used in children in the 1990s but measured a different volume of tissue, mostly bone surface, and so the data are not comparable (Wielopolski et al 1983, Rosen et al 1989). A recent study of children in China (Specht et al 2014) did publish tibia data in children down to age 1, but did not measure the calcaneus.

In this study we measured both tibia and calcaneus because they represent different types of bones, with different levels of blood perfusion and different turnover rates. The tibia is predominantly a compact, cortical bone while the calcaneus contains more spongy, trabecular bone. By studying two bones, which represent different bone types, with different turnover rates, and linking to blood and serum data, we were aiming to try and formulate a better understanding of the overall metabolism and biokinetics of lead in the body across the lifespan.

The surprising result from the studies presented here is that the ratio of lead to bone mineral measured in the calcaneus of young children is very high compared to that in children older than 6. Differences were significant, and were tested using group averages and by fitting different mathematical models to the data, but this is a result from a relatively small \((n = 16)\) group of children aged 1–5, that should be verified in further studies. Possible reasons for higher calcaneus Pb levels may be (a) higher bone turnover in early childhood, which assumes the lead was incorporated into bone more strongly in children under age 6 than in children over age 6 (our results suggested that the lead to bone mineral ratio measured by this system falls as children age), (b) the system is measuring not only the calcaneus bone but some mixture of bones and other tissues in the foot or (c) maternal lead status is the most important determinant of the body burden of lead in early life. Further research is required to differentiate among these alternative explanations.

Our data can be compared with earlier studies of children over the age of six. A comparison of tibia data assessed in children between the ages of 6 and 19 between 2009 and 2011 with the studies performed in Hamilton, Ontario in the early 1990s (Gamblin et al 1994, Roy et al 1997) found no statistically significant evidence at the 95% confidence level \((p = 0.07)\) that tibia lead content is less now in Ontario children and teenagers than two decades ago. However, that comparison was between two relatively small groups and the variance may be mask-
ing an underlying reduction. In order to investigate this, we looked at data from similar studies performed in Boston and Pittsburgh in the 1990s (Hoppin et al. 1997, Needleman et al. 2002). In Boston a total of 168 students at a suburban Boston high school had their tibia lead content assessed by $^{109}$Cd K XRF. The children ranged in age from 13.5 to 19 years of age. The average tibia lead content found in Boston Rosen et al. (1989) in the early 1990s was $4 \pm 0.3 \, \mu g \, Pb \, g^{-1}$ bone mineral (mean \pm SEM). When we compare the mean (\pm SEM) tibia content in an age matched group from the current study (13.5\ – \ 19.0 \ years), the mean tibia content of $1.1 \pm 1.1 \, \mu g \, Pb \, g^{-1}$ bone mineral is significantly lower than in Boston 15\ – \ 20 \ years ago ($p = 0.01$). The Pittsburgh data are drawn from a case-control study of lead and delinquency conducted in 1996 (Needleman et al. 2002). Our data are not significantly lower than the control group data of 150 teenagers from that study. However, figure 9 shows the data (for all children aged 6\ – \ 19) from Hamilton 1991\ – \ 1994, Pittsburgh 1996, Boston 1995 and the mean of these three studies compared to data from this study (Toronto) 2009\ – \ 2011. Boston is significantly different from Toronto 2009\ – \ 2011 and the average of Boston, Pittsburgh or Hamilton 1991\ – \ 1994 is significantly different ($p = 0.046$) than Toronto 2009\ – \ 2011. Nonetheless, this might suggest that tibia lead content may also have fallen in children and teenagers in the North East USA and Ontario Canada over a 15\ – \ 20 \ year period as blood lead levels have fallen.

**Conclusions**

Studies that incorporate bone lead measurements in people across the lifespan are feasible at sites distant from home laboratories even with present-day low levels of lead exposure.

Tibia and calcaneus lead levels were higher in older adults than in young adults and linear regressions of tibia lead content and calcaneus lead content versus age were significant. The slope of the relationship between tibia lead and age has fallen by a factor of 2 in the last two decades. This indicates that health measures taken to protect Canadians from exposure to lead have been extremely effective. It also indicates that exposures that previously might have been considered ‘usual’ (as assessed by $^{109}$Cd K XRF bone measurements) may need to be re-categorized to permit better identification of ‘high’ exposure individuals. Current ‘usual’ exposure can be defined as having a bone lead level within the 99% confidence interval of the slope of the regression of bone lead versus age presented in this work.

Bone lead levels in older individuals have fallen since 1993. It appears that the reduction in lead exposure in Canada has been so significant that lead is now being released from the tibia through bone remodeling faster than new lead is being incorporated into the bone mineral matrix. Our data support estimates determined in smelter workers that there may be some age related variation in the half-life of lead in the tibia: this requires further research.

Tibia lead levels were found to be low in children. The difference between children aged 6\ – \ 19 years of age measured between 1991 and 1994 and this study (2009\ – \ 2011) was suggestive but not significant at the 95% confidence level. However, using data from other studies in the northeastern USA, there is some evidence that childhood tibia stores are now also lower than in the 1990s.

Calcaneus lead levels were found to increase in younger children compared to teenagers. It is not known whether this is an effect of metabolism differences, or arises from maternal exposure or is an artifact caused by
geometry of the bones in the infant foot. This requires further research before calcaneus measurements can be used in children under age 6.

The data in this study show that bone lead levels are low in the Canadian population and are significantly lower than two decades ago. As a consequence ‘usual’ or average lead body burden needs to be redefined to 50% of the level used for the last two decades. This reduction is the outcome of the very effective implementation of regulations and policies to reduce lead exposure in Canada.

Acknowledgments

This work was funded by Chemicals Management Plan at Health Canada. We wish to thank all of the staff whose skills and hard work contributed to the success of the study and the participants who took the time to contribute to this study.

Declaration of interests

The authors declare that they have no competing interests.

Authors contributions

FM: lead author on the paper and participated in the design and conduct of study.
MF: participated in the writing of the paper, design and coordination of the study.
DC: participated in the design, conduct and reviewing of manuscript.
MI: participated in study design, conducted the study training of blood collection materials and writing of manuscript.
NH: participated in the study design, reviewing of manuscript.
CW: was involved in the study design.
RB: was the study doctor and participated in the participant follow-up, and reviewing of manuscript.
LM: was involved in the design of the study and reviewing of the manuscript.
TA: involved in the design, coordination and reviewing of the manuscript.

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