Toxic Metals and Essential Minerals in the Hair of Children with Autism and their Mothers

J.B. Adams[1]; C.E. Holloway[1]; F. George[2]; D. Quig[3]
[1] Arizona State University, Tempe, Arizona
[2] Holistic Osteopathic Medical Care, Cave Creek, Arizona
[3] Doctor's Data, St. Charles, Illinois

Abstract:
The levels of 39 toxic metals and essential minerals in hair samples were determined for children with autism spectrum disorders and a subset of their mothers compared to controls. The major findings are:

1) Iodine levels were 45% lower in the children with autism (p=0.005).
2) Lithium levels were 30% lower (p=0.04) in the younger children with autism . The mothers of young children with autism had especially low levels of lithium (-56%, p=0.005).
3) Children with pica had a 38% lower level of chromium (p=0.002), and also 58% lower levels of sodium (p=0.05).
4) Children with low muscle tone had very low levels of potassium (-66%, p=0.01), high zinc (+31%, p=0.01), and high levels of barium (+109%, p=0.03).

Keywords: autism; hair analysis; iodine; lithium; potassium;

Corresponding author:
James B. Adams
Arizona State University
PO Box 876006
Tempe, AZ 85287-6006
(480) 965-3316
(480) 965-0037 (fax)
jim.adams@asu.edu

Introduction

It has been estimated that approximately 25% of children and adolescents exhibit psychological disorders that may significantly impair daily functioning[1 2]. While numerous factors may be involved, excessive levels of toxic metals and deficiencies of some essential minerals have been implicated in neurobehavioral and cognitive dysfunction in some children [3 4 5 6 7 8 9]. For example, low levels of calcium, as assessed in scalp hair, were significantly correlated with problem behavior and distractibility in students attending grades K through four in Victoria, British Columbia[10]. High levels of potentially neurotoxic manganese and cadmium have also been correlated with learning problems [10 11], dyslexia [12], and inversely with intelligence scores in children and adolescents[13]. Lead, a widely recognized neurotoxic metal, continues to be a significant problem in children in the United States today [8 9 14]. Further, population studies clearly indicate adverse neurological effects in children associated with exposure to methylmercury from maternal fish consumption [15].

While the cause of autism is unknown, there are suggestions that mercury or other toxic metals may play a role in its pathogenesis. A recent review by Bernard et. al.[16] discusses over 75 similarities between autism and prenatal/infantile mercury exposure. Some of the major similarities include a much higher prevalence/sensitivity in males vs. females, decreased language/communication, decreased social relatedness, repetitive behaviors, and gastrointestinal problems. Similarly, a study of monkeys who were prenatally exposed to mercury found that they exhibited decreased social play and increased passive behavior.[17]

The amount of toxic metals and essential minerals can easily be assessed by blood, urine, and hair. Hair is especially useful for toxic metals, as they are much more concentrated in the hair. Since hair grows at a rate of 1-1.5 cm/month, a 2-3 cm sample from next to the scalp can provide an average over 2-3 months. It provides a measure of what is being transported in the body during that time, but will not be able to detect earlier exposures. There has been some recent well-publicized criticism of hair analysis studies [18], but the major criticism centered on differences in results between different labs, some of which were using inferior equipment and less rigorous preparation techniques. Those concerns can be addressed by using a single high-quality lab, with blinded testing of subjects vs. controls. In a classic review of over 250 reports, the EPA concluded that hair is “a meaningful and representative tissue” for measuring toxic metals and selected nutrients.[19]

There have been several previous studies of the levels of toxic and essential minerals in the hair of children with autism [20 21 22 23]. Those studies are summarized in Table 1. There was also one study by Kimhi et al [24], which measured only vanadium concentrations in 10 autistic adults vs 10 controls; they found no significant difference in vanadium levels. As can be seen in Table 1, many abnormalities have been reported, but the results of these studies sometimes contradict one another. Most of those studies suffer from relatively small sample size. Also, they did not differentiate between the children who had pica (eating non-food items) and those who did not, which is common in autism and could account for an increased toxic metal burden. A limitation with one study [22] was that most of the controls were siblings of children with autism. Also, the studies were done before 1985, when measurement techniques were not as advanced as they are today. Only one of the studies (Wecker et al [23]) mentions the use of a special common shampoo, but they only used it for 3 days prior to collecting the hair sample; this is important to avoid contaminants through hair care products, which can invalidate results. Therefore, it is clear that a larger study, with more rigorous avoidance of contaminants and an assessment of pica, is necessary to resolve the discrepancies in the previous studies.

In addition, there was a recent study by Holmes’ et. al [25] of the level of mercury in the hair of infants (aged 12-24 months) who later were diagnosed with autism compared to controls (n=94 and 45, respectively). This study found that the autism group had 1/8 of the normal amount of mercury in their baby hair compared to controls, which suggests an inability to excrete mercury. They also found that the severity of autism had a strong inverse relationship with the level of mercury, with the most severe group having the lowest levels of mercury in their hair. This is consistent with the hypothesis that the group with the most inhibition of mercury excretion would be the most severely affected.

There has never before been an attempt to analyze the hair of the mothers of children with autism. Since the mothers are the major source of exposure to toxic metals, and also the source of essential minerals during gestation and breastfeeding, we felt that it would be important to also analyze the levels in their hair.

Therefore, this study was designed to measure the amount of toxic metals and essential minerals that were excreted into the hair of both the children with autism and their mothers, and compare those groups to neuro-typical controls. For the mothers, the limitation is that this is done many years after pregnancy, but we think that some general trends due to diet and environment may remain observable. A prospective study would be preferable, but the cost and time involved is prohibitive compared to this study. Regarding the children, although it is important to determine the levels at the onset of autism, it is also useful to know them at later times. This is partially accomplished by analysis of the autistic children as a group, and a sub analysis of the youngest children aged 3-6 years.

Participant Selection

The participants were invited to participate by a mass mailing sent to approximately 1000 families of people with autism in the state of Arizona, using the mailing lists of the Greater Phoenix Chapter and the Pima County (Tucson) Chapter of the Autism Society of America. The inclusion criteria for the children was age 3-15 years, with a diagnosis by a psychiatrist or developmental pediatrician of Autism Spectrum Disorder (ASD), including autism, PDD/NOS, and Asperger's Syndrome.

Parents of the participants with ASD asked friends and neighbors to act as controls for the study. The criteria for the controls were that they: 1) be mentally and physically healthy individuals aged 3-15 years without any developmental delays, illness, or other medical conditions, and 2) be unrelated to a person with ASD. Also, there was an attempt to match the ages and genders as closely as possible.

There were 51 children with ASD enrolled in the study, including 48 with autism, 2 with PDD/NOS, and one with Asperger's. (Some of the children had received multiple diagnoses, in which case they were counted in the most severe category). However, in Arizona, only children diagnosed with autism receive significant services from the state (respite, behavioral therapy, speech therapy, occupational therapy, physical therapy, music therapy); children with diagnoses of Asperger's or PDD/NOS do not receive such services. Therefore, even though qualified psychologists, psychiatrists, or developmental pediatricians gave the diagnoses, there is some question as to their validity. That is a limitation of this initial study, and future studies can include a more rigorous assessment of the child's diagnosis.

There were 40 neurotypical children enrolled as controls in the study. There were 12 girls and 10 girls in the ASD and control groups, respectively, comprising 23.5% and 25% of their groups. There were two sets of twins in the ASD group, and one set of twins and one pair of siblings in the control group. Ages ranged from three to 15 years of age, with a mean age of 7.1 and 7.5 years for the children with ASD and the controls, respectively, with standard deviations of 3.0 years for each. Thus, there was overall a good match between the groups in terms of gender and age.

In the ASD group, 16 of the children (32%) were reported by their mothers to exhibit moderate to severe pica, including eating sand, dirt, paper, wood, painted objects, and other inedible substances. It was expected that this would affect their toxic metal burden, so they are analyzed as a subgroup. Also, analysis of their essential minerals might suggest if a deficiency of certain essential minerals could account for their pica tendencies.

The children with ASD were also divided into several additional subgroups based on several other symptoms, so that it could be determined if any of the symptoms were associated with abnormal levels of elements in their hair. All of the subgroupings were based on their mothers' reports of symptoms. These subgroups included:

1. Regression: 32 children were reported to have regressive ASD at an average age of 18 months, vs. 17 that appeared to be autistic from birth, and 2 with possible regression.
2. Ear Infections: 26 were reported to have had more than 8 ear infections during their first 3 years of life, vs. 25 having had less than 8 ear infections (the typical children had on average 4 ear infections)
3. Gastrointestinal Problems: 32 children were reported to have moderate or severe chronic diarrhea and/or constipation, vs. 18 who had mild or no symptoms. (Only one of the control children had a moderate problem).
4. Sleep Problems: 31 children were reported to have moderate or severe sleep problems, vs. 17 who had mild or no symptoms. (Only one of the control children had a moderate sleep problem).
5. Muscle Tone: 15 children had moderate or severe problems with low muscle tone, vs. 35 children who had mild or no symptoms (Only one of the control children had a moderate loss of muscle tone).

6. Excessive Salivation/Drooling: 16 children had mild, moderate or severe problems with unusual salivation/drooling, vs. 34 who had no symptoms (Only two of the control children had a mild problem with excessive salivation).

Finally, the children with ASD were divided into subgroups according to whether or not they were taking nutritional supplements. 25 (49%) of the children with ASD were taking nutritional supplements, vs. 7 (17%) of the typical children. For the children with ASD, 8 were taking a generic children's multivitamin, 7 were taking Super Nu Thera, 4 were taking Spectrum Support, and the remaining 6 were taking a mixture of supplements. Super Nu Thera is a multivitamin/mineral supplement with very high levels of vitamins, and modest levels (less than the RDA) of magnesium, zinc, manganese, and selenium. Spectrum Support is also a multivitamin/mineral supplement with very high levels of vitamins, and modest levels of calcium, chromium, manganese, magnesium, selenium, and zinc. Of the 7 typical children taking a supplement, they were all taking a generic children's multivitamin/mineral supplement. The generic children's multivitamins generally contained 100% of the RDA of many vitamins, and usually only a few minerals (if any) at lower amounts.

In comparing the subgroup of children with ASD taking supplements vs. those not on supplements, there was no statistically significant difference in their level of toxic metals. There was also no statistically significant difference in their level of essential minerals, with one curious exception that was marginally statistically significant: the children on supplements had lower levels of calcium (459 vs. 762, p=0.05). This was probably because the children on supplements were more likely to be on a dairy-free diet, so they consumed less calcium, and most of the supplements contained little or no calcium. Overall, since the modest amounts of minerals in the supplements did not have a statistically significant effect on mineral levels, they will not be considered further in the analyses discussed below. It should be pointed out that lithium and iodine, which are discussed below in detail, were not present in any of the supplements. Potassium was present in a few of the supplements, but only at very low levels (below 100 mg, which is only a few % of the RDA) due to federal regulations.

Mothers were asked to participate as well, but their participation was optional. Only mothers who had not dyed or permed their hair within two months of collecting samples were included in the data. (None of the children had had their hair permed or dyed). A total of 29 mothers of children with ASD and 25 mothers of typical children were enrolled in the study.

Hair Sampling

All participants (children and mothers) were asked to wash their hair for two weeks with Johnson's and Johnson's "No Tears" Formula Baby Shampoo, without the use of any other hair care products (no conditioner, gel, hairspray, etc.). After two weeks, a sample of hair was cut using stainless steel scissors. The hair sample was taken from the nape of the neck, taking the one-inch closest to the neck.

The samples were sent to Doctors Data Lab for analysis in a blinded fashion. In the laboratory, the hair specimens were further cut and washed using a modified method developed by the International Atomic Energy Agency[26]. The hair specimens were cut into approximately 0.3 cm pieces and mixed to allow a representative subsampling of the hair specimen. After cutting, each sample was washed four times with a 1:200 v/v dilution of Triton X-100, then rinsed with acetone and allowed to drain. Samples were then rinsed three times with ultra-pure deionized water and two times with acetone. The dried samples were weighed prior to nitric acid / microwave digestion as described in detail by Puchyr et al [27]. After digestion, the samples were cooled and a 500 microliter aliquot of an internal standard was added and mixed with 50 ml of ultrapure, deionized water. The samples were then analyzed for element content using ICP-MassSpec. To ensure validity, calibration verifications, a certified hair reference control, in-house controls, spiked hair samples and other appropriate control samples were analyzed. Results are expressed as mcg / gm.

The results are reported in Tables 1, 2 and 3. Statistical analysis of the data was carried out with an unmatched t-test, assuming 2-sided normal distributions of unequal variance. Since a total of 39 elements were examined, it is to be expected that random chance alone would result in 2 values with a p-value of 0.05. Therefore, although we report values of up to p=0.05, some of those could be due to random chance, and only p values below 0.01 should be considered conclusive.

Summary of Statistically Significant Results

Toxic Metals

Aluminum: The children with ASD had slightly lower levels of aluminum (16%, p=0.05), and the difference was slightly more pronounced in the 3-6 year old group (24% lower, p=0.04). The pica subgroup was generally higher in aluminum, so that the decrease is mostly due to the non-pica group.

Arsenic: The pica subgroup had a 25% lower level of arsenic (p=0.05), whereas the non-pica group had near-normal levels of arsenic.

Uranium: The non-pica subgroup had a 27% lower level of uranium (p=0.05), whereas the pica group had higher, near-normal levels.

Barium: The pica subgroup had a 99% higher level of barium than the control group (p=0.04), whereas the non-pica subgroup had near-normal levels of barium.

Overall, the pica subgroup had higher levels of aluminum, uranium, and barium, presumably due to increased consumption. However, their arsenic level was slightly lower, and the reason for this is unclear. It could be a random result, or perhaps arsenic is being less well excreted due to competition with higher levels of other toxic metals.

In contrast, the non-pica subgroup generally had normal levels of toxic metals, except for slightly lower aluminum and uranium levels.

In terms of other subgroups, a ttest comparison of the subgroups did not reveal any statistically significant differences in any of the toxic metals for the regression, gastrointestinal, ear infection, or salivation subgroups. The Sleep disorder subgroup had slightly lower levels of arsenic than the non-sleep disorder ASD subgroup (0.08 vs .11, p=0.02), but the controls had an average level of 0.095, so this is likely to be a random result. Similarly, the Muscle Tone subgroup had lower levels of bismuth (0.10 vs 0.35, p=0.05), but the controls had an average level of 0.28, so again this may be a random result.

In the mothers of children with ASD, there was no statistically significant difference in the level of heavy metals in their hair. There was a trend (p=0.06) that the level of barium was higher in the mothers of children with ASD. However, when the subgroup of mothers of children ages 3-8 was considered, there was no significant difference in their barium levels, and the mean levels were closer, which suggests that barium is probably not a maternal risk factor for having a child with ASD. It is interesting to note that the high level of barium in the ASD mothers is consistent with high levels in their children.

Mercury: Since mercury is of great interest as a possible cause of ASD, it is worthwhile to note that the mothers of children with ASD had 57% more mercury in their hair on average than the typical mothers, but this difference was not statistically significant (p=0.22). When the subgroup of mothers of young children was considered, there was less difference. In terms of the validity of our testing, it should be pointed out that the median values we found for the typical mothers (0.18 mcg/g) are consistent with those of a recent large NHANES study of 702 women of age 16-49 years (0.2 mcg/g)[28]. Both of those median values for typical women are much lower than the median we found for the mothers of children with autism (0.40 mcg/g).

Also, the children with ASD had nearly normal levels of mercury in their hair, with the pica subgroup having 24% more than the controls, and the non-pica subgroup having 10% less. These results were not statistically significant. So, mercury levels did not appear abnormal in this group of children. However, it should be pointed out that this is long past their primary exposure to mercury (from thimerosal-containing vaccines, maternal seafood consumption, and maternal mercury dental fillings), so this hair measurement would not reflect such a long-previous exposure.

Our results are not necessarily inconsistent with the results of Holmes et al. [25], which found unusually low levels in baby hair, as the ages of their group (12-24 months) are quite different than ours (age 3-15 years). Actually, if both sets of data are valid, then they suggest a temporary loss of the ability to excrete mercury in young infants. This temporary loss could be explained by the excessive use of oral antibiotics in children with autism [29], as oral antibiotics have been shown to dramatically inhibit mercury excretion to 1/10 of normal in rats [30].

In summary, there were only minor differences in levels of heavy metals between the non-pica children with ASD vs. the control group. The pica subgroup of the children with ASD did have elevated levels of some heavy metals, primarily barium, but slightly below normal levels of arsenic. The mothers of children with ASD did not have any statistically significant difference in the amount of heavy metals in their hair.

Essential Minerals

Iodine: For the children with ASD, the mean level of iodine was much lower (45%) than for the control children, and the difference was highly statistically significant (p=0.005). When the subgroup of age 3-6 years was considered, the magnitude of the difference was almost identical (47%), although the difference was not statistically significant due to the smaller number of children in the subgroup. This suggests that iodine could be an important factor in the early development of autism, presumably through its effect on thyroid function. Iodine deficiency was extremely common in parts of the US in the early 1900's, and caused many cases of goiters (enlarged thyroid) and cretinism (a form of mental retardation due to iodine deficiency). This prompted the federal government to mandate that iodine be added to salt (iodinized salt). However, based on our informal phone survey of several major snack food and fast food manufacturers, non-iodinized salt seems to be the form primarily used in french fries, potato chips, and other snack foods commonly eaten by young children. So, it is plausible that a small fraction of children in the US could still be marginally deficient in iodine, and that this could significantly affect their mental status. Also, it should be pointed out that according to the NHANES surveys I and III[31], average iodine levels in the US (measured in the urine) have declined more than 50% during the 20 year period from 1971-1974 to 1988-1994, so that an increasing fraction of the population has low levels of iodine that are likely to increase the risk for mental retardation. Thus, low iodine levels could be a cause or exacerbating factor for autism. However, it needs to be pointed out that hair measurements have not been validated for iodine as reflective of body status, so future studies of iodine levels in blood are warranted, as well as studies of thyroid function in autism.

Phosphorus: There was a small, but very statistically significant, difference in the level of phosphorus, with the children with ASD having a 12% lower value than the controls, with a p=0.001. When the subgroup of children age 3-6 was considered, the magnitude of the difference was almost identical (11% lower), although the difference was not statistically significant due to the smaller number of children in the subgroup. The importance of this small difference in phosphorus level is unclear.

Strontium: The form of strontium measured was the stable and most common isotope (88), not the radioactive isotopes. Strontium levels were 56% higher in the ASD group (p=0.03), and the difference was even larger in the 3-6 year old group (71% higher) although it was not quite statistically significant (p=0.08). In comparing the pica vs non-pica subgroups, it is clear that the strontium levels were much higher in the pica group, and the pica subgroup accounted for most of the difference with the typical children.

Strontium has not been shown to be essential for humans, but more study is needed to be conclusive. The non-radioactive isotopes (the form measured in this study) appear to have a very low toxicity, unlike the radioactive isotopes which are highly radioactive and carcinogenic. So, an increase in the stable isotopes is probably reflective of pica, but unlikely to have much clinical significance.

Lithium: In the subgroup of children ages 3-6, the children with ASD had a 30% lower level of lithium with a marginal statistical significance (p=0.04). For the full group of children (ages 3-15), the difference was less (15%) and it was not statistically significant.

Sodium: The pica subgroup had 58% less sodium than the typical children (p=0.05). The subgroup of non-pica autistic children had 32% less sodium than the typical children, which was not significant. This is somewhat surprising, as there are anecdotal reports of children with ASD preferring salty foods such as french fries and potato chips.

Chromium: The pica subgroup had much less chromium than the non-pica subgroup (0.28 vs 0.42, p=0.004) or the typical children (0.28 vs 0.45, p=0.002). These results are highly statistically significant, and suggest that chromium deficiency may play a role in pica.

Sulfur: The pica subgroup had slightly lower sulfur levels than the non-pica subgroup (46,600 vs 48,700, p=0.02), but the controls had an intermediate value of 48,120, so again this may just be a random fluctuation of little significance. Overall, this result is somewhat surprising, as two previous studies found that children with autism have excess urinary excretion of sulfate and very low plasma levels of sulfate. [32] [33]

Copper: The pica subgroup had much more copper than the non-pica subgroup (55 vs 25, p=0.03), whereas the typical children had an intermediate value of 33.

Overall, the pica subgroup had low levels of sodium, chromium, and sulfur, with the low chromium level being the most statistically significant, and hence most likely to be a possible factor in the etiology of pica. The pica group also had elevated levels of strontium and copper, presumably due to increased consumption.

In terms of other subgroups, a ttest comparison did reveal some difference in levels of essential minerals:

1. Regression: the children who had regressed had a higher level of magnesium (72 vs. 39, p=0.03), with the controls having an average of 51. This may be due to random chance, since the two groups are clustered around the average value.
2. Gastrointestinal: there was no statistically significant difference in levels of essential minerals between those who had gastrointestinal problems and those who did not.
3. Sleep: The children with sleep disorders had lower levels of selenium (0.99 vs 1.19, p=0.007, with controls =1.06), higher levels of zinc (178 vs 140, p=0.04, with controls=147), and higher levels of phosphorus (193 vs 177, p=0.05, controls = 213). The magnitude of these differences is not large compared to the controls, so it seems likely that they are due to random chance.
4. Ear Infections: The children with fewer infections had slightly lower levels of phosphorus (175 vs 200, p=0.004, controls =213). This result is highly statistically significant, but its importance is unclear.
5. Muscle Tone: The children with low muscle tone had very low potassium (16 vs 61, p=0.01, vs controls =47), high copper (56 vs 26, p=0.05, controls =33), high zinc (193 vs 150, p=0.01, controls=147), and high barium (2.03 vs 0.95, p=0.03, controls = 0.97). Potassium is needed for muscle contractions, so very low levels of it are likely to result in low muscle tone. The significance of the other results is unclear.
6. Salivation: There were not any statistically significant differences in this subgroup.

Overall, we think the most important results are the low level of iodine in the group as a whole, the low level of lithium in the younger children, and the low level of potassium in the children with low muscle tone. These findings suggest that deficiencies of these minerals could be part of the underlying cause of autism. Also, supplements of iodine and lithium could be beneficial to children with autism, and increased potassium could be useful to children with low muscle tone For potassium, increased consumption of fruits and vegetables may be a safer source of potassium than supplements, due to the concern of potassium supplements on heart conditions.

Lithium in Mothers: In the mothers of children with ASD, the level of lithium was 40% lower than the mothers of typical children, and the result was marginally statistically significant (p=0.05). Also, when the subgroup of mothers of children ages 3-8 was considered, the difference was more pronounced (56% lower) and more statistically significant (p=0.005). Since low lithium was also observed in the younger children with ASD, this makes the result especially interesting. It should be pointed out that hair has been found to be a reliable method to assess lithium deficiency in goats, in agreement with measurements of blood, milk, and several other organs.[34] Lithium concentrations are highest in the brain,[35] and are highest during the first trimester,[36] so a deficiency of it during pregnancy could adversely affect fetal development, and especially brain development. Also, low levels of lithium in humans has been found to correlate with a wide range of behavioral problems, including aggression and decreased sociability. [37] [38] [39] One placebo-controlled treatment study found that low dose supplementation (400 mcg/day) was beneficial to drug addicts, resulting in increases in the subcategories of happiness, friendliness, and energy. [40] It should be noted that lithium is also used at dramatically higher doses (of the order of 1,000,000 mcg/ day) as a psychiatric medication for mood stabilization.

In addition, goats on a lithium-deficient diet were found to suffer from lowered immunological status and chronic inflammations, they had less lithium in their milk, and their infants were found to have reduced growth rates.[34] We hypothesize that the low levels of lithium in the ASD mothers results in lower levels in their children, which may explain why the children suffer from a much higher level of ear infections in their first three years of life [29]. In turn, that much higher level of ear infections results in much higher oral antibiotic use, which results in a temporary decrease in the ability to excrete mercury [30], and can also contribute to gastrointestinal problems by eliminating normal gastrointestinal flora. So, a low lithium level is plausible as an important factor in the etiology of autism.

There were no other statistically significant differences in the levels of toxic metals or essential minerals between the mothers of children with ASD and mothers of typical children. Only the lithium levels were abnormal in the mothers of children with ASD.

Comparison with Previous Studies

Table 1 compares the results between our study and the previous studies. There are some areas of agreement, and some areas of disagreement. We believe that the present study is generally more reliable because it involved a larger number of participants, the controls were unrelated, subjects pre-washed their hair for 2 weeks with the same contaminant-free shampoo, and pica tendencies were taken into account in the subgroups.

Reference Ranges for Adults vs. Children

It should be pointed out that the levels of toxic metals and essential minerals for the control mothers were often quite different from the levels of the control children (see Tables 2 and 3). Also, the levels of the younger control children were often quite different from those of the children group as a whole. So, in interpreting the results of hair analysis studies, it is important to ensure that the reference group is age-appropriate.

Limitations of the Present Study

This study has several limitations, including:

1) Sample size: A larger sample size, from multiple sites, is needed to validate or refute the preliminary findings of this study.
2) Sample bias: The participants knew that this study involved an investigation of toxic metals including mercury, with fish being a common source. Therefore, it is possible that autism participants who consumed fish were somewhat more likely to participate. This may partially explain our mercury findings.
3) Autism Severity: This study did not evaluate the severity of autism. It would be interesting to determine if the severity of certain symptoms of autism correlated with hair levels.
4) Contamination: Contamination of hair samples is always a concern. Washing with a common shampoo, and washing by the lab, can help to reduce those concerns. With a large enough sample size, contamination of samples should be fairly similar for the autism and control groups.
5) Correlation of hair levels with body levels: Although hair is known to be a good indicator for body levels of some toxic metals and essential minerals, that is not true for all cases, and in some cases we do not know.

Summary
We have evaluated the levels of 39 toxic metals and essential minerals in children with ASD, including the first evaluation of the hair of mothers of children with ASD. We believe that the most important results are:

1) Low iodine levels (-45%, p=0.005) in the children with ASD, including in the young subgroup (-47%, not significant). Low iodine may result in significant impairment of thyroid function, and iodine deficiency is a known cause of cretinism, a type of severe mental retardation.

2) Low lithium levels in the young children ages 3-6 years (-30%, p=0.04), in the mothers of children with ASD (-39%, p=0.05) and especially in the mothers of young children ages 3-8 years (-56%, p=0.005). The consistency of this result in the mothers and the children, and the high statistical significance in the young mothers, suggests that this is an important factor in ASD.

3) Phosphorus levels are consistently low (-11%, p=0.001) in children with ASD, including young children. Although the magnitude of the effect is small, it is highly statistically significant. It is unclear if this small difference would have an effect on the symptoms of ASD, but it may be a clue to some other important mechanism. Also, the autistic children with fewer ear infections tend to have less phosphous than those with many (175 vs 200 mcg/g, p=0.004, controls = 213), but the meaning of this is also unclear.

4) Pica is strongly associated with a low level of chromium (-38%, p=0.002), and moderately associated with low levels of sodium (-58%, p=0.05). This suggests that nutritional supplements of chromium, and possibly sodium, could be beneficial in the treatment of pica. Pica is also (less strongly) associated with high levels of copper, strontium, uranium, and barium, presumably due to increased consumption.

5) Low muscle tone was associated with very low levels of potassium (-66%, p=0.01), high zinc (31%, p=0.01), high levels of barium (+109%, p=0.03), and possibly high copper. This suggests that potassium levels in blood should be checked, and supplementation may be beneficial.

6) Mercury levels were not significantly different in the children with ASD, although it should be pointed out that this is long past their primary exposure to mercury (thimerosal in vaccines, maternal seafood consumption, maternal dental amalgams). Their mothers had a 57% higher level of mercury on average, but it was not statistically significant (p=0.22).

7) Age-appropriate reference ranges are important for interpreting the results of hair analysis, as we found that the levels of many toxic metals and essential minerals were quite different for the control mothers than for the control children.

All of the results discussed above should be investigated in a larger study, to confirm the findings. These findings may be significant in terms of pointing to nutritional deficiencies (especially lithium, iodine, and potassium) as a contributing factor in the etiology of autism. Dietary supplementation with those minerals may help treat the some of the symptoms of ASD in some children. Also, prenatal supplementation with lithium could possibly reduce the incidence of autism, and more investigation into maternal lithium levels in ASD is needed.

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Table 1: Toxic and Essential Elements in Children with ASD vs Controls: Percentage differences between children with ASD vs. controls are listed if they were statistically significant; otherwise "n.s." is used if the element was tested for but the difference was not statistically significant. A blank space means that the element was not tested for. In the present study, we list results for both the entire group of children (ages 3-15) and for the younger subgroup (ages 3-6).

	Shearer	Gentile	Marlowe	Wecker	Present study (all ages, then 3-6 yr only)
Sample size (autism/controls)	12/12	47/37	28/18 (14 controls were siblings)	12/8/22 (autism/pervasive disorder/controls)	51/40, 23/16
Mean age (autism/controls)	8.0/8.4	Children – age not stated	8.8/10.8	5.7/5.7/4.2	7.1/7.5, 4.6/4.6
Testing method	Atomic Absorption	Not stated	Atomic Absorption	Atomic Absorption	ICP-MassSpec
Avoidance of hair products	No?	No?	No?	Yes (3 days)	Yes (2 weeks)
Aluminum		n.s.	n.s.		-16% / -24%
Antimony		n.s.			n.s.
Arsenic			n.s.		n.s.
Beryllium			n.s.		n.s.
Bismuth					n.s.
Cadmium	-62%	n.s.	n.s.	n.s./-34%	n.s.
Lead	n.s.	n.s.	n.s.	n.s./n.s.	n.s.
Mercury			n.s.	n.s./n.s.	n.s.
Platinum					n.s.
Thallium					n.s.
Thorium					n.s.
Uranium					n.s. (see pica)
Nickel		n.s.	n.s.		n.s.
Silver					n.s.
Tin		n.s.			n.s.
Titanium		n.s.			n.s.
Calcium	n.s.	n.s.	-76%	-64%/n.s.	n.s.
Magnesium	n.s.	Higher	-69%	-82%/-84%	n.s.
Sodium		n.s.	n.s.	n.s./n.s.	n.s. (see pica)
Potassium		lower	n.s.	n.s./n.s.	n.s.
Copper	n.s.	n.s.	n.s.	-53%/n.s.	n.s. (see pica)
Zinc	n.s.	n.s.	n.s.	n.s./n.s.	n.s.
Manganese		n.s.	n.s.	-27%/-26%	n.s.
Chromium		n.s.	n.s.	-36%/n.s.	n.s. (see pica)
Vanadium		n.s.	n.s.		n.s.
Molybdenum		n.s.	n.s.		n.s.
Boron		n.s.			n.s.
Iodine					-45% / n.s. (see pica)
Lithium			n.s.	+50%/n.s.	n.s./-30%
Phosphorus		n.s.	+16%		-12% / n.s. (see pica)
Selenium			n.s.		n.s.
Strontium		n.s.			+56% / n.s. (see pica)
Sulfur					n.s.
Barium		n.s.			n.s. (see pica)
Cobalt			n.s.	n.s./-46%	n.s.
Iron		n.s.	n.s.	n.s./n.s.	n.s.
Germanium					n.s.
Rubidium					n.s.
Zirconium		n.s.			n.s.
Silicon		n.s.	n.s.		

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