Hair Minerals, Diet and Behavior of Prader-Willi Syndrome Youth

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Abstract

This study investigated the relationships between mineral elements and Prader-Willi syndrome (PWS) and determined which minerals, if any, separated a group of PWS individuals (N= 19) from a non PWS mentally retarded control group (N = 60). The PWS group had significantly raised hair-magnesium levels and significantly lower hair-silicon levels than controls. The PWS group was also elevated in hair-calcium, magnesium, and copper in relation to laboratory standards, while their hair silicon, chromium and lithium levels were deficient in relation to laboratory norms. Decreasing hair-chromium and increasing hair-copper levels correlated with various nonadaptive behaviors characteristic of PWS. Discriminant function analysis revealed that by using 16 hair-minerals subjects could be correctly classified as PWS or non PWS with 89.5 percent and 95.0 percent accuracy respectively. It is concluded that continuing research is needed to study the relationship between mineral element patterns and PWS.

Prader-Willi Syndrome (PWS) was described in 1956 by Doctors A. Prader, A. Labhart, and H. Willi. It is considered an uncommon condition resulting from a birth defect. The pattern of characteristics associated with PWS is remarkably consistent among those affected by it. PWS is believed to be among the five most common syndromes in most birth defect clinics.

According to the Prader-Willi Syndrome Association (1984), features considered essential for a diagnosis of PWS include hypotonia, feeding problems, mild dysmor-phism and delayed motor development. Childhood features include obesity/hyper-phagia, intellectual impairment/school problems, and abnormal pubertal development/ hypogonadism. The significant manifestations of PWS are abnormal growth (short stature), hypotonia (lack of muscle tone, size and strength), hypogonadism (under developed or small genitals, lack of complete sexual development), and central nervous system performance dysfunction (some degree of mental retardation in 80-95% of the cases, insatiable appetite that leads to obesity, if uncontrolled.)

Other characteristics are small hands and feet, which may be puffy in appearance, and a narrow bifrontal forehead diameter. Somnolence (excessive sleepiness) is frequently reported by parents even in children who are not obese. Reduced sensitivity to pain and scratching and picking at sores or insect bites is common. Strabismus and myopia are also common. Some individuals develop diabetes, heart problems, and scoliosis. Personality problems develop in late adolescence. Temper tantrums, stubbornness, and depression are typical, and may be part of the CNS dysfunction.

The etiology of PWS is largely unknown and at the present time there is no known cure for this syndrome. Abnormal mineral metabolism can cause a variety of illnesses (Underwood, 1977), and scalp hair has been proposed as a convenient sampling tissue for screening an individual's burden of certain minerals (Laker, 1982, Passwater and Cranton, 1983). Minerals comprise five percent of the molecular composition of our bodies and serve a wide range of functions. They may be crucial for the functioning of the central nervous system or unwanted and toxic. Additionally, there are many minerals of hypothesized yet undemonstrated value or detriment.

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The first purpose of this research was to determine if concentrations of certain minerals in the hair of PWS individuals were different from a control group. The second purpose was to determine the relative importance of each mineral to the discrimination of the two groups. A third purpose was to examine the daily nutrient intake of PWS individuals. A fourth purpose was to correlate hair minerals with the behavior of PWS youth.

METHOD

Subjects

Parent members of the Prader-Willi Syndrome Association in Wyoming and Minnesota were contacted and asked to allow their PWS child to participate in the research. Nineteen parents consented resulting in a PWS sample population of 19. The mentally retarded control subjects were randomly drawn from public schools and group homes in Wyoming. Case histories/ school records of the control subjects were surveyed to determine whether there was a known or highly probable medical reason for their retardation. Only subjects with unremarkable medical histories for mental retardation were included in the study (N = 60). None of the controls were diagnosed as PWS.

The mean age of the PWS subjects was 21.68 ± 7.68 and the mean of the mentally retarded "etiology unknown" control group was 22.08 ± 15.99 (p = NS). There were 10 males in the PWS group compared to 40 males in the control group (p = NS). All 79 subjects in the study were Caucasian.

The mean social class of the PWS was 2.2 (Hollingshed and Redlich, 1958) compared to a mean social class of 2.9 in the controls (p = NS). One of the PWS individuals was reported to engage in pica, the behavioral habit of ingesting inedible materials, such as paper or plaster, while four of the controls had histories of pica. Pica has been shown to substantially contribute to increased metal burdens.

Classification of Hair Mineral Levels

Every part of the human body contains at least a few atoms of every stable element in the periodic table. Although a large number of these elements are found in detectable amounts in human tissue, blood, and urine, hair, in particular, contains a higher concentration of many of these elements. Trace elements are accumulated in hair at concentrations that are generally higher than those present in blood serum, provide a continuous record of nutrient-mineral status and exposure to toxic minerals, and may serve as a probe of physiologic functions (Passwater and Cranton, 1983). Scalp hair has several characteristics of an ideal tissue for epidemiologic study — namely that it is painlessly removed, normally discarded, easily collected, and its contents can be easily analyzed.

Hair samples (about 400 mg) were collected from the nape of the children's neck, as close to the scalp as possible by the senior author. The hair samples were then submitted to Doctor's Data, Inc., a state and Center for Disease Control licensed laboratory in West Chicago, where they were analyzed with three instruments (the atomic absorption spectrophotometer, the graphite furnace, and the induction coupled plasma torch) to determine 6 toxic mineral levels and 15 nutrient mineral levels. The six toxic mineral levels tested for were: lead, mercury, cadmium, aluminum, nickel, and beryllium; whereas the 16 nutrient mineral levels tested consisted of: calcium, magnesium, sodium, potassium, copper, zinc, iron, manganese, chromium, phosphorous, molybdenum, silicon, lithium, cobalt, and vanadium.

Laboratory techniques used to assure the reliability of results and to meet reproducibility requirements included:

1. A blind sample was run from the initial steps through the entire procedure to assure reproducibility of methods;
2. At least one of every three tests was a standard. Working standards were made to assure proper values;
3. The in-house pool was completely remade and analyzed monthly to eliminate the possibility of precipitating elements and to assure reproducibility;
4. Temperature and humidity were controlled to assure reliability and consistency of testing instruments;
5. The hair samples were weighed to the thousandths of a gram (.001g is equal to approximately four hairs), 1 inch (.0254 m long); and only volumetric flasks, the most

accurate available, were used for diluting the ashed sample;

6. Lot-number control sheets for all reagents were used to assure uniformity, and records were kept and available for inspection;
7. All glassware was acid washed in-house before use and between each use, including acid prewashed disposable test tubes;
8. The water used was virtually mineral free, rated at 18+ MEG; and
9. Upon receipt, the hair sample was washed thoroughly with deionized water, a non-ionic detergent, and an organic solvent to remove topical contaminants.

Reports summarizing the significant findings of the hair analysis for each subject were received from Doctor's Data, Inc. Findings consisted of two main sections. The first summarized the significant findings related to the 15 nutrient-mineral levels. These findings were based on a statistical comparison of the levels determined in the present analysis with those observed in a normal population. The second dealt with the six toxic minerals. This section listed both the observed hair level and suggested upper limit for each toxic mineral and plotted each level in relation to the upper limit.

Food Intake Evaluation

A 24-hour diet record was completed for each PWS subject. For institutionalized subjects, a staff dietitian completed the records for each subject. Subjects were each assigned meals with an estimated calorie intake to control for weight according to their sex and age. The dietitian was asked to record items served for the subject's meals and snacks. The amount of food and method of preparation was recorded by the dietitian. Subjects ate all food given to them.

For non-institutionalized subjects, the senior author interviewed the subject's parent or person responsible for meal preparation by a 24-hour recall. Food eaten, amount, and method of preparation were recorded.

Diet records from both the institutionalized and non-institutionalized subjects were analyzed by a computer program using nutrient values from U.S.D.A. Handbook No. 72 (Adams and Richardson, 1981).

PWS Behavior Rating Scale

An informal 12-item behavior rating scale was developed by the senior author which listed nonadaptive behaviors associated with PWS. The twelve items were (1) slow moving, (2) stubborn, (3) sleepy, (4) belligerent (food related), (5) irritable; (6) impulsive, (7) lazy, (8) belligerent (non-food related), (9) anti-social with peers, (10) moody, (11) antisocial with family, and (12) physically aggressive. Each PWS subject was rated by a primary caretaker, e.g., mother, group home staff member, on each of the 12 items using a one to four point scale with "1" denoting never observed, "2" denoting rarely observed, "3" denoting sometimes observed, and "4" denoting frequently observed.

RESULTS

The two groups were compared for hair mineral concentrations. As shown in Table 1, the PWS group's mean hair concentration of magnesium was significantly higher than the control group, while the PWS group's mean silicon, cadmium, aluminum, and nickel levels were considerably below that of the controls. The data were analyzed with the t-test for two independent samples design of SPSS (Nie, Hull, Jenkins, Steinbrenner, and Bent, 1975), yielding significant **t** values for magnesium ($t = 2.07, 77, p. < .05$), silicon ($t = -1.98, 77, p. < .05$), cadmium ($t = -2.99, 77, p. < .01$), aluminum ($t = -2.85, 77, p. < .01$), and nickel ($t = -3.44, 77, p < .01$). The results of the t tests on the other nutrient and toxic elements were nonsignificant.

As also shown in Table 1, the PWS group's mean hair magnesium, calcium and copper levels were more than one standard deviation above the theoretical normal range established by Doctor's Data, Inc. (1985), while their mean hair silicon, chromium, and lithium levels were more than one standard deviation below the theoretical normal range of Doctor's Data, Inc. The control group's mean hair cadmium level was above the normally tolerated limit established by Doctor's Data, Inc. (1985)

A discriminant analysis was then performed using a program from SPSS (Nie et al., 1975). The stepwise method using Wilks, Lambda was employed to ascertain the relative contributions of the 21 minerals to

Table 1
Results of Trace Mineral Analysis Mean S.D.

Trace Mineral	PWS Group (ppm)	Mentally Retarded Control Group (ppm)	Normal Range[a] (ppm)
Calcium	1585.360 ± 1936.56	1326.65 ± 3894.38	503.000 - 1436.000
Magnesium	183.570 ± 256.34*	82.30 ± 158.61	40.000 - 158.000
Sodium	72.840 ± 106.22	84.00 ± 134.12	32.000 - 136.000
Potassium	24.100 ± 39.64	26.51 ± 44.82	12.000 - 106.000
Copper	103.780 ± 136.25	53.86 ± 158.82	13.000 - 81.000
Zinc	173.780 ± 58.05	178.65 ± 153.22	125.000 - 183.000
Iron	12.210 ± 11.92	15.56 ± 17.13	8.700 - 17.000
Manganese	1.320 ± 1.34	2.13 ± 2.48	0.298 - 1.540
Chromium	0.590 ± 0.02	0.81 ± 0.72	0.593 - 1.270
Cobalt	0.260 ± 0.13	0.48 ± 0.16	0.139 - 0.352
Lithium	0.001 ± 0.003	0.05 ± 0.13	0.012 ± 0.084
Molybdenum	1.050 ± 0.26	1.56 ± 1.04	0.180 - 1.900
Phosphorus	144.470 ± 30.53	133.05 ± 91.85	115.000 - 209.000
Silicon	0.630 ± 0.61*	1.35 ± 1.53	4.570 - 12.000
Vanadium	0.150 ± 0.09	0.50 ± 1.60	0.030 - 0.200
Lead	2.840 ± 3.70	9.33 ± 14.70	15.000[b]
Mercury	1.240 ± 0.56	1.48 ± 1.51	2.500[b]
Cadmium	0.360 ± 0.24**	1.64 ± 1.85	1.000[b]
Aluminum	3.780 ± 5.66**	10.55 ± 9.80	30.000[b]
Nickel	0.450 ± 0.26**	0.96 ± 0.62	2.200[b]
Beryllium	0.010 ± 0.01	0.04 ± 0.99	0.100[b]

a Theoretical Normal Range (+ ISD) established by Doctor's Data, Inc. (1985) b Normally tolerated limit established by Doctor's Data, Inc. (1985) * p < .05 by two tailed test. ** p < .01 by two tailed test.

the separation of the groups.

The combination of nickel, magnesium, cadmium, calcium, phosphorus, lead, mercury, molybdenum, potassium, manganese, copper, aluminum, silicon, and beryllium in order of entry into the discriminant function significantly separated the PWS and control groups (F 16, 63 = 7.75, $p<.001$). As shown in Table 2 each of these minerals contributed uniquely over and above the previously entered minerals to the discrimination between the groups. On the basis of the discriminant function 89.5% of the PWS subjects and *95%* of the controls were correctly classified. These percentages are optimistic however, since the function was applied to the data that produced it. A cross-validation of the discriminant function is expected to result in somewhat smaller percentages.

As shown in Table 3, nutrient intake data revealed that subjects from both the institutionalized and non-institutionalized settings consumed a relatively low fat diet.

Generally, Americans consume 40% of their calories as fat. The American Heart Association recommends an intake of 30-35% calories as fat. Subjects from both settings appeared to consume adequate amounts of calcium, phosphorus, vitamins Bl, B2 and B12 according to the Recommended Daily Allowance (RDA, 1980). Sodium and potassium intakes were well within the Estimated Safe and Adequate range (RDA, 1980). However, the remaining vitamins and minerals were well below the RDA except for vitamin A in non-institutionalized subjects, which had one in-

Table 2
Summary of the Stepwise Discriminant Analysis

Step	Mineral	Λ^{a}	R^{2b}	ΔR^{2c}	F^{d}	d^{e}
		.86	.14	.14	11.85*	1,77
		.75	.25	.11	12.53*	1,76
		.66	.34	.09	12.36*	1,75
		.64	.36	.02	10.32*	1,74
		.60	.40	.04	9.33*	1,73
		.58	.42	.02	8.44*	1,72
		.55	.45	.03	8.10*	1,71
		.49	.51	.06	9.10*	1,70
		.46	.54	.03	8.89*	1,69
		.44	.56	.02	8.58*	1,68
		.42	.58	.02	9.19*	1,67
		41	.59	.01	8.71*	1,66
		39	.61	.02	8.58*	1,65
		37	.63	.01	8.16*	1,64
		37	.63	.00	8.41*	1,63
		36	.64	.01	8.05*	1,62

Note: N = 79
a Wilks lambda (A = 1-R^2)
b Squared multiple correlation
c Incremental increase in R contributed by the variable entered at that step
d Test of the significance of the variable entered at that step
e Degrees of freedom
*** F<.01**

dividual consuming a large amount of vitamin A. For institutionalized subjects, zinc intake was below 50% of the RDA of 15 mg/d, and for the non-institutionalized subjects, zinc intake was less than 2/3 of the RDA. Likewise, iron intakes were marginal for both institutionalized and non-institutionalized subjects. Folic acid intake was about 1 /3 of the RDA for institutionalized subjects (RDA = 400 ug for folic acid), and 3/4 of the RDA for non-instutionalized subjects.

Table 4 contains the results of the PWS Behavior Rating Scale. SPSS partial correlations, controlling for age and sex, were run between elements upon which the PWS subjects were elevated (magnesium, calcium, and copper) or depressed (chromium, silicon, and lithium) in relation to laboratory norms and the 12 items of the behavior rating scale. Significant correlations occurred between chromium and sleepy (-.40, p<.05), belligerence-food related (-.51, p < .05), irritable (-.32, p< .10), impulsive (-.37, p< .10), and anti-social with peers (-.34, p< .10). Decreasing lithium correlated with belligerence-food related (-.37, p<.10), while increasing copper correlated with impulsive (.33, p <.10), moody (.33, p< .10), and anti-social with family (.40, p < .05). Increasing calcium correlated with moody (.40, p<.10); all other correlations were nonsignificant.

DISCUSSION

The value of the above study is that it contains the baseline concentrations of some nutrient and toxic minerals in the hair of PWS individuals. The literature on the subject contains no data for direct comparison.

Although the number of PWS subjects in

Table 3
Daily Nutrient Intake of Prader-Willi Subjects for both Institutionalized and Non-Institutionalized Subjects

Nutrient	**Institutionalized (N = 15)**			**Non-Institutionalized** (N = **4)**		
	Mean	S.D.	**Range**	**Mean**	S.D.	Range
Kcal	1568	360	1171-2326	1819	158	1636-2018
Protein (%Kcal)	17.9	1.9	17-20	18.8	1.7	17-21
Carbohydrate (VoKcal)	55.4	4.3	48-60	50.3	8.3	42-61
Fat (%Kcal)	26.7	2.7	23-32	30.8	9.0	20-40
Sodium (mg)	3020	668	2373-4437	1748	243	1547-2041
Potassium (mg)	3150	480	2607-4264	3724	1269	2688-5524
Calcium (mg)	1197	219	958-1560	1098	270	796-1344
Phosphorus (mg)	1411	358	1063-2078	1572	365	1215-2075
Magnesium (mg)	252	34	214-327	215	35	183-261
Iron (mg)	10.6	2.6	7.9-16.2	12.6	2.6	10.8-16.4
Zinc (mg)	6.3	1.2	5.2-8.7	9.0	1.1	8.1-10.7
VitA(I.U.)	3265	584	2661-4542	6459	4464	3242-13050
VitD(I.U.)	261	53	206-309	292	86	179-361
Vit E (mg)	4.1	0.8	2.6-6.0	13.1	15.1	3.4-35.6
Vit C (mg)	5.5	23	54-83	157	44	103-203
VitBl(mg)	1.4	0.4	1.1-2.3	1.5	0.7	1.1-2.5
Vit B2 (mg)	1.8	0.4	1.4-2.4	2.1	0.2	1.8-2.2
Vit B3 (mg)	15.0	4.7	10.7-24.6	19.8	3.8	16.8-25.2
Vit B6 (mg)	1.4	0.3	1.0-2.0	2.2	0.9	1.4-3.4
Folic acid (ug)	137	19	112-179	301	84	236-425
VitB12(ug)	3.8	0.8	3.0-5.0	4.4	1.3	2.9-6.1

Table 4
Results of PWS Behavior Rating Scale Mean S.D.

1. Slow Moving	2.38	±	0.78
2. Stubborn	2.94	±	0.80
3. Sleepy	2.83	±	0.78
4. Belligerent (food related)	2.61	±	0.85
5. Irritable	2.50	±	0.85
6. Impulsive	2.77	±	0.87
7. Lazy	2.72	±	1.01
8. Belligerent (non-food related)	2.11	±	0.96
9. Anti-social with peers	1.83	±	0.92
10. Moody	3.16	±	0.61
11. Anti-social with family	3.33	±	0.76
12. Physically aggressive	2.61	±	0.85

the present study was somewhat small, there were several interesting findings. First, the PWS subjects' mean hair-magnesium level was significantly higher than in the control group and was more than one standard deviation above laboratory norms. Signs and symptoms of elevated magnesium include hypotonia, central nervous system depression, somnolence and listlessness, and reduction of sensitivity to pain (Venugopal and Luckey, 1982). Each of these symptoms are also features of PWS.

The PWS group was also elevated in hair copper and hair calcium in relation to laboratory norms. Like increased magnesium, increased hair-copper has been linked to central nervous system depression (Pfeiffer, 1974). Elevated hair copper has also been linked to idiopathic scoliosis, (Pratt and Phippen, 1980), a condition common to PWS (Holm and Laurnen, 1981).

According to Bland (1979), in the absence of excessive dietary intake elevated hair calcium levels lead to a clinical suspicion of metabolic deposits in soft and connective tissues of the body. This is the process associated with osteoporosis, and osteoporosis due to lack of puberty development is found in some PWS individuals (Prader-Willi Syndrome Association, personal communication).

The PWS group was significantly lower than controls and more than one standard deviation below laboratory norms in hair-silicon. Hair silicon deficiency in animal studies is associated with retarded growth, abnormalities in skeletal development, and abnormalities in tooth-enamel development (Passwater and Cranton, 1983). Each of these features is also present in PWS.

The PWS group was also deficient in hair-lithium and hair-chromium in relation to laboratory norms. Although the clinical significance of hair-lithium levels is largely unknown, decreased hair-lithium has been associated with central nervous system dysfunctions (Phil, Drake and Vrana, 1980).

Decreased chromium uptake has been linked to heart disease and diabetes, conditions common to PWS. Adequate chromium is also required for the proper use of blood sugar to produce energy, and PWS individuals are frequently described as unenergetic.

The mentally retarded "etiology unknown" group was significantly higher in hair concentrations of nickel, aluminum, and cadmium. Their mean hair-cadmium level was above the normally tolerated limit established by laboratory norms. These data support other recent studies that have correlated increased hair metal levels with mental retardation (Marlowe, Folio, Hall and Errera, 1982; Marlowe, Errera and Jacobs, 1983). Studies of general school populations have also linked increasing hair-metal levels and their interactions to decrements in psychometric intelligence (Thatcher, Lester, McAlaster, and Horst, 1982; Moon, Marlowe and Errera, in press), adaptive behavior (Marlowe et al., 1985), and academic achievement (Moon, Marlowe, Stellern and Errera, 1985).

Diet records represent only one day's eating pattern and may not be representative. However, the day chosen was random and perhaps typical of the eating pattern. A cause for concern is the low levels of diet zinc in both the institutionalized and non-institutionalized subjects. Zinc is important for proper taste sensations, growth and development, reproductive functions, and epidermal integrity (Guthrie, 1983). Since many of the subjects would tend to have problems in these areas, proper zinc nutriture is important. The best sources of diet zinc include seafood and meat. Cereals and legumes are also good sources. Increased meat in the diet would also increase the amount of dietary iron, which was marginal in the subjects studied.

The correlations between element levels and the PWS behavior rating scale must, of course, be viewed with considerable caution, given the very small sample size (N=19). Nevertheless, the direction of the relationships between chromium and copper and the various behavioral items are biologically plausible and warrant further investigation.

Prior to summarizing the findings, it must be reiterated that hair element analysis is only a screening test and not a precise method of determining what is occurring in the body. Suspicions raised on hair analysis must always be confirmed by more precise and scientifically validated forms of testing before an exact diagnosis can be made.

Twenty-one hair elements were examined here. According to Passwater and Cranton's (1983) review of the literature, hair elements of proven clinical significance determined in this study are calcium, magnesium, zinc, copper, cadmium, chromium, nickel, lead and mercury. Hair elements suggested to have possible clinical significance are sodium and potassium, while the other hair elements determined here have an unknown clinical significance because of an absence of scientific data.

In summary, a discriminant function analysis revealed that by using 16 hair minerals subjects could be correctly classified as PWS or non PWS with 89.5 percent and 95.0 percent respectively. Stepwise discriminant analysis revealed that nickel accounted for 13 percent of the variance between the two groups, magnesium accounted for an additional 11 percent, cadmium another 9 percent, and calcium 2 percent. Overall, the 16 elements that were entered into the discriminant function accounted for about 65 percent of the variance in the two groups. There are a variety of possible theories as to why this occurred. We simply present this evidence to encourage others to examine mineral metabolism in Prader-Willi syndrome.

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