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Relation between Dietary Manganese Intake and Biological Markers of Manganese Exposure

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Relation between Dietary Manganese Intake and Biological Markers of Manganese Exposure

Abstract

Manganese is an essential trace element, yet can be toxic in excess. The inter- and intra-individual variations in manganese levels in different biological media make research difficult. This study examined the relationship between dietary Mn intake and blood Mn. ***Methods:*** Subjects were recruited through the Cincinnati Lead study cohort. The Youth/Adolescent Questionnaire was used to evaluate the dietary intake of manganese. ***Results:*** The mean manganese dietary intake was 3.25 mg±2.06 (SD). The mean blood manganese concentration was 0.8mg/dl±0.36 (SD). The Spearman rank correlation coefficient between dietary manganese intake and blood manganese was not significant (*P* value= 0.99). ***Conclusions***: We found no correlation between dietary manganese intake and blood manganese. This is due to our small sample size and the use of the YAQ that has not been designed specifically to measure manganese. More studies are needed to better understand the effect of dietary manganese intake on blood manganese.

Relation between Dietary Manganese Intake and Biological Markers of Manganese Exposure

Introduction

Manganese (Mn) is an essential trace element for normal health and functions of different systems such as the skeletal, immune, nervous and reproductive systems. It is found in blood in the form of metalloproteins and is part of many enzymatic systems such as pyruvate carboxylase and superoxide dismutase (MnSOD), an anti-oxidant enzyme of mitochondria in lymphocytes.

Manganese is essential for different functions of various systems in the body. It is important for normal bone development where 25-40% of the total body burden of Mn is found[1]. It plays important roles in the immune and reproductive systems. Manganese is involved in vitamin K dependent blood clotting mechanism, defense against free radicals, glucose tolerance and energy production through metabolism of carbohydrates, amino acids and lipids.

A major source of manganese is diet. Manganese is found in cereals, fish, meats, vegetables, nuts, spices and beans as well as beverages such as tea, coffee and wine.[2]

The recommended dietary allowance (RDA) of manganese is difficult to establish but the US National Research Council has estimated a safe and adequate dietary Mn intake (ESADDI) of 2-5mg/day for adults.[3]

The adequate Mn intake (AI) is 2.3 mg/day for men and 1.8 mg/day for women with a maximum limit of 11 mg/day as reported by the Food and Drug Administration (FDA) and the National Academy of Sciences (NAS).[4] The adequate intake of Mn depends on age. (Table 1)Infants in the first year have the highest manganese AI compared to children and adults. Pregnancy and lactation increase the AI manganese requirement.[5]

The individual's dietary intake of Mn depends on the type of food consumed, water Mn concentration and supplement use. The average dietary Mn intake is 3-9 mg/day which makes dietary overdose extremely rare[6]. Mn concentration in supplements ranges from 0.6-8.1 mg/day[4] and in some supplements as high as 5-20 mg according to the National Academy of Sciences in 2001.

There is substantial intra-individual as well as inter-individual variation in retention and excretion of manganese as well as large variation in inter-laboratory manganese measures from the same biological material.[6]There is variation in the concentration of manganese depending on the biological material used for measurement from the same individual and even between individuals.

Much debate concerning the normal range of manganese in the body has been published in the literature due to wide variation in the concentration of this element in our diet, water supply and environment. Many biological materials are available to measure manganese levels in the body. None of them has been validated as the best biomarker of manganese.

It is important to identify a valid, reliable and sensitive biomarker of manganese exposure in order to easily diagnose Mn toxicity or deficiency before the clinical signs and symptoms become apparent. Development of this biomarker relies upon standardized methods of Mn collection and analysis in order to interpret and understand the results.

Background

A. Manganese Exposure Source

We are exposed to manganese from different sources. Mn is naturally found in rocks, water and plants. Primary exposure to manganese occurs through diet, drinking water and in

supplements. Another source of manganese exposure is inhalation of manganese dust particles either by environmental (within normal concentration of manganese in air) or occupational exposure from certain industries. Mn is used in many industries that manufacture products such as batteries, ceramics and pigments and is released into the air as small dust particles[1]. Interest in Mn has developed recently due to the health hazards associated with Mn aerosol inhalation from the combustion of methylcyclopentadienyl manganese tricarbonyl (MMT), an anti-knock agent found in unleaded gasoline.[7]

Non-traditional exposure pathways include infant formula and parenteral nutrition[8]. In medical practices, patients are exposed to manganese either through its use as a contrast material or as a constituent of parenteral nutritional solutions.[9]

Total Parenteral Nutrition solutions (TPN), commonly given to neonates, contain 25 µg of Mn/ ml which is retained at a 100 % rate. This delivers approximately 100 times more Mn to these neonates compared to neonates fed human milk. Taking in consideration that neonates retain this high dose, pass little or no stool and often have signs of liver dysfunction and cholestasis, there is a huge risk of Mn toxicity in neonates on TPN.[10]

Average air manganese levels are approximately 0.02µg/m³.[11] In Cincinnati, the mean air manganese concentration is 0.004µg/m³. The lowest observable adverse effect level (LOAEL) of manganese in water is estimated by the Environmental Protection Agency (EPA) to be 4.2 mg/day.[3] In the US, water manganese levels typically are <0.05ppm. [12]

B. Manganese Health Effects

There have been no reports of adverse health effects associated with over-exposure to Mn in the diet. However, there have been a few reports of over-exposure to Mn in drinking water.

Kondakis et al has reported an increase in manganese hair level that is correlated with high manganese levels in drinking water. This was confounded by older age.[13] In children, exposure to high levels of manganese in drinking water resulted in lower scores on tests of short term memory, visuo-perceptual coordination and manual dexterity. [14]

Although rare, dietary Mn deficiency can result in adverse effects. In animals, manganese deficiency was associated with growth retardation, impaired reproduction in females and testicular degeneration in males as well as skeletal abnormalities, ataxia and imbalance in carbohydrate and lipid metabolism.[15]

Human studies have included a case study where an individual developed scaly dermatitis, decreased serum cholesterol, decreased levels of clotting proteins and slowed growth of hair and nails[16]. Imbalance of tissue concentration of Mn is reportedly associated with several disorders such as acromegaly, epilepsy, amyotrophic lateral sclerosis and catabolic disease.[17]

Exposure to high levels of manganese is neurotoxic and occurs mainly through inhalation rather than dietary intake. Environmental exposure to high levels of manganese is mainly observed in workers in mines and welders. It results in a Parkinson's-like neuromuscular disorder known as Manganism.[18]Most cases of Manganism are due to occupational exposures to high levels of manganese but some cases have been reported in liver cirrhosis patients and those receiving parenteral nutrition[19, 20]. Under such conditions, manganese is deposited in the brain in areas that notably have high concentrations of non-heme iron such as globus pallidus, caudate-putamen, substantia nigra and subthalamic nuclei.[21] The severity of manganism has been reported to be positively correlated to the duration of manganese exposure and the total cumulative manganese exposure.[21]

Magnetic Resonance Imaging (MRI) has been reported in many studies to be sensitive in detecting manganese deposits in the brain. In rats, manganese significantly alters T1-weighted MRI signal intensity (T1 relaxation time within the globus pallidus), unlike other elements, such as iron and copper. [22] Human studies have also demonstrated significant correlation between manganese blood concentration and MRI signal intensity ($r = 0.77$).[23]

Manganism presents with psychiatric and extra-pyramidal motor dysfunction symptoms such as reduced response speed, irritability, intellectual deficits, mood changes, compulsive behaviors, slurred speech, excessive salivation, sweating and disturbance of balance. Manganism is related to degeneration of GABAminergic neurons within the globus pallidus.[1, 24] Manganism symptoms stabilize but rarely disappear after cessation of manganese exposure. [21]The Occupational Safety and Health Administration (OSHA) has set the permissible occupational atmospheric Mn exposure limit to 5 mg/mm³.

In both case control and longitudinal studies, manganese exposure has been found to be an independent factor to the risk of Parkinson disease.[25] High dietary intake of iron, especially in combination with high dietary manganese intake, resulted in almost doubling the risk of Parkinson disease (odds ratio=1.9, 95% CI 1.2-2.9)[26]. Levo-Dopa is the current standard in the treatment of Parkinson disease. In a double blinded randomized clinical trial, Levo Dopa therapy has been reported to be ineffective in the management of manganese induced Parkinsonism. Thus, Levo Dopa can be used to clinically distinguish manganism cases from Parkinson disease.[27]

There is limited and debatable evidence concerning the carcinogenesis of manganese exposure in both animals. The Environmental Protection Agency (EPA) has classified

manganese as a Group D substance (i.e. "not classified" with regards to human carcinogenicity).[28]

C. Manganese Transportation

Manganese is transported in blood by albumin and macroglobulins, predominantly when tightly bound to transferrin.[7, 29, 30] On the cellular level, Mn transport involves Ca+2 channels especially across the blood-brain barrier. [1]

Normal tissue levels of Mn following dietary intake is regulated by intestinal absorption and hepatic biliary elimination[31] where only 1- 5% of the total dietary Mn intake ends up in the blood (mean ± standard deviation=5.9% ±4.8%) [3, 30]. Therefore, manganese toxicity occurs when manganese is administered directly into the blood, i.e. parenterally or in subjects with hepatic or biliary dysfunctions.[4]It has also been reported that there is latent manganese toxicity associated with congenital biliary atresia correction surgery.[32]

D. Normal Metabolism of Manganese

Manganese balance and retention in the body is affected by several factors such as dietary content of carbohydrates, animal proteins, manganese as well as other minerals (iron, calcium and phosphorus) and phytate (wheat bran) dietary content [3, 33]. Moreover, the variation in dietary Mn intake affects manganese metabolism.

Manganese absorption is affected by body weight when it is estimated in women and men separately, but was found to be not significantly related to weight when the data from both sexes were pooled together .[34]There is also enhanced manganese absorption with alcohol consumption.[35]

It is reported that there is a significant decrease in Mn blood level and a significant increase in urinary and hair Mn levels among other minerals in diabetic patients compared to healthy individuals.[36]

From animal studies, it was suggested that the rate of reduction/ oxidation reactions involving manganese is an important factor in manganese retention and its clearance from the body. It was shown that the oxidized state of manganese Mn (III) bound to transferrin has a slower rate of clearance compared to Mn (II) bound to macroglobulins. [37]

The nutritional status of manganese is affected by its bioavailability in the diet for absorption. Consumption of a vegetarian diet such as leafy green vegetables and whole grains does not affect manganese status since these types of food are also rich in non-heme iron. Other dietary factors also influence manganese nutritional status such as calcium, phosphorous and ascorbic acid content. [29]

In animal and in vitro human studies[38, 39], it was found that the placenta has low permeability to manganese transportation to the developing fetus. Despite this finding, high environmental manganese exposure was found to be teratogenic and resulted in low birth weight.[40]

Manganese and Iron

Manganese has a similar absorption mechanism as does iron (Fe) which is the most important variable for Mn uptake in healthy adults.[4] Divalent metal transporter-1 (DMT-1) mediates the relationship of Mn and iron in the gastrointestinal tract. In humans and animals, Mn absorption is extremely low (<10 %) and is similar to non-heme iron absorption.[30] Intestinal Mn absorption is significantly increased in the presence of anemia induced by low dietary iron intake but not due to bleeding.[33] There is a significant negative association between Mn absorption and

ferritin concentration in whole blood. It is reported that women absorb a higher percentage of Mn than men due to lower Fe stores in their bodies. [34]

The interaction between manganese and iron is dependent on the form of iron in diet. It has been reported that dietary non-heme iron has a negative effect on manganese absorption in the intestines, while dietary heme iron did not affect the nutritional status of manganese. [29]It has also been reported that iron supplementation (60mg/day for 4 months) resulted in higher fecal manganese excretion, decreased blood manganese levels and decreased MnSOD activity in lymphocytes due to lower Mn retention. [29]

In addition, one of the Mn metalloenzymes, arginase, has a decreased activity in the presence of high ferritin in the blood due to the lower absorption of Mn under this condition.[33]These results have been shown to be consistent in both animal and human studies. Diet high in saturated fat tends to increase Mn absorption by the same mechanism that it increases iron absorption.

Manganese and Calcium

There is a competitive interaction between Mn and calcium (Ca) in which dietary Ca supplements greatly reduce the bioavailability of Mn by reducing intestinal absorption.[41] A possible relation between Mn deficiency and osteoporosis has been suggested in several studies.[42]

Manganese and Other Supplements

Wedekind et al has reported a significant absolute decrease in the absorption of manganese in the presence of excess inorganic phosphorus supplement in chickens. [43]

Other minerals such as copper (Cu) did not significantly affect Mn plasma levels in humans, although dietary copper induced lower Mn absorption and higher Mn turnover in rats. [44]

Zinc (Zn) is reported to increase Mn plasma levels while Cadmium inhibits Mn intestinal uptake in rats. [45]Ascorbic acid supplement greatly enhances iron absorption from diet. Thus, dietary manganese absorption is reduced under this condition. [46]

Phytate (inositol hexaphosphate), is found in soy formula and infant cereals. It forms insoluble complexes with manganese and other minerals which then pass through the gastrointestinal tract unabsorbed. Thus, phytate in soy formula inhibits manganese absorption.[8]

E. Potential Biomarkers of Manganese Exposure

The best biomarker of exposure is the measurement of the substance itself or its specific metabolites in body media. However, this can be confounded by several factors, such as exposure to the same substance from different sources, finding a specific metabolite that is exclusive to that substance and the bioavailability of the substance at the time of sampling depending on its half life, duration and route of exposure. Different biological materials can be used to measure Mn such as plasma, serum, whole blood, hair, urine and spinal fluid. [47, 48]

Bile

Ninety percent of the absorbed Mn is excreted in the bile. [10] Biliary excretion of Mn may be considered a potential biomarker of manganese levels but is difficult to measure since excretion is rapid, especially with large oral doses of Mn. Biliary excretion does not reflect on the body stores of Mn. [3, 6] It is reported that concentration of biliary manganese is correlated with and is equivalent to the manganese concentration excreted in the urine. [49]

Although, Mn balance in men and women did not differ in terms of excretion of Mn in the feces following an acute high oral dose of manganese[34], women have a lower Mn half life and absorb significantly more Mn than men in general.

Davidsson and colleges demonstrated in a human study using a single oral dose of radio-labeled manganese that the rate of fecal excretion of manganese as a marker of manganese absorption and retention in the body is not reliable. This may be explained by low Mn absorption following a high acute oral dose of manganese and the possibility of constipation. Also, fifty percent of the retained Mn is excreted after 10 days. They also reported that organs other than the liver are involved in the excretion of manganese after systemic Mn administration.[6]

Since inhaled manganese goes directly to the blood from the lungs, it bypasses the normal metabolism pathway of the liver and intestines; it gets deposited in the brain in the globus pallidus as demonstrated by Magnetic Resonance Imaging (MRI). Thus, biliary manganese level is not a good biomarker of occupational Mn exposure.[50]

Tissues

Tissues with high melanin concentration, such as dark skin and the retina, as well as tissues with high energy demand, such as the brain, have high Mn concentrations. Also, the liver, kidneys, bone and pancreas tend to have high Mn concentration. [51]

Tissue manganese concentration is the best biomarker of Mn but measurement of tissue concentrations requires biopsies (such as liver biopsy) which are not readily accessible. Acute high manganese exposure may result in a temporary elevation in manganese tissue concentration that returns to normal once exposure is stopped. However, the availability of tissue for manganese measurement is not readily accessible since biopsies are invasive, expensive and sometimes impractical, such as in the case of brain biopsy.

The reported "normal" range of Mn concentration in tissue is 0.3-2.9 µg/g wet tissues weight. [51]In human fetal tissues, manganese concentration ranged from 0.35- 9.3 µg Mn/g dry weight.[52]

Dorman et al demonstrated an age related higher manganese concentration in testes and lower Mn in pancreas of male rats that are independent of inhaled manganese and reflect dietary manganese intake. [53]

Enzymes

Lymphocyte manganese superoxide dismutase (MnSOD) activity has been studied as a potential biomarker of dietary manganese intake and was found to be correlated with Mn blood concentration.

In a longitudinal study of 47 women, both serum manganese and lymphocyte MnSOD activity were sensitive to moderate dietary manganese supplementation and reflected manganese exposure. [54] There was a delayed but significant increase in MnSOD activity as well as serum manganese concentration after 89 days of supplementation with Mn. This increased activity was more apparent in subjects who consumed >31% of their energy requirement from polyunsaturated fats, which seems to make manganese more bioavailable for absorption. In this same study, the urinary excretion of manganese was not affected by manganese and/ or iron supplementation. [54]

In an animal study, arginase, an enzyme activated by manganese, has reportedly decreased activity in liver and kidneys in association with consumption of a manganese deficient diet.[55]

Hair

Manganese has an affinity for melanin and that is why it is found in human hair. Hair Mn level can be used to measure long term exposure to Mn but is very sensitive to collection methods, which may vary in terms of external contamination. Heavy metals occur in hair in higher concentrations than in blood or urine which makes this medium more accurate and

sensitive. Hair manganese is influenced by hair color, age of the hair and the use of hair dyes. Hair Mn is usually higher in females than in males, but hair Mn levels are independent of the age of a person.

High levels of hair manganese were reported in prisoners that were more violent than those with normal hair Mn levels. [4]It is reported that manganese down-regulates dopamine in the brain and leads to anti-social and aggressive behavior, violent crimes and a tendency toward drug abuse and/or depression.

Normal values of Mn in the hair of non-exposed individuals ranges from 200-4400 μg per Kg according to the ATSDR report on hair trace element analysis.[56]

Hair as a medium for measuring manganese represents a very reliable and valid method, but it has certain advantages and disadvantages that should be taken in consideration. Hair is an easy and non-invasive medium to obtain and it is easy to store. It reflects long term exposures to trace elements. On the other hand, hair is very sensitive to collection and preparation methods that should be standardized to maintain the accuracy, reliability and reproducibility of the analysis. The protocols for hair collection recommend using clean hair that has not been bleached, dyed or permed for 3 months, as well as using the newest hair growth in the sample. It is not recommended to wash the hair sample with shampoo, especially those containing selenium sulfide or lead acetate, which would then contaminate the sample and distort the analysis. [57]

In addition, hair color is a confounding factor in the analysis of the manganese levels in hair since it has a strong affinity to melanin and is thus found in higher concentration in African and Hispanic peoples than in light color hair within the same exposure levels. Therefore, hair manganese can be used as a biomarker of manganese exposure but it does not necessarily reflect the absorbed dose and has limited benefit extending beyond a few months due to hair growth and

loss cycles. In addition, there is confounding by external contamination, hair color and the presence of other heavy metals deposits on the hair.

Urine

Manganese can be measured in urine despite low urinary excretion and a short half- life. It can be used to discriminate between an environmentally exposed population and a non-exposed population but has limited value with regards to the exposure-response relationship on the individual level.

Urinary excretion of Mn is reportedly a doubtful biomarker of dietary Mn intake, as it fluctuates with the amount of Mn intake in the diet. In the general population, urinary Mn is < 3 µg/L.[7]The urinary manganese half-life is reported to be less than 30 hours.[58]

A Chinese study tested the significance of urinary manganese as a valid biomarker of manganese exposure in welders. In this study, the investigators compared welders given ethylene-diamine-tetra-acetic acid (EDTA) chelation treatment to control welders, both exposed to the same levels of occupational manganese. They reported that the use of EDTA chelation treatment in exposed welders resulted in a two fold increase in the urinary manganese excretion compared to their control counterparts. Thus, the authors concluded that EDTA can be used as a challenge test to define recent exposure to manganese.[9]

Urinary manganese excretion level is the best biomarker indicating recent exposures, especially on a group but not on an individual basis due to the huge inter-individual variability within the normal range.

Oral Tissues

Manganese is found in the oral cavity as a constituent of teeth, saliva and calculus deposits on the teeth.

Manganese has been detected in the saliva of welders in amounts that are significantly correlated to inhalation Mn levels in an exposure-dose relationship. Saliva samples can be used as a non-invasive approach to measure Mn, although salivary Mn levels have not been validated as a reliable measure of dietary Mn. However, a significant correlation between salivary Mn level and blood Mn level has been reported ($r = 0.57$, $p < 0.05$). [59]

It has been found that there are significantly higher manganese concentrations in supra-gingival calculus compared to the sub- gingival calculus deposited around the teeth. [60]

Manganese, a heavy metal that fights against caries has not been studied in teeth either in children or adults as a marker of exposure.[61] In a study of children between the age of 4 and 11 years, it has been reported that there is a lower concentration of manganese in the roots of primary (deciduous) teeth than in permanent (adult) teeth within normal values. In carious teeth, the concentration of manganese was found to be higher in permanent teeth compared to primary teeth. [61]

In the case of primary teeth, mineral deposition starts in the fourth month in- utero and continues until they are replaced by permanent teeth. Thus, the primary teeth can be useful to evaluate the long term exposures to heavy metals such as lead, zinc and copper. Permanent teeth can be used as a medium for measuring heavy metal exposures when they are lost due to caries, periodontal disease or during orthodontic treatment.[61]

It has been reported that the concentration of manganese among other trace elements such as iron, calcium and lead in teeth is dependent on the type of the tooth (incisors, canines and molars) as well as the health status of that tooth (carious versus sound). [62]

Teeth are very reliable media to evaluate long term exposures and are fairly easy to obtain in the case of primary teeth. However, it is ethically unacceptable to measure any trace element in permanent sound teeth since this would involve extraction and loss of the tooth.

The enamel of the first deciduous molar provides long term manganese exposure at the 20th gestational week, while the root tip provides manganese exposure at 7 months postnatal[63]. Using this method, Ericson et al reported a significant correlation between high enamel manganese content and behavioral dysfunctions in children. This is explained by DAergic neuronal damage and number reduction as a result of increased manganese exposure during formation of the neurons at the 20th gestational week. [64]

In a case control study, irreversible pulpitis (inflammation of the dental nerve tissue in the pulp) has been associated with significant increase in the transcription of MnSOD and other superoxide dismutase enzymes.[65]

Blood

Manganese concentration in whole blood is higher than in serum or plasma (Table2). Milne and colleges measured manganese concentration in different cellular components of blood. They reported manganese content in whole blood was primarily due to the high concentration of manganese in erythrocytes (66%), while platelets and leukocytes can contribute 30% of the manganese content in whole blood. Although RBC's have higher manganese content, they are not very good indicators of the status of manganese in the body due to their longer half-life compared to platelets and leukocytes.[66]

The mean biological half-life of Mn in blood is 10.5±0.6 days and 37±7 days after oral ingestion and parenteral administration of Mn, respectively. [30]

A study of children on long term TPN attempted to correlate whole blood Mn level with the amount of Mn deposited in the globus pallidus of the brain, as measured by T1-weighted MRI. This study found that the correlation was not significant and whole blood Mn level does not reflect on the amount of Mn deposited in the brain. [67] Interestingly, whole blood Mn level was significantly correlated to Mn deposits in the brain of adult patients with liver disease who were environmentally exposed to Mn as measured by the signal intensity of T1-weighted MRI. This indicates lower manganese excretion in bile.[68]

There is a well documented although not clearly explained correlation between low blood manganese and presence of convulsions in epileptic patients [69] as well as low blood manganese level in diabetic patients. [70]

It is estimated that Mn blood levels range between 6 and 9 μg/L in the general population. [7] ATSDR reports a 4-14 μg/L range for Mn levels in blood.[56]

In an animal study, whole blood manganese concentrations remained unchanged when manganese deficient rats were given manganese balanced diets and blood sampled 24 hours later. This indicates that whole blood manganese is not a good marker of recent dietary manganese history but is reflective of the manganese tissue levels.[71]

Some investigators have reported that the blood Mn level is not a good biomarker of Mn since Mn has a short half life and thus any Mn detected in blood will not reflect the Mn body stores or dietary intake. Both urinary and blood manganese levels reflect the amount of Mn absorbed during the sampling frame or shortly before. [48]

In conclusion, there is no specific biomarker that is sensitive to long chronic exposures to high doses of manganese.

Human Milk

Human milk contains manganese in very low concentration (4-8 μg/l) which provides the infant with 0.4-1.6 μg/kg/day. Human milk does not show significant fluctuation in manganese concentration throughout lactation. It is reported that maternal dietary intake of manganese is not correlated to the amount of manganese in human milk[8] Formula fed infants receive 10 – 50 times more manganese than breast fed infants. On the other hand, manganese absorption is higher from human milk than from soy formula. [8],[72]Hair manganese in 4 months old breast-fed infants was significantly lower than in formula-fed infants ($P < 0.01$).[8]

E. Potential Markers of Manganese Effects

A biomarker of manganese effects, as measured by alteration in system functions or disease status, can be used to detect over-exposure to manganese before the clinical symptoms are apparent. In this regard, low dopamine excretion in the urine has been reported in manganese-exposed workers. Also, decreased production of testosterone in Manganism patients resulted in low excretion of 17-ketosteroids in urine, but the correlation to the time of onset of clinical symptoms is undetermined. Both these biomarkers of effect still need to be tested for their reliability and specificity to manganese exposure.

Moreover, workers exposed to occupational levels of Mn had higher serum prolactin that was positively correlated to blood and urine Mn. This represents an indirect manganese effect due to similar inhibition of dopamine neurons by both Mn and prolactin. [73]

Thus, the best available biomarkers of manganese effect are serum manganese levels in conjunction with lymphocyte MnSOD activity levels in the presence of low to moderate exposure.

F. Susceptible Population to Manganese Exposure

Decreased efficacy of absorption and excretion mechanisms of manganese make certain individuals more susceptible to adverse effects of manganese exposure. This exposure can be within normal ranges for healthy non-susceptible individuals. Thus, elderly and very young populations are more susceptible to Mn exposure.

High Mn exposure in children affects their overall school performance, dexterity and attention span as well as behavior. [74] Barlow et al., in a matched case controlled study, have reported high hair Mn levels in hyperactive and learning disabled children. [75] This can be explained by the shared dopaminergic and GABAergic systems in both hyperactivity and Mn toxicity.

Children are mainly exposed to manganese through drinking water with a high Mn concentration and/or through their diet, especially from infant formula. [76] It has been established that formula has a higher concentration of manganese than does human milk. In addition, it is reported that neonates retain more and excrete less manganese than adults. This makes formula-fed infants at a greater neurotoxicity risk, especially since Mn homeostasis mechanisms are not fully developed. However, these risks have not been proven by direct evidence. [8]

Kondakis et al reported that elderly persons are susceptible to developing neurological side effects more than their younger family members exposed to the same levels of manganese in drinking water. This can be explained by poorer organ functioning with increased age, any accumulated toxic damage to the nervous system and altered nutritional status. [13] Also, Tanaka and colleges reported that older welders represent most of the cases of manganese neurotoxicity. [77]

Also, liver disease patients are more susceptible to manganese accumulation in the brain due to impaired body manganese clearance. The ante-descendant relationship of high blood manganese and liver disease is very difficult to establish. In a clinical trial on children in the intensive unit receiving parenteral nutrition, there was a decrease in the liver enzymes such as AST and plasma bilirubin after cessation of manganese supplementation. [78]

One potential cause for a lack of a consistently good biomarker for air Mn exposure may be the role of dietary Mn intake. Dietary Mn intake may be a confounding factor for Mn exposure. The purpose of this thesis is to explore potential biomarkers of Mn exposure and to determine the relationship between dietary Mn and blood Mn.

In this study, we are attempting to correlate the dietary Mn intake with Mn blood levels in an effort to establish a valid Mn biomarker that can be used as a measure of dietary Mn deficiency or toxicity, taking into consideration the effect that other minerals have on Mn levels.

Hypothesis

There is a positive correlation between the dietary manganese intake and the amount of Mn in blood. This correlation is affected by the presence of other trace elements in the diet.

Specific Aims

Specific Aim 1: Evaluate the correlation between dietary manganese intake and blood Mn**.**

Specific Aim 2: Determine the role of other nutrients, such as Calcium and Iron, on the biological markers of Mn.

Methods

Study subjects

This study will utilize data collected through the "Neurobehavioral Effects of Low Level Manganese Exposure" (Haynes, PI). Study participants were recruited from the Cincinnati Lead Study (CLS) cohort, a birth cohort of approximately 300 subjects.

CLS cohort subjects were recruited from consecutive births between 1979-1984 from obstetrical clinics located in the geographic areas within Cincinnati, Ohio. All CLS subjects were formula fed.

Exclusion criteria included:

- Women known to be addicted to alcohol or drugs or those who were diabetics.
- Women with psychoses or neurological disorders.
- Thirty five weeks or less gestational aged babies.
- Babies less than 1500 grams in birth weight.
- Infants with defined genetic syndromes or other serious medical conditions at birth.

This cohort is 56% female; 90% African American; and currently between the ages of 21-26. Approximately 96 subjects participated in this study.

Nutritional intake was determined through the Youth/Adolescent Food Frequency Questionnaire (YAQ). This survey has been developed and validated by the Channing Laboratory researchers, Brigham & Women's Hospital, Boston, Massachusetts (Pearson correlation, $r = 0.54$).[79]

The YAQ contains information regarding Mn dietary intake that will allow the examination of the correlation of Mn intake and blood manganese concentration. The survey refers to dietary intake over the past year before completing the questionnaire. The YAQ is based

on the validated adult Nurses' Health study semi-quantitative food frequency questionnaire but in a simpler form.

Blood Manganese

Whole blood samples were collected on the 23 year old subjects for Mn analysis. Blood was collected by venipuncture by a certified phlebotomist using techniques to ensure minimal extraneous lead (Pb) contamination. Mn was analyzed in blood using a method previously described. [10]To quantify Mn in the blood sample, 100 µl of blood was suspended in 900 µl of 0.1% Trition X-100. Aliquots of 20 µl were injected into the atomic absorption spectrophotometry (AAS) system. The detection limit for this method is 0.05µg/dL. The imprecision within series (5.3%) and between series (10.4%) were evaluated using spiked blood samples (Mn concentration: 25µg/dL).

Blood samples were analyzed by the H&E Laboratory at the University of Cincinnati (UC) under specific standard procedures for accuracy and validity of the data. For quality measures, 5% of the samples analyzed at UC were sent to the Channing Laboratory, Harvard Medical School for analysis.

Statistical Analysis

SAS version 9.3.1 program was used for all statistical analyses. Means of Mn dietary intake and Mn blood levels were calculated. Nonparametric correlation analysis was used to calculate the correlation between Mn dietary intake and Mn blood levels (Spearman's Correlation Coefficient). The manganese blood values were log transformed so that this variable's distribution was approximately normally distributed. The Pearson correlation coefficients between log-transformed blood Mn and dietary Mn as well as other minerals that affect Mn blood levels were calculated. Multiple regression analysis was used to include the

other dietary covariates in the model (such as iron, calcium, potassium and phosphorus), to determine their effect on Mn in blood. An $\alpha = 0.05$ was used to judge statistical significance.

Results

Ninety six subjects were included in this study. Fifty four were females (56%) and forty two were males (44%). Ninety percent of the subjects were African American. Subjects were 21-26 years of age at the time of the YAQ completion. All the subjects were 23 years old at the time of blood sample collection.

The mean manganese dietary intake was 3.25mg/dl ±2.06 (mean ± SD). The minimum and maximum intakes were 0.57 mg/dl and 12.4 mg/dl, respectively.

The mean blood manganese concentration was 0.80 µg/dl ±0.36 (mean ± SD). The minimum and maximum concentrations were 0.06µg/dl and 2.03µg/dl, respectively.

We looked at other elements in the subjects' diet. The mean ±SD calcium intake was 927.2 mg//dl ± 485.1. The mean ±SD iron intake was 79.4 mg/dl ± 32.5. Potassium mean ±SD intake was 2859 mg/dl ± 1604, while phosphorus mean ±SD intake was 1425 mg/dl ± 714.5.

The Spearman Rank correlation coefficient between dietary manganese intake and blood manganese levels was not significant ($r = 0.0002$, P value =0.99).

There were no statistically significant correlations between blood manganese and dietary calcium, iron, potassium and phosphorus using the Spearman rank correlation coefficient (r = 0.02, -0.2, 0.01 and 0.03, respectively; P value = 0.81, 0.06, 0.93 and 0.81, respectively).

The log transformed blood manganese concentration was not correlated to nutritional manganese intake. The Pearson correlation was not statistically significant ($r = -0.16$, $P = 0.87$).

Correlations between log transformed blood Mn with the other dietary elements were calculated. Log transformed blood Mn levels were not significantly correlated with dietary phosphorus (r =0.05, P = 0.66), calcium (r = 0.1, P = 0.33), iron, (r = -0.2, P = 0.08) or potassium (r = 0.03, P = 0.74).

Multiple regressions of log transformed blood Mn and blood Mn levels were performed using the above mentioned dietary elements as predictors. None of these elements was statistically significant on the log transformed blood Mn or the untransformed blood Mn levels.

The model for log transformed blood Mn explained only 5% of the variation in this outcome, while the model for blood Mn levels explained 6.3% of the variation in the untransformed outcome.

Backward elimination of insignificant dietary elements was performed. A model with only calcium and iron as predictors of log transformed blood Mn explained only 4% of the variation in this outcome. In the final model, with only iron regressed on log transformed blood Mn levels, iron approached significance with a P value = 0.057.

Discussion

We have found no significant correlation between dietary manganese intake and manganese blood levels. Also, we did not find a significant correlation between blood manganese levels and other elements that are known to influence manganese absorption and retention in the body. The mean blood manganese concentration of 0.80mg/dl in our sample is in agreement with the reported mean blood manganese in literature.

Our study is limited by several factors. Manganese is found in trace amounts in blood and other biological media and since our sample size was small (n=96), it might have not been

sufficient to capture significant changes in blood manganese due to dietary manganese intake. We used the YAQ, which has been validated as a food frequency questionnaire, but it was not designed specifically to monitor dietary manganese intake. It might have missed some of the major and important sources of manganese in the diet, making the association of manganese enriched food with blood manganese levels difficult to establish.

Air manganese levels for CLS subjects is considered to be low (0.004 $\mu g/m^3$), but this can be a confounder of the blood manganese levels that has not been controlled for in the analysis. In addition, the subjects of this study are 90% African American who have almost the same dietary habits. This might have resulted in missing certain types of food that are rich in manganese.

It is unknown what level of prolonged and low level exposure to manganese is linked with Mn toxicity symptoms and neurological outcomes. It is difficult to determine a critical effect level of manganese exposure versus the essential dietary level due to the wide variation in intake from all sources.

There is no recommended dietary allowance (RDA) for manganese since it is a trace element that is available in very minute amounts in the body and diet. There is also a wide range of manganese values in the blood and urine, which makes determination of the level at which the preclinical symptoms of toxicity become apparent very difficult to estimate. In addition, the symptoms of manganese toxicity are vague, irreversible and progressive and resemble other neurological disorders such as Parkinson's disease.

We are still in need of a stable biomarker of manganese that would reflect the body stores as well as the exposure levels to manganese that would enable us to define a normal range of manganese intake and body levels and identify the level at which subclinical symptoms begin.

The association of hair manganese with violent behavior and hyperactivity in children and adults has been shown to be statistically significant, but a cause and effect relationship has not been demonstrated, since other agents might also be involved, such as lead.

Many studies in animals, especially rodents, attempted to simulate the effects of environmental manganese exposures in the diet. It was reported that there is no definitive motor dysfunctions similar to those seen in humans. Thus, these animal studies may not be good models, and extrapolation of the results to humans, especially a dose- response relationship, may not be possible.

Other animal studies on non-human primates (such as monkeys) reported the same neurological injuries following exposure to manganese similar to those seen in humans, but at different exposure levels or doses.

In conclusion, we are in need of studies that can precisely determine the baseline range of manganese in different biological materials, the exposure levels at which subclinical symptoms begin to occur, studies that will allow us to better understand and learn more about the ways our bodies deal with manganese exposure and whether the exposure is environmental or dietary. More research needs to be performed to study absorption and excretion, as well as studies that could more accurately correlate manganese with other metals that occur concomitantly in the diet or from environmental exposure sources.

Table (1) Adequate Intake (AI) of Manganese according to Age.*

Adequate Intake (AI) for Manganese			
Life Stage	**Age**	**Males (mg/day)**	**Females (mg/day)**
Infants	0-6 months	0.003	0.003
Infants	7-12 months	0.6	0.6
Children	1-3 years	1.2	1.2
Children	4-8 years	1.5	1.5
Children	9-13 years	1.9	1.6
Adolescents	14-18 years	2.2	1.6
Adults	19 years and older	2.3	1.8
Pregnancy	all ages	-	2.0
Breastfeeding	all ages	-	2.6

* Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes for Vitamin A, Vitamin K, Boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academy Press. 2001:394-419.

Table (2) Baseline Manganese Concentration in Different Human Biological Media*.

Biological Media	Whole blood	Serum	RBC	Urine	Hair	Human Milk
Mn concentration range	8.0-18.7 µg/L	0.54-1.78 µg/L	16 µg/Kg	0.5-9.8 µg/L	200-4400 µg/kg	4-8 µg/L

* Iyengar V and Woittiez J. Trace elements in human clinical specimens: evaluation of literature data to identify reference values. Clin Chem. 1988;34:474-481.

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