Efficacy of Hair Mineral Analysis for Assessing Zinc Status

by

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ABSTRACT OF THE THESIS

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The objective of this research is to seek information on the efficacy of hair mineral analysis for assessing zinc status by determining the concentrations of zinc in hair from two anatomical locations, scalp and pubis, and from two keratinized tissues, hair and nail. This was accomplished using hair and nail samples from several individuals and analyzing those samples using atomic absorption spectroscopy. The analysis of the zinc concentration in scalp hair compared to the zinc in pubic hair yielded a p-value of 0.0471, a Pearson's correlation coefficient of 0.301, a linear regression r^2 value of 0.0904 using GraphPad, and a t value of 2.01. The analysis of the zinc concentration in scalp hair compared to the zinc concentration in finger nail yielded a p-value of 0.766 using GraphPad, a Pearson's correlation coefficient of 0.108, a linear regression r^2 value of 0.0117, and a t value of 0.307. The statistical analysis does not support a linear or non-linear correlation between the level of zinc in human scalp hair and human finger nail and does not support the existence of a linear correlation but indicates a possible non-linear relationship zinc concentrations in scalp hair and pubic hair. The existence of this non-linear relationship could indicate the presence of a regulated metabolic process for the deposition of zinc in these two keratinized tissues.

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INTRODUCTON

Since the middle of the 20th Century, the determination of trace element levels in human scalp hair has become increasingly popular for monitoring environmental exposures, evaluating heavy metal poisonings, assessing nutritional status and diagnosing diseases. Blood and urine analysis are the more traditional approach to evaluating trace element levels in the human body, but the biokinetics as well as changing environmental factors often result in fluctuating trace element concentrations in blood and urine. Hair, on the other hand, is considered by some to be a metabolic end product providing a more permanent record of the trace elements associated with health and disease and of trace elements assimilated from the environment.

In 1942, Schoenheimer described the body tissues as being in a state of dynamic equilibrium or homeostasis which tended to maintain stability with coordinated responses to compensate for changes in the physiological system. Hair is an exception. Unlike the other body compartments, hair is a metabolic end product that is thought to incorporate trace elements into its structure during its growing process. During the growth phase of a hair cycle, the matrix cells at the papilla of the hair follicle show intense metabolic activity and produce hair at a rate of approximately 0.3 mm/day as shown in Figure 1. The developing hair is exposed to this metabolic milieu for only a short period of time during which trace elements from the blood are partitioned into the nascent hair. As the growing hair approaches the skin surface, it undergoes a hardening process, or keratinization, and the trace elements accumulated during its formation are sealed into the protein structure of the hair. It is in this way that the trace element concentrations of the hair are possibly related to the trace elements in the body. Flesch (1954) recognized this

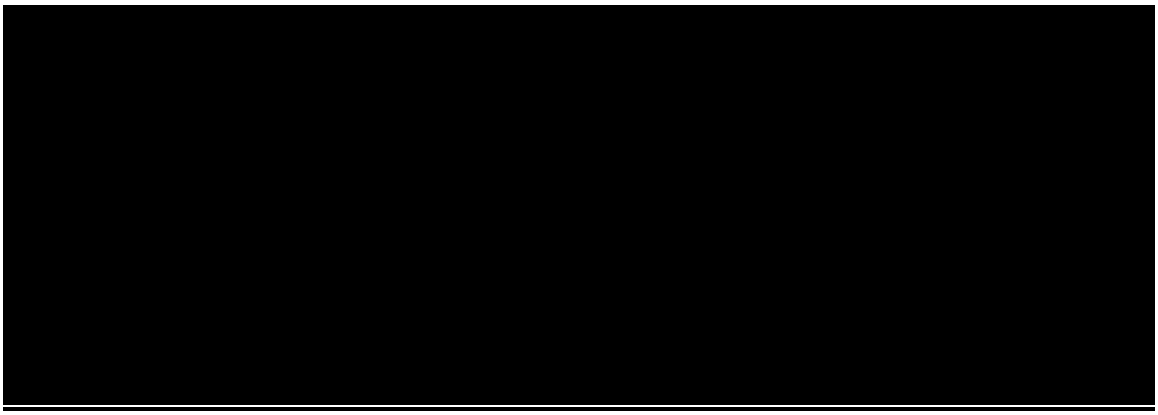
possibility more than fifty years ago when he suggested that hair functioned as a minor excretory organ for arsenic and possibly for other toxic elements. Hopps (1977) and Chittleborough (1980) have speculated on the possibility of interpreting the results of hair analysis for information about trace elements in other body compartments.

Figure 1: Active Hair Follicle showing a connection between blood supply and dermal papila

The popularity of using hair as a biopsy tissue for trace elements resides, in part, is due to the ease with which samples may be collected and in the stability of the samples after collection. In addition, the concentrations of most elements are frequently two or three orders of magnitude greater in hair than they are in more conventional tissues such as blood or urine. Some typical values are compared in Table 1.

LEAD

Table 1. Frequent "Normal" Values for Some Essential and Some Toxic Trace Elements in Blood, Urine and Hair (Iyengar and Wolttiez, 1988)



Investigations of systemic intoxication and nutritional deficiency were among the early applications of hair mineral analysis. Kopito and his colleagues reported correlations between the concentrations of lead in the hair of children and the clinical signs of lead poisoning (Kopito, *et al*., 1967). Strain and his coworkers reported zinc – deficient Egyptians were found to have a mean hair zinc level approximately half that of the normal Egyptian population (Strain, *et al*., 1966).

Lead poisoning from a variety of domestic and industrial sources is a subject of concern because it affects the nervous system of children given to eating nonfood items such as dirt and clay and because its effects are so severely and permanently damaging to the fetuses and newborns of mothers ingesting lead.

The signs and symptoms of acute lead poisoning are a sweet metallic taste, salivation, vomiting, intestinal colic and lowered body temperature. In addition, there may be cerebral edema, convulsions and coma. Kidney damage is frequent, and peripheral neuropathy often causes wrist drop. When death occurs, it is usually due to cardiovascular collapse.

Chronic lead poisoning has CNS manifestations. These are most pronounced in children, and they include irritability, headache, insomnia, restlessness and ataxia. Later, confusion, delirium, convulsions and coma may develop. Muscle paralysis involving the exterior muscle of the wrist and foot may develop also. The gastrointestinal signs of chronic lead poisoning often include stomach distension after meals, constipation, nausea, vomiting and colic. Appetite loss, weight loss and fatigue usually follow. A black or purple line sometimes forms at the margin of the gums where lead in the saliva may precipitate with sulfide produced by gingival bacteria. Some other heavy metals, such as arsenic, bismuth, tin and mercury, produce similar precipitates at the gingival border. Hematological characteristics of chronic lead poisoning are basophilic stippling and elevated lead content. In addition, the urinary lead concentration is elevated as are the concentrations of urinary coproporphyrins (Kalman, 1977).

Kopito and his colleagues (Kopito, *et al*., 1967) reported finding relationships between the concentrations of lead in the hair of children with suspected lead poisoning and the major clinical and laboratory findings associated with chronic plumbism in children. The mean lead concentration in the hair from 17 children demonstrating these signs and symptoms was 282 ± 226 mg/kg compared to a mean value of 24 ± 24 mg/kg for 41 control children. Radiographic examinations showed increased density in the line of provisional calcification at the metaphyses of the long bones in 13 of the 17 patients, and large quantities of lead were excreted in the urine in response to treatment with chelating agents in 16 of the 17 patients. The source of the lead exposure was attributed to pica.

The data collected in a lead poisoning surveillance project established a correlation between concentrations of lead in the blood, BPb, and the concentrations of lead in the hair, HPb, of children with confirmed plumbism; BPb = 0.02757 HPb + 39.79, where BPb is the whole blood lead concentration expressed as μg/100 mL and HPb is the concentration of lead in the hair expressed as mg/kg. For the 11 data points reported, the correlation coefficient, r, was 0.854 with p less than 0.001 (Marzulli, et al., 1978).

On the basis of data from a study of lead concentrations in the blood and hair from 121 children living within 500 meters of a secondary lead smelter and their urban and rural controls, Chattapadhyay (Chattapadhyay, *et al*., 1977) and his collaborators established an exponential relationship, *log* HPb = 0.8 BPb + 0.025 where HPb is the concentration of lead in the hair expressed as μg/g and BPb is the concentration of lead in the blood expresses as μg/100 mL. An exponential relationship was reported by Niculescu and coworkers (Niculescu, *et al*. 1983) too, HPb = 1.502 $e^{0.018BPb}$ where HPb is the concentration of lead in the hair expressed as ng/cm and BPb is the concentration of lead in the blood expresses as μg/dL. The data from which this relationship was derived were collected from two groups of male workers with different occupational exposures to airborne lead identified only as high and low. Regression analysis showed a significant correlation between HPb and BPb for the workers exposed to high airborne lead concentrations, $r = + 0.72$ and $p < 0.001$. For the workers exposed to low airborne lead concentrations, the correlation was less strong, $r = + 0.03$ and $p < 0.05$.

Additional reports of correlations between HPb and exposure are listed in Table 2.

Table 2. Some Reported Environmental Sources of Elevated Lead in Hair

Lead Exposure	Number	HPb	Reference
Pottery Worker	**181**	**43 ± 6 ppm**	**Weber, *et al.* (1984)**
Control	**24**	**17 ± 2 ppm**	
Gas Station Workers	**53**	**48.7 ± 17.5 ppm**	**Burguera, *et al*., (1987)**
Controls	**53**	**17.2 ± 8.1 ppm**	
Near By Lead Smelter[1]	**263**	**588 ± 664 ppm r = 0.38, p < 0.0001**	**Tavares and Branado, (1989)**

[1] **BPb vs. *log* HPb**

MERCURY

The acute toxicity of mercury was identified in the workplace before its chronic toxicity to the general public was suspected. Beginning in 1953, increasing numbers of adults and children residing near Minamata Bay showed loss of coordination, numbness of the limbs, partial blindness and partial loss of hearing. Convulsions, coma and death followed in 46 of 125 cases. By 1956, a congenital Minamata disease was observed in offspring of symptom – free parents. The disease was subsequently diagnosed as acute methyl mercury poisoning and traced to the consumption of fish that had concentrated, or "biomagnified", mercury from industrial wastes discharged into Minamata Bay (Horvath, 1976).

Katz and Katz (1992) have reviewed many correlations of hair mercury concentrations with blood mercury concentrations and of hair mercury concentrations with dietary and occupational exposures to mercury. Among those cited were HHg = 0.367 BHg + 0.694 Sherlock, et al., 1982) and HHb = 289BHg + 63.4 (Hansen, et al., 1983). In the former, HHg is the mercury concentration in the hair expressed as mg/kg, and BHg is the blood mercury concentration in μg/L. In the latter, the correlation, r, was 0.9222 with HHg expressed as μg/kg and BHg as μg/L. From their review, Katz and Katz

(1992) concluded scalp hair analysis was probably valid for evaluating environmental exposure to and/or systemic intoxication with mercury.

ARSENIC

As originally suggested by Flesch (1954), scalp hair analysis may be applicable to evaluating arsenic poisoning too. It is interesting to note that the health insurance plans from Aetna (Aetna, 2007) will authorize payment for hair analysis only for the diagnosis of arsenic poisoning while those from Cigna (Cigna, 2008) do not cover chemical analysis of hair because it is considered experimental, investigational or unproven.

In chronic arsenic poisoning, diarrhea and vomiting occur. Tremors and peripheral neuritis are present in some cases. The afferent and motor sensory nerves in the lower extremities are attacked. Ankle jerk disappears, and leg muscle atrophy begins. Arsenic stimulates the horny layer of the skin, which leads to the appearance of dark brown scales. Skin keratoses frequently result from prolonged arsenic exposure. These may become malignant. Chronic arsenic poisoning has been caused by drinking water contaminated with arsenic. Hindmarsh and his collaborators have reported one such instance in Waverly, Nova Scotia (Hindmarsh, *et al*., 1977).

The work of Valentine and her coworkers (1979) shows the efficacy of hair analysis for evaluating systemic arsenic intoxication. Samples of blood, urine, hair and tap water were obtained from some 150 residents of two California and three Nevada communities where the ground water supplies contained 6, 51, 98, 123 and 393 μg As/L. The arsenic concentrations of these samples were determined by atomic absorption spectrometry of the gaseous hydride. The results showed dose – response relationships

for regressions of the logarithms of the concentrations arsenic in the urine and in the hair with the logarithms of the concentrations arsenic in the tap water. The dose – response for the regression of the logarithm of the concentrations arsenic in the blood with the concentrations of arsenic in the tap water was not observed at tap waster concentrations below 100 µg As/L. For this reason, they concluded, "… blood does not seem to be useful in assessing the degree of exposure to arsenic. Hair and/or urine are useful."

A similar study (Olguin, *et al*., 1983) involving 50 residents of a Mexican village where the potable water supply contained 0.41 mg As/L and an equal number of control subjects whose water supply contained 0.005 mg As/L had similar results and a similar conclusion. The results are reproduced in Table 3. The authors noted the exposed subjects with the highest hair arsenic concentrations displayed the cutaneous signs of arsenic poisoning including stomach pain, headaches, diarrhea and vomiting.

Table 3. Arsenic Concentrations in Blood, Urine, Hair and Nails from Inhabitants of the Study Populations

	Blood, µg/L	Urine, µg/L	Hair, µg/kg	Nail, µg/kg
Exposed	**8 ± 5**	**300 ± 180**	**1240 ± 610**	**4550 ± 3250**
Control	**2 ± 1**	**10 ± 10**	**60 ± 20**	**420 ± 240**

More recently, Vahter and her coworkers (Concha, et al., 2002) reported on the arsenic concentrations in the urine and hair of female subjects from eight locations in Argentina exposed to arsenic in drinking water. The average arsenic concentrations in the water from these eight locations and the average concentration of arsenic in the urine of the women from these eight locations are summarized in Table 4.

Table 4. Average Concentrations of Arsenic in Drinking Water and Urine

Location	Water, µg/L	Urine, µg/L
Rosario de Lerma	**0.6**	**7.2**
Tolar Grande	**2.5**	**15**
Joaquin V. Gonzalez	**6.4**	**10**
Olacapato	**14**	**26**
Santa Rosa de los Pastos Grande	**31**	**55**
Anta	**187**	**141**
San Antonio de los Cobres	**189**	**265**
Taco Pozo	**215**	**366**

There was a significant correlation between the average arsenic concentrations in drinking waters and the average arsenic concentrations in urine; $r = 0.96$, and $p < 0.001$, but marked variations in the urinary arsenic concentrations among the individuals from each location. In addition, there was a significant correlation between the average arsenic concentrations in urine samples and the average arsenic concentrations in hair: $r = 0.64$, and $p < 0.001$. As was the case with the drinking water correlation, there were variations in the hair arsenic concentrations for similar concentrations in urine. In one case where the hair contained 1500 µg As/kg and the corresponding urine contained 64 µg As/kg, the donor was suspected of bathing in the arsenic-rich water, 6000 µg/L, at the thermal spa in San Antonio de los Cobres.

The possibility of exogenous contamination was explored further by two of the investigators by measuring the arsenic concentrations in their own hair before and after washing in the arsenic-rich water at San Antonio de los Cobres. Pre-washing arsenic concentrations were 33 and 78 µg/kg. Corresponding post-washing arsenic concentrations were 395 and 461 µg/kg. This observation clearly supports the external rather than the internal (i.e., metabolic) deposition of arsenic in the hair. The inability to differentiate between endogenous and exogenous deposition is one of the most compelling reasons to

reject hair analysis as an indicator of body burdens of heavy metals. It is for this reason the authors conclude, "Arsenic concentrations in drinking water and hair do not provide reliable measures of individual arsenic exposures." They recommend, "The arsenic concentration in urine seems to be a better marker of individual arsenic exposure than concentrations in drinking water and hair."

"Some form of laboratory washing of hair samples has been considered necessary to remove metal – containing dirt and fluids added by nature or by hair treatments." (Hinners, *et al*., 1974) as attempts to differentiate between endogenous and exogenous depositions. Consequently, laboratories involved in hair analysis employ a variety of washing procedures to remove external contamination. None has been entirely successful in differentiating between endogenously and exogenously deposited heavy metals. Even with this obvious shortcoming, hair analysis can be useful as a screening agent for exposures to toxic elements such as arsenic, mercury and lead, and an indicator that additional clinical studies should be undertaken.

ZINC

Four years before Kopito and his colleagues (Kopito, *et al*., 1967) reported correlations between the concentrations of lead in the hair of children and the clinical signs of lead poisoning, Prasad and his coworkers (1963) reported the first conclusive evidence of zinc deficiency in Egyptian dwarfs based on: decreased plasma, erythrocyte and hair zinc concentrations, increased zinc (^{65}Zn) plasma turnover, decreased 24 - hour exchangeable zinc pool and decreased urinary and fecal zinc excretion.

Prasad (1966) reported that dietary zinc deficiency was responsible for retarded growth and delayed sexual maturation in Iranian and Egyptian males. As a result of a 100-mg $ZnSO_4$ supplement to their daily diets, nine of nine affected subjects developed normal sexual function and grew in stature by an average of 10.5 cm during a 59-day confinement period. Eight similarly affected subjects fed the same diet without the zinc supplement grew in stature by an average of only 4.2 cm during the 59-day confinement period. They required 244 days to develop sexual maturity.

Zinc has been identified as a cofactor in more than a hundred human and animal enzyme systems (Sandsted, 1984). Among these systems are: acid phosphatase, alkaline phosphatase , glucokinase, enolase, carboxypeptidase A, carboxypeptidase B, alcohol degydrogenase, the glutamate, lactate and malate dehydrogenases and carbonic anhydrase (Kruse-Jarres, J.D., 1987).

One of the major clinical abnormalities associated with zinc deficiency is acrodermatitis enteropathica. Acrodermatitis enteropathica is inherited as an autosomal recessive trait. Infants are the most frequent victims of this disease some characteristics of which include chronic diarrhea and seborrheic skin lesions usually located near body orifices. Zinc supplementation has been reported to clear the skin lesions and normalize the bowel function (Neldner and Hambidge, 1975). Hypogeusia is another consequence of zinc deficiency. This disorder is responsive to zinc supplementation too.

The RDA (recommended daily allowances) for zinc is: infants < 1 year, 5 mg; children < 4 years, 8 mg; adults and children > 4 years, 15 mg; pregnant or lactating women, 15 mg. The LD_{50} (rabbit) for zinc is 2 mg/kg. Dietary zinc is absorbed from the duodenum and from the jejunum as complexes with mucosal proteins. Many proteins and

amino acid complexes are involved in zinc transport. A 14 – compartment model for zinc metabolism has been developed using stable isotope techniques. This model assisted in the validation of mathematical models for homeostasis (Miller, *et al*., 2000) involving transfer from stomach to intestine, equilibria between blood plasma, erythrocyte, liver and intestine, the colon and fecal excretion and the kidney and bladder followed by urinary excretion. Hair was not among the compartments considered. This model is shown in Figure 2. Stable isotope techniques have been applied to an investigation of the dose dependent absorption and excretion of zinc in the human (Tran, *et al*., 2004). The resulting dose – response model indicated that doses greater than 20 mg result in relatively small and progressively diminishing increases zinc absorption by the healthy adult human.

The blood reference range for zinc is from 785 – 1112 μg/L. Seminal zinc concentrations are more than two orders of magnitude greater, 80 – 230 mg/L. Zinc may be a factor in male fertility (Marmar, *et al*., 1975).

Figure 2. 14-compartment model for human zinc metabolism showing compartments as circles except for the rectangle representing the colon, a non-mixing delay compartment. (Miller, *et al*., 2000)

Following the announcement confirming zinc deficiency in man cited above (Prasad, *et al*., 1963) Strain and his collaborators proposed the use of hair as a biopsy tissue for the diagnosis of zinc deficiency in males (Strain, *et al*. 1966). They found the mean zinc concentration of zinc in hair from 22 normal Egyptian males was 103.3 ± 4.9 mg/kg while that from ten "untreated" dwarfs was 54.1 ± 5.3 mg/kg. The mean zinc concentration in hair samples from eight "treated" dwarfs who had undergone from six to twelve weeks of oral zinc sulfate therapy (30 mg t.i.d.) was 121.1 ± 4.8 mg/kg. Four of the eight "treated" subjects were originally in the "untreated" group and had subsequently received the oral therapy. The hair samples from all four demonstrated marked increases in zinc concentration: from 30 to 114, from 37 to 110, from 40 to 112 and from 75 to 122 mg/kg. The conclusion drawn from this study was, "Hair analysis appears to be a reliable,

simple and atraumatic method of assessing zinc body stores." This statement may have been the beginning of using hair mineral analysis for assessing zinc status.

Although endorsed by some commercial laboratories and nutritional counselors, the efficacy of hair mineral analysis for assessing zinc status has become the subject of conflict and controversy. A typical announcement for commercial hair analysis is reproduced in Figure 3, and summaries of some commercial services are listed in Table 5.

Figure 3. Internet Advertisement from Optimum Solutions, LLC, holistic Health Consultants (210 Elmwood drive, Lafayette, LA 70503) offering a report and diet plan for $199 with a notation indicating individualized supplements are extra.

Table 5. Some Commercial Laboratories Selling Hair Analysis, Nutritional Counseling and Dietary Supplements

Vendor	Cost	Provides Counseling?	Sells Supplements?
Advanced Family Health	**$125**	**no**	**yes**
Analytical Research Labs		**yes**	**yes**
Complete Nutrition ….		**yes**	
Dallas Massage & Therapist	**yes**	**yes**	
Graceful Earth, Inc	**$185**	**yes**	**yes**
Dr. Janet Starr Hull	**$180**	**yes**	**no**
Nutritionally Yours	**$159**	**yes**	
Optimum Solutions, LLC	**$199**	**yes**	**yes**
Reynolds Health & Nutrition	**$140**	**yes**	**yes**
Sanascan	**$84**	**no**	**no**
Test Country	**$79.95**	**no**	**no**
Trace Elements			**yes**
Lawrence Wilson, MD	**$295**	**yes**	**yes**

Animal experiments in which 28 rats were maintained on a low zinc (~ 1.54 ppm) diet for 50 days prior to plucking newly – grown hair showed lower mean hair zinc concentrations, 101 ± 13.8 mg/kg, than did an equal number of control rats receiving more than ten times more (~ 18.8 ppm) zinc in the diet, 164 ± 17.7 mg/kg. The daily mean increase in body mass for the animals maintained on the zinc deficient diet was approximately one third less than that measured for the rats receiving more dietary zinc, 0.58 ± 0.30 versus 0.83 ± 0.13 g/day. Because some animals receiving the low zinc diet demonstrated growth rates did not differ from those of the rats receiving more dietary zinc, it was concluded that although zinc concentrations in hair are dependent upon zinc intake, they do not necessarily reflect the severity of the of the metabolic effects of zinc deficiency (Reinhold, *et al*., 1968). Rats maintained on graded diets containing 3, 15, 35 and 68 ppm zinc from zinc carbonate for five weeks prior to sacrifice and tissue analysis showed correlations between zinc intake and zinc concentrations in the hair ($r = 0.681$ and $p < 0.001$) and between zinc intake and zinc concentrations in the bone ($r = 0.616$ and $p < 001$) but no correlations with zinc intake and plasma zinc concentrations ($r =$

0.0.032 and $p = 0.808$) or with zinc concentrations in the hair and plasma zinc concentrations ($r = 0.154$ and $p = 0.678$) (Deeming and Weber, 1977). Deeming and Weber concluded, "Therefore, hair zinc analysis can be used to aid in diagnosis of a deficiency or evaluate dietary intake for the rat but can not be used to assess the state of zinc metabolism." Their latter observations may reflect that blood serum is not a major compartment in zinc homeostasis.

In the course of a nutritional survey in the Republic of Panama, Klevay (1970) collected 433 blood and hair samples, 210 female and 223 male, for zinc analysis. Erythrocytes were separated from plasma, and each was analyzed separately. No differences in zinc concentrations on the basis of gender were observed. Plasma zinc concentrations were about ten times less than the zinc concentrations in the erythrocytes, 95 ± 125 μg/100 mL versus 968 ± 245 μg/100 g for males and 76 ± 111 μg/100 mL versus 961 ± 246 μg/100 g for the female subjects. Statistically significant correlations between the zinc concentrations in the hair and the zinc concentrations in the erythrocytes were found for only 41 of the 433 samples For these 41 samples, $r = 0.381$ and $p < 0.05$. That the correlation was observed for only ten percent of the subjects may be an additional indication the blood is not a major compartment in zinc homeostasis.

Gibson (1984) reported no significant correlations related hair and serum zinc values for 68 elderly (58 – 89 years old) Canadian women, and Hunt and her research group (Hunt *et al*., 1983) found zinc supplementation (20 mg Zn as the acetate) did not increase mean zinc concentrations in serum or in hair from 213 Mexican women.

Like Klevay (1970), Erten and his collaborators (Erten, *et al*., 1978) examined hair samples from 115, 50 male and 65 female, healthy children and adolescents. They

found no difference in zinc concentrations on the basis of gender or on the basis of hair color, nor did they observe a correlation between serum zinc and hair zinc in seven protein-calorie malnourished children. They did, however, observe the zinc concentrations in the hair samples from the malnourished children were greater that those of a control population, 170.9 ± 17.5 versus 109.3 ± 19.5 μg/g. It is important to note zinc deficiency was not confirmed in the protein-calorie malnourished children.

In response to a letter to the editor written by José Dória (Dória, 1981), Erten and his collaborators (Erten, *et al*., 1978) identified the healthy group of children as students in kindergartens, elementary and secondary schools in around Ankara and the malnourished children as a separate group selected from pediatric clinic patients. They suggested the higher zinc concentrations in the hair from the malnourished children could be attributed to a slower than normal formation and growth of the hair fiber. Dória was of the opinion, the younger children inherently had higher hair zinc concentrations than did older children; viz. hair zinc concentrations were age dependent.

Subsequently, Dória and his collaborators (Dória, *et al*.,1982a, 1982b) confirmed there were indeed differences in the concentrations of zinc in hair of different colors. Their data are reproduced in Table 6.

Table 6. Hair Zinc Concentrations for 147 Brazilian Children, 1 – 12 years of Age, According to Color

	Black	Brown	Blonde
Number of Subjects	**28**	**80**	**29**
Mean Hair Zinc, μg/g	**158**	**152**	**122**
Standard Deviation	**± 57**	**± 46**	**± 39**

Hair color was determined by visual examination and later confirmed by determinations of its melanin contents. Nutritional status was evaluated using height/age and mass/age parameters and found to be unrelated to hair zinc concentrations.

McKenzie (1979) went one step further and wrote, "It was concluded that the measurement of zinc in serum, urine, hair and toenails did not provide a sensitive indication of zinc status." Her results for 96 New Zealand university students are summarized in Table 7. Also included in Table 7 are results for small samples of some other subgroups of New Zealanders.

Table 7. Zinc Concentrations from New Zealand Subjects

Subjects	Serum µg/mL	Urine µg/24 hr	Hair µg/g	Nail µg/g
42 male students	**84 ± 15**	**578 ± 259**	**180 ± 25**	**120 ± 140**
54 female students	**121 ± 30**	**334 ± 168**	**195 ± 23**	**118 ± 53**
14 adult ER patients	**83 ± 14**	**650 ± 532**	**183 ± 27**	**113 ± 32**
13 oyster openers	**108 ± 18**	**505 ± 234**	**169 ± 24**	**108 ± 33**
4 electroplaters	**79 ± 11**	**585 ± 314**	**182 ± 34**	**122 ± 24**
3 galvanizers			**4210**	**2000**
19 dermatol. clinic patients	**122 ± 19**	**691 ± 441**	**176 ± 25**	**112 ± 33**

Unlike the findings with Panamanian (Klevay, 1970) and Brazilian (Dória, *et al*.,1982a, 1982b) children, the zinc concentrations in hair from New Zealand university students showed a gender – based difference, 180 ± 25 male versus 195 ± 23 female. Serum zinc concentrations were negatively correlated with hair zinc concentrations for both the male and female students, $r = -0.35$ and $p < 0.05$. The oyster openers, who consumed several dozen, zinc-rich (335 ppm zinc on a dry mass basis), raw oysters each week during season, did not show elevated zinc concentrations in serum, urine, hair or nail, but the zinc concentrations of the hair and toenail from the galvanizers were quite remarkable. Individual hair and toenail zinc concentrations were 1150, 1160 and 10320 and 1160, 2240 and 2600 µg/g, respectively. The most senior galvanizer demonstrated the highest hair and toenail zinc concentrations. While the occupational setting could have made an exogenous contribution to the high hair zinc concentration, this is an unlikely explanation for high concentrations of zinc in the toenails. However, no information on

differences, if any, between the biokinetics of galvanizing zinc and nutritional zinc is available. Hair may reflect environmental exposures to zinc as well as the more toxic elements arsenic, mercury and lead.

Reilly (1981) reported no differences in the concentrations of zinc in hair on the basis of age, gender or domicile with one exception. Mean hair zinc concentrations in Brisbane women more than 65 years old were lower than those of Brisbane women in the 18 to 65 years old group. The data are reproduced in Table 8.

Table 8. Hair Zinc Concentrations Showing Lack of Age, Gender or Domicile Influence

	Oxford Residents		**Brisbane Residents**	
Age Group	Male	Female.	Male	Female
1 – 15 years	**154.4 ± 2.0**	**165.9 ± 13.6**	**163.3 ± 18.6**	**173.4 ± 13.9**
15 – 18 years	**169.0 ± 13.3**	**162.2 ± 12.2**	**168.4 ± 11.5**	**154.4 ± 24.2**
18 – 65 years	**163.8 ± 4.2**	**180.0 ± 7.9**	**171.9 ± 11.7**	**186.5 ± 10.3**
> 65	**169.0 ± 13.3**	**162.2 ± 12.2**	**168.4 ± 11.5**	**154.4 ± 24.1**

In spite of much evidence to the contrary, the similarities between the concentrations of zinc in hair from populations as geographically separated as England and Queensland may be indicative of a yet – to – be defined biokinetic pathway. Similarly, the values for zinc in reference materials IAEA HH – 1 (M'Baku and Parr, 1982) and NIES No. 13 (Yoshinaga, *et al*., 1997) are 174 ± 8.7 and 172 ± 11 mg/kg, respectively. These reference materials were prepared using hair from Austrian and Japanese sources, respectively.

Lacking defined biokinetics, the use of hair mineral analysis as a biomarker to assess nutritional status as well as to monitor environmental exposures, evaluate heavy metal poisonings and diagnose diseases came under increasing criticism.

In 1982, Hambidge (Hambidge, 1982) published his opposition to using hair mineral analysis for the assessment of nutritional status citing contamination from the

external environment, uncertainties about the effectiveness of washing procedures for removing exogenous trace elements, effects of hair cosmetics, variations in trace element concentrations due to hair color, hair location, hair length, *et cetera*, variations in trace element concentrations associated with the age and/or gender of the donor, variations in the growth rate of hair, uncertainties associated with the measurement techniques, poorly defined correlations with other tissues and a lack of clearly defined normal ranges for trace element concentration in the hair as his reasons. He maintained commercial exploitation of hair analysis was out of proportion to the scientific justification for its use, and he urged health care professionals and the public to be aware of the limited value of hair mineral analysis until and unless scientific validation was forthcoming.

Barrett's (Barrett, 1985) criticisms were based on the divergent results and the divergent interpretations he obtained from commercial laboratories to which he submitted hair samples from the two healthy teenagers. He was particularly concerned by the lack of consistent "normal values" for the minerals determined, allusions to various metabolic disorders and recommendations of dietary supplements to manage nutritional imbalances. Duplicate hair samples from a teen – aged female were submitted to one of these laboratories under different names. The reports contained similar divergences. In Barrett's opinion, the commercial use of hair analysis in this manner was, among other things, "unscientific".

In the summary of their review on hair analysis, Mason and Zlotkin (Mason and Zlotkin, 1985) wrote:

> The analysis of hair for trace elements is potentially a safe, noninvasive and extremely useful diagnostic tool, but it has not yet been proven to be reliable or to reflect the status of trace elements elsewhere in the body. As well, little is known about the normal ranges of concentrations of elements in the hair or

> about the physiologic and pharmacologic factors that affect the concentrations. Until these problems have been resolved satisfactorily, the diagnostic use of hair analysis performed by commercial laboratories cannot be justified in clinical practice.

This is exactly what Katz and Chatt (Katz and Chatt, 1988) meant when they said, "At present, the research is incomplete, and the assessment of nutritional status on the basis of trace elements in human scalp hair is premature."

Continued research, however, has not produced consistent results. A study of 691 Finnish children and adolescents showed no significant relationship between hair and serum zinc concentrations for 407 female subjects, but zinc concentrations were found to be associated with hair color and serum zinc concentrations in 284 male subjects (Laitnen, *et al*., 1988). Significant increases in plasma and hair zinc concentrations were reported for Chinese preschool children after receiving 3 – 6 month zinc supplementation as 3 – 5 mg $ZnSO_4$/kg/day for retarded growth, anorexia or pica (Chen, et al., 1985). These increases are presented in Table 9.

Table 9. Plasma and Hair Zinc Concentrations Before and After Treatment

	Before Treatment		**After Treatment**	
	Plasma µg/dL	**Hair µg/g**	**Plasma µg/dL**	**Hair µg/g**
control	**99.8 ± 15.7 n = 30**	**152.9 ± 32.0 n = 94**		
pica	**90.4 ± 38.1 n = 47**	**78.1 ± 25.7 n = 47**	**109.1 ± 32.4 n = 47**	**134.1 ± 59.4 n = 47**
anorexia	**79.3 ± 24.0 n = 69**	**81.2 ± 26.9 n = 91**	**108.2 ± 32.5 n = 69**	**131.5 ± 32.7 n = 69**
retarded growth	**83.2 ± 26.3**	**82.3 ± 29.4**	**101.0 ± 30.9**	**119.5 ± 35.0**

HAIR ANALYSIS

Perhaps as an attempt to justify the use of hair mineral analysis for the assessment of nutritional status, Doctor's Data (Doctor's Data, 1988) published a summary of the

favorable literature regarding hair analysis including the statement, "Over the past 12 years, Doctor's Data, Inc. has performed close to a million hair element tests, accumulating what is probably the largest body of information regarding this procedure." (Over four million tests are claimed in 2002 (http://www.doctorsdata.com/test_info.asp).) However, the issues raised by Barrett (1985) and by Mason and Zlotkin (1985) have remained unresolved.

With the growth of the internet, the pros and cons of hair mineral analysis moved from the printed word to the electronic word. Lawrence Wilson (Wilson, 2007) and Janet Hull (Hull, 2007) are among the more vocal proponents of commercial hair mineral analysis (See Table 5.)

The controversy returned to the scientific literature with a report from Seidel and her colleagues at the California Department of Health Services on an assessment of commercial laboratories performing hair mineral analysis (Seidel, *et al*., 2001). They submitted samples of scalp hair collected from one volunteer to the six largest U.S. commercial laboratories engaged in hair mineral analysis. These laboratories were reported to analyze some 225,000 hair samples each year at a cost of nine and a half million dollars. The average cost of analysis, $42.25, is inconsistent with the fees cited in Table 5. The report cited a number of significant differences between the results obtained by the laboratories that conducted the tests. The laboratories used different analytical instrumentation, either ICP-AES or ICP-MS. A variety of washing procedures were used for preparation of the samples. One laboratory did not wash the hair at all as part of the test preparation. Several of the laboratories claimed to use a Chinese certified reference material that was certified for seventeen elements; however, there was no certified

reference material for all the elements analyzed. In addition, the certified values for these commercial reference materials can be up to seven times greater than the concentrations of the laboratories in-house reference ranges. A final comparison of the results from all six laboratories showed very little agreement about which element concentrations or element ratios were indicative of disease. For example, one laboratory placed a great deal of emphasis on hair sodium levels due to the association of high sodium levels with cystic fibrosis (Bisse, *et al*., 1996), but two other laboratories dismissed hair sodium levels as unreliable. Four laboratories recommended vitamin and mineral supplements with three of those four suggesting a product line that could cost up to as much $100 per month for an indeterminate period of time. Laboratories even gave completely opposite dietary recommendations with one laboratory suggesting that the donor had a 'high metabolism' and should increase intake of purine-containing foods and vitamin A while another laboratory suggested the exact and total opposite. One of the recommendations made in this report was, "Primary care physicians refrain from using hair analysis to assess environmental exposures or nutritional balance." It would seem that not much has changed since Barrett's reservations in 1985, and hair analysis remains unreliable.

Steindel and Horwanitz (Steindel and Horwanitz, 2001) published their opinions on hair analysis for trace metals. Among the shortcomings they associate with hair mineral analysis are: [1] the absence of uniform methodologies, [2] the inability to distinguish between endogenous and exogenous trace elements, and [3] the poorly – defined normal values. Faced with these uncertainties, utilizations of hair mineral analysis for assessing nutritional status and/or basing nutritional counseling should be reconsidered "… unless and until the reliability of hair analysis value is established and

evidence becomes available that clinical recommendations based on hair analysis improve patient outcomes."

Drasch and Roider submitted scalp hair samples from two volunteers to seven laboratories offering commercial hair mineral analysis in Germany (Drasch and Roider, 2002). Six weeks later, additional samples of the hair from one of the two volunteers were submitted to the same seven laboratories. The results reported by these seven laboratories showed little agreement between the laboratories. In addition, there was little agreement within the laboratories on the results for the samples submitted six weeks apart. Consequently, Drasch and Roider concluded the results from these laboratories were unreliable, and recommended refraining from using such analyses to assess individual nutritional status or suspected environmental exposure.

The commercial laboratories, nutritional consultants, practitioners of alternative medicine and chiropractors and other proponents of hair analysis responded to these criticisms with a series of letters to the editor (Lurie and Zylke, 2001) and statements on the Internet (Doctor's Data, 2001; Townsend Letter, 2001) as well as published reviews on the subject (Bass, *et al*., 2001) .

Frisch and Schwartz (Frisch and Schwartz, 2002) continued to urge caution in interpreting hair mineral analysis results based on their experiences with patients who suspected environmental exposures to heavy metals and who had undergone hair analysis independently. They reported case histories of three patients who had come to their clinic at the Johns Hopkins Bloomberg School of Public health seeking chelation therapy for heavy metal intoxications. Complaints included fatigue, muscle aches, joint pain and depression. Neither the clinical signs and symptoms nor the histories of environmental or

occupational exposures were indicative of heavy metal intoxications, and chelation therapy was not warranted. The complaints were attributed to fibromyalgia in one case. In the others, the complaints were not resolved. The report also reviewed the pitfalls of hair analysis for toxicants in the clinical practice, and attributed the promotion of hair analysis to commercial laboratories, nutritional consultants, practitioners of alternative medicine and chiropractors who's Internet Web Sites imply hair analysis can be useful in diagnosing metabolic disorders and promote the use of dietary supplements.

The Agency for Toxic Substances and Disease Registry (ATSDR) explored the potential use of hair analysis as a tool to assess exposure in 2003 (Harkins & Susten, 2003). The goal of the panel of seven experts was to determine the utility of hair analysis as an assessment of exposure to harmful waste sites. The group addressed several questions such as for what substances are reliable hair analysis methods available, what situations are appropriate for the use of hair analysis, and what gaps in data exist that limit the interpretations of the results of hair analysis. The panel came to several conclusions on many of the most pressing issues regarding hair analysis. The panel concluded that aside from drugs of abuse and methyl mercury, there is no accurate method to determine the source or impact of trace elements on the health of an individual. For the question of when it is appropriate to use hair analysis, the panel concluded that due to the variation in the growth rate of human hair, it is not generally useful for determining recent exposure or exposure greater than one year prior. As to the question of what gaps exist in the current available data, the panel concluded that a standard methodology needs to be developed and implemented to ensure accurate and reliable results. The panel also stated that more needs to be done to establish a normal reference

range to aid in the interpretation of data, to develop a method for the differentiation of exogenous contamination from endogenously deposited samples, and to understand the biological mechanism of the uptake of these substances and the lack of correlation between hair levels and those in blood and other target organs. In general, the ATSDR recommended that before hair analysis can become a reliable and useful tool for the assessment of exposure to hazardous materials, more research is needed for establishing normal reference ranges, greater understanding of the biology of hair, and establishing if and when hair may serve as a better indicator of disease than another biological media such as blood or urine. In the meantime, the panel is concerned about the potential for medical fraud and misuse of hair analysis for unwarranted and unethical medical treatment. The ATSDR has plans to develop and distribute health education materials to educate the public about the information that can and cannot be gleamed from hair analysis testing.

Hair and nail are both keratinized tissues. Like hair, nail may be considered to be a metabolic end product. The data presented in Table 3 show elevated concentrations of arsenic in both the hair and the nail of exposed individuals, and the data presented in Table 7 show similar elevations of zinc. While exogenous contributions cannot be ruled out, it may be that the mineral analysis of nail has utilization for assessing trace elements in the body.

While both hair and nail are both keratinized tissues, they differ both in structure and composition. The active hair follicle is shown in Figure 1. The genesis for the fingernail is shown below in Figure 4.

Figure 4. Finger Nail Showing Connection to Viable Tissue

Nail is generated by living cells that are located at the ends of fingers and toes. Human nails consist of several parts which include the nail plate which is the visible portion of the nail, the nail bed which is the skin located underneath the nail plate, and the matrix which is where the cells that actually produce the nail are located. The remaining parts of the nail are the lumala, the cuticle, and the nail folds (American Academy of Dermatology [AAD], 2004).

The growth rate of nail is unknown, but it is thought to be about ten times slower than the growth rate of hair (Rodushkin & Axelsson, 2000; Rodushkin & Axelsson, 2003). Furthermore, the rate at which different nails grow varies due to several factors. The nails on the dominant hand, i.e., the nails on the right hand of a right handed person, grow faster than the nails on his/her non-dominant hand. There is a difference in the growth rate of fingernails compared to toenails with fingernails growing at a faster rate. Further variance in nail growth rate includes the time of year, gender, and age of the person (AAD, 2004).

The amino acid compositions of hair and nail are compared in Table 10. One difference between hair and nail appears to lie in the percentage of the sulfur – containing amino acid cystine (Block, 1939).

Table 10. Amino Acid Composition of Hair and Nail (Block, 1939)

	Hair, %	Nail, %
Nitrogen	**15.4**	**14.9**
Sulfur	**5.0**	**3.8**
Histidine	**0.6**	**0.5**
Lysine	**2.5**	**2.6**
Arginine	**8.0**	**8.5**
Cystine	**15.5**	**12.0**
Tyrosine	**3.0**	**3.0**
Tryptophan	**0.7**	**1.1**
Phenylalanine	**2.6**	**3.6**
Glycine	**4.3**	**5.7**
Other	**62.8**	**63.0**

The issues underlying the rival attitudes and diverse opinions about the efficacy of hair mineral analysis for assessing nutritional status have yet to be resolved. The conflict and controversy will undoubtedly continue.

RESEARCH OBJECTIVES

The objective of this research is to seek information on the efficacy of hair mineral analysis for assessing zinc status by determining the concentrations of zinc in hair from two anatomical locations, scalp and pubis, and from two keratinized tissues, hair and nail. If it is assumed the hair and the nail are among the body compartments involved in zinc homeostasis and in the biokinetics of zinc, quantitative relationships between the zinc concentrations in hair from the two anatomical sites and/or in the two keratinized tissues should be observed. If, on the other hand, these zinc concentrations are unrelated, zinc deposition may be a random process unrelated to zinc metabolism.

Zinc was selected as the focus of this research for several reasons. Zinc is an essential trace element. The biokinetics of zinc have been defined with blood plasma as the central compartment, but the hair was not considered among the other compartments for zinc. Attempts to establish correlations between the concentrations of zinc in blood and those in the hair have led to contradictory conclusions perhaps because blood is a transient compartment while hair is a permanent, one-way compartment. Nail was not considered in the biokinetic model. The available resources are amenable to the quantitative determination of zinc.

INSTRUMENTATION

The zinc concentrations in the keratinized tissues were determined by atomic absorption spectrometry. The instrument used for all analyses was a Varian SpectrAA 250+ atomic absorption spectrometer which is a P.C. – controlled, double-beam spectrophotometer utilizing a zinc hollow cathode lamp. The software selects wavelength and slit width and controls gas flow. In a double-beam instrument, the beam of radiation that is emitted from the hollow cathode lamp is split into two beams, reference and sample, by a chopper which is half mirrored and deflects a portion of the beam and allows the other portion of the beam to pass through the sample. The two beams are then recombined to form one beam by a half-silvered mirror, and which then passes through a Czerney-Turner grating monochromator. The beam then proceeds to travel through a photomultiplier tube (PMT) which acts as a transducer, and the output from the PMT passes into a synchronizer which is synchronized with the chopper. The ratio of the sample signal to the reference signal is amplified and the measurement is sent to a computer as an absorbance measurement. With atomic absorption spectrometry, only one element can be detected at a time. This is done by first aspirating the aqueous sample into a flame generated by a mixture of gases, acetylene and air in this case. The sample is atomized in the flame and the atoms are struck by the radiation emitted from the hollow cathode lamp which is emitted at a specific wavelength specific to the element to be detected. This wavelength of energy is absorbed by the atoms in the flame which in turn excites these atoms. The ratio of the amount of energy that is absorbed is proportional to the concentration of the atoms in the sample (Skoog, *et al*., 1998).

The instrument utilized a volatile gas flame to atomize the sample. The flame may be replaced by an electronically heated graphite furnace which may offer greater sensitivity and require less sample, but can require a greater degree of operator skill to establish ideal conditions for the measurement of each sample. Also, where the flame atomizes the sample in a single step the graphite furnace heats the samples in three steps. First the sample is dried, then charred or pyrolyized and then finally atomized. Another instrument that may be utilized to perform this type of analysis is inductively coupled plasma mass spectrometer (ICP-MS). One of the advantages of ICP is that the plasma is nearly twice as hot as a conventional flame and the residence time of the atoms of the analyte is also about twice as long which leads to a more complete atomization and an enhanced signal. The output of the ICP can be sent to a mass spectrometer which separates and measures the ions formed in the plasma based on their mass-to-charge ratios allowing for simultaneous, multi-element, as many as 70, determinations (Harris, 2003).

REAGENTS

Zinc calibration standards were prepared from a stock solution made by dissolving 1.004 grams of zinc in 40 mL of 1:1 HCl solution and then diluting the resulting solution to 1 liter to yield a 1000-ppm zinc standard solution. A second set of zinc standards also ranging in concentration from 0.5-ppm to 2.0-ppm was prepared by dilution of a commercially – available, 1000 – ppm stock solution (Acros Organics, Lot #A0224266). A typical calibration curve is shown in Figure 5.

All dilutions were made with water purified by all – glass distillation after a prepurification by reverse osmosis.

The CRM NIES No. 13 was used for data validation. This reference material was prepared by the Japanese National Institute for Environmental Sciences and certified for homogeneity and trace element concentrations. The value for zinc is of 172 ± 11 mg/kg (Yoshinaga, *et al*., 1997).

Reference materials Hair – 30 and Nail – 30 were prepared in – house by grinding and homogenizing in a Wiley mill pooled samples of the respective tissues collected from volunteers. This material was used to evaluate precision of the zinc determinations and equivalency of the sample preparation methods.

Trace metal grade nitric acid (Fisher Scientific) was used to dissolve the samples.

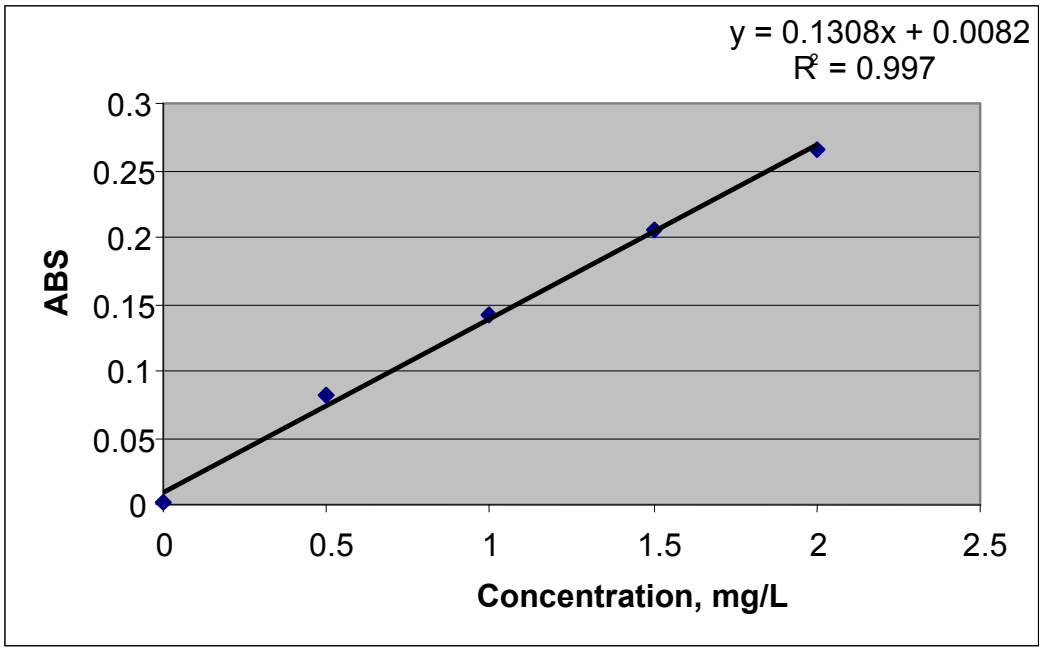


Figure 5. Typical calibration curve for the determination of zinc

SAMPLE COLLECTION

Samples of hair and nail were collected from volunteers under the guidance of the Institutional Review Board (IRB), Office of Research and Sponsored Programs, Protocol #: 07-396M.

Hair samples were cut from the occipital region close to the scalp and from the pubic region with stainless steel scissors and stored at ambient temperature in numbered Ziplock® bags. Forty-three different individuals provided hair samples. Samples were labeled only with the date the sample was taken, scalp or pubic, and a number in order to match samples from the two anatomical sites. As required by the IRB, no connection between sample and subject could be made to ensure anonymity of the participants.

Samples of scalp hair and finger nail were collected from ten additional individuals following IRB approval. All samples were collected within a three day time period following a two week growth period. The samples immediately placed in plastic Ziplock® bags. The bags were stored at ambient temperature in large unmarked brown envelopes until needed. All materials contained no record of any kind of the donor in order to ensure anonymity of donors.

SAMPLE PREPARATION

Individual samples were cleaned using a 1% Triton X-100 detergent solution. The samples were placed in an ultrasonic cleaner along with 25 mL of detergent solution and washed for approximately 30 seconds. Ultrasonic cleaning with nonionic detergent was used by the Japanese National Institute for Environmental Sciences in the preparation of the hair samples for CRM NEIS No. 5 (Okomoto, *et al*., 1985). Individual samples were removed using stainless steel tweezers and placed into open topped plastic containers. Plastic containers were numbered for sample identification and topped with a Kimwipe®. Sample containers were then placed in a vacuum desiccator with calcium chloride desiccant and evacuated. Samples were left to dry for one week to ensure all samples were fully and completely dried.

As stated above, the determination of zinc by flame atomic absorption spectrometry begins with the aspiration of the aqueous sample into a flame generated by combustion of a mixture of gases, acetylene and air in this case. This makes dissolution of solid samples necessary. Two approaches to dissolving the samples in nitric acid were evaluated; [1] decomposition of the protein matrix in sealed, heated Teflon® vessels, and [2] hot plate digestion in watch glass covered beakers.

Replicate 200 mg specimens of washed and dried Hair – 30 were placed in the Teflon® vessels. Two mL of concentrated nitric acid (Fischer Scientific Trace Metals Grade lot # 1106020) was added. Several acid – only blanks were included. The Teflon® vessels were then sealed inside a stainless steel bomb apparatus and placed in an oven overnight at approximately 100^{o}C. The Teflon® vessels were removed after the bomb assemblies cooled. The digested samples were then transferred to separate 10 mL Class A

volumetric flasks. The Teflon® vessels were rinsed with high purity water, and the rinses were added to the flask. The digested samples were diluted to 10 mL and then analyzed by atomic absorption spectrometry using a Varian SpectrAA 250+ spectrometer.

Replicate 200 mg specimens of washed and dried Hair – 30 were placed in separate 15 mL beakers. Two mL of concentrated nitric acid (Fischer Scientific Trace Metals Grade lot # 1106020) was added to the contents of each beaker. Two mL of acid was added to several additional beakers to serve as blanks. A small glass bead was placed in each beaker to prevent bumping, and the beakers were covered with watch glasses. The beakers were placed along the edge of a large hot plate set to 'Low' heat to ensure equal heating of all samples and allowed to digest overnight. Once all samples appeared to be fully digested, the beakers and their contents were allowed to cool. The digested sample was then transferred to separate 10 mL Class A volumetric flasks. The beakers were rinsed with high purity water, and the rinses were added to the flasks. The sample was diluted to 10 mL and analyzed for zinc by atomic absorption spectrometry using a Varian SpectrAA 250+ spectrometer.

The results of the two approaches to dissolving the samples for the determination of zinc are compared in Table 11.

Table 11. Comparison of Sealed Vessel and Open Beaker Hair – 30 Sample Dissolution

Sealed Vessel				Open Beaker	
#	ppm Zn	#	ppm Zn	#	ppm Zn
1	199	8	216	1	239
2	200	9	220	2	212
3	207	10	219	3	225
4	179	11	211	4	203
5	200	12	193	5	200
6	208	13	202	6	218
7	192			7	219
Mean ± SD	**205 ± 9**			**217 ± 13**	

Application of the t test to the results in Table 11 gave a t value of 2.2870 corresponding to a two – tailed P value of 0.0345. By conventional criteria, the difference between the means is considered to be not statistically significant. Literature values for zinc in human scalp hair range from 150 to 250 ppm. Application of the F test gave a value of 2.0 which is less than the critical value of 2.8 at p = 0.05. Consequently, it can be concluded the two approaches to dissolving the hair samples are of equal precision.

Samples of Nail – 30 were dissolved in sealed vessels and in open beakers using the same procedures as those described above for the Hair – 30 samples. The results for the nail – 30 samples are compared in Table 12.

Table 12. Comparison of Sealed Vessel and Open Beaker Nail – 30 Sample Dissolution

Sealed Vessel		Open Beaker	
#	ppm Zn	#	ppm Zn
1	112	1	134
2	118	2	121
3	114	3	118
4	101	4	110
5	105	5	123
6	139	6	129
7	123		
8	118		
Mean ± SD	116 ± 11		122 ± 8

Application of the t test to these results gave a t value of 1.1097 corresponding to a two – tailed P value of 0.2889. By conventional criteria, the difference between the mean zinc concentrations is considered to be not statistically significant. Literature values for zinc in human finger nail range from 80 to 191 ppm. Application of the F test gave a value of 1.9 which is less than the critical value of 4.15 at p = 0.05. Consequently, it can be concluded the two approaches to dissolving the nail samples are of equal precision.

The average value for the blanks was 0.03 ± 0.01 ppm zinc based on calculations assuming a sample mass of 100 mg.

DATA VALIDATION

The data presented in Tables 11 and 12 show coefficients of variation from 5 to 10% for the determination of zinc in the reference materials Hair – 30 and Nail – 30.

The accuracy of the zinc determinations was evaluated using two certified reference materials, NIES CRM 13 and Bowen's kale. NIES CRM 13 is a reference material for trace elements in human hair prepared by the Japanese National Institute for Environmental Sciences (Yoshenaga, 1997). Bowen's kale was prepared and certified by Professor H.J.M. Bowen as reference material for trace elements in a botanical matrix (Katz, 2002).

Replicate 200 mg specimens of each CRM were prepared for the determination of their zinc contents by atomic absorption spectrometry as described above. The results of these determinations are listed in Table 13.

Precision of the results for the zinc determinations in these certified reference materials show coefficients of variation between 5 and 10%. The relative errors are, respectively, 10 % and approximately 6 %. The difference between the experimental mean for CRM 13 and its reference value is not quite statistically significant by conventional criteria; i.e., $t = 1.8$, and $p = 0.094$. Limitations on the quantity of CRM 13 precluded additional measurements on the zinc concentration in this material.

Table 13. Results of Zinc Determinations in NIES CRM 13 and Bowen's Kale

	NIES CRM 13		Bowen's Kale	
	Sample	ppm zinc	Sample	ppm zinc
	1	142	1	34.7
	2	166	2	35.9
	3	166	3	27.5
			4	38.0
			5	37.7
			6	41.9
			7	35.6
			8	36.8
			9	32.9
			10	32.5
			11	34.4
Mean	158 ppm		36.2 ppm	
± SD	± 14		± 2.6	
Reference Values	172 ± 11 ppm		30 – 38 ppm	

MEASUREMENTS OF ZINC IN HAIR AND NAILS

The scalp hair and finger nail samples collected from the ten volunteers were washed, dried, weighed and dissolved as described above. Reagent blanks were included. The zinc concentrations in the resulting solutions were determined by atomic absorption spectrometry. The results of these measurements are listed in table 14.

Table 14. Zinc Concentrations in Scalp Hair and Finger Nail, ppm

Subject	Hair	Nail	Subject	Hair	Nail
1	254	124	6	203	164
2	175	181	7	176	176
3	452	211	8	168	256
4	198	173	9	191	157
5	218	153	10	233	210

The scalp and pubic hair collected from the 43 volunteers were prepared similarly prior to the determination of their zinc contents. The results of these measurements are shown in Figures 12 and 13, respectively, and they are listed in Table 15.

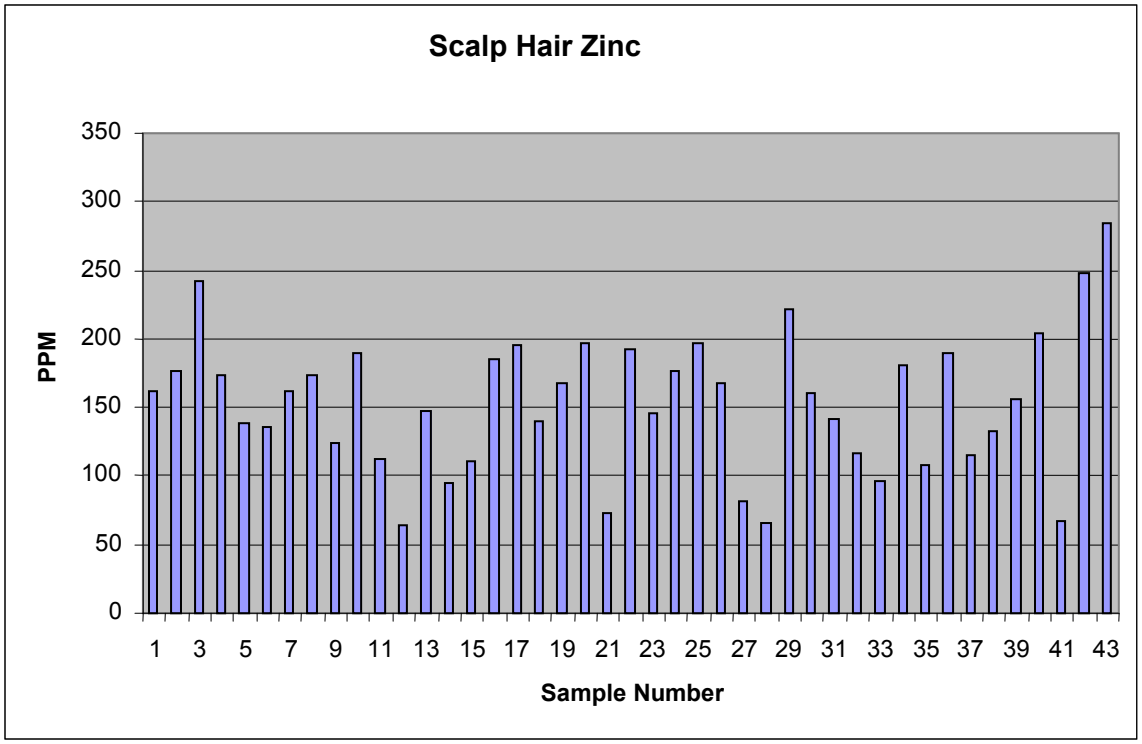


Figure 6. Zinc Concentrations in Scalp Hair Samples

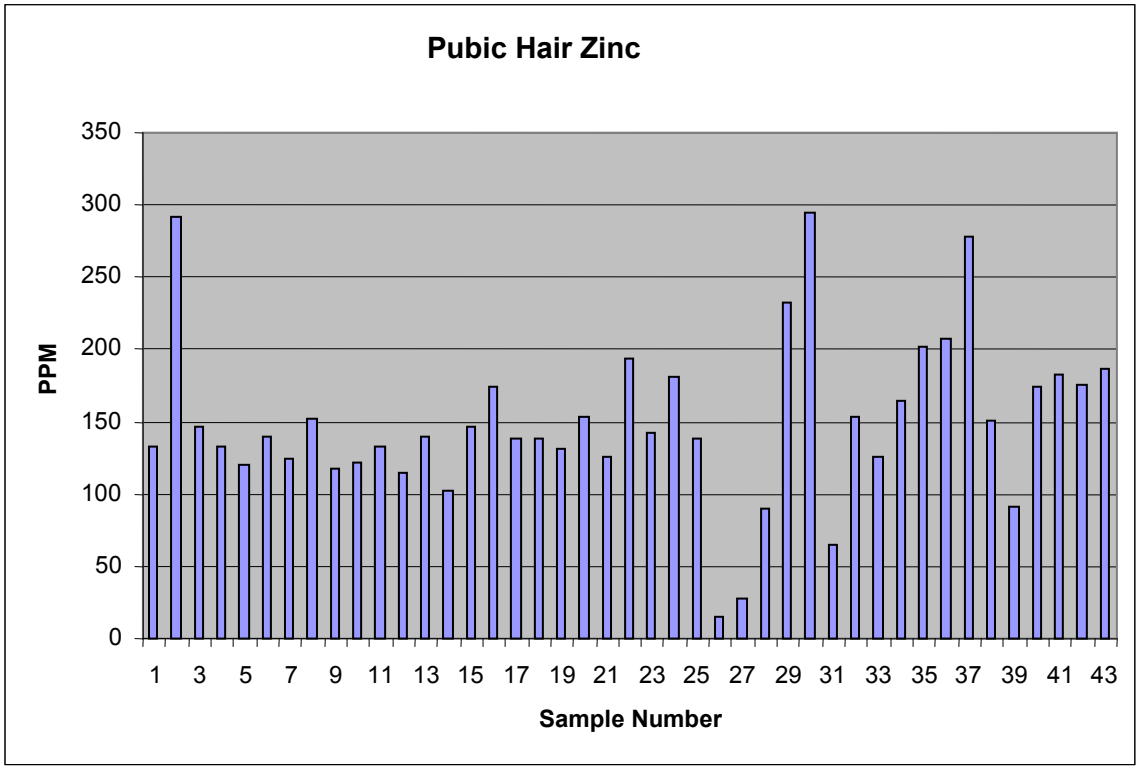


Figure 7. Zinc Concentrations in Pubic Hair Samples

Table 15. Zinc Concentrations in Pubic and Scalp Hair Samples, ppm

Subject	Pubic Hair ppm Zinc	Scalp Hair ppm Zinc
1	133	162
2	292	177
3	147	242
4	133	173
5	120	138
6	140	136
7	125	162
8	152	173
9	118	124
10	122	190
11	133	112
12	115	64.4
13	140	148
14	102	95.0
15	146	111
16	175	185
17	138	196
18	138	140
19	132	168
20	153	197
21	126	72.4
22	194	193
23	142	146
24	181	177
25	139	197
26	15	167
27	27.1	81.5
28	89.6	65.8
29	233	222
30	294	160
31	65	141
32	153	117
33	126	96
34	164	181
35	202	108
36	208	189
37	278	115
38	151	132
39	91.2	156
40	175	204
41	182	67.5
42	176	248
43	187	285

The mean values and standard deviations for these data are 150 ± 57 ppm zinc in the pubic hair samples and 154 ± 51 ppm zinc in the scalp hair samples. Application of the t test to these results gave a t value of 0.3250 corresponding to a two – tailed P value of 0.7460. By conventional criteria, the difference between the mean zinc concentrations is considered to be not statistically significant.

The mean values and standard deviations for the data presented in Table 14 are 227 ± 84 ppm zinc for scalp hair and 181 ± 37 ppm zinc for finger nail. Application of the t test to these results gave a t value of 1.601 corresponding to a two – tailed P value of 0.1269. By conventional criteria, the difference between the mean zinc concentrations in scalp hair and in finger nail is considered to be not statistically significant. However, the difference between the mean zinc concentrations in the scalp hair of the ten subjects listed in Table 14, 227 + 84 ppm, is significantly greater than that of the 43 subjects listed in Table 14, 154 ± 51 ppm. The t value and the corresponding two – tailed P value are 3.753 and 0.0008, respectively. No explanation of this statistical difference is attempted.

DISCUSSION

While the literature appears to be supportive of the idea hair, and perhaps nail, are minor excretory organs for the toxic elements lead, mercury and arsenic, the literature on the nutritional element zinc is contradictory at best. This has led to a long – standing division of opinion on the efficacy of hair mineral analysis for assessing zinc status. Support for hair mineral analysis is found in numerous Internet web sites such as those featuring the holistic health practioners Alan Ashby and Carol Dewey (hairanalysisreport.com), the physicians Alan Greenberg (scienceformulas.com) and Lawrence Wilson (drlwilson.com) and the certified nutritionist Janet Starr Hull (hairanalysisprogram.com). There are many more. The opposition to hair mineral analysis resides in [1] a lack of reference ranges in which to frame the interpretation of results, [2] difficulties in distinguishing between exogenously and endogenously deposited zinc, [3] a lack of understanding on how and to what extent the endogenous zinc is incorporated into the hair and [4] a lack of consistent correlations between zinc in the hair and zinc in the blood or other tissues (ATSDR, 2001). This opposition is reflected in the American Medical Association's Policy Statement H – 175.995 Hair Analysis – A Potential for Medical Abuse which states, "The AMA opposes chemical analysis of hair as a determinant of the need for medical therapy and supports informing the American public and appropriate governmental agencies of this unproven practice and its potential for health care fraud. (Sub. Res. 67, I – 84; Reaffirmed by CLRPD Rep. 3 – I – 94: CAS Rep. 6, A – 04)" (AMA, 2007).

Nitric dissolution prior to flame atomic absorption spectrometry is suitable for the determination of zinc in hair. The coefficient of variation for replicate determinations of

zinc in Hair – 30 (and in Nail – 30) is less than ± 10 %. The relative error is within 10 % of the CRM NIES 13 reference value for zinc.

The mean value for scalp hair samples from 43 subjects (154 ± 51 ppm) did not differ significantly from the mean value for pubic hair samples from the same 43 subjects (150 ± 57 ppm). DeAntonio and her coworkers, (DeAntonio, *et al*., 1982) also found no significant difference between the concentrations of zinc in pubic and scalp hair. They reported mean values of 158 ± 50 ppm and 155 ± 50 ppm, respectively, for 73 subjects. On the other hand, Wilhelm and his collaborators (Wilhelm, *et al*., 1990) reported the mean concentrations of zinc in public and scalp hair, 133.3 and 148.8 ppm, respectively, were statistically different based on their analyses of samples from 41 subjects. The absence of a difference in the zinc concentrations in hair from two anatomical sites would be indicative of endogenous deposition by the same biokinetics and supportive of using hair mineral analysis for the assessment of zinc status. However, there is inconsistency in making this observation.

When the data for the concentrations of zinc in the scalp and pubic hair are compared on a subject – by – subject basis marked differences are observed. Inspection of Figure 8 reveals little agreement between the zinc concentrations in hair from these anatomical sites. Some of the paired samples differ by more than 100 ppm in their zinc concentrations. Others show much better agreement. If the values represent endogenously deposited zinc, these subject – by – subject variations tend to support randomness rather than biokinetic control.

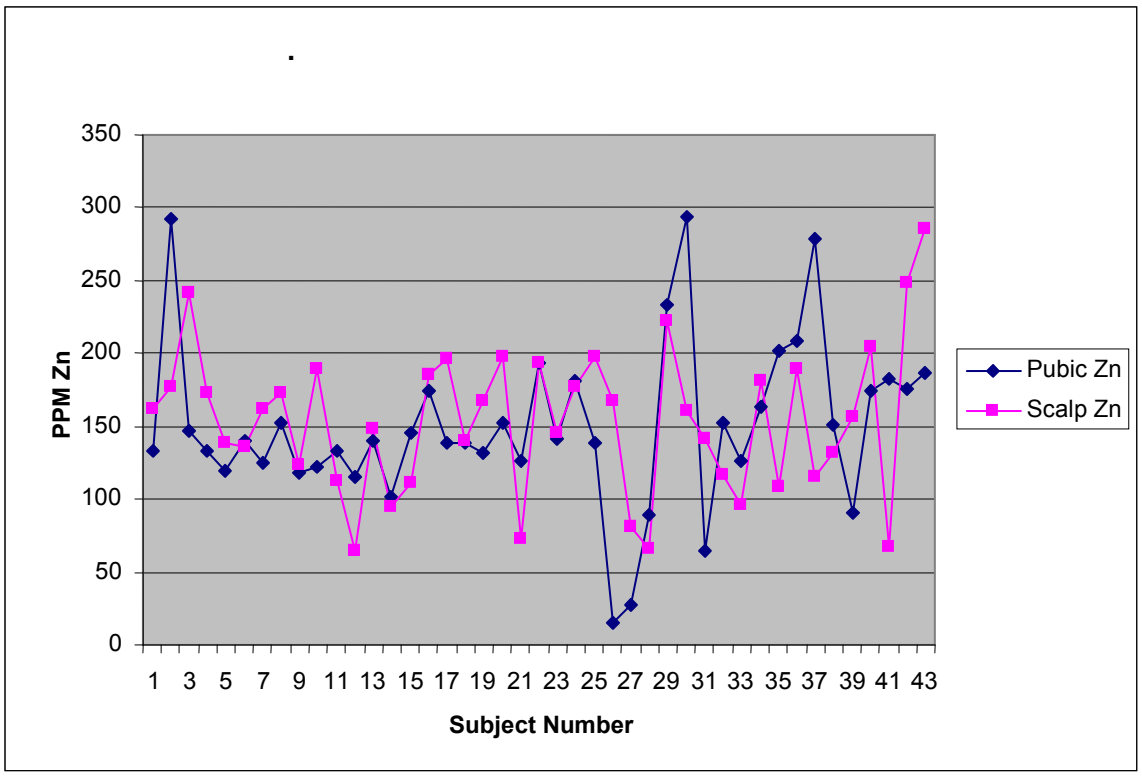


Figure 8. Subject By Subject Comparison of Zinc Concentrations in Pubic and Scalp Hair

Sometimes data points that are as much as more than two times higher than the average can be explained. A pubic hair zinc concentration of greater than 1000-ppm may be due to contamination from semen which has a very high zinc concentration (Emsley, 2001). Extremely high or extremely low data points can also be due to hair cosmetic treatments or simply to the brand of shampoo an individual uses. Differences in the zinc concentrations in scalp and pubic hair from a given individual can be attributed to different cosmetic use at the two anatomical sites, i.e., talcum powder. This possibility again raises the question of endogenous verses exogenous deposition.

Statistical analysis was conducted using a combination of web-based statistical software (GraphPad) and Microsoft® Excel®. Linear regression R^2 values and Pearson's correlation coefficients were generated using Microsoft Excel® statistical functions. T

values were calculated by hand using set equations (Harris, 2003). T_c values at the 95% confidence level were used. If an exact t_c value was not available for the degrees of freedom, the next lowest available t_c value was used. The student's t is a statistical analysis tool that is used to express confidence intervals and to compare results. A t test is used to test the null hypothesis which states that the mean value from two sets of values is not the same. It is conventional to reject the null hypothesis if there is less than a 5% chance that the difference is due to random error (Harris, 2003). Pearson's correlation coefficients range from a value of -1 to a value of +1. A Pearson correlation coefficient of 0 would mean the values are not correlated at all. A Pearson's correlation coefficient of +1 indicates a perfect positive correlation and a -1 indicates a perfect negative correlation.

The p-values (probability values) were generated using a web-based statistical program based on r values and degrees of freedom (GraphPad). P-values are used as a measure of the strength against the null hypothesis. The null hypothesis, termed H_0, can be stated that the means of two populations that are the same and any observed difference in the means of those two populations is due to random chance. P-values have a range from a value of 0 to a value of 1. The significance of a p-value, 0.03 for example, can be expressed as a 3% chance that any random sampling of data from identical populations will result in a higher observed difference in the means of the populations and a 97% chance that a smaller difference will be observed in the means. A threshold value is typically set to determine the significance of the p-value. A p-value of 0.05, or 5%, is a typical cutoff value with values less than the cutoff value indicating a rejection of the null hypothesis and a p-value higher than the cutoff value indicting the null hypothesis may

not be rejected and that the difference in the population means is not statistically significant (GraphPad). The rejection of the null hypothesis is an indication that a correlation exists between the means of the sample data. It is important to distinguish here that the p-values in this case are a measure of correlation of the means of the concentrations for the two sites, not the individual samples from which the means were calculated.

In Figure 9, the scalp hair zinc concentrations and the pubic hair zinc concentration are paired on a subject-by-subject basis and regressed. A p-value of 0.0471 for the comparison of zinc in scalp hair to zinc in pubic hair was obtained (GraphPad) and a Pearson's correlation coefficient of 0.301. Zinc concentrations in scalp hair and pubic hair were correlated with a linear regression R^2 value of only 0.0904. A t value of 2.01 was calculated with 42 degrees of freedom. This wide subject – by – subject variations in the zinc concentrations in both hair samples are clearly seen in Figure 8 also. While some values are very close to one another in a matched set, another matched set can differ by more than 150-ppm.

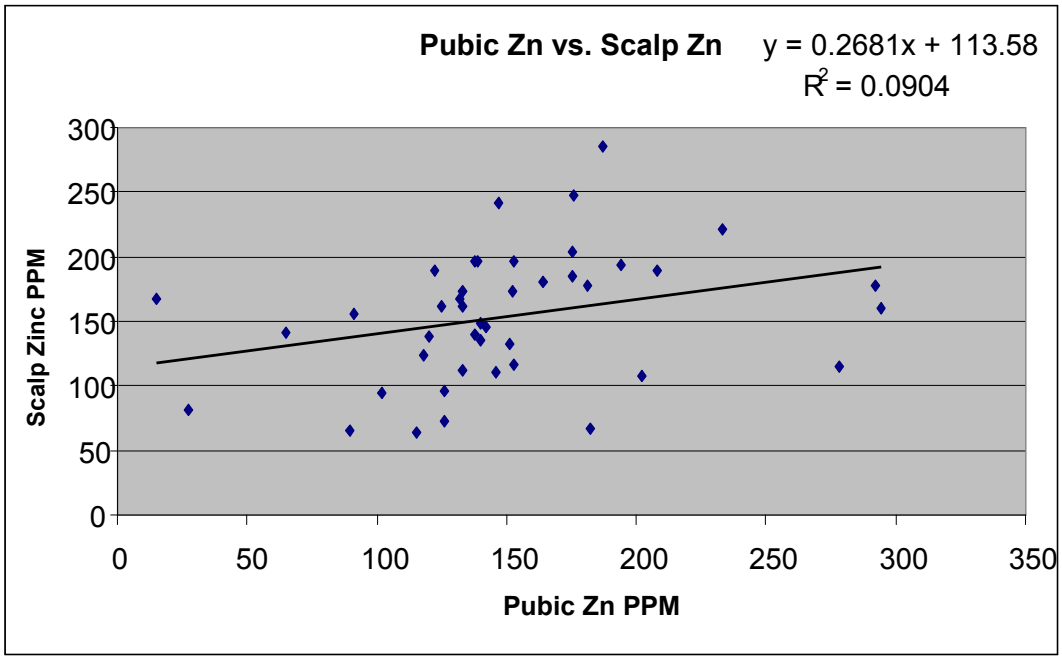


Figure 9. Linear Regression of Scalp Hair Zinc Concentrations Against Pubic Hair Zinc Concentrations on a Subject By Subject Basis

An R^2 value for zinc in scalp hair and pubic of only 0.0904 and a Pearson's correlation coefficient of only 0.301 based on the p-value would suggest the lack of a linear relationship. The p-value of 0.0471 for the comparison of zinc in scalp hair to zinc in pubic hair is very close to the cut off of 0.05, and may justify rejection of the null hypothesis and suggest that there exists a non-linear correlation between these two values. A calculated t value of 2.01 while extremely close to the t_c of 2.02 on a student's t table, is still less than the t_c value of 2.02 for the given degrees of freedom at the 95% confidence level. Even though the p-value suggests the existence of a non-linear correlation between these two anatomical sites for zinc concentration, a value of 0.0471 is very near the cutoff value stated previously. The strength of this non-linear correlation may be questioned given the proximity of the p-value to the cutoff value for the rejection of the null hypothesis. It can also be debated whether any data points should be

eliminated based on a q-test since they do reflect the concentration of zinc in that individual's hair at that specific time.

Linear regression of these data show little, if any, linear correlation between the concentrations of zinc in the pubic hair with the concentrations of zinc in hair from the pubis. Figure 9 shows this linear regression line, scalp hair Zn concentration = 0.2681 pubic hair zinc concentration + 113.58. A slope of unity is expected when zinc is deposited in pubic hair and in scalp hair by the same biokinetics. A slower growth rate in the pubic hair could result in a slope of greater than unity, but this is not observed. The correlation coefficient, R^2, and the Pearson's correlation coefficient would suggest the lack of a linear relationship the p-value may indicate the existence of a non-linear relationship between the concentrations of zinc from these two anatomical sites. However, as stated before, the strength of this relationship may be questionable given the p-values proximity to the established cutoff point.

The subject – by – subject comparison for zinc in hair and nail samples shown in Figure 10 indicates the concentrations of zinc in hair are higher than the concentrations of zinc in nail in eight of the ten subjects. However, the t test gave a t value of 1.601 corresponding to a two – tailed P value of 0.1269. By conventional criteria, the difference between the mean zinc concentrations in hair and in nail is considered to be not statistically significant. The lack of a linear correlation in the zinc concentrations of these two tissues is demonstrated by an extremely low linear regression R^2 value of only 0.0117 and a Pearson's correlation coefficient of only 0.108 obtained in the linear regression shown in Figure 11. A p-value of 0.766 for the comparison of the zinc concentration in hair to the concentration of zinc in nail was obtained (GraphPad). With

a p-value of 0.766, there is a 77% chance that there will be a greater difference in the means of randomly sampled identical populations. This p-value is far from the established cutoff value of 0.05 and is evidence against the existence of a non-linear relationship. A t value of 0.307 was obtained with 8 degrees of freedom.

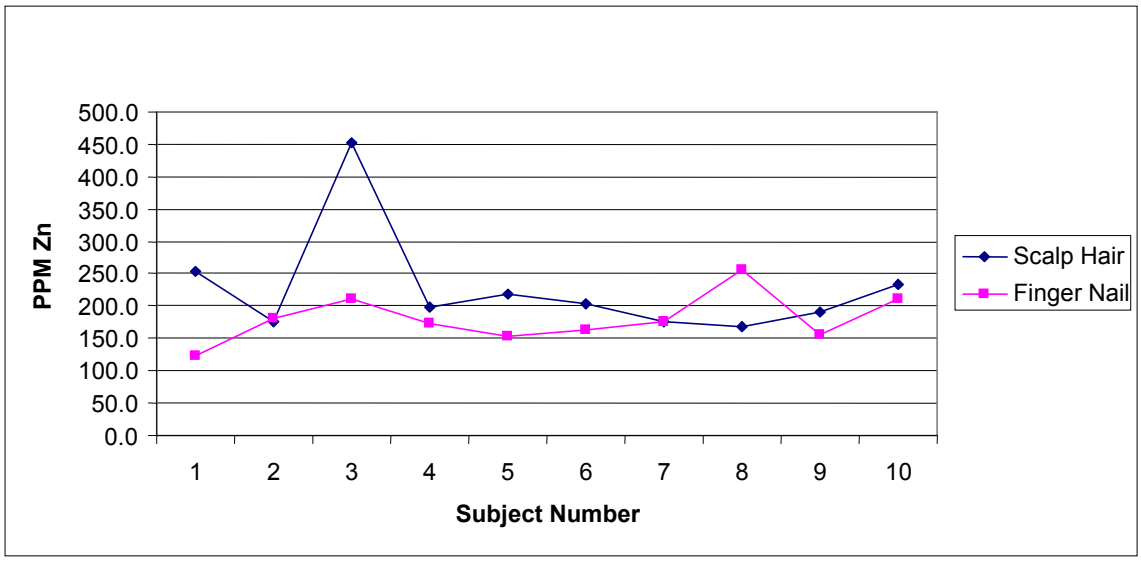


Figure 10. Comparison of Zinc Concentrations in Hair and Nail on a Subject By Subject Basis

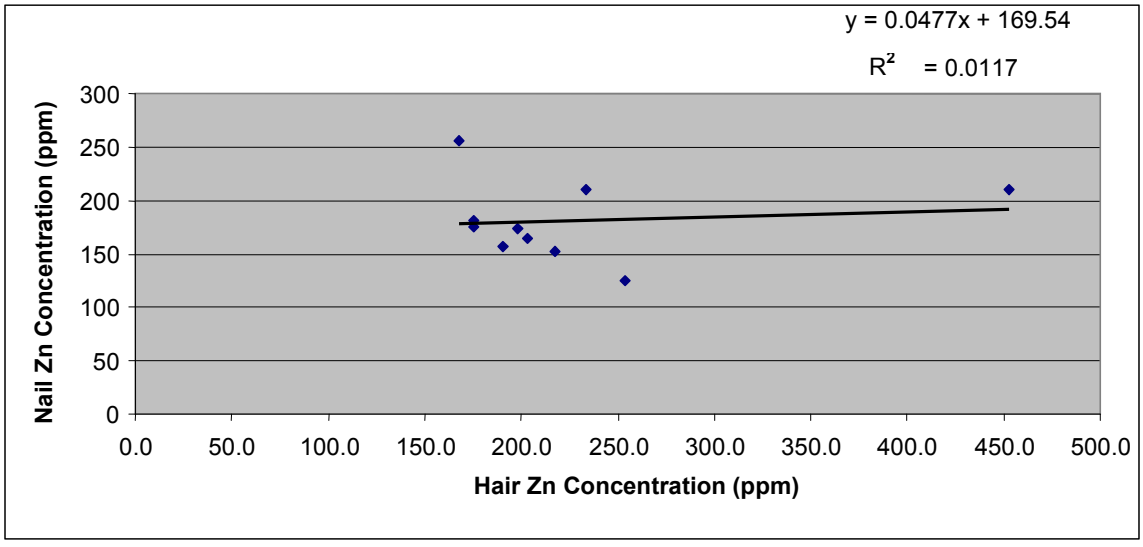


Figure 11. Linear Regression of Concentrations of Zinc in Nail Against Concentrations of Zinc in Hair on a Subject By Subject Basis

CONCLUSION

There is no correlation, linear or non-linear, between the amount of zinc in scalp hair and finger nails. There may be a non-linear correlation between the amounts of zinc in scalp hair compared to pubic hair. The poor r^2 value and Pearson's correlation coefficient suggest a lack of linear relationship but the p-value would be indicative of a non-linear relationship. However, given the proximity of the p-value to the established cutoff value for the assessment of a correlation, the strength of this relationship may be questioned. While this non-linear relationship may exist, using it as evidence for the existence of a regulated metabolic process for the deposition of zinc in scalp hair and pubic and thus the use of trace element analysis of zinc to assess medical status may be premature.

Continued utilization of hair and nail analysis for such purposes as the assessment of medical or nutritional status should wait until more information about the mechanism of trace element uptake in keratinized tissues such as hair and nail becomes known, a standardized methodology is developed, and a reliable and accurate method for the removal of exogenous contamination is developed. These factors remain problematic and keep trace element analysis from being a viable tool for the assessment of zinc status in the human body.

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