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Analyses of Toxic Metals and Essential Minerals in the Hair of Arizona Children with Autism and Associated Conditions, and Their Mothers

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ABSTRACT

The objective of this study was to assess the levels of 39 toxic metals and essential minerals in hair samples of children with autism spectrum disorders and their mothers compared to controls. Inductively coupled plasma–mass spectrometry was used to analyze the elemental content of the hair of children with autism spectrum disorders (n=51), a subset of their mothers (n=29), neurotypical children (n=40), and a subset of their mothers (n=25). All participants were recruited from Arizona. Iodine levels were 45% lower in the children with autism (p=0.005). Autistic children with pica had a 38% lower level of chromium (p=0.002). Autistic children with low muscle tone had very low levels of potassium (–66%, p=0.01) and high zinc (31%, p=0.01). The mothers of young children with autism had especially low levels of lithium (56% lower, p=0.005), and the young children (ages 3–6 yr) with autism also had low lithium (–30%, p=0.04). Low iodine levels are consistent with previous reports of abnormal thyroid function, which likely affected development of speech and cognitive skills. Low lithium in the mothers likely caused low levels of lithium in the young children, which could have affected their neurological and immunological development. Further investigations of iodine, lithium, and other elements are warranted.

Index Entries: Autism; hair analysis; iodine; lithium; potassium.

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INTRODUCTION

Autism is a developmental disorder that occurs in children under 3 yr of age and involves three major areas: impaired language/communication, abnormal or stereotypic behaviors, and impaired social interactions. Its diagnosis is based solely on the observation of these symptoms, as there is no known cause or biological marker. It is a spectrum disorder, ranging from classic autism (most severe), to Pervasive Developmental Disorder Not Otherwise Specified (PDD/NOS), to Asperger's syndrome (least severe). Studies of identical twins have clearly demonstrated that both genetic and environmental factors are important, but the genes and environmental factors have not yet been identified.

Regarding environmental factors, either the exposure to toxins or lack of essential nutrients could be important. There have been suggestions that mercury or other toxic metals might play a role in the pathogenesis of autism *(1)*. Similarly, the lack of essential minerals is known by definition to cause many health problems, and a lack of them (or in some cases, an excess of them) could contribute to the etiology of autism. The amount of toxic metals and essential minerals can be assessed by blood, urine, and hair. Hair is useful for measuring metals, as they are much more concentrated in the hair. Because hair grows at a rate of 1–1.5 cm/mo, a 2- to 3-cm sample from next to the scalp can provide an average over 2–4 mo. It provides a measure of what is being transported in the body during that time, but it will not be able to detect earlier exposures. There was a recent well-publicized criticism of hair analysis studies *(2)*, but the major criticism centered on differences in results among 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 *(3)* concluded that hair is "a meaningful and representative tissue" for measuring toxic metals and selected nutrients. Similarly, the National Health and Nutrition Evaluation Study *(4)* continues to use hair as one method to evaluate levels of metals such as mercury.

In the early 1980s, there were several studies of the levels of toxic and essential minerals in the hair of children with autism *(5–8)*. Several abnormalities were reported, but the results of the studies are generally inconsistent. There was also one study *(9)* that measured only vanadium concentrations in 10 autistic adults vs 10 controls; no significant difference in vanadium levels was found. Most of these studies suffer from a relatively small sample size. Also, they did not differentiate between the children who had pica (eating nonfood 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 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 *(8)* mentioned the use of a special common shampoo, but the subjects only used it for 3 d 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.

A recent study *(10)* measured mercury levels in the blood and hair of children with autism (*n*=82; meanage =7.2 yr) compared to controls (*n*=55) living in Hong Kong. The authors found that children with autism had slightly elevated levels of mercury in their blood, but the difference was not statistically significant (*p*=0.15). They did not find a significant difference in hair mercury levels, but the levels were unusually high in both populations (2.26 and 2.07 ppm for the autism and control groups, respectively); the authors suggested that the reason for the high levels is that the "Hong Kong Chinese are famous for eating seafood."

In addition, there was a recent study *(11)* of the level of mercury in the baby hair of infants (aged 12–24 mo) who later were diagnosed with autism compared to controls (*n*=94 and 45, respectively). This study found that the autism group had one-eighth of the normal amount of mercury in their baby hair compared to controls. In the control group, the amount of mercury correlated with the mother's seafood consumption and number of mercury amalgam dental fillings, but that was not true of the children with autism, who had a low level regardless of their mother's seafood consumption or number of dental fillings, which suggests a general 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. Because 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 investigate the hypothesis that children with autism and/or their mothers had abnormal levels of toxic metals or essential minerals in their hair. This will not necessarily indicate cause and effect, but it will allow us to look for possible associations that can be further investigated.

PARTICIPANT SELECTION

This study was conducted with the approval of the Human Subjects Institutional Review Board of Arizona State University. All parents and (where possible) children signed informed consent forms. The autism participants were families of people with autism in the State of Arizona, contacted using the mailing lists of the Greater Phoenix Chapter and the Pima County (Tucson) Chapter of the Autism Society of America. The inclusion

Table 1
Characteristics of Participants

	Autism Spectrum	Controls
Number	51	40
Age (range is 3-15 years)	7.1 +/- 3.0	7.5 +/- 3.0
Number of Males/Females	39/12	30/10
Autism/PDD-NOS/Asperger's	48/2/1	n/a
Number of twin/sibling pairs	2/0	1/1
Fraction with Pica (moderate or severe)	32%	0%
Major Developmental Regression	63%	0%
Excessive Ear Infections (>8 during first 3 years)	51%	15%
Chronic Diarrhea/Constipation	63%	2%
Sleep Problems (moderate or severe)	61%	2%
Low Muscle Tone (moderate or severe)	29%	2%
Number of mothers who contributed hair samples	29	25

criteria for the children was age 3–15 yr, 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 yr 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. Table 1 lists the characteristics of the cases and controls.

The children with ASD were also divided into several subgroups based on 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 and included pica (eating of nonfood items, such as paper, sand, etc.), major developmental regression (occurring on average at 18 mo), excessive ear infections (>8 during first 3 yr of life), gastrointestinal problems (chronic diarrhea/constipation), sleep problems (moderate/severe), and low muscle tone (moderate/severe). Table 1 gives the numbers in each subgroup. Also, a subgroup of children aged 3–6 yr was analyzed, which included 23 children with autism and 16 controls.

Mothers were asked to participate as well, but their participation was optional. Only mothers who had not dyed or permed their hair within 2 mo of collecting samples were included in the data. (None of the children had had their hair permed or dyed.) A subgroup of mothers of younger children (ages 3–8 yr) was also analyzed, and it included 22 mothers of children with autism and 15 controls.

HAIR SAMPLING AND ANALYSIS

All participants (children and mothers) were asked to wash their hair for 2 wk with Johnson's and Johnson's "No More Tears" Formula Baby Shampoo, without the use of any other hair care products (no conditioner, gel, hairspray, etc.). After 2 wk, a sample of hair was cut using stainless-steel scissors. The hair sample was taken from the nape of the neck, taking the 1 in. 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 *(12).* The hair specimens were cut into approx 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 and then rinsed with acetone and allowed to drain. Samples were then rinsed three times with ultrapure 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. *(13).* After digestion, the samples were cooled and a 500-µL 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 inductively coupled plasma–mass spectrometry. 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 micrograms per gram, equivalent to parts per million.

The results are reported in Tables 2–5. Statistical analysis of the data was carried out with an unmatched *t*-test, assuming two-sided Normal distributions of unequal variance. Because a total of 39 elements were examined, only *p*-values of 0.01 or lower will be discussed, although Tables 2–5 highlight values up to p=0.05.

Table 2
Toxic Metals: Children with Autism vs Controls

	Al	Sb	As	Be	Bi	Cd	Pb	Hg	Pt	Tl	Th	U	Ni	Ag	Sn	Ti
AGES 3-15																
autism avg	17.5	0.19	0.088	0.00	0.28	0.126	0.62	0.29	0.00	0.00	0.00	0.21	0.24	0.42	0.30	1.92
control avg	20.9	0.08	0.095	0.00	0.28	0.138	0.81	0.29	0.00	0.00	0.00	0.28	0.22	2.13	0.28	1.90
ttest	**0.05**															
AGES 3-6																
autism avg	16.29	0.30	0.09	0.00	0.46	0.069	0.50	0.38	0.00	0.00	0.00	0.22	0.21	0.33	0.24	2.13
control avg	21.47	0.10	0.11	0.00	0.45	0.124	0.68	0.21	0.00	0.00	0.00	0.24	0.22	0.55	0.32	2.11
ttest	**0.04**															
PICA Subgroup																
pica avg	19.11	0.09	0.071	0.00	0.15	0.081	0.65	0.36	0.00	0.00	0.00	0.25	0.25	0.79	0.25	1.48
non-pica avg	16.69	0.23	0.095	0.00	0.34	0.15	0.60	0.26	0.00	0.00	0.00	0.19	0.23	0.25	0.33	2.13
Ttest pica vs nonpica																
ttest pica vs controls			**0.05**													
ttest np vs con.												**0.05**				
st. dev (autism)	8.13	0.58	0.05	0.00	0.61	0.21	0.63	0.41	0.00	0.00	0.00	0.16	0.23	1.05	0.55	1.41
st. dev (controls)	8.22	0.07	0.05	0.00	0.86	0.14	0.73	0.35	0.00	0.00	0.00	0.20	0.18	6.15	0.17	1.39

Note: Units are micrograms per gram. *t*-Test values are given if they statistically significant. The pica subgroup is a subgroup of the total.

Table 3
Essential Minerals: Children with Autism vs Controls

	Ca	Mg	Na	K	Cu	Zn	Mn	Cr	V	Mo	B	I	**Li**	P	Se	Sr	S	Ba	Co	Fe	Ge	Rb	Zr
AGES 3-15																							
autism avg	614	63	59	48	34.6	163	0.38	0.38	0.20	0.12	2.51	0.62	0.045	188	1.04	3.92	48,059	1.27	0.03	14	0.073	0.065	0.86
control avg	491	51	98	47	33.4	147	0.33	0.45	0.21	0.11	2.71	1.13	0.053	213	1.06	2.51	48,120	0.97	0.04	16	0.064	0.046	0.76
ttest												**0.005**		**0.001**		**0.03**							
AGES 3-6																							
autism avg	408	44	54	56	26	147	0.35	0.43	0.19	0.13	1.89	0.59	0.031	187	1.06	2.61	47,987	0.86	0.02	13.62	0.08	0.07	0.91
control avg	323	33	38	23	31	134	0.31	0.59	0.26	0.09	2.70	1.12	0.044	210	0.99	1.53	49,181	1.04	0.04	16.65	0.07	0.04	0.99
ttest													**0.04**										
PICA Subgroup																							
pica avg	836	85	41	40	55	177	0.43	0.28	0.20	0.13	1.60	0.64	0.052	185	1.07	5.35	46663	1.93	0.03	13.98	0.06	0.07	0.79
non-pica avg	512	53	67	52	25	156	0.35	0.42	0.20	0.11	2.92	0.61	0.041	189	1.03	3.26	48697	0.96	0.03	14.04	0.08	0.07	0.90
Ttest pica vs nonpica					**0.03**			**0.004**									**0.02**	**0.03**					
ttest pica vs controls			**0.05**					**0.002**				**0.04**		**0.01**		**0.02**		**0.04**					
ttest np vs con.												**0.01**		**0.01**									
st. dev (autism)	556	67	64	82	39	54	0.26	0.21	0.16	0.14	3.08	0.48	0.04	31	0.27	3.74	2964	1.22	0.03	5.67	0.07	0.10	0.48
st. dev (controls)	369	50	163	99	31	41	0.19	0.32	0.15	0.14	2.27	1.02	0.04	36	0.43	2.09	3299	1.29	0.04	9.45	0.01	0.05	0.46

Note: Units are micrograms per gram. *t*-Test values are given if they were statistically significant. The pica subgroup is a subgroup of the total.

Table 4
Toxic Metals: Mothers of Children with Autism vs Controls

	Al	Sb	As	Be	Bi	Cd	Pb	Hg	Pt	Tl	Th	U	Ni	Ag	Sn	Ti
average autism	13.4	0.05	0.06	0.00	0.21	0.07	0.89	0.55	0.00	0.00	0.00	0.25	0.28	1.77	0.31	1.06
average controls	10.6	0.04	0.06	0.00	0.36	0.06	0.60	0.35	0.00	0.00	0.00	0.32	0.22	0.41	0.31	1.76
ttest																
ages 3-8																
avg autism	10.7	0.05	0.06	0.00	0.24	0.06	0.53	0.48	0.00	0.00	0.00	0.24	0.22	1.43	0.33	0.97
avg control	11.1	0.03	0.05	0.00	0.15	0.04	0.58	0.44	0.00	0.00	0.00	0.36	0.23	0.18	0.32	1.77
ttest																
St.Dev. (autism)	10.5	0.04	0.05	0.00	0.59	0.06	1.68	0.65	0.00	0.00	0.00	0.18	0.22	5.50	0.25	0.66
St.Dev. (controls)	4.1	0.03	0.04	0.00	0.97	0.05	0.52	0.48	0.00	0.00	0.00	0.22	0.15	1.16	0.24	2.27

Note: Units in micrograms per gram. *t*-Test values are blank because none were statistically significant.

Table 5
Essential Minerals; Mothers of Children with Autism vs Controls

	Ca	Mg	Na	K	Cu	Zn	Mn	Cr	V	Mo	B	I	**Li**	P	Se	Sr	S	Ba	Co	Fe	Ge	Rb	Zr
average autism	1,620	136	75	38	32	197	0.24	0.35	0.17	0.05	1.35	1.55	**0.041**	195	1.88	9.59	46,397	2.74	0.04	13.0	0.06	0.04	0.74
average controls	1,373	100	162	34	21	181	0.16	0.30	0.12	0.05	1.75	1.08	**0.067**	203	0.99	7.27	44,964	1.53	0.04	10.2	0.06	0.05	0.65
ttest													**0.04**										
ages 3-8																							
avg autism	1,534	120	84	46	28	191	0.23	0.35	0.15	0.05	1.19	1.50	**0.038**	189	2.11	8.80	45,104	2.13	0.04	11.3	0.06	0.05	0.70
avg control	1,767	113	177	34	26	180	0.18	0.31	0.14	0.05	1.40	1.16	**0.085**	203	1.02	9.26	44,427	1.96	0.04	10.3	0.06	0.05	0.62
ttest													**0.005**										
St.Dev. (autism)	986	106	118	68	29	74	0.20	0.27	0.13	0.02	1.60	1.69	**0.03**	53	3.16	6.18	4034	2.79	0.03	10.2	0.01	0.06	0.63
St.Dev. (controls)	1232	94	231	50	12	47	0.11	0.09	0.14	0.02	1.37	0.93	**0.06**	32	0.73	7.56	3839	1.70	0.04	3.6	0.01	0.05	0.51

Note: Units in micrograms per gram. Only the Li values were statistically significant.

LIMITATIONS OF THE PRESENT STUDY

This study has several limitations, including the following:

1. Sample size: A larger sample size, from multiple sites, is needed to improve the statistical power of this study and validate or refute its findings.
2. Sample bias for cases: The participants represented 51 families out of approx 1000 in the Arizona area who were contacted by mail. The reason for the modest participation rate was that the study included several other parts [reported in a separate article, *(14)*] and a modest participation rate is typical for medical studies that require substantial time commitment. Because participants generally did not know their elemental status prior to the study, this was probably a minor effect.
3. Sample bias for controls: The controls were chosen from the friends and neighbors of the cases, which allows for an easy way to obtain a reasonable match of geographic location and socioeconomic status, but is not the most rigorous method.
4. Prevalent vs incident cases: This study involves prevalent cases, not incident ones, so the results are not necessarily indicative of mineral status during the development of autism.
5. Autism severity: This study did not independently evaluate the severity of autism. It would be interesting to determine if the severity of certain symptoms of autism correlated with hair levels.
6. Contamination: Contamination of hair samples is always a concern. This study involved washing with a common shampoo and washing by the lab, which reduces those concerns. With a large enough sample size, contamination of samples should be fairly similar for the autism and control groups.
7. 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.

RESULTS

Toxic Metals in Children with Autism

As shown in Tables 2 and 3, none of the children with autism had abnormal levels of toxic metals with a p-value of 0.01 or less. Some possibly abnormal levels (p=0.05) are listed in Tables 2 and 3 and a larger study is needed to investigate those.

Toxic Metals in the Mothers

In the mothers of children with ASD, there was no statistically significant difference in the level of heavy metals in their hair.

Essential Minerals in the Children with Autism

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 yr was considered, the magnitude of the difference was almost identical (47%), although the difference was not statistically significant because of the smaller number of children in the subgroup.

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 yr was considered, the magnitude of the difference was almost identical (11% lower), although the difference was not statistically significant because of the smaller number of children in the subgroup.

Lithium: In the subgroup of children aged 3–6 yr, 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 (aged 3–15 yr), the difference was less (15%) and it was not statistically significant.

Chromium: The pica subgroup had much less chromium than the nonpica subgroup (0.28 vs 0.42, p=0.004) or the typical children (0.28 vs 0.45, p=0.002).

In terms of other subgroups, there were some differences in levels of essential minerals:

1. For the regression and gastrointestinal subgroups, there were no differences that reached a statistical significance of p=0.01 or lower.
2. Sleep: The autistic children with sleep disorders had lower levels of selenium than the autistic children without sleep disorders (0.99 vs 1.19 PPM, p=0.007, with controls, p=1.06 PPM).
3. Ear infections: The autistic children with fewer infections had slightly lower levels of phosphorus than the autistic children with more infections (175 vs 200 PPM, p=0.004, controls p=213 PPM).
4. Muscle tone: The autistic children with low muscle tone had very low potassium compared to the autistic children with normal muscle tone (16 vs 61 PPM, p=0.01, vs controls =47 PPM) and high zinc (193 vs 150 PPM, p=0.01, controls =147 PPM).

Essential Minerals in the Mothers

The only statistically significant differences in the levels of essential minerals between the mothers of children with ASD and mothers of typical children was in their lithium levels. 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). When the subgroup of mothers of children aged 3–8 yr was considered, the difference was more pronounced (56% lower) and more statistically significant (p=0.005).

DISCUSSION

Toxic Metals in the Children with Autism

Overall, it appears that the children with autism do not have major differences in their levels of toxic metals compared to controls. Because mercury toxicity has been suggested as a cause of autism, it is worthwhile to note that the autistic children in this study had levels that were very similar to those of the typical children. In terms of the validity of our testing, it should be pointed out that the mean values we found for the typical children aged 3–6 yr (0.21 μg/g) are similar but somewhat higher than those of the 1999–2000 NHANES study *(4)* of 838 children aged 1–5 yr (0.12 μg/g). In terms of our results, our finding of similar values of mercury for autistic children and controls is consistent with the study by Ip et al. *(10)*, which found similar (albeit much higher) levels in the autism and control children in Hong Kong. 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.

Thus, our results are not necessarily inconsistent with the results of Holmes et al. *(11)*, which found unusually low levels in baby hair, as the ages of their group (12–24 mo) are quite different than ours (age 3–15 yr). 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 higher use of oral antibiotics (for ear infections) in children with autism as we found here, as Rowland et al. *(15)* showed that oral antibiotics dramatically inhibit mercury excretion to one-tenth of normal in rats.

Toxic Metals in the Mothers

Overall, the mothers of children with autism did not have any statistically significant differences in the level of toxic minerals in their hair. Because 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 mean values we found for the typical mothers (0.35 µg/g) were similar but somewhat higher than those of the 1999–2000 NHANES study *(4)* of 1726 women of age 16–49 yr (0.20 µg/g). Because the average value of the autism mothers is somewhat higher than that of the controls in our study and the sample size is small, a larger study might be warranted.

Essential Minerals in Children with Autism

Iodine: The low levels of iodine in the hair of children with autism suggests that iodine could be important in the etiology of autism, presumably through its effect on thyroid function. There have been several studies of thyroid function and autism, including a report of a high incidence of thyroid abnormalities in parents of children with autism *(16),* abnormal thyroid function in young adults with severe autism correlating with impaired verbal communication *(17),* and reduced thyroid-stimulating hormone (TSH) levels in children with autism (n=41) vs controls *(18).* There was also one small study (n=14) that found normal levels of TSH in children with autism, but that study did not have a control group *(19).* Overall, the reports of abnormal thyroid function in most of the studies are consistent with our findings of low iodine, and it is possible that impaired thyroid function is a cause of some of the symptoms of autism, especially language impairment and mental retardation.

Iodine deficiency was extremely common in parts of the United States in the early 1900s and caused many cases of goiters (enlarged thyroid) and cretinism (a form of mental retardation resulting from iodine deficiency). This prompted the federal government to encourage the addition of iodine to salt (iodinized salt). According to Hollowell et al.'s analysis *(20)* of the NHANES surveys I and III, average iodine levels in the United States (measured in the urine) have declined more than 50% during the 20-yr period from 1971 to 1974 to 1988–1994, presumably resulting from decreased use of table salt (which is one of the major sources of iodinized salt). It is possible that the decreasing level of iodine in the United States is causally related to the large increase in autism during the last 20 yr.

One study found that iodine levels in hair did increase after exposure to iodine, but the hair was also susceptible to external contamination (such as from sweat) and loss of iodine because of washing *(21).* In the present study, all samples were washed in the same manner, so external contamination effects were minimized; thus, the results should be reasonably reflective of excretion rates of iodine. Future studies should use blood, urine, or saliva for increased reliability.

Lithium: In the subgroup of children aged 3–6 yr, 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 (aged 3–15 yr), the difference was less (15%) and it was not statistically significant. Low lithium was the only statistically significant finding in the mothers of children with autism, so we hypothesize that low levels in the mothers was the cause of low lithium in the young children, which tended to normalize as they grew older. Anke et al. *(22)* found that hair is a reliable method to assess lithium deficiency in goats, in agreement with measurements of blood, milk, and several other organs. Lithium concentrations are highest in the brain *(23)* and are highest during the first trimester *(24)*, so a deficiency of it during pregnancy could adversely affect fetal development and especially brain development. Also, low levels of lithium in humans have been found to correlate with a wide range of behavioral problems, including aggression and decreased sociability *(25–27)*. One placebo-controlled treatment study by Schrauzer and de Vroey *(28)* found that low-dose supplementation (400 μg/day) was beneficial to drug addicts, resulting in increases in the subcategories of happiness, friendliness, and energy. It should be noted that lithium is also used at dramatically higher doses (of the order of 500,000 μg/day) as a psychiatric medication for bipolar disorder.

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. We hypothesize that the low levels of lithium in the ASD mothers result in lower levels in their children, which might explain why the children suffer from a higher level of ear infections in their first 3 yr of life. In turn, higher level of ear infections results in higher oral antibiotic use, which results in a temporary decrease in the ability to excrete mercury 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.

Phosphorus: The significance of the slightly lower phosphorus level in children with autism is unclear. It might be an indicator that children with autism tend to consume foods with lower nutritional content (less fruits and vegetables).

Chromium: The finding of low chromium in the pica subgroup of autistic children was highly statistically significant. However, it is unclear if it is a cause of pica or the result of it. Low levels of iron and zinc have previously been reported as being associated with pica, possibly as a cause of it *(29)*.

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 because of increased consumption.

For the subgroup with low muscle tone, the finding of low potassium was large and statistically significant. Potassium is needed for muscle contractions and is released during periods of activity. Low potassium in the hair is an indication of low muscle activity. The low muscle activity could be the result of low potassium in the body overall, but hair measurements are inconclusive regarding this point.

For the other subgroups, the differences in essential minerals were generally minor.

Essential Minerals in the Mothers

The very low level of lithium in the mothers of children with autism is interesting because that was the only abnormal finding in the mothers. The significance of this is discussed in the preceding subsection.

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