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In vivo measurement of bone manganese and association with manual dexterity: A pilot study

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ABSTRACT

We used neutron activation analysis (NAA) to measure hand bone manganese (BnMn) in 19 adult males. Median BnMn was 0.89 μg/g dry bone (interquartile range = 1.07). After adjustment for age and occupation, higher ln (BnMn) was significantly associated with lower manual dexterity based on the Purdue Pegboard assembly task: β = −1.77, standard error = 0.79, p = 0.04. Due to the small sample size, these results should be interpreted cautiously. BnMn appears to be a promising biomarker, and should be further studied.

1. Introduction

Manganese (Mn) is an essential element, but overexposure to Mn can result in neurotoxic manganism, a condition similar to but distinct from Parkinson's disease (Chen et al., 2016). Chronic occupational exposure to Mn has been associated with impairments in behavior, cognition, and motor function (Chen et al., 2016); however, these relationships are still not fully understood (Chen et al., 2016).

Biomarkers of exposure and effect are a key tools in environmental health research. Mn has been measured in a variety of matrices including blood, urine, nails, and hair (Zheng et al., 2011); however, these have been limited by issues with variability, short half-lives, and external contamination (Zheng et al., 2011). Thus, there is a need for development of a cumulative biomarker for Mn.

As approximately 40% of Mn in the body is stored in bone, bone has been suggested as a potential cumulative biomarker for Mn (ICRP, 1975). A recent toxicology study has determined that the average half-life of Mn in rat bone is 143 days, roughly equivalent to 8.6 years in humans (O'Neal et al., 2014), supporting the concept that BnMn may represent cumulative Mn exposure. Additionally, bone manganese (BnMn) concentrations were significantly correlated with the amount of Mn in brain tissue (O'Neal et al., 2014), suggesting BnMn also may be indicative of Mn dose to target tissues.

A method for measuring BnMn with neutron activation analysis (NAA) was first developed by a team from McMaster University, and later used to assess BnMn in humans (Aslam et al., 2008a, 2008b; Pejović-Milić et al., 2009). In Pejović-Milić et al. (2009) the authors report a significant correlation between BnMn and a cumulative exposure index, which is additional evidence that BnMn may reflect long-term exposure.

Recently, our team has developed a much more compact NAA system for noninvasive, *in vivo* measurement of BnMn (Liu et al., 2013, 2014, 2017). It is based on a compact deuterium-deuterium (DD) neutron generator system and is able to be transported to field sites, which makes it a potentially useful tool for epidemiologic studies. To test the feasibility of using this technology in human studies, we assessed BnMn exposure in male adult volunteers and determined whether BnMn is associated with self-reported exposure sources or fine motor dexterity.

2. Materials and methods

In 2015, twenty study participants were recruited using advertisements through a university-based online newsletter and directly contacting welders who had participated in another research study and indicated a willingness to be contacted about future research opportunities. Participation was limited to males to reduce potential confounding by sex. One participant was excluded due to external contamination of the BnMn signal from a wristwatch, thus 19 participants are included in analyses.

Abbreviations: BnMn, Bone manganese; Ca, Calcium; DD, Deuterium-deuterium; HPGe, High purity germanium; IQR, Interquartile range; IVNAA, *In vivo* neutron activation analysis; Mn, Manganese; NAA, Neutron activation analysis; SD, Standard deviation; SE, Standard error

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The NAA procedure results in a small dose of radiation to the participant: the whole body effective dose was calculated to be 0.017 mSv, roughly one-fifth of that obtained from a full standard chest x-ray (Liu et al., 2014). This exposure was reviewed by Purdue's Radiation Safety Committee and Biomedical Institutional Review Board and was determined to be not more than minimal risk to participants. Participants signed an informed consent document. This study was approved by Purdue University's Biomedical Institutional Review Board.

A self-administered questionnaire was used to collect demographics and activities which have been previously associated with manganese exposure, specifically: “Have you done any of the following in the past 10 years, as a job or as a hobby?” Activities included welding, soldering, smelting, and other work with metals (ATSDR, 2012; Chen et al., 2016). Occupation was used as a proxy for socioeconomic status, defined as “student/faculty” or “worker.”

Participants also completed the Purdue Pegboard test of manual dexterity, a test which has been used previously in research on manganese neurotoxicity (Cowan et al., 2009). The Pegboard test includes timed tests for the right hand, left hand, both hands, and an assembly task which also involved both hands. Each test is completed three times, and the average score is used in analysis. Right and left hand scores were summed to determine a combined score and self-reported handedness was used to classify trials as using dominant vs. non-dominant hands. Lower scores indicate poorer performance, i.e., fewer pegs placed within the time limit. These scores were normally distributed.

In vivo BnMn in the participant's right hand was measured using a deuterium-deuterium neutron generator based NAA system (Liu et al., 2013, 2014). The general premise of NAA is that stable isotopes of elements are converted into unstable isotopes by neutron capture, and as these unstable isotopes decay, the characteristic γ-rays emitting from the target can be collected to quantify elemental concentrations. After cleaning their hand with 50% alcohol to remove Mn contamination and washing their hands with soap and water, participants sit with their hand in an irradiation cave and their hand is irradiated for 10 min. The arm was held in place using a water-filled sleeve, which also served to reduce the radiation dose. Following irradiation, roughly 3 min was spent transferring the participant from the irradiation cave to a seated position in front of the γ-ray detection system. The placement of the detection system far from the generator, along with shielding the detection system, also reduced the amount of background radiation. The participant then places their hand in a γ-ray detection system consisting of one high purity germanium (HPGe) detector with relative 100% efficiency for one hour. A spectrum is collected and ^{56}Mn signal with relatively long half-life of 2.58 h is determined from the count of 847 keV γ-rays, which are characteristic of ^{56}Mn (Liu et al., 2013). BnMn concentration is then calculated from Mn γ-ray signal and a calibration line generated from a set of Mn-doped bone equivalent phantoms. To correct for the variation of neutron flux, hand palm attenuation, and counting geometry, Mn/Ca ratio, as opposed to Mn net signal, is used for system calibration. The limit of detection for this method was 0.34 μg/g dry bone (Liu et al., 2017). To convert units of μg/g dry bone to μg/g Ca, multiply by 3.94; this incorporates the Ca concentration in dry bone of a standard reference man.

Similar to bone lead measurements using *k*-x-ray fluorescence, the NAA method provides a continuous estimate varying around the true bone manganese value; thus, negative estimates are occasionally derived. The recommendation for analysis of bone lead is to retain negative values to minimize bias from left censoring (Kim et al., 1995). Therefore, we retained estimates below the limit of detection (n = 5), including negative BnMn values (n = 4). BnMn data was relatively normally distributed in this sample based on examination of density plots (Fig. 1) and quantile-normal plots. However, a log transformation is also viable, and as it is common to transform manganese biomarker data, and transformation would reduce the influence of extreme observations, we present medians and interquartile ranges (IQR) in addition to means and standard deviations for BnMn data and use a natural log transformation of BnMn in regression analyses. As one cannot take the natural log of negative values, a constant of 0.75 was added to all observations prior to transformation. This affects the absolute value of BnMn measurements, but not the results from a regression analysis.

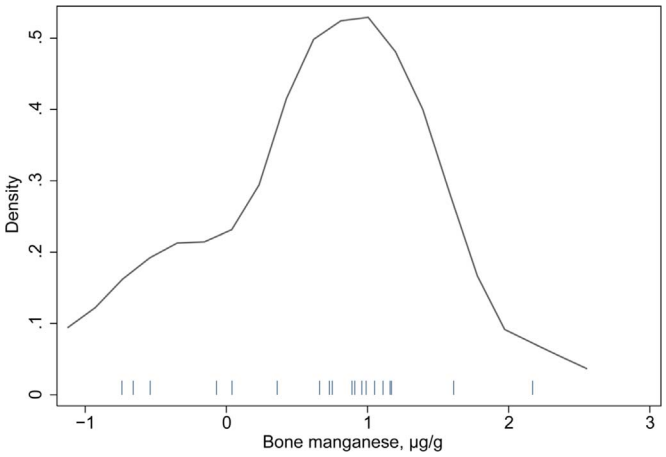

Fig. 1. Kernel density plot of bone manganese concentrations, n = 19.

Statistical analyses were completed using Stata 14.0 (College Station, TX). A p-value < 0.05 was considered statistically significant. Mann-Whitney and Student's t-tests were used to assess the association between demographics and self-reported activities with BnMn and Purdue Pegboard test scores. Linear regressions were used to compare BnMn with Purdue Pegboard scores, adjusting for age and occupational status.

3. Results

Average age was 37.2 years (standard error (SD): 16.1) and ranged from 18 to 62. More participants were students or faculty (n = 12, 60%) compared to workers (n = 7, 40%). Workers consisted of individuals who worked at a trailer manufacturer, welding or metal fabrication laboratories; one had an administrative position.

Median BnMn was 0.89 μg/g dry bone with an interquartile range of 1.07 μg/g (Table 1). BnMn ranged from −0.74 to 2.17 μg/g and the mean was 0.66 μg/g. Higher median BnMn was observed among those who were less than 40 years old (vs. ≥ 40), students/faculty versus workers, and those who self-reported welding, working with metals, or both. However, none of these differences were statistically significant. The same pattern was observed with mean BnMn.

Mean (SD) pegboard test scores were 14.3 (1.96) for dominant hand; 14.1 (1.87) for non-dominant hand; 39.6 (5.26) for combined dominant and non-dominant hand scores; 11.2 (1.69) for both hands; and 28.2 (5.51) for the assembly task. Values for the assembly score compared with demographics and hobbies are shown in Table 1; results for other test scores were similar.

Table 2 presents linear regression results. In unadjusted models, higher BnMn was related to worse performance in all Pegboard tests, but this was not statistically significant. In models adjusted for age and occupation, higher BnMn was significantly associated with worse performance on all Pegboard tests except for the non-dominant hand. When models were run using untransformed BnMn, there were no statistically significant associations of BnMn with Pegboard outcomes (data not shown).

4. Discussion

In this small pilot study, we used NAA to measure *in vivo* BnMn among 19 adult males. Our results demonstrated somewhat higher BnMn among students/faculty versus workers as well as individuals