A COMPARISON OF HAIR CHROMIUM CONCENTRATIONS IN COLLEGE STUDENTS

By

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STUDENTS

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CHAPTER I

INTRODUCTION

Since the late 1950's, nutritionists and others have become interested in the biological functions of chromium, as well as its essentiality in human nutrition. The relationship between chromium deficiency and glucose tolerance, probably due to chromium being a cofactor for insulin, has been established first in animal studies and later in human adults and in juvenile diabetics. Surveys have revealed that body tissues from people of other areas in the world have 2.5 to 13 times as much chromium as those from the United States.

Chromium is thought to be involved in carbohydrate and lipid metabolism and in protein synthesis, leading to assumptions as to the trace element's role in atherosclerosis, hypercholesteremia, and in the functioning of DNA and RNA. Chromium concentrations in the human body have been found to be inversely related to age.

Hair is a particularly stable biopsy material and is high in chromium. It is also relatively convenient to obtain. Thus hair is highly adaptable for use in chromium assay.

The purpose of this study is to determine and compare hair chromium levels of three groups of students at Oklahoma State University. The groups included Caucasians, diabetics, and Chinese.

There are six assumptions for this study. They are:

1. Chinese subjects have more hair chromium than Caucasians or diabetics.

2. Diabetics have the lowest hair chromium level among the three groups.

3. There are minimal differences in concentration between insulin-dependent and non-insulin-dependent diabetic students.

4. There is very little difference in concentration for different hair colors.

5. There is little difference in concentration on the basis of age within a given age group.

6. There is little difference in concentration between male and female subjects.

CHAPTER II

LITERATURE REVIEW

Chromium

Chromium, the fifteenth most abundant metal in the human body (35), is the latest essential trace element found to participate in the normal functioning of metabolic processes in man (33, 39). The possibility that chromium might have biologic value was first suggested in 1954, when the trace element was found to enhance cholesterol and fatty acid synthesis in rat liver (3, 35). Nutritionists and other researchers have since become interested in the function of chromium and the amount required in the body.

Chemistry and Occurrence

Chromium is quite unique and is different from other trace metals in several respects. It has six valence states, the trivalent and hexavalent states being more common. The hexavalent state is stable in water. The trivalent form of chromium is most stable, and this is the form that chromium is found in foods and tissue stores (25, 35, 39). There are various opinions concerning the active form of chromium. It has been shown that chromium as a supplement to rats must be

given in the trivalent form for it to be biologically active (43), yet another report mentioned effective cholesterol biosynthesis with divalent, trivalent, and hexavalent chromium (3).

Knowledge about the valence states of naturally occurring chromium is incomplete. In biological materials, chromium is present in the trivalent state. In aqueous solutions, such as in blood, chromium occurs mostly as a compound which is stable in acids. It also appears as olates, which are less stable complex forming compounds with hydroxyl and water. Olation occurs mostly in an alkaline environment, such as in the intestines (25, 39).

Chromium storage in man is estimated to average about 5.78 mg, one-third of which is found in the skin. About 15 percent of the body chromium is found in muscle, 10 percent in whole blood (of which 9 to 30 percent is in serum), 5 percent in fat, and very little in bone. Chromium exists in two forms in the blood, either as free (or dialyzable) chromium or bound to plasma protein as Cr (III) (39). The chromate ion can penetrate the red blood cell membranes easily, thus accounting for its use in labeling erythrocytes (25).

Hair chromium is probably in the trivalent state, since in previous studies with hair, oxidation of the trace element to the hexavalent state was necessary (4). The chromium level in hair is about ten times that found in circulating blood levels (11). Investigators have shown

different chromium levels in human hair of various age groups; Mertz has found a range of 0.20 to 20 ppm for hair chromium concentrations (25), Schroeder reports 0.39 to 0.96 ppm in hair (40), and Hambidge reports 0.052 to 3.00 ppm (13, 14).

Brain tissue has the highest chromium concentration of all human organ tissues, with about 80 ppm in the cerebellar cortex. Heart tissue has a concentration of 0.0017 to 0.0195 ppm, while that of the liver and lungs is 0.179 to 0.852 ppm. Chromium in the aorta, lungs, heart, and spleen was shown to decline during infancy; but chromium in kidney and liver tissue was stable until after the first decade of life (25). The decline of chromium in the lungs was observed to reverse and increase after the second decade of life. There is no equilibrium between chromium in tissue stores and that in circulation, because, except in chromate form, chromium does not stay long in the blood, whereas there is storage in the body organs.

Metabolism

Trivalent chromium is poorly absorbed in the alimentary tract in humans (25). About one percent of an oral dose of trivalent chromium is absorbed and appears in urine. Absorption is probably mostly in the hexavalent state, as shown in a study in which about 3 to 6 percent of an oral dose of chromate was absorbed and eliminated in the urine. Absorption is affected by the acidity of gastric juice and the

amount of food in the gastrointestinal tract, since both factors will reduce the amount of chromate absorbed. Chromium in the form of an organic complex, on a _glucose tolerance_ factor, is better absorbed than inorganic salts. However, the site of chromium absorption and the absorbability of different chromium complexes are not now known (25, 27).

Nature provides three barriers to protect man from indirect absorption of excess amounts of chromium from the soil, thus maintaining a low level of this metal in the human body (25). These include:

1. Geochemical barrier. Plants have a low toxic limit for chromium absorption, uncharacteristic of most other trace metals.

2. Phytochemical barrier. Some plants absorb, while others reject, soluble chromium in the soil by some unknown mechanism.

3. Biochemical barrier, probably due to an exclusion mechanism in the gastrointestinal tract or liver, which seems to regulate chromium accumulation.

Absorption and transport of chromium in serum is due partly to siderophilin (25, 39), before it is stored in tissues. About 80 percent of the chromium is excreted in the urine. There is probably some reabsorption of the trace element before excretion, which is uninfluenced by urine volume (39). The daily urinary chromium excretion is about 2 to 6 ug/l. However, man seems to excrete more chromium in the

urine than the amount absorbed, considering an average dietary chromium intake of about 80 ug/day, with a 0.5 percent, or 0.4 ug absorption. Urinary chromium probably occurs as an inorganic salt, together with the glucose tolerance factor, rather than as plasma protein chromium. The site of chromium excretion into the intestine is still unknown (25).

Functions

Chromium probably functions as a catalyst to enhance several body processes (35). Most of the early experimentation for the biologic role of chromium was done with animals, which have higher tissue chromium levels than man (35). Studies have shown that supplements of both Cr (III) and Cr (IV) accelerated growth, increased body size, and prolonged the life of rats (36, 38).

Insulin and Carbohydrate Metabolism. Trivalent chromium has been established as a glucose tolerance factor for rats: a dietary ingredient required for the maintenance of normal glucose uptake and utilization (43). Polarographic studies have suggested the function of chromium as a cofactor for insulin. This function can be illustrated in the following diagram:

$$\text{Intra-chain disulfide of insulin + Sulfhydryls in the cell membrane} \xrightarrow[\text{in a complex}]{\text{Cr (III)}} \text{Effective glucose uptake by cells.}$$

For animals on a low chromium diet for a few weeks, symptoms resembling those of diabetes mellitus developed.

Twice as much time was required for their glucose tolerance curve to come back to the fasting level when compared with the control rats. Supplementation of 2 ppm of chromium helped to improve the impairment overnight (38). In vitro, chromium also helped to decrease the amount of insulin needed for glucose uptake of rat eye lens (25, 39).

Recently, human studies have provided data to support findings from animal studies. Oral chromium supplements for human studies are made with trivalent chromium, mostly in the form of hexa-aquo chromic chloride and chromic acetate, which is also used sublingually to avoid olation by bypassing the alkaline small intestines. Hexavalent chromium can be used as a supplement after being reduced industrially to chromic oxide with molasses and to chromic acetate with glucose (39).

Studies with rats and human subjects have shown that chromium in the blood rose after insulin or glucose administration (39). The increase was thought to come from tissue stores of chromium, aided by the presence of glucose or insulin. The increased amount of chromium, probably in the free form, was calculated to be about 1.5 percent of estimated body chromium stores.

A correlation between an increase in serum chromium and plasma immunoassay insulin was shown in 70 percent of tests given (10). A glucose load administered to healthy subjects increased their plasma chromium levels, but not those of diabetic patients, who had the same response only after chromium supplementation and improved glucose tolerance (7).

Chromium deficiency can sometimes be a complication for impaired glucose tolerance in "protein-calorie" malnutrition. One example of this deficiency is a study done with children from Jerusalem and Nigeria with kwashiorkor (39). Single doses of 250 ug of Cr (III) were able to bring back to normal their fasting hypoglycemia and postintravenous glucose hyperglycemia with low removal rates.

In three studies with diabetic patients, some showed improvement in glucose tolerance after taking 1 mg chromic chloride daily for six months. In addition, some of them were even able to reduce or cease their insulin medication. Adult patients with atherosclerosis, but without diabetes mellitus, had their diabetic-like glucose tolerance curves restored to normal by chromium supplementation (39).

Chromium determination in hair was made on insulin-dependent juvenile diabetic patients by Hambidge, _et al_ (11), who found these subjects to have much lower chromium concentrations than the control children. The difference was thought to be caused by body chromium deficiency, or a depletion of chromium due to abnormal carbohydrate metabolism, as indicated by excessive loss of urinary chromium after an oral glucose load.

Lipid Metabolism. Studies with rats have shown that chromium enhances the function of insulin as much as 94 percent in the utilization of glucose carbon for fat synthesis (24). It also is involved with insulin for the oxidation of glucose to carbon dioxide (27).

Chromium supplementation of 1 to 5 ug/ml in drinking water given to laboratory rats from weaning to two years of age suppressed serum cholesterol levels, thus suggesting Cr(III)'s role in serum cholesterol homeostasis (39). The contribution of chromium towards fatty acid metabolism can also be illustrated by the much lower incidence (2% vs 19%) of aortic plaques in rats fed a chromium-supplemented diet (37).

In a human study with no diet restriction, after five months of a supplement of 2 mg/day of chromium acetate, a decline of about 14.2 percent of serum cholesterol was shown in some subjects. The decline is comparable to results from a saturated-fat-restricted diet. In one patient who was on 1 mg chromium supplements, his serum cholesterol level fell 26 percent in seven weeks (35).

Protein Synthesis. Chromium was first found to be associated with protein in the chrome tanning of skin, in which a reaction takes place mainly between Cr(III) and the free carboxyl group of the acidic amino acids (glutamic and aspartic) of the protein (25).

Chromium enhances the action of insulin on protein synthesis and in the swelling of liver mitochondria (25). Chromium deficient rats on a low protein diet could not synthesize some amino acids into heart protein. A supplement of 2 ug/g chromium stimulated the growth of rats somewhat, probably due to its function in increasing tissue protein in the animals.

It was found that nucleic acids from beef liver, in comparison to other subcellular elements, contain extremely high concentrations of chromium (45, 46). Thus it was suggested that chromium may have a functional relationship to DNA and RNA.

<u>Chromium Deficiency</u>

Chromium deficiency implies body chromium levels at suboptimal levels, rather than a total absence of the trace element (39). Increasing depletion of chromium with advancing of age has been shown. Thus it is not surprising that prolonged deficiency can threaten the health of a population (26). Some factors that contribute to chromium deficiency are as follows:

1. Loss occurs through extensive use of refined foods that make up the main portion of the caloric diet, such as flour, rice, sugar, and refined fats. There is little chromium in the endosperm of cereal and grains, but more is concentrated in the gluten (41). Thus, much of the trace elements, including chromium, are easily lost in the refining process. White bread, as compared with whole wheat bread, is found to be lower in chromium by 71.4 percent, while there is a 40 percent loss of chromium in refined wheat flour as compared to whole wheat flour. Refined white sugar is much lower in chromium as compared to raw or brown sugar (41).

2. From olation. Since an alkaline medium and high temperatures enhance olation, the process occurs favorably in the small intestine and in the canning of foods (39).

3. Dietary habits. As will be seen in the discussion of the following section, chromium occurrence in most foods is apparently not abundant. Habitual preference for foods that are lower in chromium can lead to a deficiency unless a variety of foods is consumed. Studies have shown a variation from 0.007 to 0.440 mg of chromium per day in normal institutional diets (26).

4. Repeated pregnancies. Multiparous women have been shown to have only one-third of the hair chormium level compared to that of nulliparous women (12). Studies have shown that newborns have extremely high chromium concentrations in comparison to that of any other age group (13). Thus it is assumed that the newborn obtains its chromium from the mother in the fetus stage, and continued pregancies would be expected to deplete the mother's tissue chromium stores.

5. Endocrine disturbances, such as diabetes mellitus, as discussed in the section on carbohydrate metabolism (11).

Chromium in the Diet

Information about chromium occurrence in various foods is not complete. Table I gives some chromium concentrations in foods in both the trivalent and hexavalent states.

Chromium levels are generally higher in some spices, which were probably contaminated during processing. Other

TABLE I

TRIVALENT AND HEXAVALENT CHROMIUM IN SOME FOODS

Sample	Cr(III) (ug/g)	Cr(VI) (ug/g)	Total (ug/g)	% Cr(III)
Beef liver	0.10	0.12	0.22	45.4
Chicken breast	0.06	0.10	0.16	37.5
Egg, chicken	0.19	0.03	0.22	86.4
Thyme	3.38	0.41	3.79	89.4
Black pepper	1.02	1.24	2.26	45.1
Tomatoes, stewed	0.13	0.02	0.15	86.3
Tomato, raw	--	--	0.01	--
Corn oil	0.41	0.61	1.02	40.2
Corn oil margarine	0.14	0.00	0.14	100.0
Rat diet	0.10	0.07	0.17	58.8
Commercial dog food	--	--	3.40	--

possibilities of obtaining chromium in food through contamination during processing are indicated by the unexpectedly high chromium levels in commercial dog foods, corn oil, margarine, canned tomatoes, cheese, and stewed rhubarb (35). Milk has a low chromium content, with a mean of 11.6 ng/ml milk for breast milk and 8.0 ng/ml for undiluted cow's milk (10). Several kinds of fish and lobster are very low in chromium (25). Clams have up to 0.4 ppm on a wet-weight basis. All chromium in wine is in the trivalent form.

A recommendation for daily chromium intake has not been set. In vitro, about 0.001 to 0.1 ug/g chromium is probably enough to be effective (27). There is a maximum dosage of chromium for optimal effect. After that, increased concentration only leads to decreased function. About 0.2 to 0.5 ug of chromium per gram of body weight is required if simple salts of trivalent chromium are used. However, much less is needed for chromium in the form of a natural complex--a glucose-tolerance factor. The inorganic form of chromium is poorly absorbed and accounts for only about 0.5 percent absorption (39). Not much is known about the chromium content of foods, although it is likely that a lot more chromium is absorbed from unprocessed food sources. The intestinal tract absorbs at least about 10 to 20 percent of ingested organic complex chromium.

Chromium in Foreign Tissues

Extensive surveys of chromium levels in populations from 31 areas of the world showed that tissue chromium is relatively deficient for subjects from the United States (39). By means of emission spectroscopy, 17.6 percent of Americans, as compared to 1.57 percent of foreign subjects, had very low tissue chromium concentrations. Thus, tissue chromium levels for foreign subjects were 2.5 to 13 times higher (that for liver chromium being more than two times higher) than those from the United States.

Male organs, except for lungs, of Americans had a low chromium level compared to those of the Near and Far East, with Africans ranking second in concentration. Aortic chromium concentrations of Americans were less than other white, Oriental, and Negro subjects investigated in other parts of the world (39).

Different chromium concentrations were also noted according to regional residential differences (27). The difference might originate from the concentration in soil and water, which is absorbed by plants and eventually consumed by man.

Hair

Hair has been called the "recording filament" (14). As well as being the base material for trace element detection, it is also a good means for detecting borderline malnutrition (31). Studies with different groups of people have

shown insignificant serum chromium variation among groups, and Hambidge (10) has concluded that serum is not a good material to show chromium nutritional status.

Previous studies differ in regard to using measured lengths of hair from the scalp for trace element research (6, 22). This is not necessary for chromium determination for two reasons. First, there is no metabolic turnover in the hair shaft. Hair chromium is quite stable and is unaffected by external environmental factors, provided the hair is untreated and the individual has not been exposed to a heavily chromium contaminated area (14, 15). Secondly, hair is a keratinized product of the hair follicle. The follicles have "growing" and "resting" periods at different times. Thus, there is no reason to take samples of hair that are of the same distance from the scalp when these hair shafts may not have grown for the same length of time (2).

Hair provides storage for trace elements (10, 14). The presence of chromium in hair reflects the necessity of the trace element for hair growth and the amount consumed in the diet. It is believed that different concentrations found along various distances along a shaft of hair reveal varying levels of chromium intake of that individual at certain times. It is not known how the body supplies chromium to hair follicles nor how hair chromium is related to other tissue chromium in the body (14).

Atomic Absorption Spectrometry

Atomic absorption spectrometry (or atomic absorption spectroscopy, hereafter abbreviated as AA) is the most widely used modern method for chromium analysis (25). AA was demonstrated more than a hundred years ago by Kirchhoff, a German physical chemist (48). However, the application to routine chemical analysis did not become popular until the 1950's when two Australian chemists designed a reliable hollow cathode source (23, 48). Flame emission spectrometry was at first more commonly used in flame spectroscopy (23). The two techniques differ in that emission measures excited atoms, while absorption measures the nonexcited and nonionized atoms (17).

In 1953, Walsh (48) found that AA is advantageous over emission in that AA is more stable against temperature changes as well as against interferences caused by atomic energy exchange. Its high sensitivity, convenience, and reduction of cation interference have contributed to its usefulness in trace element analysis (4).

Another highly sensitive method for trace element analysis is gas chromatography (33). Other methods for the analysis of chromium in biological materials, besides the ones mentioned, are spark source mass spectrography, coulometry, polarography, and neutron activation analysis.

The efficiency of AA analysis can be affected by flame types and nebulization conditions (17). There are three

major interferences with AA, all of which can be avoided. They are chemical, ionization, and matrix interference (17).

AA has been used to determine chromium in blood and urine, in faeces, lubricating oils, steels, aluminum alloys, and many biological materials (48). In order to increase sensitivity for the detection of chromium, the design of the burner is very important (17). Organic solvents are sometimes used to extract chromium from hexavalent to the trivalent state before measuring the concentration of atomic absorption (4).

CHAPTER III

EXPERIMENTAL PROCEDURE AND MATERIAL

Pre-experimental Study

A pre-experimental trial study was designed and carried out to find the most feasible procedure for hair chromium determination. Scrap hair from a barber shop was used. Eight hair samples were used, each weighing about one gram. Half of the samples were dry-ashed, and the others were wet-ashed. Half of the dry-ashed samples were washed with carbon tetrachloride, and half of them were not washed. The same treatment was given to the wet-ashed samples. Dry-ashing was done in a 600°C muffle furnace for about 7 hours. Then the ash was dissolved in 3 ml of diluted hydrochloric acid. Wet-ashing was done with a nitric-perchloric acid mixture, as described in a later section.

Atomic absorption spectroscopy was used for all digested samples, and the results were statistically analyzed. The better method was chosen for the thesis study as judged by statistical significance and convenience of preparation.

Description of Subjects and Groups

All subjects in this research were taken from the student population at Oklahoma State University. The subjects,

male and female students aged 18 to 25, were selected and divided into three groups. The groups consisted of ten students each as follows: Caucasians, Chinese, and diabetics. They were volunteers through personal contacts, and most of the diabetic subjects were chosen in response to an advertisement in the college newspaper. A personal data sheet was completed by all subjects; and their characteristics will be discussed in Chapter IV.

Hair Sample Collection

The experimental method used in this study was developed with reference to several previous studies (4, 5, 11, 12, 13, 14, 15, 40, 47) as well as to the trial study. Hair samples, approximately two grams, were collected from the occiput of the head, close to the scalp, with thinning scissors under sterile conditions in the laboratory. The whole length of hair shafts was used. They were then stored in plastic bags and sealed tightly. None of the hair had been dyed or bleached.

All samples were collected in a week's time during the month of May, 1972. The short period of time for sample collection was to control against the possibility of seasonal hair chromium variation, as suggested by Hambidge (14).

Sample Preparation

Duplicate samples of hair were used from each subject. All samples were prepared and analyzed randomly. Hair

samples were weighed and then washed with about 30 ml of carbon tetrachloride (Reagent grade) in a 200 ml beaker under a hood. The washing solution was shaken gently for a few minutes, then drained through a piece of unbleached muslin cloth. Each sample was dried at about 88°C, covered with watch glasses with cloth pieces still on the beakers. After about 10 minutes, the dried cloth pieces were tapped gently into the beaker to free any hair still clinging. The muslin cloth was removed, and drying continued for about three hours. The washed samples were then weighed again. The percentage weight loss after washing for all the samples is given in Table II.

Sample Digestion and Preparation for Analysis

A digestion mixture was prepared using a 3:1 mixture of Reagent grade nitric-perchloric acid. The acids were mixed with a magnetic stirrer and a Versamix. For each set of samples digested (8 to 11 samples), an acid blank was used. All glassware used was washed with a detergent and rinsed with hot tap water followed by first dionized water and then the acid mixture.

For digestion of the sample, a 20 ml acid mixture was added to a 200 ml beaker containing the hair sample and covered with a watch glass. The sample was then allowed to remain in contact for 8 to 12 hours. The mixture was heated to 270°C or until white fumes formed and the mixture turned clear. Fuming was continued to drive off the excess

TABLE II

PERCENTAGE OF WEIGHT LOSS AFTER WASHING WITH CARBON TETRACHLORIDE

Sample Number	Pre-Washed Hair Weight (g)	Washed Hair Weight (g)	Percentage Weight Loss From Washing	Average (%)
1a	1.07259	0.96594	9.94	9.31
1b	0.94140	0.85975	8.67	
2a	1.26735	1.17305	7.44	8.38
2b	1.01381	0.91935	9.32	
3a	1.00235	0.98372	1.86	4.84
3b	1.02411	0.94416	7.81	
4a	1.11615	1.04435	6.43	6.81
4b	1.16315	1.07965	7.18	
5a	1.17389	1.08609	7.48	6.72
5b	1.22734	1.15419	5.96	
6a	1.10582	1.01702	8.03	7.54
6b	1.20855	1.12335	7.05	
7a	1.08774	1.01429	6.75	7.32
7b	1.82869	1.68463	7.88	
8a	1.06296	0.98235	7.58	8.32
8b	1.07560	0.97818	9.06	
9a	1.07580	0.98455	8.48	7.98
9b	1.17811	1.09001	7.48	
10a	1.01925	0.95285	6.51	4.61
10b	1.00458	0.97749	2.70	
11a	1.05258	0.99358	5.61	6.39
11b	0.79796	0.74086	7.16	
12a	1.01485	0.92800	8.56	9.14
12b	1.11945	1.01065	9.72	
13a	1.00480	0.92732	6.72	7.45
13b	1.00065	0.91892	8.17	
14a	1.01270	0.93370	7.80	6.67
14b	1.18560	1.11990	5.54	
15a	1.05064	1.01263	3.62	5.27
15b	1.04150	0.96939	6.92	

TABLE II (Continued)

Sample Number	Pre-Washed Hair Weight (g)	Washed Hair Weight (g)	Percentage Weight Loss From Washing	Average (%)
16a	1.20108	1.09870	8.52	6.60
16b	1.06153	1.01180	4.68	
17a	1.11569	1.03674	7.08	7.26
17b	1.13887	1.05418	7.44	
18a	1.03864	0.96250	7.33	7.73
18b	1.02326	0.94016	8.12	
19a	1.07900	1.04723	2.94	4.85
19b	1.06885	0.99660	6.76	
20a	1.00600	0.97982	2.60	5.12
20b	1.02632	0.94796	7.64	
21a	1.10405	1.00085	9.35	9.31
21b	1.14830	1.04200	9.26	
22a	1.02380	0.94123	8.07	8.66
22b	1.05865	0.96070	9.25	
23a	1.05139	0.95534	9.14	8.44
23b	1.02141	0.94212	7.74	
24a	1.15125	1.06099	7.84	7.17
24b	1.27426	1.19156	6.49	
25a	1.02955	0.95440	7.30	7.47
25b	1.17110	1.08165	7.64	
26a	1.22915	1.17347	4.53	6.98
26b	1.00618	0.91126	9.43	
27a	1.01140	0.93440	7.61	5.29
27b	1.00216	0.97254	2.96	
28a	1.21434	1.11382	8.28	8.36
28b	1.08780	0.99611	8.43	
29a	0.96430	0.88355	8.37	8.73
29b	1.15415	1.04779	9.08	
30a	1.31607	1.15722	12.07	11.49
30b	1.42942	1.27347	10.91	

perchloric acid, which might interfere with AA analysis. If caramelization took place, when the digestion mixture thickened and/or turned black, more of the oxidizing acid mixture was added to all samples and the digestion completed.

Sides of beakers and watch glasses were then rinsed with deionized-distilled water and evaporated almost to dryness. Sides of the beaker and watch glass were then rinsed three times with water again and then filtered into a 10 ml volumetric flask and brought up to volume. The samples were then stored in plastic bottles in the refrigerator until ready for analysis.

Working standard solutions with concentrations of 0.01 to 0.40 ug/ml (ppm) chromium were prepared, using chromium standard stock solution[1] with a concentration of 1000 ppm. The solutions were stored in polyethylene bottles and kept in the refrigerator until ready for analysis.

Analyses of Samples

Both sample and working standard solutions were analyzed at room temperature (25°C) by taking them from the refrigerator about two hours before analysis. Analyses were conducted using a Perkin-Elmer 303 atomic absorption spectrophotometer at a wavelength of 3574 Å, using an air-acetylene flame and a three-slot burner. The sensitivity for a reading of seven was 0.01 ug/ml. The following instrumental settings were used:

---

[1]Fisher Scientific Company---Chromium Standard Solution.

Scale------------------5.0

Air pressure level----9.0 (inches of water)

Acetylene level-------8.5 (inches of water)

Gain------------------4.3 (approximate)

Meter response--------1.0

Slit------------------3.0

Data Calculation and Analyses

A standard chromium curve was drawn for chromium concentration versus absorption of standard solutions. A computerized linear program was used with a correlation coefficient of 0.996, indicating high significance for the curve. Two to three readings were taken for each sample solution, and the average was taken for the final absorption. Sample concentrations in micrograms of chromium per gram of hair (dry weight) were calculated. Statistical tests of AOV, LSD Multiple Comparison, and the t-test were used to analyze data.

Post-Thesis Study

Five more hair samples from hair cuts were obtained and analyzed after the thesis study to reinforce experimental findings. The five subjects consisted of a Caucasian student as the control, a middle-aged Caucasian who had been a diabetic for 39 years, a Caucasian obese student, a Jamaican black student, and a Thai student. Samples were prepared, digested, and analyzed similarly to those in the thesis study.

CHAPTER IV

RESULTS

Chromium

Chromium concentrations were calculated in micrograms per gram of hair (dry basis). Tables III through VIII and Figure 1 show a summary of hair chromium concentrations for the three groups with a comparison of various parameters.

A general description of donors of hair samples and of their hair chromium concentrations is shown in Table III. The chromium level ranged from 0.38217 ug/g for one female Caucasian to 1.81319 ug/g for a male diabetic subject. In Figure 1, a three-dimensional picture comparing hair chromium concentrations of the 30 subjects categorized under the three groups is given.

Table IV gives a comparison of mean hair chromium concentrations for the three groups. The levels show an increase in the order of Caucasian, Chinese, and diabetic subjects, with values of 0.62678, 0.69579, and 0.96855 ug/g, respectively. By using the AOV (Analysis of Variance) test, the difference in concentrations among the three groups is shown to be significant at the 0.03 level. Further statistical analysis with LSD (Least Significant Difference) Multiple Comparison showed that there is no significant difference

TABLE III

A LIST OF HAIR CHROMIUM CONCENTRATIONS FOR ALL 30 SUBJECTS AND A DESCRIPTION OF DONORS

Sample Number	Hair Color	Age	Sex	U.S. Residence (years)	OSU Residence (years)	Hair Cr concn (ug/g)
			Caucasian Non-Diabetic			
1	Brown	20	M	20	2	0.74141
2	Brown	22	M	22	5	0.60787
3	Brown	21	F	21	4	0.69768
4	Brown	20	F	20	2	0.38217
5	Brown	20	F	20	2	0.64856
6	Red	20	F	20	2	0.53168
7	Blond	21	M	21	1	0.48306
8	Black	23	M	23	4	0.83581
9	Brown	24	M	24	6	0.51349
10	Blond	23	M	23	4	0.82609
			Chinese Non-Diabetic			
11	Black	19	F	1/2	1/2	0.48983
12	Black	25	M	6	5	0.66665
13	Black	24	F	1/3	1/3	1.12001
14	Black	25	M	5	4	0.65234
15	Black	25	F	5	4	0.48240
16	Black	25	M	6	4	0.68663
17	Black	21	F	3/4	3/4	0.56943
18	Black	24	F	3	3	0.66053
19	Brown	25	F	4	2 1/2	0.80101
20	Black	21	M	3	3	0.82902
			Caucasian Diabetic			
21	Brown	23	M	23	4	0.78207
22	Brown	19	M	19	1	0.80093
23	Brown	21	M	21	2	0.50866
24	Brown	20	F	20	1 1/2	0.74151
25	Brown	25	M	25	7	1.81319
26	Brown	18	M	18	1	1.19160
27	Brown	18	M	18	1	0.77156
28	Red	19	F	19	2	1.22295
29	Blond	19	M	19	2	0.63411
30	Brown	22	M	22	4	1.21887

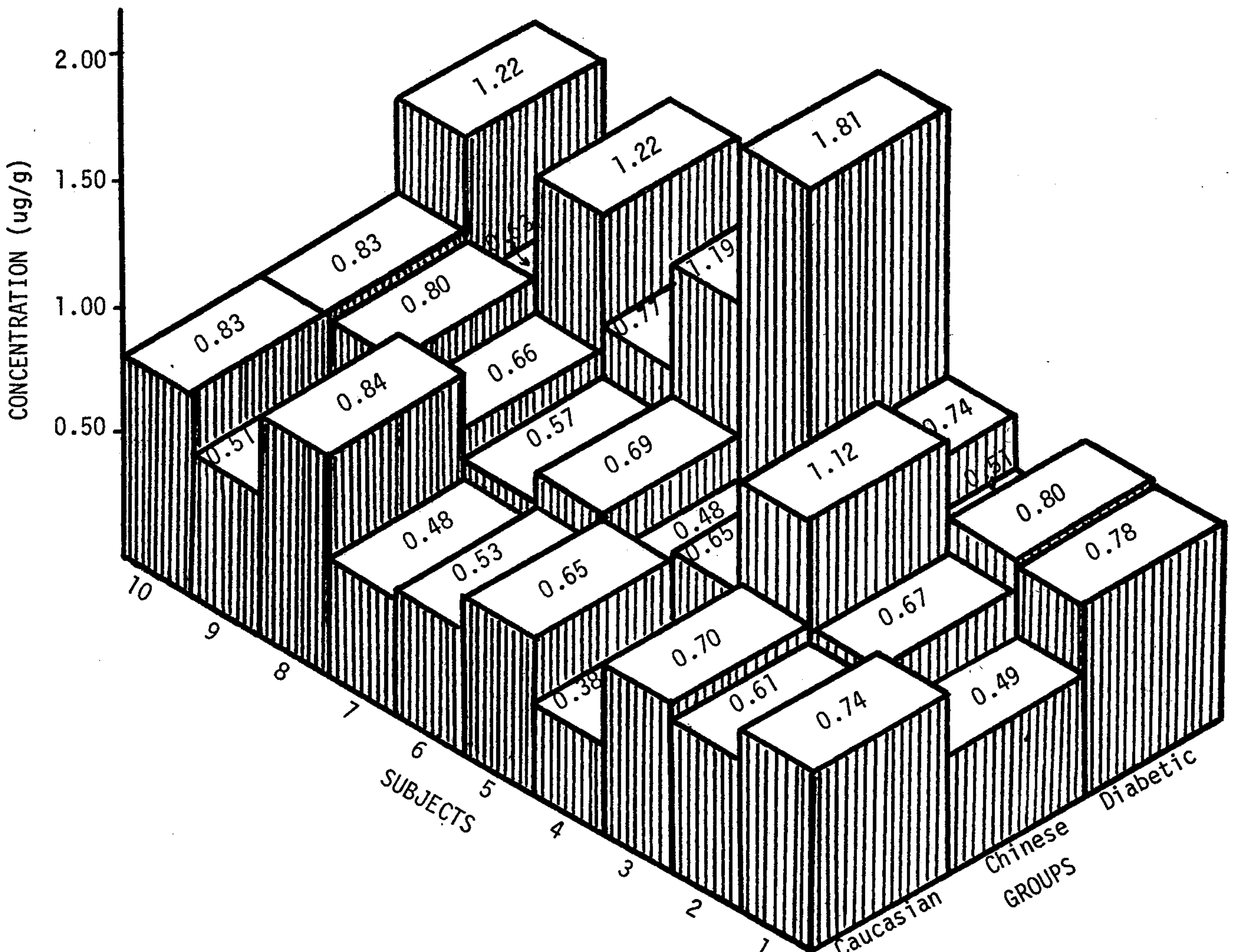

Figure 1. Hair Chromium Concentrations of All 30 Subjects

between the Caucasian and Chinese groups. However, the difference in chromium concentrations between diabetic and Caucasian and between diabetic and Chinese were found to be significant at the 0.025 level. Descriptions of AOV and LSD Multiple Comparison are given in Tables XVI and XVII in Appendix B.

TABLE IV

A COMPARISON OF MEAN HAIR CHROMIUM LEVELS FOR ALL CAUCASIANS, CHINESE, AND DIABETICS

Groups	Hair Cr Concn* (ug/g)
Caucasians	0.62678
Chinese	0.69579
Diabetics	0.96855

*Difference between diabetic and other two groups is significant at the 0.025 level.

A comparison of insulin-dependent and non-insulin-dependent diabetic subjects is given in Table V. The mean hair chromium concentration for the seven insulin-dependent subjects is shown to be slightly higher (0.99278 ug/g versus 0.91201 ug/g) for the three non-insulin-dependent diabetics.

TABLE V

COMPARISON OF HAIR CHROMIUM CONCENTRATIONS AMONG DIABETIC SUBJECTS

Subject Number	Years as Diabetic	Medication and Years	Hair Cr Concn (ug/g)	Mean Hair Cr Concn* (ug/g)
		Insulin-Dependent		0.99278
21	19 1/2	NPH-U80 19 1/2	0.78207	
22	16	Ultra Lente 16	0.80093	
23	7	Lente U80 7	0.50866	
25	14	Lente U80 14	1.81319	
26	7	NPH-U80 7	1.19160	
29	4	NPH-U80 4	0.63411	
30	11	Lente NPH-U80 11	1.21887	
		Non-Insulin-Dependent		0.91201
24	1 1/2	---	0.74151	
27	4 1/2	---	0.77156	
28	1 1/4	---	1.22295	

*No significant difference by t-test.

This difference was not shown to be significant with the t-test. Details of the t-test are given in Table XVIII in Appendix B.

Further comparisons of hair chromium concentrations based on hair color, age, and sex of the subjects are shown in Tables VI, VII, and VIII. Due to the small number of subjects in each category and the closeness of values, the differences are assumed to be statistically insignificant, and no test was conducted for these data.

TABLE VI

COMPARISON OF MEAN HAIR CHROMIUM CONCENTRATION BASED ON HAIR COLOR

Hair Color	Non-Diabetic Subjects		Diabetic Subjects	
	Cr Concn (ug/g)	Number of Subjects	Cr Concn (ug/g)	Number of Subjects
Black	0.69927	10	---	0
Blond	0.65458	2	0.63411	1
Brown	0.62746	7	1.80910	8
Red	0.53168	1	1.22295	1

Other Elements in Hair

Results for concentrations of other elements besides chromium, namely calcium, zinc, copper, iron, and manganese, were determined with AA and are shown in Tables IX to XI.

TABLE VII

COMPARISON OF MEAN HAIR CHROMIUM CONCENTRATIONS BASED ON AGE

Age	Non-Diabetic Subjects		Diabetic Subjects	
	Cr Concn (ug/g)	Number of Subjects	Cr Concn (ug/g)	Number of Subjects
18	---	0	1.96316	2
19	0.48983	1	1.94049	3
20	0.57596	4	0.74151	1
21	0.64480	4	0.50866	1
22	0.60787	1	1.21887	1
23	0.83095	2	0.78207	1
24	0.76468	3	---	0
25	0.65781	5	1.81319	1

TABLE VIII

COMPARISON OF MEAN HAIR CHROMIUM CONCENTRATIONS BASED ON SEX

Sex	Non-Diabetic Subjects		Diabetic Subjects	
	Cr Concn (ug/g)	Number of Subjects	Cr Concn (ug/g)	Number of Subjects
Female	0.63833	10	0.98223	2
Male	0.68424	10	1.76434	8

TABLE IX

CONCENTRATION OF ELEMENTS OTHER THAN CHROMIUM IN HUMAN HAIR

Subject Number	Concentration (ug/g)				
	Ca	Cu	Fe	Mn	Zn
1	233.880	31.0704	13.4172	1.20365	128.841
2	333.927	56.6500	9.2527	0.66605	126.891
3	312.425	16.0256	12.9622	1.30398	151.915
4	302.689	14.4249	14.3676	0.53494	137.385
5	274.797	13.8889	20.2865	0.60674	124.534
6	586.417	49.0062	14.0004	0.96734	120.323
7	774.619	13.0112	9.4687	0.84878	98.104
8	259.090	14.8945	15.5598	1.02209	127.945
9	222.868	11.7349	13.6384	0.65203	126.875
10	484.477	24.8264	24.7268	0.74467	138.648
11	497.971	13.6548	22.1151	1.21845	159.598
12	295.409	18.0335	10.3353	2.73216	122.243
13	558.381	33.8649	25.3225	3.87598	143.281
14	170.817	14.7339	15.7206	0.93298	120.354
15	729.534	27.2212	11.9008	2.39187	119.195
16	219.077	12.8039	14.9926	0.90759	119.285
17	484.876	49.5965	11.4950	1.96816	126.193
18	616.432	33.3968	22.2621	3.48662	114.365
19	472.704	18.5292	18.7578	2.69893	101.259
20	393.486	15.1654	26.0359	1.30312	121.983
21	268.696	20.1639	18.3691	1.90011	120.538
22	398.385	18.3814	17.9847	3.21874	129.088
23	223.556	22.9523	10.9597	0.98256	130.468
24	189.837	12.0942	14.6478	0.49909	119.698
25	339.422	13.6494	18.1975	0.90451	137.725
26	389.625	23.9093	28.9429	2.22750	141.260
27	446.199	41.2205	15.5337	1.46990	138.640
28	340.699	35.5770	27.5832	2.66021	125.808
29	244.296	10.2329	30.8808	1.59350	127.133
30	135.127	32.7091	73.1310	2.26598	114.416

TABLE X

COMPARISON OF ELEMENTS OTHER THAN CHROMIUM IN HUMAN HAIR BETWEEN DIABETIC AND NON-DIABETIC STUDENTS

Group	Concentration (ug/g)				
	Ca	Cu	Fe	Mn	Zn
Control	378.519	24.5533	14.7768	0.85503	128.146
Diabetic	297.584	23.0890	25.6230	1.77221	128.477

TABLE XI

COMPARISON OF ELEMENTS OTHER THAN CHROMIUM IN HUMAN HAIR AS A FUNCTION OF RACIAL CHARACTERISTICS

Group	Concentration (ug/g)				
	Ca	Cu	Fe	Mn	Zn
Caucasian	378.519	24.5533	14.7768	0.85503	128.146
Chinese	443.869	23.7000	17.8938	2.15158	124.775

A comparison of the five element concentrations in hair for all 30 subjects is given in Table IX. Mean values for each group were obtained and are reported in Table X as a comparison between diabetic and non-diabetic students and in Table XI as a comparison based on racial characteristics.

Of the five elements, calcium was highest in concentration, as is shown in Tables X and XI. Chinese subjects had the highest mean calcium level with a concentration of 443.869 ug/g, as compared to 378.519 ug/g for Caucasians and 297.584 ug/g for diabetics.

The element with the next highest concentration was zinc. Diabetics had the highest concentration, 128.477 ug/g, but were very close to that of Caucasians, 128.146 ug/g. Zinc in Chinese hair was slightly lower, 124.775 ug/g. These differences are probably not significant.

The mean concentration of copper among the three groups was fairly consistent. The highest concentration was found among Caucasians, that of 24.5533 ug/g, the lowest concentration was among diabetics, or 23.0890 ug/g. The mean copper level for Chinese subjects was 23.7000 ug/g.

Iron was present at approximately the same concentrations as copper. The mean iron concentration for the diabetic group was 25.6230 ug/g, which was the highest concentration for all groups, as compared with 14.7768 ug/g for Caucasians, and 17.8938 ug/g for Chinese.

Hair manganese concentration was present only in trace quantities. The Chinese subjects were shown to have the

most manganese in hair with a mean of 2.15158 ug/g. Hair from diabetic subjects contained less manganese, that is, 1.77221 ug/g, and the least manganese was present in hair from non-diabetic Caucasians, only with a concentration of 0.85503 ug/g.

CHAPTER V

DISCUSSION

The experimental method developed in this study is feasible in that sample collection is convenient and sample handling and storage are easy. The procedure to detect chromium is not complicated and can be performed within the limitation of available equipment. Above all, the detection is sensitive enough to yield data with chromium concentrations in nanogram (ng) units or fractional parts of a microgram (ug).

Flynn *et al* (6) have discussed the importance of taking hair samples a controlled distance from the scalp for trace element studies. However, another study by Hambidge (14) showed inconsistent differences in hair chromium levels from various sections of the same hair shafts. No controlled length of hair was used in this study since external environment does not seem to have an effect on hair chromium contamination or loss.

Different washing solutions have been used by experimenters to remove loosely bound external contamination. Hambidge used hexane (an organic fat solvent), ethanol, and deionized water (11, 12, 13, 14). Not much difference was noted in terms of chromium concentrations when detergent

(commercial shampoo) and organic solvents (such as hexane and ethanol) were used, indicating very little difference with the type of washing solution. For this study, carbon tetrachloride, as suggested by Schroeder (40), was used to remove grease and dust in hair since it is simple to use. The washing procedure is important in order to obtain a true sample size for a more accurate calculation of element concentration.

A pre-experimental trial study was designed to compare dry-ashing with wet-ashing to digest organic matter in hair. The higher chromium concentration in wet-ashed hair samples was found to be significant at the 0.05 level with AOV. With the unavailability of a large furnace, only a small number of samples can be dry-ashed at one time, making dry-ashing more time consuming than wet-ashing. Dry-ashing is also disadvantageous in its ease of sample loss during the process.

Sulfuric acid is often used in wet-ashing to raise the boiling point of the acid mixture. If care is taken, this extra acid can be omitted. About 98 percent of sample digestion is done by nitric acid, in the nitric-perchloric procedure, which oxidizes the aliphatic organic molecules. Perchloric acid is used to break up benzene rings and to oxidize chromium from the trivalent to the hexavalent state. It is important to evaporate digestion solutions almost to dryness to release the perchlorate anion (as indicated by the white fume) because perchlorates may interfere or cause

errors in atomic absorption. Feldman (4) suggested the use of methyl isobutyl ketone for extraction after digestion, to obtain more concentrated sample solutions. Methyl isobutyl ketone was not used here because its addition caused a wild, dangerous flame in the atomic absorption and defeated the original purpose.

Feldman (4) suggested preparing working standards on the day of analysis. Working standards in this study have been kept for almost one and a half months before the final analysis and yielded a good standard curve for all concentrations. Some sample solutions have been kept for almost two months before the final analysis, yet gave steady values for repeated readings. This finding that standard and sample solutions can keep would be helpful for studies that require lengthy preparation of large quantities of samples.

Differences between duplicate samples from the same subject were observed among some subjects. This may be due to the heterogeneity of individual hair samples causing uneven distribution of chromium in the duplicates. The assumption is supported by the difference in percentage weight loss in duplicate samples after washing with carbon tetrachloride, as illustrated in Table II.

Another factor that contributes to the dissimilar values of sample duplicates may be caused by the presence of white, sand-like materials in digested solutions. These materials were identified to be silica and could have influenced sample weight, which served as a basis for calculating hair chromium

concentration. Samples observed to contain more silica gave a lower chromium concentration than their duplicates from the same subject.

Another observation that may have caused differences in chromium concentrations for duplicate samples lies in caramelization, a reaction which could be caused by malanin, which is responsible for the dark pigment in hair. Caramelization was found to be more common for dark-colored hair samples. Caramelization indicates incomplete acid digestion by forming a thick, gluey substance toward the end of the digestion period. For some unexplained reason, all samples with more severe caramelization turned out to have higher chromium concentrations than their duplicates.

After washing with solvent and drying, quite a number of hair samples were observed to contain white, dandruff-like deposits. These foreign materials may have an inverse effect on hair chromium levels chemically or physically. Thus, uneven distribution of these deposits in turn may contribute to a difference in chromium concentrations of hair sample duplicates.

Hair Chromium Concentrations

Hair samples for this study were collected in the month of May. Hambidge (14) found in one study that hair chromium levels were lower in the month of May than in the other months. Findings in this research, however, fall within the range of concentrations of one previous study (12)

and were comparatively higher than another study (14) when the subjects were compared under similar conditions.

The higher tissue chromium concentration of Orientals as compared to Caucasians has been suggested by Schroeder (35) in a large survey. The same result is true here, although the difference is not significant. The exact reason for the difference is not known. It may be caused by a difference in diet for the two cultures, as chromium levels differ widely in foods. On the other hand, as shown in Table III, for Chinese subjects there was not a consistent decrease of chromium levels with an increase of residence in this country, indicating factors other than diet are most likely involved.

Chromium levels in the hair of diabetic students were found to be significantly higher than those of either Chinese or Caucasians, unlike the assumption made at the beginning of the study. The reason for the difference may lie in the function of chromium as a cofactor of insulin in the body. In the case of diabetics, more chromium may be stored in the hair when there is less insulin to react with the trace element than would be in the body of control Caucasians. The mechanism for the transfer of chromium to this body pool, if it is true, cannot yet be explained.

These findings are contrary to Hambidge's on juvenile diabetics, whose hair chromium concentrations are lower than normal healthy children's (11). The higher hair chromium concentrations with college-aged diabetics, as compared with

juvenile diabetics, may have something to do with the age difference. Being young adults, yet past the fast growing age of children, they may not need to use as much chromium as do the juvenile diabetics; thus, more is stored in the hair.

As was assumed, there is no significant difference in hair chromium levels between diabetics that are insulin-dependent and those that are not. However, insulin-dependent diabetics do have a slightly higher concentration of chromium than those that are non-insulin-dependent. This difference, however, is statistically insignificant.

As shown in Table VIII, there are differences in mean hair chromium concentrations for male and female subjects, being higher in the male subjects. However, the difference is not consistent when compared on an individual basis, as shown in Table III. It is thus considered possible that sex can be a factor in body chromium level in individuals because of differences in physical build and dietary habits.

All subjects in this study actually belong to the same age group, with a total difference in age of only seven years. Thus, it is reasonable to assume that not much difference in hair chromium concentration would be expected due to age.

There is not much difference in hair chromium concentrations among the four groups of hair colors. The same result held true for diabetic and non-diabetic subjects. Thus, the color of an individual's hair may not have an influence on chromium concentration.

Post-Thesis Study

In a post-thesis study done on five individuals, the hair chromium level was lowest for a Thai female student and exceptionally high in a Caucasian who was overweight, as is shown in Table XII. The other three subjects, a control Caucasian student, a middle-aged male who had been a diabetic and on insulin for 39 years, and a Jamaican Negro student, all had similar levels. The very high chromium level in the obese Caucasian was twice that of the control subject in this group and more than 11 times as much as that of one Caucasian subject in the thesis study. This may be caused by his greater dietary intake with an excess for storage. This possibility has yet to be proved in future work.

TABLE XII

HAIR CHROMIUM CONCENTRATION FOR POST-THESIS STUDY

Sample Number	Donors and Description	Hair Cr Concn (ug/g)
X1	Caucasian control	1.96410
X2	Caucasian insulin-dependent diabetic (for 39 years)	0.92316
X3	Caucasian college student (overweight)	4.39655
X4	Jamaican Negro	0.83769
T	Thai college student	0.32943

Other Elements in Hair

Since this study is concerned primarily with the methodology and comparison of hair chromium concentrations, no extensive discussion of the other five elements analyzed will be attempted.

Calcium was the most abundant of the six elements studied in hair. There was much individual variation in hair calcium concentration. Among the three groups, Chinese hair had the most calcium, about 60 ug/g more than Caucasians. Diabetics had the lowest concentration, about 80 ug/g less than non-diabetic Caucasians.

Human hair was quite rich in zinc. Not much difference in zinc concentration between the three groups was shown.

Hair copper concentration was much lower than zinc or calcium, yet high enough to be assayed without much difficulty. Like calcium, it differs among individuals; but there was little real variation among the three groups.

Iron in hair showed a distinct difference among groups, being highest for diabetics and lowest in Caucasians. Like chromium, the difference between diabetics and non-diabetics was considerably greater than that between Caucasians and Chinese. By rough comparison of most samples, there seemed to be a correlation between hair chromium and iron concentrations.

Manganese was present only in minute concentrations in hair, being only slightly higher than chromium. It was

highest in concentration in Chinese hair, lower in that of diabetics, and lowest in non-diabetic Caucasians.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Summary

Hair samples collected from Caucasian, Chinese, and diabetic college students were wet-ashed and analyzed by atomic absorption spectrometry for hair chromium concentrations. Results from this study can be summarized as follows:

1. There was no significant difference in hair chromium concentrations between Caucasian and Chinese subjects.

2. Diabetic subjects had the highest hair chromium level among the three groups studied. The difference between diabetic and the other two groups was highly significant.

3. There was no significant difference in hair chromium concentrations between insulin-dependent and non-insulin-dependent diabetic subjects.

4. There was some difference in mean hair chromium concentration between male and female subjects. The difference was not consistent when compared on an individual basis.

5. There was little variation in hair chromium concentration for different hair colors.

6. There was little difference in hair chromium concentration when different age levels were compared within the same age group.

Conclusions

As a result of reviewing related previous works, careful observation, and from the outcomes of this study, the following conclusions can be drawn:

1. Hair is a valuable biopsy material for chromium study and is a promising tool to compare chromium nutritional status of different groups of individuals. As discussed in Chapter II, chromium is stored in hair in the trivalent state.

2. The wet-ashing method is feasible for hair sample digestion. The stability of the hexavalent state of chromium in water prolongs the storage period of hair sample solutions.

3. If hair chromium concentrations are studied in diabetic subjects, only age-matched individuals or groups should be used. That age is important is reflected in the difference in values between juvenile, college-aged, and middle-aged diabetics.

Suggestions

There is a sizeable listing of chromium research projects awaiting further investigation. They could be important in the health of the world populations. Further study is needed for an understanding of the exact functioning of chromium in the human body to reveal the mechanisms for uptake, storage (including that in hair), excretion, site of action with body insulin or insulin supplements, and its

relationship with other nutrients in the body. Other suggestions for research are as follows:

1. A greater number of subjects in each group, larger hair sample size (thus enabling an increase in the final volume without a decrease of concentration for AA analysis), and homogenized individual hair samples for well-mixed duplicates are needed. Further refinements in the procedural manipulation include:

(a) Use of a washing solvent other than carbon tetrachloride to eliminate dandruff-like materials in hair samples.

(b) Procedures to control silica deposits in sample solutions.

(c) Procedures to overcome the problem of severe caramelization in the wet digestion procedure.

(d) Use of non-static chromium-free containers (the plastic bags used here are very static to hair).

(e) Weighing hair samples in those containers to be ashed and/or digested together with the hair samples so as to eliminate errors in sample transfer and weighing.

2. Hair chromium concentrations in obese subjects need to be determined against a control group.

3. Hair chromium concentrations need to be determined between different age groups, such as young subjects against older subjects, both non-diabetic and diabetic.

4. Hair chromium concentrations of different races in the same country, such as American Negroes against Caucasians, need to be checked in an attempt to discern genetic variation in a population.

5. Hair chromium level research by sex and hair color would be of great value in an attempt to determine sex-linked genetic factors with other variables being controlled.

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APPENDIX A

PRE-EXPERIMENTAL STUDY DATA

TABLE XIII

WET ASHING OF HAIR SAMPLES, PRE-EXPERIMENTAL STUDY

Sample Number	Type	Hair Cr Concn (ug/g)	Average Concn (ug/g)
1	Washed with CCl_4	1.60759	
			1.94180
2	Washed with CCl_4	2.27209	
3	Not washed	1.46523	
			1.36990
4	Not washed	1.40154	

TABLE XIV

DRY ASHING OF HAIR SAMPLES, PRE-EXPERIMENTAL STUDY

Sample Number	Type	Ash Weight (%)	Percent Ash	Hair Cr Concn (ug/g)	Average Concn (ug/g)
5	Washed with CCl_4	0.01194	1.21	---	
					0.40316
6	Washed with CCl_4	0.00986	1.05	0.40316	
7	Not washed	0.01255	1.24	1.09561	
					0.84960
8	Not washed	0.01365	1.18	0.60395	

TABLE XV

AOV FOR PRE-EXPERIMENTAL' STUDY

Source	df	SS	MS	F*
Total	7	3.79749		
Ash	1	2.69595	2.69595	25.36640
Wash	1	0.10020	0.10020	1
Ash and wash	1	0.57622	0.57622	5.42170
Error	4	0,42515	0.10628	

*ash is significant at the 0.05 level

APPENDIX B

STATISTICAL TESTS USED IN THE THESIS RESEARCH

TABLE XVI

AOV FOR CAUCASIAN, CHINESE, AND DIABETIC SUBJECTS

Source	df	SS	MS	F*
Total	29	2.58149		
Group	2	0.65320	0.326602	4.57310
Error	27	1.92828	0.071418	

*significant at the 0.03 level

TABLE XVII

LSD MULTIPLE COMPARISON FOR CAUCASIAN, CHINESE, AND DIABETIC SUBJECTS

	Diabetic	Chinese
Caucasian	0.341963	0.069003
Chinese	0.272760	

$LSD_{0.05} = 0.24994$

Significant to the 0.025 level for:

Diabetic and Caucasian group,

Diabetic and Chinese group.

TABLE XVIII

t-TEST FOR INSULIN-DEPENDENT AND NON-INSULIN-DEPENDENT DIABETIC SUBJECTS

	Insulin-Dependent	Non-Insulin-Dependent
Mean Cr Concn (ug/g)	0.99278	0.91201
Number of Subjects	7	3

$$t_8 = \frac{0,99278 - 0.91201}{Sp \; \frac{1}{6} + \frac{1}{2}}$$

$$= 0.26948*$$

*The difference in hair chromium concentration is not significant.

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