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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 BnMn was significantly associated with lower manual dexterity based on the Purdue Pegboard assembly task: $\beta=-1.77$, 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.

Keywords

manganese; *in vivo* neutron activation analysis (IVNAA); biomarkers; motor skills; environmental exposure

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

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Review Board Approval
This study was approved by Purdue's Biomedical Institutional Review Board; IVNAA protocols were also reviewed by Purdue's Radiation Safety Committee.

Conflict of Interest
The authors declare that they have no competing financial interests.

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., the authors report a significant correlation between BnMn and a cumulative exposure index, which is additional evidence that BnMn may reflect long-term exposure (Pejović-Milić et al. 2009).

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.

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 minutes. The arm was held in place using a water-filled sleeve, which also served to reduce the radiation dose. Following irradiation, roughly 3 minutes 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 hours 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 (Figure 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) 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.

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 (SE): 3.69) and ranged from 18–62. 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.

Mean (SE) pegboard test scores were 14.3 (0.45) for dominant hand; 14.1 (0.43) for non-dominant hand; 39.6 (1.21) for combined dominant and non-dominant hand scores; 11.2 (0.39) for both hands; and 28.2 (1.27) 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.

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 who reported welding or working with metals versus not doing these activities; however, none of these were statistically significant. Higher BnMn was significantly associated with lower performance on most skills of fine motor dexterity after controlling for age and occupational status.

The research team from McMaster University has completed several studies which assess BnMn concentrations. They estimated a mean of 0.63 µg/g Ca (standard deviation (SD) = 0.30) among non-exposed individuals from cadaver study (Aslam et al. 2008a), and 0.12 µg/g Ca (SD = 0.68) from a pilot study of eight healthy males, also without occupational exposure (Aslam et al. 2008b). An additional study reported mean BnMn among 30 welders (2.9 µg/g Ca, SD=0.4) and 10 controls (0.1 µg/g Ca, SD=0.7) (Pejović-Milić et al. 2009). After converting our results to the same units, mean BnMn in this study is 2.6 µg/g Ca (SD = 2.99); this is higher than estimates among non-exposed individuals (Aslam et al. 2008b,

2008a; Pejović-Milić et al. 2009) but similar to estimates from estimates among welders (Pejović-Milić et al. 2009). Thus, our results are within the range of the prior reports of BnMn.

However, whereas Pejović-Milić et al. documented a significant difference between BnMn of welders and controls (Pejović-Milić et al. 2009), we did not see a significant difference between those who reported welding and those who did not. This difference could be explained by the fact that we requested information about either hobbies involving welding as well as jobs. While we attempted to recruit career welders, it is likely that some of those reporting welding only welded intermittently. Additionally, one respondent reported smelting, but not welding. Thus, our inability to detect a significant difference between welders and non-welders could also reflect that some non-welders may have had manganese exposure from other sources.

Our results demonstrating that BnMn is associated with lower performance on Purdue Pegboard trials is consistent with prior literature (Cowan et al. 2009; Ellingsen et al. 2014; Bowler et al. 2016). However, we cannot rule out the possibility that the association of BnMn with Pegboard scores may, in part, be due to our use of a convenience sample or uncontrolled confounding, as described more below.

There are limitations to this study. As this was a pilot study, we were limited to a small convenience sample and did not have sufficient resources to assess additional Mn biomarkers. Many comparisons are reliant on self-reported data without an indicator of the length (years) of exposure. We were also not able to control for multiple potential confounders, such as educational status. The small number and convenience sampling limits the external validity of these results, i.e., the ability to which these results can be extrapolated to the general population.

However, this analysis does have several strengths. Our team has successfully developed a transportable method for *in vivo*, noninvasive measurement of BnMn concentrations with sufficient detection limits to quantify BnMn in both environmental and occupationally-exposed individuals. Comparisons of BnMn with self-reported work or hobbies, although not statistically significant, are consistent with what would be expected from a Mn biomarker. Taking into account the associations of BnMn with motor function BnMn may be useful as a biomarker for Mn exposure or effect. Additionally, given the long half-life of Mn in bone, and Pejović-Milić et al.'s finding that BnMn was correlated with a cumulative exposure index (Pejović-Milić et al. 2009), BnMn should be investigated as a potential biomarker of cumulative exposure. To ensure that this NAA method to measure BnMn is robust, future work should include a larger sample of individuals with a wider range of Mn exposure and compare multiple biomarkers and a more robust exposure history with bone Mn measurements.

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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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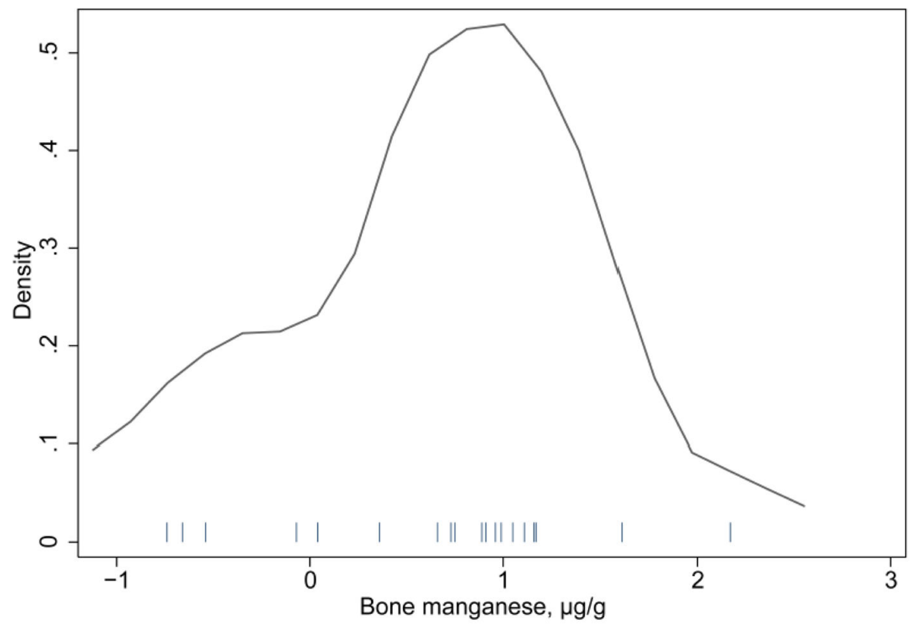

Figure 1.
Kernel density plot of bone manganese concentrations, n=19.

Table 1

Average bone manganese concentrations and pegboard assembly scores by population characteristics

Variable	Category	N	Bone manganese, μg/g		Assembly test	
			Median (IQR)	p^a	Mean (SE)	p^b
All		19	0.89 (1.07)		28.2 (1.27)	
Age	< 40 years	12	0.94 (0.75)	0.311	28.5 (1.35)	0.773
	≥ 40 years	7	0.66 (1.03)		27.7 (2.69)	
Occupation	Worker	7	0.66 (1.06)	0.311	25.1 (1.78)	0.053
	Student/faculty	12	0.93 (0.76)		30.1 (1.51)	
Welded	No	12	0.82 (1.16)	0.866	30.1 (1.50)	0.046
	Yes	7	0.91 (0.69)		25.0 (1.78)	
Worked with metal	No	13	0.75 (1.12)	0.380	29.3 (1.28)	0.235
	Yes	6	0.94 (0.50)		26.0 (2.87)	
Welded or worked with metal	No	10	0.74 (1.18)	0.514	29.8 (1.36)	0.204
	Yes	9	0.96 (0.39)		26.5 (2.14)	

IQR = interquartile range; SE = standard error

[a]Wilcoxon rank-sum test

[b]Student's *t*-test

Table 2

Regression results for the association of Purdue Pegboard scores with bone manganese

Pegboard test	Unadjusted model		Adjusted model[a]	
	β (SE)	*p*	β (SE)	*p*
Dominant	−0.43 (0.33)	0.204	−0.59 (0.27)	0.047
Non-dominant	−0.36 (0.32)	0.273	−0.49 (0.29)	0.105
Combined	−1.25 (0.87)	0.169	−1.67 (0.74)	0.039
Both hands	−0.47 (0.27)	0.104	−0.59 (0.23)	0.022
Assembly	−1.44 (0.90)	0.127	−1.77 (0.79)	0.040

Values are coefficients for natural-log transformed bone manganese compared to Purdue Pegboard score. SE = standard error

[a]Adjusted for age (continuous) and occupational status (student/faculty vs. worker).