Effect of Washing on Trace Element Content of Human Hair

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ABSTRACT. Three individual studies were undertaken to determine the effects of washing a human head hair sample in water, before analyzing for 15 minerals by atomic absorption.

Calcium, magnesium, sodium, and potassium levels were most affected by washing. Longer washing time produced more mineral loss. Sectioning the hair before washing produced slightly lower average mineral loss. Percentage of washout for each mineral was erratic in both cut and uncut samples. Samples from females lost more mineral than samples from males.

METHODS AND MATERIALS.

STUDY A

A single sample of about 8.0 grams of human hair was divided into three equal portions. Portion 1 was left unwashed. Portion 2 was washed in distilled water for 10 minutes. Portion 3 was washed in distilled water for 30 minutes. Portions 2 and 3 were shaken, for the times indicated, on a variable speed mechanical shaker at 30 strokes per minute.

After washing, the two washed samples were decanted and rinsed twice in 500 ml of distilled water in a Gooch type, glazed porcelain crucible with perforated bottoms, rinsed twice again in flowing deion-ized water for one minute and placed in a drying oven for 3 hours at 110 degrees C. The washed samples were removed from the oven, lightly covered and allowed to equilibrate overnight. The following day all three samples were cut into 1.0 mm lengths or less, using surgical dissection scissors.

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STUDY B

Ten randomly-selected samples which had previously been analyzed in the unwashed condition were chosen for study B. Each sample was divided into two equal portions and cut into 1 mm or less lengths, *before washing.* The samples were then washed for 10 minutes in flowing deion-ized water, drained and dried for three hours at 110 degrees C. The samples were removed from the oven, lightly covered and allowed to equilibrate overnight.

STUDY C

Ten randomly-selected, uncut samples which had previously been analyzed in the unwashed condition were used. Each sample was washed and dried exactly as in study B, but the samples were *not cut.* Following the washing and drying procedures, the samples were cut into 1.0 mm lengths, or less, and then analyzed.

ANALYSIS PROCEDURE

A. Weighing Out:

Study A. Two 300 mg portions were weighed out of the unwashed sample. Five 300 mg portions were weighed from the 10-minute wash sample. Five 300 mg portions were weighed from the 30-minute wash sample.

Study B and Study C. One 300 mg portion was weighed out from each sample in Study B and Study C.

B. Digestion:

All of the digestion tubes are acid washed in 10% HC1 before use. To each tube in the study was added 2.0 ml of a 3:1 solution of nitric acid/perchloric acid (Baker Instra-analyzed) and tubes were placed under a hood for 30 minutes. The

tubes were successively heated at 95 degrees C. for thirty minutes and 210 degrees C. overnight.

C. **Trace Element Sample:**

Following digestion, the tubes were re-hydrated with 6.0 ml of 0.9% HCl solution and vortexed. This is the trace element sample (Cu, Fe, Mn, Ni, Pb, P, Cd, Al).

D. **Macro-element Sample:**

0.8 ml of the trace element sample was diluted to 4.0 ml with a 0.2% cesium chloride solution. Cesium chloride is added to reduce the ionization effect of a nitrous oxide flame.

E. **Phosphorus Determination:**

0.4 ml of the trace element solution was added to 1.0 ml of a vanadomolybdophosphoric acid reagent and read in a Gilford 300N Spectrophotometer, equipped with a flow-through cuvette, at 400 nm.

F. **Mercury Determination:**

10.0 mg of hair was dissolved in 0.3 ml of nitric acid in a 16 x 125 tube (Baker Instra-analyzed) and diluted to 10.0 ml with 0.9% HCl solution. The cold vapor method using sodium borohydride (Aid-rich) at 253.6 nm. was employed.

INSTRUMENTATION AND EQUIPMENT

All determinations except phosphorus were made on a Perkin-Elmer 5000 Atomic Absorption unit equipped with an AS 50 Auto Sampler, a Data System 10 computer, a PR-100 printer, and a MHS 10 borohydride generation system. Phosphorus determination was performed on a Gilford 300N Spectrophotometer (see above).

CALIBRATION AND QUALITY CONTROL

Calibration of the instrument was achieved using Fisher Atomic Absorption Standards.

Quality control encompasses four separate preparations:

1) A check sample is prepared from the Fisher A. A. Standards at a concentration about equal to the average patient results.
2) An in-house hair control is prepared in the laboratory and is repeatedly analyzed until enough data is accumulated to extract a mean and one standard deviation.
3) A National Bureau of Standards bovine liver preparation is used as a control.
4) A blank solution of the acids used in digestion.

All of the above preparations are analyzed for every mineral, every time a batch is run. This data is collected and available.

All mechanical pipetting equipment is checked monthly for accuracy, as is the electronic balance used in weighing the samples.

DATA

STUDY A

In table 1, data from the samples — 2 unwashed, 5 washed for 10 minutes, and 5 washed for 30 minutes — are averaged for each category. Individual variation was < 5% for all elements. Therefore, I believe this to be a valid method of reporting.

STUDY B

Results of Study B are reported in Table 2. Only calcium, sodium, and potassium are reported in studies B and C, these being the only elements which appear to be affected by the one one-minute washing. The percent variation of the other elements (Cu, Fe, Mn, Pb, Ni, Cd, Mg, Cr, Al, P, Zn, Hg) was 3% or less and is considered instru-mentational. Copies of the full reports are available.

Three samples from study B have been deleted. They were found to be two horses and a dog. As they did not fit our criteria they were dropped.

RESULTS

In Study A, significant change occurred in potassium, sodium, calcium and

TABLE 1. STUDY A. UNWASHED, 10-MINUTE WASH, 30-MINUTE WASH

ELEMENT	UNWASHED	10 MIN. WASH	30 MIN. WASH
Cu	1.1	1.1	1.1
Fe	3.4	3.3	2.2
Mn	.09	.09	.05
Ni	0.1	0.2	0.1
Pb	2.2	2.1	2.0
Cd	.06	.06	.05
Ca	62	43	43
Mg	9	7	8
Na	38	7	2
K	17	2	2
Zn	11	12	12
Al	4.2	4.2	4.1
Hg	.06	.05	.05
P	9.8	9.7	9.7
Cr	.12	.14	.12

All results are in mg%.

magnesium, and to a lesser extent iron, manganese, and nickel levels. Extended washing time resulted in more mineral loss.

In Studies B and C, there was variable loss of calcium, sodium, and potassium from sample to sample, with no constant pattern. Overall, there was more loss in the samples which were not sectioned before washing (Study C). For each mineral studied in both Study B and Study C, samples from females incurred more loss than samples from men. These results are summarized in tables 4 and 5.

DISCUSSION

CONTAMINATION VS. ENDOGENOUS MINERALS

Contamination of a laboratory sample is a constant worry for all laboratory workers, particularly when the sample is exposed to the environment as is human head hair.

The most often-quoted reference for washing is that reported by G. S. Kennington in *Science Magazine* (3) on his study of the effects of soaking antelope hair in a solution of radioactive 22Na (1 uc/ml) for ten days.

While Dr. Kennington shows with this study that hair can be contaminated with 22Na and cleaned with repeated washings of distilled water, and that 24Na is removed under the same conditions, he does not characterize nor differentiate a sodium contamination from the endogenous sodium. The industry has assumed the contamination, which this study does not support.

His comments at the end of the paper, referring to ionic radius and charge, are undoubtedly correct as the residual elements following an eighty-minute wash in distilled water would be the insoluble ones. However, as the body requires soluble inorganic forms, these forms should invariably be present in the tissues formed by that body. Hair is no exception, and Study A would appear to support this observation.

PERCENTAGE OF LOSS AND CHEMICAL SOLUBILITY. Based on the cation solubilities of their compounds, five major solubility groups can be defined, from the least soluble to the most soluble: (4).

1) Lead — (least soluble)
2) Copper, cadmium, mercury
3) Aluminum, chromium, iron, zinc, nickel, and manganese
4) Calcium and magnesium
5) Sodium and potassium — (most soluble)

TABLE 2.
RESULTS OF STUDY B. SAMPLES CUT TO 1 MM BEFORE WASHING.

CALCIUM:				
SAMPLE #	UNWASHED	WASHED	% CHANGE	SEX/AGE
1	23	15	35	F/43
2	118	94	20	M/34
3	82	70	15	F/25
4	82	70	15	F/34
5	91	80	12	M/39
6	30	27	10	M/53
7	11	10	unchanged	M/51
SODIUM:				
SAMPLE #	UNWASHED	WASHED	% CHANGE	SEX/AGE
1	10	5	50	F/43
2	7	4	43	M/34
3	5	3	40	M/39
4	7	5	29	F/25
5	11	7	27	F/34
6	28	21	25	M/51
7	26	20	19	M/53
POTASSIUM:				
SAMPLE #	UNWASHED	WASHED	% CHANGE	SEX/AGE
1	4	1	75	F/43
2	4	1	75	M/39
3	26	22	8	M/53
4	8 8	8 7	unchanged	M/51 F/34 F/25 M/34
5	2 2	3 3	unchanged	
6			unchanged	
7			unchanged	

All Results are in mg%.

By comparing the 30-minute wash results of Study A with the five solubility groups, a definite correlation between washout and solubility group can be seen. Likewise, Study B and Study C show the same pattern for calcium, sodium, and potassium in varying degrees. In short, the degree of washout correlates well with chemical solubility. We can speculate that washout probably has less to do with physiology or external contamination of the hair, and more to do with chemical solubility of the elements involved.

CUTTING THE HAIR BEFORE ANALYSIS. Studies B and C were undertaken to compare the effect of sectioning the hair before and after washing. One minute was chosen as the shortest practical time unit for washing, without making the wash procedure too labor intensive. The data presented in tables 2 and 3 show erratic results. While these results may be due to incomplete washing, they may also indicate the individual biochemistry of the samples used.

Overall, no consistent percentage of loss was apparent for any of the three minerals during washing in either study. Sectioned samples showed overall slightly lower average percentage of mineral loss during washing. In Study B, the calcium results for samples 3 and 4 are the exceptions.

TABLE 3.
RESULTS OF STUDY C. SAMPLES LEFT UNCUT BEFORE WASHING

CALCIUM:				
SAMPLE #	**UNWASHED**	**WASHED**	**% CHANGE**	SEX/AGE
1	282	95	66	F/27
2	195	69	66	F/50
3	97	64	34	F/29
4	242	164	32	F/-
5	269	196	27	M/56
6	39	29	26	M/67
7	80	64	20	M/29
8	37	31	16	M/38
9	88	83	6	M/9
10	15	16	unchanged	M/41
SODIUM:				
SAMPLE #	**UNWASHED**	**WASHED**	**% CHANGE**	SEX/AGE
1	45	18	60	M/56
2	25	13	48	F/29
3	6	4	33	F/50
4	11	8	27	M/9
5	34	30	13	M/38
6	23	20	13	M/67
7	26	24	8	F/-
8 9	18	19	unchanged	M/41
10	4	4	unchanged	F/27
	3	3	unchanged	M/29
POTASSIUM:				
SAMPLE #	**UNWASHED**	**WASHED**	**% CHANGE**	**SEX/AGE**
1	4	1	75	F/27
2	7	2	71	F/29
3	31	18	42	M/56
4	7	5	29	M/9
5	48	39	19	M/67
6	48	40	17	M/38
7	16	14	12	M/41
8 9	4 3	4 3	unchanged	F/-
10	2	1	unchanged	M/29
			unchanged	F/50

All results are in mg%.

VARIATION IN MALE AND FEMALE HAIR MINERAL LOSS.

Study B and Study C both showed samples from females lost more mineral during washing than samples from males. This finding supports studies which indicate female hair is more porous than male hair. The increased porosity could account for a more rapid loss of mineral from hair cut from women. While more studies are necessary, Study B and Study C indicate sex may be another important variable that must be taken into account if hair is washed.

TABLE 4. RANGE OF MINERAL LOSS IN STUDY B AND STUDY C.

	STUDY B (cut)	STUDY C (uncut)
MINERAL	**RANGE OF LOSS**	**RANGE OF LOSS**
CALCIUM	0-35%	0-66%
SODIUM	29-50%	0-66%
POTASSIUM	0-75%	0-75%

TABLE 5. AVERAGE LOSS FROM MALE AND FEMALE SAMPLES

MINERAL	LOSS IN FEMALES	LOSS IN MALES
STUDY B.		
CALCIUM	21.6%	10.5%
SODIUM	35.3%	31.75%
POTASSIUM	25%	20.75%
STUDY C.		
CALCIUM	49.5%	15.83%
SODIUM	22.25%	18.83
POTASSIUM	36.5%	19.83%

CONCLUSIONS

There is no doubt washing a sample removes quantities of certain minerals, specifically calcium, sodium, and potassium. Studies A, B and C indicate the pattern of mineral removal most closely correlates with the chemical solubility of the elements tested.

Some have judged these losses constitute "contamination". I believe, as Rob-bins (5) has stated, that we are dealing with highly soluble compounds of calcium, sodium, and potassium, which are required by our body chemistry, and these losses constitute an integral part of the hair fiber.

These studies also indicate more washing removes more mineral, the percentage of loss of mineral is erratic from sample to sample, and overall, samples from females lose more mineral than samples from males.

Based on the studies presented, I believe washing of the hair before analysis should be discontinued as a common practice, until we can positively state where mineral contamination stops and endogenous mineral starts.

ACKNOWLEDGEMENTS

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