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Determination of Trace Elements in Human Hair

Reference Intervals for 28 Elements in Nonoccupationally Exposed Adults in the US and Effects of Hair Treatments

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ABSTRACT

The concentrations of 28 elements in hair of three populations of non-occupationally exposed adults in the US (n = 271) were determined. The 10th, 50th, and 90th percentiles, and geometric means for these data were obtained to define reference intervals for these elements. The effects of various hair treatments, age, and sex on concentrations of 17 selected elements in hair were determined for these populations. Age had little effect on elemental concentrations. Males tended to have higher Cd and Pb levels, but lower Mg and Ti levels than females. Males using dandruff shampoo had significantly higher concentrations of Na, Se, and Ti than those using only regular shampoo and/or conditioners. Ba, Ca, Cu, Mg, Na, and Sr were all elevated in females using permanents or color treatments, compared to those using only dandruff shampoo, regular shampoo, and/or conditioners.

Index Entries: Analysis; inductively coupled argon plasma (ICAP); atomic absorption; reference intervals.

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INTRODUCTION

Hair is potentially of much value as an indicator of the elemental status of a clinical subject. It has relatively high concentrations of many elements of interest to clinical chemists, toxicologists, and others, can be collected non-invasively, and is easy to store. Although the analysis of hair for trace elements is relatively simple and straightforward, considerable controversy exists over the interpretation of elemental hair data generated by such analyses. Thus, the usefulness of hair for exposure assessment or evaluation of nutritional status can be somewhat limited. Some of the difficulties in interpretation of trace element data for hair were reviewed by Hambridge (*1*), and include

1. External contamination;
2. Choice of washing procedure;
3. Effects of hair treatments;
4. Variations with hair color, location, diameter;
5. Variations with age, sex, season;
6. Variable (or slow) rate of hair growth;
7. Analytical problems, e.g., choice of technique, calibration, and so on;
8. Correlation of hair levels with other biological tissue levels; and
9. Lack of reference or "expected" range of values.

We have attempted to provide solutions to some of these areas of difficulty in an effort to evaluate the potential usefulness of multielement hair analysis. Problems (1) and (2) above were addressed with the use of a standardized washing technique. Similarly, problem (7) was handled by using primarily inductively coupled argon plasma, with cold vapor atomic absorption for analysis of mercury. The issues raised in (3) and (4) were approached with the use of a questionnaire (Appendix A), which asked for a detailed history of hair treatment products, and with use of a "standardized" sampling technique. Problem (6), is, of course, not amenable to analytical solution.

The objectives of this analytical work were: to evaluate the "expected" or reference range of elements in adult hair; and to evaluate the effect of various hair treatments, as well as age and sex, on elemental concentrations. The populations chosen for study were all free of obvious disease and were not occupationally exposed to the elements in question. These subjects could, therefore, be interpreted as representative of an "unexposed" or control population for studies involving environmental or occupational exposure, nutritional evaluation, or evaluation of effects of disease. With the reference intervals provided by this study, interpretation of multielement hair data is made more meaningful. Comparisons are presented between these findings and those of other workers to

evaluate our data in the context of other elemental hair data in the literature.

EXPERIMENTAL

Spectroscopy

The inductively coupled argon plasma emission spectrometer was a Jarrell-Ash Model 1160 Atomcomp, equipped with Mark III software, a Digital PDP 11/34 computer, and Plasma Therm HFP 2000 R.F. Generator operated at 27.12 MHz. Mercury was measured with an LDC Mercury Monitor. Operating parameters are summarized in Tables 1 and 2.

Reagents and Glassware

Hair digestion was accomplished with ultrapure nitric acid (Redistilled grade, cat # 63 G.F. Smith) and 30% v/v hydrogen peroxide (J.T. Baker Ultrex grade, cat # 5170-1). All dilutions were performed with water polished to 18 megaohm/cm purity with a Milli-Q system (Millipore Corp.). Analytical standards were prepared by serial volumetric dilution of J.T. Baker "Instra-Analyzed" standards, with a nominal concentration of 1000 ± 10 mg/L. Reagents used for the cold vapor determination of hair mercury were as described by Greenwood et al. (2).

Plasticware used included teflon tubes with screw caps (Cole Parmer cat # TV-6327-31) used for hair digestion, plastic 15 mL conical tubes (Falcon cat # 2095; 2057 or Corning cat # 25319) with screw caps for storage of the digestate, and plastic bags for storage of collected hair (Cole Parmer cat # TV-6503-01). All plasticware that came in contact with the specimens or digestate were either screened for the metals to be investigated or cleaned rigorously as follows. Teflon digestion tubes used were cleaned by soaking in a detergent bath (2% v/v "Isoclean" detergent) for 12–24 h, followed by rinsing with deionized water and soaking for 12–36 h in 25% v/v nitric acid. The acid cleaned teflon ware was then rinsed with copious amounts of deionized water and dried under Class 100 laminar flow air. The method of sequential lot testing, described by Wald (3) was used to screen the plasticware, with 20 tubes each of Falcon 2057 or 2095 tubes each soaked with 0.1 F HCl. After the 0.1 F hydrochloric acid solvent was allowed to remain in the container overnight (12 + h), no detectable amounts of any of the reported elements was found in the lots used. Similar results were obtained for testing the 15 mL Corning tubes, 25 of which were screened with 0.1 F HCl. Class A volumetric glassware was used, cleaned with 2% v/v "Isoclean" detergent. This detergent cleaning was followed with copious washing with deionized water, and then soaking 24 h in 25% v/v reagent grade nitric acid. The glassware was then rinsed again with copious amounts of deionized water, and then dried under Class 100 conditions.

Table 1
Instrumental Parameters for ICAP Measurements on all Elements Except Hg[a,b]

Element	Wavelength, nm	Detection limit in Hair, mg/kg[b]
Al	308.2	0.45
As	193.6	1.1
Au	242.7	0.25
B	249.7	0.25
Ba	493.4	0.02
Be	234.8	0.01
Ca	396.8	0.02
Cd	228.8	0.15
Co	228.6	0.05
Cr	205.5	0.15
Cu	324.7	0.05
Fe	259.9	0.10
Li	670.7	0.40
Mg	279.5	0.02
Mn	257.6	0.05
Mo	202.0	0.10
Na	589.0	0.45
Ni	231.6	0.15
P	214.9	5.1
Pb	220.3	1.0
Sb	217.5	0.65
Se	196.0	4.5
Sr	421.5	0.02
Ti	334.9	0.05
Tl	190.8	3.15
V	292.4	0.10
Zn	213.8	0.05

[a]Instrument: Jarrell Ash Model 1160 "Atomcomp"; RF Generator Plasma Therm Forward Power 1120 W Reflected <5 W; Specimen Flowrate: 2.0 mL/min; Nebulizer Pressure: 30 psig (207 kPa); Observation Height: 12mm.

[b]Detection limit is defined as 2 SD of ten replicate measurements of the analytical blank (3% v/v HNO3) multiplied by 50, the inverse of the average dilution factor for the hair specimen (0.20 g/10.0 g = 10 mL).

Table 2
Instrumental Parameters for Hg Measurements

Instrument:	LDC mercury monitor
Pathlength:	30 cm
Cell volume:	17.3 cm^3
Sensitivity:	0.6
Sample inlet pressure:	20 psig (138 kPa)
Carrier gas:	Nitrogen (Ultrapure)
Reference cell:	Air

Procedure

Hair was collected using a protocol (Appendix B) that requires a minimum of 500 mg of hair from the nape of the neck. The selection of this area for specimen collection is based on cosmetic considerations as well as the criterion that "new growth" hair be collected. This collection procedure is based on the assumptions that the most recent hair growth is more representative of the body's elemental status during the last few months, and that external contamination tends to increase with hair length (4). The collected hair was bundled and placed in a plastic "zip-lock" bag. About a 200 mg portion of the hair specimen was then washed in a 15 × 100 mm disposable plastic petri dish with successive portions of 1.0% w/v sodium lauryl sulfate (or ammonium lauryl sulfate). After 30 min contact with occasional agitation the hair was then rinsed 6 times with Milli-Q water, and dried under laminar flow Class 100 air.

This washed specimen was then weighed and transferred to a Teflon screw capped vial (15 mL with screw cap), and 1.0 mL of ultrapure nitric acid was added. The vials were capped, and the specimens were allowed to stand about 2 h at room temperature, after which they were heated to 80–90°C overnight on an aluminum block. The tubes were then chilled in an ice bath to avoid sudden escape of acid vapors to approximately ambient temperature, and a 0.50 mL aliquot of 30% v/v hydrogen peroxide was added. The specimens were recapped and then reheated for 2 h at 80–110°C, cooled and brought to 10 mL volume with Milli-Q water. The digested specimens were stored in tightly capped 15 mL plastic tubes until analyzed.

Calibration of the inductively coupled plasma system was accomplished with aqueous standards made to 10.0 mg/L of each analyte except K (100 mg/L) in 3% v/v nitric acid. The standards used were

1. 3% v/v nitric acid (blank);
2. 10.0 mg/L Ca, Cd, Co, Cu, Mg, Mn, Pb, and Zn in 3% nitric acid;
3. 10.0 mg/L Al, Ba, Be, Fe, Li, Mo, Na, Ni, Sb, Sr, Ti, and Tl in 3% nitric acid;
4. 10.0 mg/L As, B, Cr, P, and Se in 3% nitric acid; and
5. 10.0 mg/L Au and V in 3% nitric acid.

Calibration was monitored periodically during analysis with an aqueous solution (3% nitric acid) which contained 2.0 mg/L of each analyte. This "check standard" is part of the overall quality control for the method. A pooled hair digest was analyzed in each analytical run to establish quality control. A typical analytical run consisted of calibration followed by analysis of the 2.0 mg/L mixed standard, the pooled hair digest, and then the digested hair samples. Calibration and quality control were checked after every ten digested hair samples. A spectrum shifter was used to correct for background emission from the hair digests. Each result is obtained by averaging two 5-s emission measure-

ments from each sample. The quality control system for these determinations consisted of comparing the means and ranges of duplicate measurements with 95 and 99% control limits, calculated on the basis of previous characterization runs. All data reported in this study had quality control results that were within 99% (and in most cases, 95%) control limits for means and ranges. This general approach to quality control has been described in some detail in other published references, including our own (5).

Mercury in hair was measured by the method of Greenwood et al. (2), digesting approximately 500 mg of hair with 45% w/v sodium hydroxide, followed by reduction of mercury to vapor with stannous chloride/cadmium chloride. In this procedure, the total mercury (sum of inorganic and organic) in hair is measured. Quantification was accomplished by standards which were a 20–80 admixture of mercuric (chloride) and methyl mercury (iodide). Quality control for these determinations was evaluated by duplicate analysis of pooled hair digests, one of which was spiked with mercury. As was the case for the other reported elements, all means and ranges for these quality control samples were within 99% control limits.

Statistical Analysis

A total of 271 adults (age 20 and up) from 3 different populations were included in the study for all elements except Hg. The 3 populations were: a random sample of people in Stands 33 and 51 (Harrisonburg, VA and Rock Hill, SC) from a National Health and Nutritional Examination Survey II (NHANES) pilot study (n = 75), a random sample of people in Stand 53 (El Paso, TX) from the Hispanic Health and Nutritional Examination Survey (HHANES) plus additional HHANES participants from other sites selected to increase representation of certain hair treatment groups (n = 103), and 93 volunteers working at the Centers for Disease Control in Atlanta, GA. There were 71 males and 200 females. The age distribution was 152 persons 20–39 y old, 97 persons 40–59 y old, and 22 persons 60 y old or more.

Mercury determinations were made for 79 adults (age 20 and up) from 2 different populations. The first was a group of 49 adults whose hair was sampled as part of a larger study of mercury exposure levels in Oak Ridge, TN. The second population was a group of 30 volunteers working at the Centers for Disease Control in Atlanta, GA. All 79 adults who gave hair samples exhibited normal levels of urinary mercury (<20 μg/L) (6).

Calculated analytical concentrations between 0 and the instrument detection limit were used "as is" in the statistical analyses. Calculated values below 0 were reset to 0. The distributions of elemental concentrations were generally right skewed, so a decimal log transformation was

used on all data after adding 0.01 mg/kg to each value (because of 0 values).

Data from all 271 adults were used to calculate overall reference intervals for 27 elements; data from 79 adults for hair mercury. Only persons with complete questionnaire data on hair treatments, age, and sex were included in the analyses for the effects of these variables (n = 197).

Hair treatment groups were defined based on the use of

1. Permanents (PERM);
2. Color treatments (COLOR);
3. Dandruff shampoos (DSHAM);
4. Conditioners (COND); or
5. None of these products (regular shampoo only, NONE).

Initial examination of the data revealed that there were no significant differences (t test, $p > 0.05$) between mean concentrations for the 17 selected elements for groups (4) and (5), so group (4) was lumped into group (5) (NONE). Persons who used multiple hair treatments and could not be classified into one and only one group were deleted from analysis for hair treatment effects. The PERM and COLOR groups contained only 2 and 3 males, respectively, so no comparisons among males were made using these groups. Age groups were defined as 20–39 (n = 115), 40–59 (n = 70), and 60+ (n = 12) years. There were 61 males and 136 females in the data set. Seventeen elements which had fewer than 10% of their values below the lower detection limit were chosen for analysis of the effects of hair treatments: Al, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Na, Ni, P, Pb, Se, Sr, Ti, and Zn.

Statistical tests were performed to determine the effects of 4 factors on hair elemental concentrations: age, sex, population (NHANES, HHANES, and CDC), and hair treatment. Stratified randomized block design ANOVA's were used to provide these tests while blocking against one extraneous factor and stratifying on others. The designs were as follows.

1. Main factor-age, block-population, stratified by—sex and hair treatment;
2. Main factor—population, block—hair treatment, stratified by—sex;
3. Main factor—hair treatment, block—population, stratified by—sex;
4. Main factor—sex, block—population, stratified by—hair treatment.

If the ANOVA showed significant differences among the main factor groups (e.g., hair treatments), the differences were located by pairwise t-tests.

RESULTS AND DISCUSSION

Reference intervals from data collected from 271 individual hair specimens for 28 elements are presented in Table 3. A comparison of previously reported values from a variety of published sources reveals generally good agreement among means or ranges reported by other investigators. There are, however, a number of elements that warrant individual discussion. These fall into two general groups—those elements known or suspected to be affected by hair treatment(s), e.g., permanent, coloring or tinting, or use of products known to contain elements measured in this scheme such as dandruff shampoo (selenium) or hair coloring (lead acetate in "Grecian Formula" or similar products); and those elements that are affected by environmental exposure, presumably from "internal" exposure from diet, water, or inhaled particulate material that might serve as markers for exposure.

Randomized block design ANOVAs for the effects of age class, stratified by sex and hair treatment, showed no significant ($p > .05$) differences for any of the 17 elements for males in either the NONE or DSHAM groups. For females, Se in the NONE group, and Mg, Mn, and Ti in the COLOR group showed significant differences among age classes. However, since only 4 out of 102 tests were significant at the $p = .05$ level, this is no more than would be expected by chance, and there is little evidence for consistent differences among the 3 adult age groups for the 17 selected elements. For this reason, none of the other analyses were stratified by age class.

Comparison of males and females in the NONE and DSHAM treatment groups showed several significant differences. Males had significantly higher Cd levels than did females in both NONE and DSHAM groups, and higher Pb levels in the DSHAM group (Table 4). Females in the NONE group had higher Mg and Ti concentrations than did their male counterparts (Table 4).

Analysis of the 3 different populations, performed separately for males and females and blocking against hair treatment, revealed significant differences in a number of elements (Table 5). Age was not a confounding variable since the mean ages of the 3 populations were within 2 y of each other (37 vs 39), and adult age class was shown not to be a significant factor in this data set (*see above*). There may be differences in the proportion of individuals in each population using the various hair treatments, but this factor was controlled statistically by the randomized block design. Geographic and/or ethnic differences seem likely. The HHANES group was entirely of Hispanic origin, whereas the CDC and NHANES group were of mixed ethnic origins (primarily white with a smaller black component). Because of these *statistically* significant differ-

ences among the populations, the reference intervals in Table 3 may not be strictly applicable to all other given populations or combinations of populations. However, the closeness of each of the individual population intervals to the overall (n = 271) intervals (as well as to those previously reported in the literature), suggests that they may still be of *practical* use.

Analyses of the effect of hair treatments revealed that for adult (20+) males (n = 56), significant differences were obtained between the "dandruff shampoo" (DSHAM) and "no treatment" (NONE) groups for Na, Se, and Ti (Table 4). The finding for Se was expected because of the Se content of some dandruff shampoos; that for the other elements is unexplained at present. For females, differences among hair treatment groups were significant for Ba, Ca, Cu, Mg, Na, and Sr (Table 4). These differences seem to reflect the effects of permanents or color treatments elevating the concentrations of these elements relative to the no treatment group. Dandruff shampoo had not significant effect for these elements in females. These effects of permanents and color treatments may be general for the adult population, but the small number of males reporting these treatments precluded statistical evaluation of this hypothesis. The similarity in behavior among Ca, Ba, Mg, and Sr is not completely surprising because of chemical similarity among these group II alkaline earth elements. Males using dandruff shampoo showed significant elevations of Na, Se, and Ti relative to the no treatment group.

Besides effects of hair treatments, a second consideration is accounting for the effects of environmental or other exposure on elemental analysis of hair. Elements commonly evaluated in this way include arsenic (*7,8*); cadmium (*9–11*); lead (*12–15*); selenium (*16–18*), and thallium (*19–20*). Elevated levels of these elements have been documented in a variety of "exposed" populations over a number of years. A major complication in interpretation of an elevated level of a selected element is the possibility of external deposition of the element via "air pollution" of fumes or particulates; absorption of the element from water during washing or other exposure; or absorption from sweat or oil. Except for absorption from sweat or oil, the presence of high levels of an element reflects *general* exposure, which may well be the major issue in public health considerations. One significant consideration, the effect of washing the specimen, needs to be addressed in interpreting this data. The "ideal" washing procedure would be one that selectively removes all the exogenously deposited elements and retains the endogenous. Such a procedure, unfortunately, does not exist. We have chosen a "mild" washing technique that we feel emulates that of *in situ* washing with detergent shampoo. Support for this process comes from research studies that show that a single washing with sodium lauryl sulfate removes very little (ca. 5–20%) of Fe, Cd, Zn, and Mn (*4*). More important, the amount removed is quite reproducible, allowing comparisons among specimens with the same detergent pretreatment.

Table 3
Analytical Results for 271 Adults, Ages 20–73[a,b]

	Geom. mean, mg/kg	Median, mg/kg	10th percentile, mg/kg	90th percentile, mg/kg	Other reported means and/or ranges in mg/kg, Reference
Al	3.87	4.20	1.16	13.1	<4 (*21*) 10–20 (*22*)
As[b]	< 1.10	< 1.10	< 1.10	< 1.10	<5 (*21*) <0.65 (*23*) 0.09 (*26*) 2–5 (*22*) <1 (*23,24*) 0.06–1.2
Au	< 0.25	< 0.25	< 0.25	0.49	<0.01 (*28*)
B	< 0.25	0.41	< 0.25	4.17	<1 (*28*) 0.98, 0.04–25 (*25*)
Ba	1.43	1.65	0.27	5.97	0.05 mg/d (*28*) 1.4, 0.12–29.0 (*25*)
Be	< 0.01	< 0.01	< 0.01	0.03	<0.01 (*28*)
Ca	799	892	194	2700	200–600 (*21*) 344 (*27*)
Cd	< 0.15	< 0.15	< 0.15	0.79	1–2 (*21*) 0.17–6.4 (*24*) 0.93 (*26*) 0.34–1.6 (*29*) 1.65 (*30*) 1.1 (*27*) 1.72 (*14*) 0.88 (*25*)
Co	< 0.05	0.05	< 0.05	0.18	0.04–1 (*21*) 0.2 (*27*)
Cr	< 0.15	< 0.15	< 0.15	0.52	0.2 ± 0.14 (*30*) 0.5–1.5 (*21*) 0.62, 0.06–5.3 (*25*) 0.09 (*27*)
Cu	15.7	12.7	9.00	40.1	12–35 (*21*) <15 (*28*) 18.25 (2.2–184) (*25*) 15–17 (*32*) 17 (*27*)
Fe	9.35	8.15	5.21	20.5	20–50 (*21*) 31.1, 9.5–85 (*10*) 22.3, 3.6–177 (*25*) 22 (*27*)
Li	< 0.40	< 0.40	< 0.40	0.40	0.02–0.8 (*21*) 0.4 (*27*) 0.06, 0.009–0.23 (*25*)
Mg	63.5	64.1	16.8	237	25–75 (*21*)
Mn	0.22	0.22	< 0.05	1.07	0.95, 0.34–2.67 (*25*) 1–10 (*21*) 1.45, 0–25 (*32*) 0.6 (*27*) <1 (*20*)

Mo	<0.10	<0.10	<0.10	0.12	0.1–1.0 (*21*) 0.06 (*20*)
Na	47.6	44.1	11.2	219	150–350 (*21*)
Ni	0.39	0.45	<0.15	1.50	0.3–1.0 (*21*) 2.8–3.2 (*33*)
					0.2 ± 0.1 (*22*)
					0.74, 0.045–11.0 (*25*)
P	146	147	118	181	100–170 (*21*)
Pb	2.43	2.12	<1.00	10.8	20–30 (*21*) 12.2 (2–155) (*25*)
					4.1 (*33*) <10 (*20*)
					13.0 (*26*) 4 (*27*)
Sb	<0.65	<0.65	<0.65	0.84	0.07–0.2 (*33*) <0.05 (*20*)
Se	<4.50	<4.50	<4.50	8.08	3–6 (*21*) 0.6–0.8 (*33*)
					0.3, 0.02–1.6 (*25*)
Sr	3.40	3.35	0.36	32.1	0.4 (*20*)
Ti	0.29	0.27	0.06	1.45	
Tl	<3.15	<3.15	<3.15	<3.15	0.01 (*20*)
					<0.05 (*34*)
V	<0.10	<0.10	<0.10	0.15	1–2 (*21*) <0.05 (*20*)
Zn	152	147	113	207	160–240 (*21*) 216 ± 90 (*22*)
					150 (*20*) 108, 20–313 (*25*)
					140 (*27*) 150–190 (*33*)
					182 (*34*)
Hg	3.55	3.55	2.49	5.52	0.7, 0.05–14 (*25*) 2.5–5 (*21*)
					7.6, 0.1–33 (*35*)
					5.1 (males), 6.9 (females) (*36*)
					4.3 (*37*)
					<120 (*38*)

[a]Mercury results are for 79 adults, ages 20–69. Values reported with a < symbol were less than the detection limit for the instrument.

[b]Fifty digests from the CDC volunteers were analyzed for As using Zeeman graphite furnace AAS; mean of 50 specimens was 0.15 mg/kg, with a 95th percentile of 0.28 mg/kg.

Table 4
Elemental Concentrations that Showed Significant Differences
(F Test, Randomized Block Design ANOVA Blocking Against Population, $p < .05$) Between Males and Females (Stratified by Hair Treatment), or Among Hair Treatments (Stratified by Sex)[a]

Element	Hair treatment	p	Sex		Geometric means, μg/g		n
			High	Low	High	Low	
Cd	DSHAM	.0164	M	F	0.25	<0.15	49
Cd	NONE	.0188	M	F	0.18	<0.15	66
Pb	DSHAM	.0118	M	F	4.35	1.47	49
Mg	NONE	.0411	F	M	53.1	34.2	66
Ti	NONE	.0024	F	M	0.34	0.17	66

Element	Sex	p	Hair treatment				Geometric means, μg/g				n
			High			Low	High			Low	
Na	M	.0064	D			N	54.8			26.4	56
Se	M	.0070	D			N	4.96			0.99	56
Ti	M	.0083	D			N	0.31			0.18	56
Ba	F	.0002	C	P	N	D	2.18	1.97	1.04	0.81	136
Ca	F	.0001	C	P	N	D	1392	1181	580	535	136
Cu	F	.0100	P	C	N	D	22.4	15.8	14.3	13.4	136
Mg	F	.0001	C	P	N	D	113	82.8	52.8	44.9	136
Na	F	.0203	C	P	D	N	73.8	66.0	48.3	39.0	136
Sr	F	.0017	C	P	N	D	5.35	3.84	2.60	2.02	136

[a]Values reported are geometric means (μg/g). Least square means were used to compare groups since the design was unbalanced with respect to sex and hair treatments within each population. Groups sharing a common underline are not significantly different from each other ($p > .05$, pairwise t-tests). D = dandruff shampoo, P = permanent, C = color treatment, N = no treatment.

Table 5
Elemental Concentrations that Showed Significant Differences
(F Test, Randomized Block Design ANOVA Blocking
Against Hair Treatment, ($p < 0.05$) Among Populations (Stratified by Sex)[a]

Element	Sex	P	Population			Geometric means, μg/g			n
			High		Low	High		Low	
Al	M	0.0045	C	N	H	11.0	8.53	3.70	61
Al	F	0.0005	C	N	H	7.14	3.30	3.09	1.36
Ba	M	0.0001	H	N	C	1.56	0.74	0.47	61
Ba	F	0.0001	H	C	N	2.54	1.31	0.81	1.36
Ca	M	0.0002	H	C	N	561	306	219	61
Ca	F	0.0001	H	C	N	1191	990	512	136
Cr	F	0.0001	C	N	H	0.18	0.11	0.08	136
Fe	F	0.0002	C	N	H	13.4	8.69	8.30	136
Mg	M	0.0002	H	N	C	49.5	29.9	29.5	61
Mg	F	0.0012	H	C	N	98.8	61.6	53.1	136
Mn	F	0.0214	H	C	N	0.25	0.24	0.13	136
Na	M	0.0001	C	N	H	108	44.3	25.3	61
Na	F	0.0001	C	H	N	193	33.4	25.7	136
Pb	F	0.0018	H	C	N	2.70	1.37	1.29	136
Sr	M	0.0001	H	N	C	6.08	0.77	0.54	61
Sr	F	0.0001	H	C	N	12.4	2.18	1.25	136
Ti	M	0.0016	C	H	N	0.47	0.27	0.21	61
Ti	F	0.0001	C	H	N	0.99	0.34	0.15	136
Zn	F	0.0002	N	C	H	182	143	138	136

[a]Values reported are geometric meams (μg/g). Least-squares means were used to compare groups, since the design was unbalanced with respect to sex and hair treatments within each population. Groups sharing a common underline are not significantly different from each other ($p > 0.05$, pairwise t-tests). C = CDC volunteers, H = Hispanic HANES participants, N = National HANES participants.

CONCLUSION

Interpretation of hair analysis data for elemental content will, undoubtedly, remain the subject of some controversy because of the previously mentioned areas of concern, e.g., standardization of collection, washing or other pretreatment, and choice of analytical and statistical methods. The data presented here will hopefully address the need for a preliminary estimate of "normal" or expected levels of metals in human hair specimens. As is evident from Table 3, the concentration levels found in our work are in reasonable agreement with those found in the literature. The number of specimens represented by our work, 271, is in considerable excess of the numbers reported be most other investigators. This relatively large number of specimens, coupled with the common hair preanalysis treatment and analytical method, adds to the value of the reference intervals presented.

Perhaps the most generally accepted use of hair analysis is documentation of exposure to toxic elements from the external environment. In this regard, the data presented may help establish an interval above which undue or excessive exposure may be indicated, even without defining of the mechanism of deposition (external "air pollution" or internal excretion). More controversial is the use of hair elemental data to evaluate nutritional status. With some rare exceptions, e.g., diabetes (*39*) and malabsorption or zinc deficiency (*40*), the pattern of hair trace metals is not well related to the nutritional or disease status of the individual. As previously mentioned, the population chosen for this study were presumably "normal" with respect to disease and nutritional status. The elucidation of the relationship of hair element concentrations and adverse health outcomes cannot, therefore, be predicted from these data. Further studies are needed to establish or discredit the use of hair trace elements in this regard.

APPENDIX A

Hair Collection Questionnaire

Specimen ID: _____
Age: _____
Sex: _____
Date of Collection: _____

1. When was the last time your hair was washed?
a. Today or yesterday
b. 2–6 days ago
c. 7 days ago or longer
2. The last time your hair was washed, was it washed at:
a. Home

b. Beauty shop or barber shop
3. The last time your hair was washed, what brand of shampoo was used?
a. Specify brand: _____
b. Don't know
4. Is this your regular brand of shampoo?
a. Yes
b. No. If No, specify brand:
5. When washing your hair do you ever use a conditioner or cream rinse on your hair?
a. Yes. If Yes then specify brand: _____
b. No. If No then skip to question 9.
6. How often do you use a conditioner or cream rinse?
a. Occasionally
b. Almost always
7. When washing your hair do you ever use a dandruff shampoo?
a. Yes. If Yes, then specify brand: _____
b. No. If No, then skip to question 9.
8. How often do you use a dandruff shampoo?
a. Occasionally
b. Almost always
9. Do you use any color treatment on your hair?
a. Yes. If Yes, specify: tint, color rinse, bleach, frost.
b. No. If No, skip to question 12.
10. How often do you color treat your hair?
a. Weekly
b. Two or three times a month
c. Once a month
d. Less often than once a month
Specify brand: _____
11. When was the last time your hair was color treated?
a. Less than one week ago
b. One to two weeks ago
c. Two weeks to a month ago
d. More than one month ago
12. Have you had a permanent wave in the last six months?
a. Yes. If Yes, then when?
(1) Within the last month
(2) Two to three months ago
(3) Four to six months ago
Specify brand: _____
b. No
13. Do you use hair spray?
a. Yes. If so, how often?

1. Daily
2. Once or twice a week
3. Less often than once a week

b. No

14. Do you use any other hair products not mentioned above?
a. Yes. If so, what kind of product(s)? _____
Specify brand: _____
15. Do you swim regularly?
a. Yes. If so, then where?
1. Chlorinated (or brominated) pool?
2. Lake
3. River
4. Other (specify) _____
16. Do you have diabetes or sugar diabetes?
a. Yes
b. No
17. Filled out by:
a. Sample person
b. Interviewer
c. Both

APPENDIX B

Hair Collection Procedure

1. Store the stainless steel surgical scissors, the aluminum clips, and the nylon combs in ziplock plastic bags when not in use.
2. Disinfect the scissors, clips, and combs after each use.
a. Dip the scissors, clips and combs into isopropyl alcohol (2-propanol, ACS reagent grade).
b. Rinse them with distilled water.
c. Rinse again with isopropyl alcohol from a polyethylene squeeze bottle.
d. Dry in a dust-free environment (ziplock bag).
3. Use disposable, powder-free plastic gloves to handle the hair specimens.
4. Collecting the hair samples:
a. Collect the hair samples from the nape area.
b. With a clean nylon comb, partition the hair between the ears as shown in the diagram (not included).
c. Fasten the hair above the ears, out of the way, with aluminum clips.
d. At 8–10 sites on the nape area, gather 15–20 strands of hair. Hold the end of the hair and cut the hair as close as possible

with stainless steel surgical scissors. A minimum of 500 mg of scalp hair is needed for analysis.
e. From each cutting of hair from the scalp, cut off the two inches of hair which were closest to the scalp (scalp hair) and put in a ziplock plastic bag.
f. Place a pre-printed label on the bag, seal the bag, and staple the questionnaire to the bag above the ziplock.

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