Mercury As An Indicator Of Autism Spectrum Disorder

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ABSTRACT

This study was initiated to examine if levels of excreted mercury in head hair could be used as an accurate indicator of Autism Spectrum Disorder. Cold vapor atomic absorption was used to quantitatively measure the level of mercury excreted in head hair. Cold vapor atomic absorption differs from flame atomic absorption in that a flame is not used to vaporize the sample and the Perkin-Elmer Flow Injection Mercury system used in this study is a dedicated mercury analysis system.

Hair samples were taken form both autistic and non-autistic volunteers. The parent of each autistic volunteer cut a small locket of hair from the back of the head. This was accomplished in the student's home during a regularly scheduled hair cut without any disturbance in the child's environment or routine. In addition, individual volunteers are to be followed for mercury excretion over a period of time.

The preliminary results showed that the control participants were better able to excrete mercury through their hair follicles. The data supports the hypothesis that people with Autism Spectrum Disorder developed the disorder in part, not because they were exposed to mercury, but rather they were unable to excrete mercury as well as the control volunteers.

PROBLEM

There are three primary research questions being addressed by this study:

1) Is cold vapor atomic absorption capable of quantitatively measuring the level of mercury excreted in head hair?

2) Can levels of excreted mercury in head hair be used as an accurate indicator of autism spectrum disorder?

3) Does the excretion of mercury through head hair fluctuate? Furthermore, does it fluctuate in a cyclical or seasonal pattern?

INTRODUCTION

MERCURY

Elemental mercury (Hg), atomic number 80, has an atomic mass of 200.59 and an electron configuration of 2-8-18-32-18-3. It has a melting point of −38.87°C and is subsequently found as a liquid at room temperature. Mercury, its compounds, and its vapors are highly toxic. Mercury-poisoning symptoms include, but are not limited to, loss of physical coordination, paresthesia, hearing impairment, blindness, and in the most severe cases death. Mercury is often used in a number of industrial processes including the production of batteries, thermometers, florescent lamps, barometers, and thermostats. All of these devices can be found in a typical household environment. However, due to widespread health concerns, reduction efforts are cutting back or eliminating mercury in said products. For example, laboratory thermometers now use pigmented alcohol in place of mercury without a loss of accuracy or ease of use.

Ethylmercury is a bioaccumulative organic compound. It is composed of an ethyl group and a mercury atom. Its formula is $C_2H_5Hg^+$. Contact with the chemical is not recommended due to its toxicity. Its effects may be detrimental to general health and well-being. Ethylmercury is a chief constituent of thimerosal ($C_9H_9HgNaO_2S$). Thimerosol has previously been used as a preservative agent in many vaccines. In infants it has been suggested that thimerosal in vaccines could contribute to the cause neurodevelopmental disorders, most notably, autism spectrum disorder.

Methylmercury, like ethylmercury, is a bioaccumulative organic compound. It is composed of a methyl group (CH_3-) bonded to a mercury atom. It has a structure very similar to that of methionine, an essential amino acid. Like methionine, methylmercury passes freely through the blood-brain barrier and the placenta of a developing human. Because of its ability to bind to proteins, it is not readily eliminated. In fetuses, methyl mercury can directly interfere with the proper progression of developing neurological systems.

Methylmercury is introduced into the environment in a variety of ways. The most common means include the burning of wastes containing methyl mercury and from the burning of fossil fuels, particularly coal. As it is absorbed by successively larger organisms its concentration increases exponentially. Large fish such as marlin, swordfish

and tuna tend to have the highest concentration and are the largest dietary source. Once ingested by humans, the chemical is readily absorbed by the gastrointestinal tract.

AUTISM

Autism or, Autism Spectrum Disorder (ASD), represents a broad spectrum of function, ranging from low to high. Typically, a high functioning individual will be able to attend school and live life to a certain degree of normalcy. In stark contrast, a low functioning individual may be confined to home schooling and require protective headgear at all times.

ASD is a disability resulting from a neurological disorder that affects the normal development of the brain. ASD is one of the five Pervasive Developmental Disorders that are characterized by “severe and pervasive impairment in several areas of development”. ASD is the most prevalent of the five disorders occurring in approximately 1 in 166 births (CDC, 2004). Most persons with autism develop normally from the first few months to the first few years of life. This makes early diagnosis extremely difficult.

The most common symptoms of ASD include difficulties in the areas of verbal communication, social interaction, and is usually marked by the refusal to accept any changes in routine or environment. Repetitive motion and self-injurious activity are common in severe cases. Physicians seeking a precise diagnosis look for oversensitivity or under sensitivity to touch, physical clumsiness or carelessness, a tendency to be easily distracted, impulsive physical or verbal behavior, abnormal activity levels, difficulty learning new movements, and delays in speech, language, or motor coordination. Currently there is no known cure for ASD.

Many cases are treated with Methylphenidate. Methylphenidate is a mild nervous system stimulant sold under the brand name Ritalin in North America. How Methylphenidate works is not exactly understood. It is believed to stimulate the release of certain chemicals in the brain resulting in increased alertness and awareness. However methylphenidate does not always produce the desired results. Symptoms include the development of blood in the stool or urine, nervousness, abnormal

sleeping patterns, increased blood pressure, and dizziness. Certain drug interactions have also been observed. Alternatives are currently being researched.

Chelation therapy has been used treatment of ASD. Chelation therapy is a sequence of intravenous infusions containing ethylenediaminetetraacetic acid (EDTA) and a variety of other substances. In this process, heavy metals are bound and removed from the bloodstream. Clinical trials thus far have yielded mixed results. Often, the side effects far outweigh any possible benefits. Side effects include, but are not limited to, kidney damage, decreased blood clotting ability, and severe vasculitis.

Recent scientific developments suggest that there exists a direct link between the levels of mercury exposure a person receives during early development, and their chances of developing ASD. It is believed, that when mercury enters the body, it binds to sulfhydryl groups on enzymes and other proteins. Furthermore, when the mercury enters the brain and spinal chord, it affects the uptake of dopamine, serotonin, acetylcholine, and norepinephrine. This is what is believed to cause the lack of motor coordination and difficulty with communication that is seen in many autistic people.

MATERIALS

Potassium Permanganate: a poisonous salt that forms dark purple crystals and is purple when dissolved in water; used as an oxidizing agent

Stannous-Chloride: a white crystalline solid with the formula $SnCl_2$

Argon: a colorless, odorless, inert gaseous element

Nitric Acid: (HNO_3), otherwise known as aqua fortis, is a colorless, corrosive liquid

Sodium Chloride Hydroxylamine Sulfate: colorless solution; used to reduce excess potassium permanganate in this appliction

Hydrochloric Acid: a clear, colorless, fuming, poisonous, highly acidic aqueous solution of hydrogen chloride

Reagent Grade Water: water that has been distilled, deionized, and filtered

Mercury Analyzer: an instrument used quantitatively measure mercury concentration with the use of an atomic absorption spectrometer (Perkin-Elmer FIMS-100)

BOD Bottle: (Biochemical Oxygen Demand) used to safely and securely store without unwanted exposure to air

Water Bath: temperature controlled container filled with water

Analytical Balance: a beam balance of great precision used in quantitative chemical analysis

PROCEDURE

MEHTOD ONE: Sample Collection

Informed consent is obtained from a legal guardian. The hair samples from the autistic volunteers are collected during a regularly scheduled haircut without any disturbance to the child's environment or routine. Control samples are typically taken from High School students and teachers. In both instances, a locket of hair is cut, not pulled, from the back of the head. Data regarding the participant's age, gender, and any medical treatment is recorded. The hair is stored in a Ziploc bag to reduce the risk of contamination. Digestion is typically conducted within seven days.

METHOD TWO: Sample Digestion

Step 01) A representative 0.40g portion of sample is weighed and placed in the bottom of a BOD bottle

Step 02) 5.0 mL of RGW, 1.5 mL of nitric acid, and 3.5 mL of hydrochloric acid are added

Step 03) The mixture is heated in the water bath for two minutes at 95℃

Step 04) 15.0 mL of a 5% potassium permanganate solution and 50 mL of RGW are added

Step 05) The bottle is heated additional 30 minutes at 95℃

Step 06) When the mixture is cool to the touch, 6.0 mL of sodium chloride-hydroxylamine sulfate is added to reduce the excess potassium permanganate resulting in a change of color

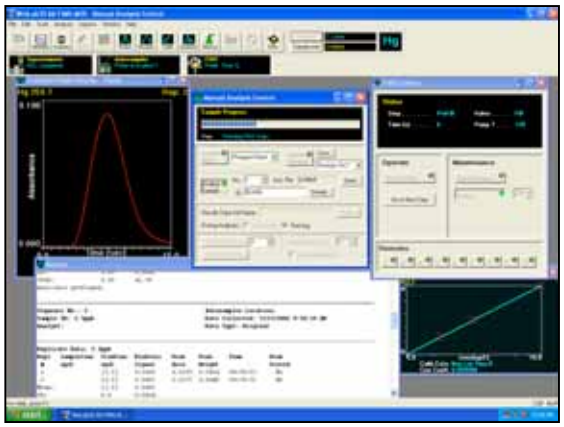
Step 07) 50.0 mL of RGW is added and the bottle is gently mixed

Step 08) Samples are filtered through qualitative filter paper into 50.0 mL sterile tubes and stored at room temperature for a maximum of 28 days

METHOD THREE: Sample Analysis

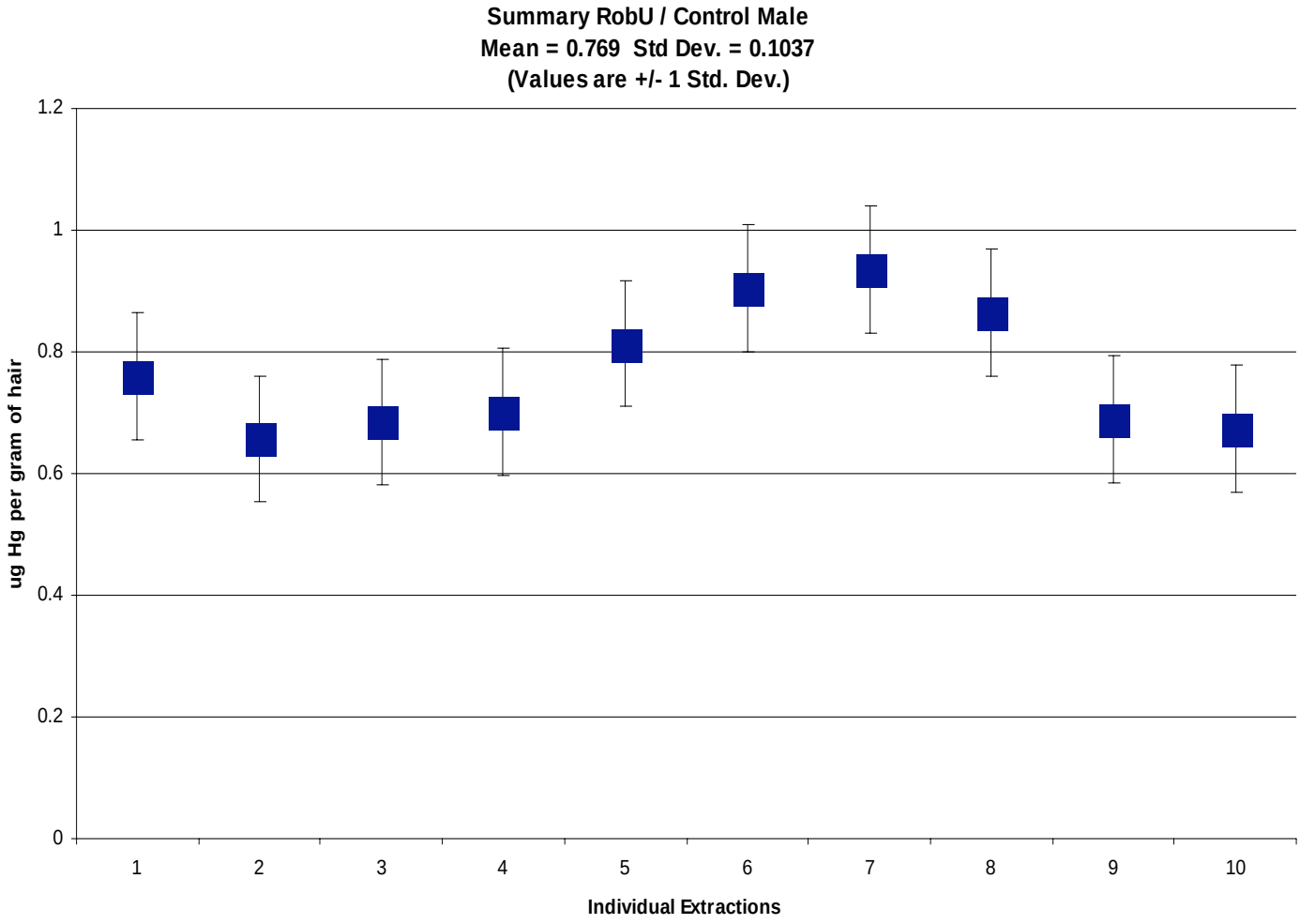
The FIMS-100 is capable of detecting total mercury in the ppb range by cold vapor atomic absorption. This is the method by which a cloud of atoms is formed from a solution containing mercury ions. Mercury in the +2 state is reduced by the addition of

stannous chloride and then delivered by a carrier gas into a quartz absorption cell that is kept at 200° C. The temperature is maintained at 200°C in order to prevent water from condensing onto the quartz cell. Measurements are reported in µg/g using a formula below that takes into account the respective mass, volume, and absorbance reading of a given sample. AA Winlab, a software suite for the windows operating system, is used in conjunction with the FIMS-100 to report measured concentrations.



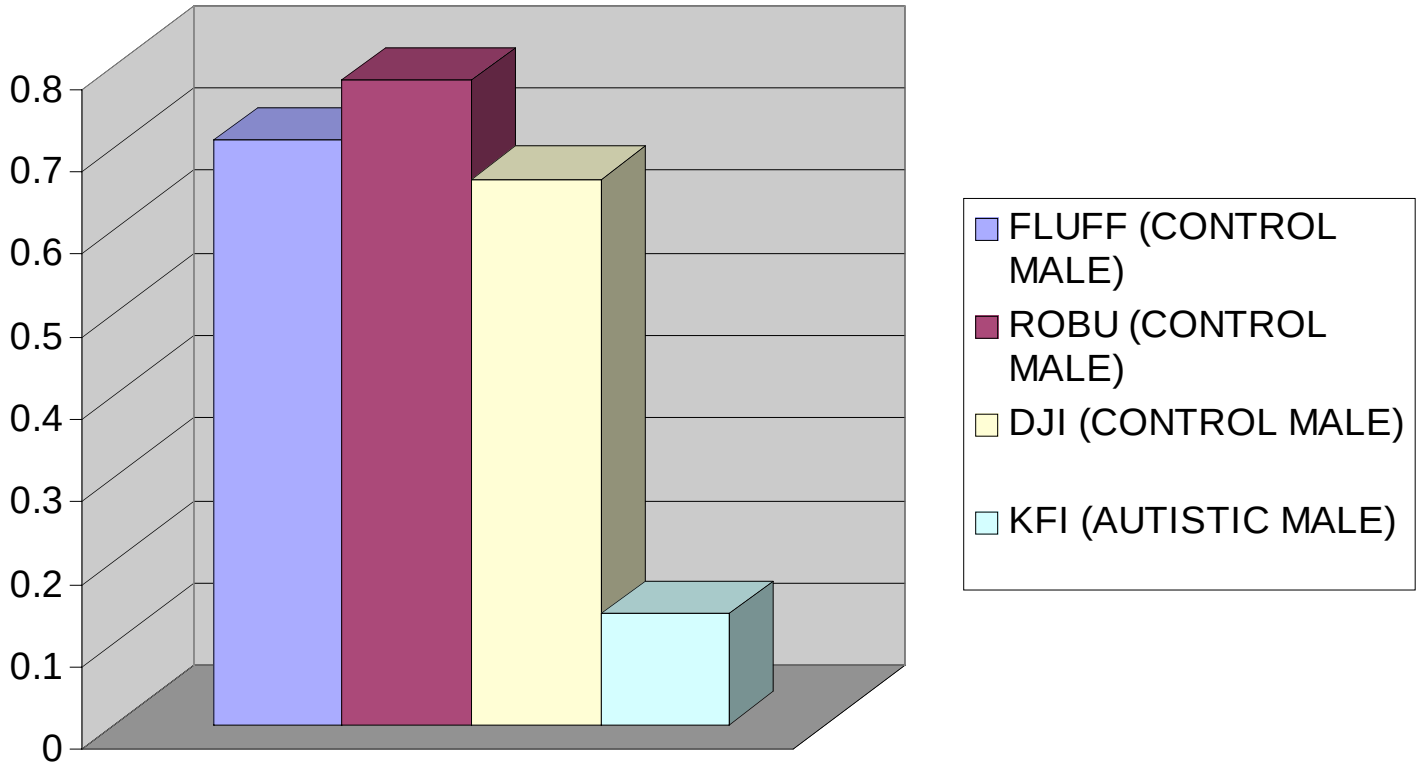
$$\mu g/g = \frac{\text{mean FIMS reading } (\mu g/l) \text{ X Volume } (0.081 \text{ L})}{\text{mass of sample in grams}}$$

RESULTS



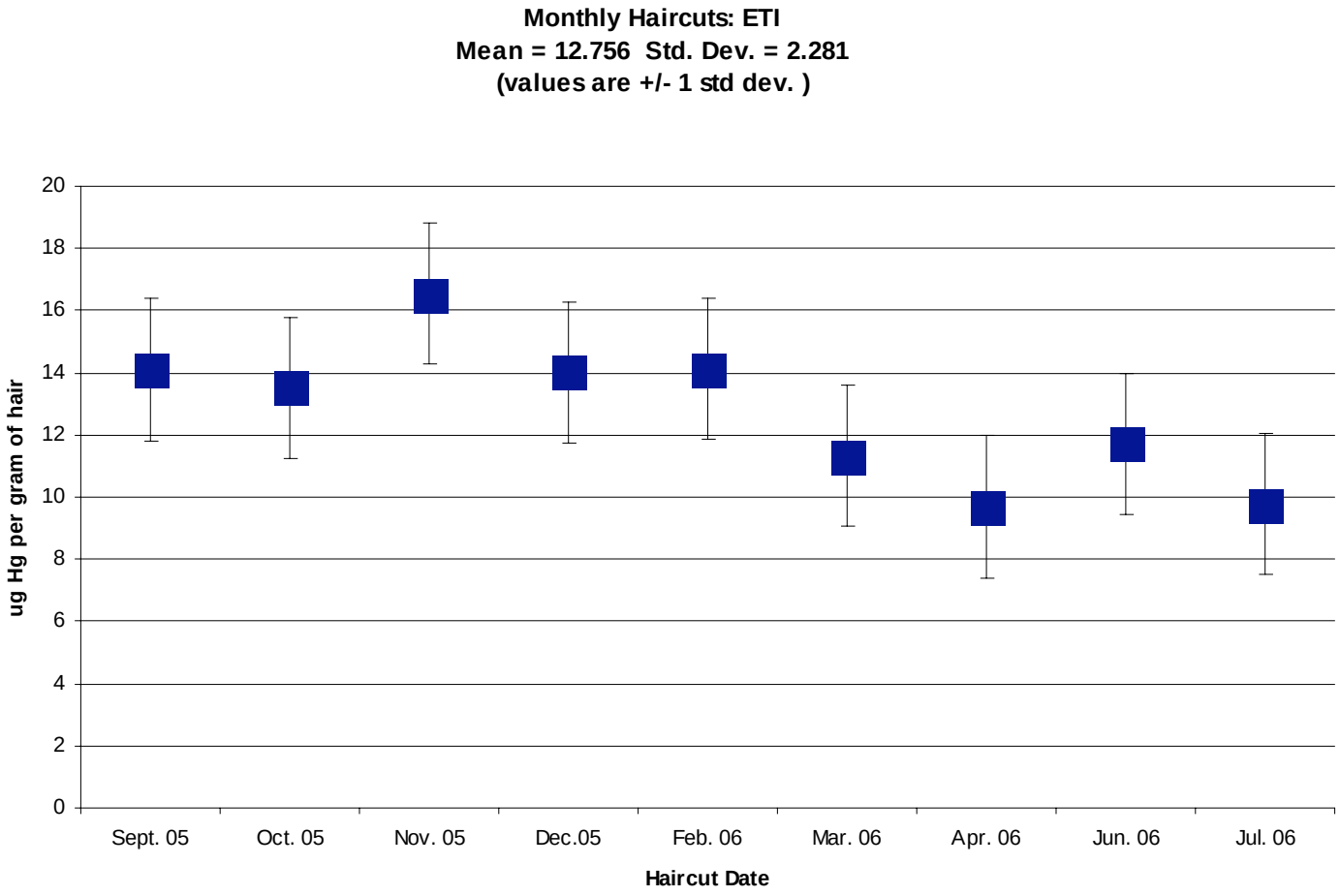
ANALYSIS: The preceding graph shows the results for one individual's hair sample that was digested a total of ten separate times. The very fact that all of the digestions are within one standard deviation demonstrates that CVAA is capable of quantitatively measuring mercury excreted in head hair.

RESULTS



ANALYSIS: The preceding bar graph demonstrates that the level of excreted mercury in the head hair of the autistic individual was significantly lower than that of the corresponding control individuals. This suggests that levels of excreted mercury in head hair can be used as an accurate indicator of ASD.

RESULTS



ANALYSIS: The preceding chart demonstrates levels of mercury excreted in one control male as observed from September 2005 through July 2006. As of yet, no cyclic or seasonal patterns have been observed. Additionally, as of June 2006, this individual has agreed to eliminate tuna fish from his diet. Additional haircuts are required in order to examine a possible decrease in excreted mercury.

DISCUSION

The results show that the control participants were better able to excrete mercury through their hair follicles. This supports the hypothesis that persons with ASD developed the disorder not due level of exposure, but rather their inability to release the mercury. This idea is further bolstered by the fact that the cases of autism have risen exponentially in countries like China since they began importing vaccines from the United States.

FUTURE WORK

More samples will be collected and analyzed. Additional aspects of the study will be explored in order to answer the underlying questions. Participants will be followed over a period of time to examine possible cyclical patterns or seasonal. At least ten male and ten female autistic volunteers will be recruited for that purpose. The new data will be further examined to find a possible correlation to hormonal imbalances. They will also be compared to behavioral charts of autistic volunteers. Additional Quality Controls will be explored and implemented to further ensure the accuracy and credibility of this study. Samples will be cross-analyzed with the help of an independent outside lab in the further pursuit of accuracy. As current diagnostic methods are often quite subjective, the results of this study will be used to design a more objective method of diagnosing ASD.

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