A COMPARISON OF HAIR CHROMIUM CONTENT IN OBESE, DIABETIC, AND NORMAL COLLEGE STUDENTS

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CHAPTER I

INTRODUCTION

Trivalent chromium is considered by many as an essential trace element. It is a co-factor with insulin and is essential in humans for normal carbohydrate metabolism. A deficiency causes a reduced sensitivity of peripheral tissue to insulin. A severe deficiency causes fasting hyperglycemia, glycosuria and mild growth retardation in rats and mice. Chromium deficiency is considered a possible risk factor with formation of cardiovascular diseases such as atherosclerosis and myocardial infarction and some forms of diabetes.

Hair is considered a stable and accurate indicator of the chromium content of the body. It can be painlessly obtained and stored for an indefinite period. Harsh chemicals, such as bleach and dye, do change the chromium content of the hair shaft, yet the external environment does not appear to affect the hair chromium. It is a good material for studies comparing the chromium in different groups.

Diabetes seems to be related to both a chromium deficiency and excessive weight. There is a possibility that they are related to each other. This study has been developed to compare the chromium level of diabetic, obese and non-diabetic normal-weight students and investigate possible relationships.

CHAPTER II

REVIEW OF LITERATURE

Introduction to Chromium

The importance of the role of chromium in nutrition is becoming increasingly evident. For example, it has been established as a co-factor with insulin necessary for normal glucose tolerance (26, 84), and it appears to affect carbohydrate metabolism, fat metabolism (70) and protein synthesis (67). As more information is known chemically, biochemically and physiologically about this element, its significance in mammalian nutrition is better recognized.

There are six forms of chromium, but the trivalent and hexavalent forms are the most common. The trivalent form is the form which is required as a co-factor with insulin for the proper utilization of glucose (52). Inorganic trivalent chromium (chromium III) is poorly absorbed from the gastrointestinal tract (53). The amount absorbed probably depends on the acidity of the gastric juice (15, 88). Organic chromium complexes are thought to be better absorbed than the inorganic forms (33). The daily intake of chromium is low (57, 51), and the percent of inorganic chromium absorbed is low, therefore, if natural complexes are not better absorbed then the body will soon be depleted of chromium.

Absorbed chromium III appears in the plasma protein fraction of

the blood. In rats it usually binds almost entirely with siderophilin but some binding occurs with plasma proteins (33, 47). In the dog a considerable amount of chromium is transported through the body in the unbound state (7). Chromium is excreted mainly through the urine (41, 47) but some studies indicate it is also excreted in the feces.

The chemical form of the complex of which chromium is a part seems to determine its biological activity. For example, when inorganic chromic chloride is mixed with brewers yeast, the chromium extracted from the yeast and pumped into the stomachs of rats is absorbed and retained better than the inorganic chromic chloride treated in the same fashion. So that this difference would not be attributed to the extra nutrients in the extract, milk is added to the inorganic chromic chloride and pumped into the stomach of rats and no significant change observed in the amount of inorganic chromic chloride absorbed (52).

Chromium Nutrition in Mammals Other Than Man

Growth and Longevity

Rats and mice are often used in the study of chromium because body functions and nutritional needs are similar to those of man, their environment can be controlled and their life span is short. While on a diet deficient in chromium, the rats and mice whose diets were supplemented with chromium showed a significant increase in growth rate compared to those without the supplement (50, 65, 67, 72). Also, the male rats and mice given the extra chromium lived longer than the male controls. There was no difference between the female controls and the test females of either species. The chromium supplements appeared to

decrease the difference in the lifespans of the male and female rats but not the mice (67, 76, 77).

Glucose Metabolism

Before 1957 impaired glucose tolerance was observed in rats raised on purified diets. After a long search for this missing "glucose tolerance factor" (GTF) (78), chromium was identified as the missing factor. Later, trivalent chromium was specifically identified as the necessary co-factor with insulin for proper glucose utilization (54, 64, 65, 78, 79), and a chromium intake of .14 ug/g or less in the diet produced a partial deficiency (74). This induced defect could be cured quickly with an oral dose of 20 ug or an intravenous doese of 0.25 ug/100 g body weight of trivalent chromium (34).

There seems to be much evidence supporting the fact that the inorganic chromium absorbed into the body is changed into an organic complex that works with insulin for proper glucose metabolism (2). The epididymal fat tissues of rats deficient in GTF have been used to demonstrate in vitro the increased effectiveness of insulin on the absorption of glucose (55) and galactose (49) when chromium was present. The mode of chromium interaction with insulin is still unknown but Mertz's studies in vitro with liver cells (46) indicate chromium forms a complex between sulfhydryl groups on the cell membranes and the sulfhydryl groups on the A chain of insulin (6).

When a severe form of chromium deficiency is induced, the resulting symptoms are identical to diabetes mellitus, complete with glycosuria and fasting hyperglycemia (65). When the rats were injected with exogenous insulin, the rate of cardiac and hepatic glycogen formed from

glucose was only half the rate of the control animals. The low response to the exogenous insulin presents the possibility that the impaired glucose tolerance may be due to the lowered response to the endogenous insulin.

Lipid Metabolism

Early in the study of chromium, Curran (9, 63) demonstrated chromium III (trivalent chromium) enhanced the synthesis of cholesterol and fatty acids from labeled acetate in the liver of rats fed a diet deficient in chromium. Yet lower serum cholesterol levels are found in rats whose chromium-deficient diet is supplemented with chromium (63). Also, the age-linked increase in serum cholesterol observed in male rats deficient in chromium tended to be eliminated with chromium supplements (64). When the aortas of rats that had died natural deaths were examined, those fed chromium were low (two percent) in aortic plaque while those on a chromium-deficient diet had a significantly higher amount (19 percent) of plaque (71). Schroeder's low chromium diet for rats caused aortic plaque, elevated serum cholesterol and elevated blood pressure. These symptoms in the rat are the same as those of the human's syndrome (71, 65). The symptoms can also be produced in diets lower in chromium with refined white sugar as the main carbohydrate. The syndrome can be prevented if raw or dark brown sugar is used instead of white in the diet (75).

Protein Synthesis

Rats fed diets low in chromium and protein are less able to incorporate several amino acids into the protein of their hearts. Insulin

slightly increased this process but a supplement of trivalent chromium significantly increased this selective method of protein synthesis. The amino acids affected were α-amino isobutyric acid, glycine, serine and methionine. None of the other amino acids seemed to be affected by the chromium-deficient state of the animals.

Corneal Lesions

Adult squirrel monkeys fed a commercial food demonstrated impaired carbohydrate metabolism. Analysis of the food and drinking water showed they contained low amounts of chromium. Tests were made and chromium (III) corrected the problem, but divalent chromium seemed to aggravate the intolerance (10). Corneal lesions have been found in squirrel monkeys (43) and rats (59) fed a low-chromium diet. When rats were fed a low-chromium and low-protein diet, ten to fifteen percent developed visible corneal lesions. The squirrel monkeys were fed a diet adequate in every respect except it was low in chromium, and 80 to 90 percent of the monkeys developed the corneal lesions. These lesions were not reversible. The contrast between the monkeys and the rats suggests the primate may be more susceptible to a dietary deficiency in chromium (43).

Chromium Nutrition in Man

Growth

Two important studies have recently been published on the effect of chromium on the growth of man (23, 24). The studies are almost alike in procedure and results. In Istanbul, 28 marasmic children between the

ages of 0 to 24 months were used in a study. They were placed in two groups, one of which was given supplements of chromium. The weight increase of the supplemented group was significantly higher ($P < 0.02$) than that of the control group. When the members of the group that received supplements were considered separately, nine of the 14 children showed a significant increase in the removal rate of glucose whereas the other five showed no change. When the weight gain of the respondents to chromium was compared statistically to the control group, the difference was highly significant ($P < 0.001$). There was no difference between the non-responding five to the control group (24). Both studies provide good evidence that a chromium supplement could cause increased growth in marasmic man (23, 24).

Glucose Metabolism

As soon as a relationship between chromium deficiency and diabetes mellitus was discovered, research was begun to learn more about the possible role of chromium in glucose metabolism. Several studies were made by supplementing the diet of diabetics with doses of chromium (III). The results suggested glucose tolerance was improved in mild cases of diabetes mellitus if the symptoms were due to a deficiency of chromium and if the chromium supplement was given for an extended period of time (14). Chromium does not change normal glucose tolerance when it is given to chromium-sufficient subjects. As stated by Mertz and quoted by Underwood (84, p. 260), "Chromium is not considered a hypoglycemic agent, a substitute for insulin or a cure for diabetes."

It has long been known infants suffering from protein-calorie malnutrition often have accompanying symptoms of fasting hypoglycemia

or impaired glucose tolerance or both. In studies from Jordan (26, 47), Nigeria (26, 33, 47), Egypt (3, 26, 47), and Turkey (23, 24, 26), children suffering from protein-calorie malnutrition were given oral doses of trivalent chromium. The results of these experiments indicate in some geological regions, protein-calorie malnutrition is complicated by chromium deficiency. For example, the children in the Nile Valley in Egypt (3) failed to respond to the chromium substitute, probably because the common foods eaten there are high in chromium. The Nigerian children (33), whose food is low in chromium, responded with an increased rate of glucose removal even though the low mean fasting level was not affected (26, 89).

Lipid Metabolism

Atherosclerosis is more common in countries where low tissue levels of chromium are found. The countries that are low in chromium are usually the more advanced countries such as England and the United States. Tests have shown that people who died of atherosclerosis had lower levels of aortic chromium than subjects who died in an accident or of other cardiovascular diseases (69). Since chromium is necessary for proper glucose and lipid metabolism (75), low chromium levels in the human may cause the abnormal metabolism and plaque formation of the atherosclerosis syndrome (68). Hospital patients with high cholesterol levels were given chromium supplements for over six months. The chromium supplement produced a decline in blood cholesterol similar to dietary restrictions of saturated fats (70).

Obesity, Chromium and Diabetics

Chromium deficiency is considered as a possible cause of diabetes in humans. Chromium is necessary for proper glucose metabolism. Since rats deficient in chromium develop diabetes (67), deficient humans may also develop diabetes. As a person grows older, the amount of chromium stored in the body decreases, and his chances of becoming diabetic increase. Therefore, some maturity onset diabetes could be caused from deficiency. Some evidence indicates that the low levels of chromium found in elderly people and insulin-dependent diabetics are from their inability to change inorganic chromium to GTF (46, 96).

There are many other factors that are believed to cause or contribute to the development of diabetes mellitus. Obesity has long been considered a major contributing factor. At least three out of four adults who become diabetic were overweight before they developed the disease (16). Continuous overeating is believed to cause the pancreas to overwork until it can no longer supply the insulin needed for proper glucose metabolism. Little research has been done investigating a possible relationship between body weight and amount of body chromium.

A large amount of chromium is lost from refined foods such as rice, flour, and especially sugar. Consumption of refined carbohydrates can reduce the chromium intake by one-half to one-fourth of that present in less refined or natural products (46). Refined foods make up a large portion of carbohydrates in the American diet and chromium is necessary for proper metabolism of carbohydrates. It has been suggested that excessive use of refined foods in the diet could be a factor in the depletion of chromium. Schroeder (67) theorizes that if glucose mobilizes chromium from tissue stores into the bloodstream and if a

portion of this mobilized chromium is lost in the urine, it would not be replaced by the refined chromium-poor foods. If this assumption is true, obese people that eat large amounts of refined foods would have low chromium levels and possibly form diabetes.

There are two chromium studies in which people with excess weight differed from normal weight individuals. In one study Hopkins (46) used middle-aged laboratory personnel who had a moderate glucose tolerance impairment when the daily diet was supplemented with 150 ug of chromium chloride. The subjects that responded to the chromium supplementation were of normal weight, while the non-responders were overweight. In the other study Chan (4) analyzed the hair of an obese Caucasian college student. It contained more than twice as much chromium as the Caucasian control and more than 11 times as much as that of one Caucasian subject in the study.

Location of Chromium in Human Tissues

At birth the chromium in human tissues is at a high level and rapidly decreases to a lower level. Then the decline in the total level of chromium in the body continues to a lesser degree during the first and second decades of life before leveling off later (67). The lungs are the only organs in which concentration of chromium rises in later life. However, when looked at separately, the various organs lose chromium at different times. The lungs, aorta, heart and spleen lose their chromium early, while the kidney and liver keep their neonatal level of chromium until the second decade of life (70).

Comparisons have been made on the amount of chromium in the body tissues of people in the United States with people living in other

countries. For example, African tissues had 1.9 times as much, Near East tissues had 4.4 times as much, and Far Eastern had five times as much chromium as people in the United States (67, 81).

Chromium in Food

As the importance of chromium in human nutrition became apparent, research was begun to determine which foods contained chromium. Most foods were found to contain some chromium but at the beginning there was no method to determine whether the chromium was biologically active or not. It was soon discovered that the refining of flour, rice, sugars, and fats (major sources of calories) caused the loss of a substantial amount of chromium. In one study 53 ug of chromium/100 kcal were found in whole wheat flour, whereas in white flour only 6.6 ug/100 kcal were found (67). The refining of foods is believed to be a major cause of low chromium levels in the tissues of people in the United States (42, 67, 68).

In studies comparing the amount of chromium found in different types of sugar, molasses was found to be highest in chromium, followed by unrefined and brown sugar. Refined sugar was the lowest in chromium content (42, 75, 86). A study was conducted on the effect of these three sugars on serum cholesterol and glucose tolerance on rats. Rats were placed on a chromium-deficient diet with refined white, dark brown or raw sugar added as the variable. Another group was given refined white sugar and a chromium supplement. All except the refined white sugar group retarded the rise of serum cholesterol, characteristic of a chromium deficiency. Also, the rats on the refined white sugar became hyperglycemic with age (75).

A significant relationship between the consumption of refined foods (especially sugar) and cardiovascular mortality has been reported (42). The diet of the average person in the United States is thought to be low in chromium (57, 61) and high in refined sugar (approximately 120 g/day/person. Glucose is believed to mobilize body stores of chromium and increase its excretion in the urine. If this is true, the high levels of refined sugar will help deplete an already low intake of chromium (21, 42, 67). It has been speculated that if unrefined sugar replaced the refined sugar in the United States diet, the daily intake of chromium would increase by a third and there would be less chromium loss from the chromium-depleting action of glucose (42).

Methods are now being developed to measure the biological activity of food. Alcohol extraction is used to remove chromium from foods, which is then tested for glucose oxidation activity on chromium-deficient adipose tissue of rats. These experiments indicate there is no significant relationship between the total chromium content of food and its biological activity. The chromium in meats, fungi and grains were found biologically active but some foods, such as egg yolk, were biologically inactive even though high in total chromium content (82).

Causes of Chromium Deficiency

The exact cause of chromium deficiency is unknown but there are probably one or more factors that work together for a low chromium state. Listed below are factors thought to contribute to a low chromium state (47).

1. The level of chromium in the body declines with age. This could be due to the fact that less chromium is needed after growth and the body

loses the extra unneeded chromium (47). Arguments against this theory include the fact that wild animals and mature humans from other countries maintain a much higher body chromium level (70). The chromium tissue level is probably due to a lifetime of insufficient intakes of chromium (57, 61).

2. In many cases insulin-dependent diabetics have been shown to have lower chromium levels than non-diabetics of the same age (14, 31). There are many theories and some disagreement on why this happens.

3. Pregnancy has been shown to deplete the mother of chromium (30, 70). The placental transfer of chromium is selective and only chromium as GTF, not as a simple inorganic salt, is transported to the young (52). The more pregnancies, the greater the depletion in the mother.

4. A diet of refined carbohydrates and foods low in chromium are believed to be the major causes of chromium deficiency in this country. The use of much refined carbohydrates not only adds little chromium to the diet but also is believed to mobilize chromium into the blood (21). In a recent test it was demonstrated that the plasma chromium of normal men and women decreased after a glucose load (contrary to earlier studies) (11). However, in pregnant chromium-deficient women the plasma chromium content increased slightly but not significantly. The plasma chromium fall that occurred in the normal subjects was never lower than the low fasting plasma chromium level of pregnancy. Davidson and Burt (11) believe chromium is absorbed into the tissue with a glucose load. If this study is duplicated, some ideas on chromium responses will be changed.

Hair as a Biopsy Material

Hair can be used for detecting borderline malnutrition and for determining the trace element content in the body (94). There has been some argument on the necessity of using hair that is only a short measured distance from the scalp in trace element research (19, 40). This procedure is not necessary when chromium is the element being studied. The results of numerous tests have indicated hair is a stable and accurate indicator of the chromium content of the body. It is also easily and painlessly obtained from the subjects being tested. Listed below are some of the reasons hair is considered a good source from which to determine the chromium content of the body.

1. The chromium content of hair is relatively stable due to the lack of turnover of chromium along the hair shaft (28).

2. The external environment does not appear to add or remove the chromium or other trace minerals from the hair shaft. But if hair is subjected to harsh chemical treatment such as bleaching or dyeing, chromium will be lost from the hair shaft (30).

3. Chromium concentration in the hair shaft varies at increasing distances from the scalp. The concentration of chromium does not appear to be related to the time the hair has been exposed to the external environment (28).

4. The decline in body chromium that occurs during the first year of life can be demonstrated by analysis at different distances from the scalp of the same hair sample. In this case, the farther the hair is from the scalp, the greater the concentration of chromium.

Atomc Absorption Spectrometry

Trace element research increased the need for better control of experimental variables, now techniques and more accurate instruments (45). Precipitation and colorimetric techniques were among the first used and they were useful in the determination of several elements (36). Later, flame emission became the most popular method, now atomic absorption spectrometry (AA) is taking its place.

AA is a popular and important tool in trace mineral analysis. Its advantages include its high sensitivity, its uncomplicated method of sample preparation and few interferences (17, 18, 36). The interferences in AA are chemical, ionization and matric, and all can be overcome. In chromium research AA is preferred over flame emission because "atomic absorption is independent of the excitation potential of the transition involved and it should be less subject to temperature variation and interference from extraneous radiation or energy exchange between atoms" (85).

Gas chromatography is another very accurate method of determining chromium content, but it is not as sensitive in measuring trace element content as AA or flame emission. Spectrophotometry, spark source mass spectrography, coulometry, polarography and neutron activation analysis are other techniques that have been used to detect and measure chromium (62).

CHAPTER III

EXPERIMENTAL PROCEDURE AND MATERIAL

Since chromium has been identified as an insulin co-factor, there has been much research comparing the chromium levels of diabetic and non-diabetic subjects. Obesity and diabetes are also related, but there has been little research comparing the chromium levels in human subjects. This study was developed to investigate and compare chromium levels in the hair of diabetics of normal weight, obese non-diabetics and normal weight non-diabetics. The experimental procedure and materials used are outlined in this chapter.

Subject Selection and Characteristics

The subjects were male and female Caucasian students between the ages of 18 and 27. The subjects fit into one of the three groups: (1) the Diabetic, Normal Weight Group, (2) the Obese, Non-diabetic Group, and (3) the Control Group, which consists of normal weight non-diabetics. There were 11 juvenile diabetics in the Diabetic Normal Weight Group. The Obese, Non-diabetic Group consisted of 15 subjects. There were 15 subjects in the Control Group. Two female obese diabetics were also studied and compared to the other groups, although they did not constitute a study group as such.

Subjects were obtained by non-random technique, i.e., through personal contacts and newspaper advertisements. None of the subjects had

bleached, dyed, lightened or permanented hair and none of the female subjects were or had been pregnant.

Each subject was individually interviewed on personal information, measurements and instruction on hair collection could be made. Height, weight, skinfold measurements and all other information pertinent to this study were recorded on a questionnaire (Appendix A). Each subject was given instructions on collecting hair. Two grams of hair samples were collected by saving the hair from hair cuts or removing it from hair brushes. The subjects collected the hair for up to four months. The estimated time necessary to work with each subject through the complete hair analysis was 12 hours.

Since weight was such an important factor in this study, an accurate and scientific method had to be used. The method used was to determine percent of body fat from skinfold measurements. Men and women store fat in different places so two methods were used. For men the Borzch and Keys (12) method was used and for women the Sloan, Burt and Blyth method (13) was used. When a Lnage Skinfold Caliper, skinfold measurements were made on the triceps, chest and abdominal areas for men and for women the triceps and iliac chest area. Normal weight was set at less than 15 and 21 percent body fat for men and women, respectively. Obese weight was set at more than 25 and 30 percent body fat for men and women, respectively (12). (Both methods are in Appendix B.) At the conclusion of the study individual information was given the subjects if they wished to have it.

Experimental Procedure

All glassware, plastic storage bottles and other equipment used

in this study were thoroughly cleaned in the following manner:

1. washed with soap and hot water,
2. rinsed three times with double distilled de-ionized water,
3. soaked for 10 or more hours in a solution of 10 percent 6N hydrochloric acid (Reagent Grade) and 90 percent double distilled de-ionized water,
4. rinsed three times with double distilled de-ionized water and then,
5. covered and dried in a drying oven.

To remove any extra chromium that might have etched into the beakers in which hair was to be digested, two extra steps were added to their cleaning. After steps 1 and 2, 15 ml of 3:1 nitric perchloric was boiled in the beakers to volatilize any remaining chromium. The beakers were then cooled, rinsed three times with double distilled de-ionized water and the above procedure was continued.

The hair samples had to be clean before they were wet ashed. Each sample was checked for lint and any other visible contaminants. The samples were washed in a beaker twice with 20 ml of hexane (Reagent Grade). The hexane was vigorously shaken and swirled for at least five minutes and then drained. The hair was washed for a third time with 15 ml of 95 percent ethanol, after which it was rinsed three times with double distilled de-ionized water and dried overnight in a drying oven. The hair was washed and dried in the same beaker to decrease the chance of contamination. After the samples were dry, they were removed one at a time and weighed immediately to prevent a weight change due to the absorption of moisture from the air. The sample was then placed in a specially cleaned 250 ml pyrex beaker, ready for the digestion process.

Digestion and Preparation for Analysis

In this study the method selected for digestion of the hair samples was a wet-ashing method with a mixture of 3:1 nitric perchloric acid. The digestion mixture was prepared using 3:1 mixture of Reagent Grade nitric and perchloric acid. These acids were mixed with a magnetic stirrer and a Versamix.

Twenty ml of digestion acid was added to each member of a run (approximately 8 samples and one acid blank). The samples were then covered with a watch glass and allowed to stand at room temperature for 12 or more hours. They were then heated to 120° temperature for two hours. By this time the samples were usually clear; if not, 10 ml of the digestion mixture were added to the clear samples. At this time the hot plate temperature was increased to give a sample temperature of 250°.

When a clear sample was near dryness, the sides of the beaker and the watch glass were rinsed with 5 ml of 1:1 hydrochloric acid. This solution was added to drive off excess perchloric acid which might interfere with AA analysis. It was prepared from one part 6N hydrochloric acid (Reagent Grade) and one part double distilled de-ionized water. If the sample turned yellow with the addition of 1:1 hydrochloric acid, the digestion process was continued. If the sample stayed clean it was returned to the hot plate until near dryness.

The sides of the beaker and watch glass were then rinsed three times with double distilled de-ionized water, funneled into a 10 ml volumetric flask and brought up to volume. The samples were stored in a polyethylene bottle and refrigerated until time of analysis.

Analysis of Samples

Standard solutions with chromium concentrations of 0.0, 0.1, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ug of chromium/ml were prepared from a standard stock chromium solution of 1,000 ppm. The standards, like the samples, were stored in polyethylene bottles and refrigerated until analysis. Both were analyzed at room temperature. After the samples were analyzed, they were then spiked with 2 ml of 1.5 ug of chromium/ml and again analyzed. The analysis was made on a Perkin Elmer 303 atomic absorption spectrophotometer, using an air-acetylene flame and a Boling burner. The instrumental settings used for chromium analysis were:

Wave Length	3574A
Meter Response	1
Gain	4
Slit	3
Range	UV
Air Flow	6
Fuel Flow	6-1/2

The AA spectrophotometer ran on 14 milliamps. A curve was made from the AA measurements of the standard solutions. The chromium in the samples was determined from this curve. Two lamps were used in this study. The first curve and two sample sets were determined by lamp serial number 9227 and the next two curves and five sample sets were determined by the second lamp number 1085.

Statistical Analysis of Data

A Fortran computer card was prepared for each subject. Subject

number, group, hair color, sex, run, curve, percent of body fat and chromium per gram of hair were coded on each card. The Analyses of Variance (AOV) test was used to statistically analyze the data.

CHAPTER IV

RESULTS

A general description of each subject, the percent body fat, the run (groups of samples on the hotplates) and AA curve and hair chromium concentration are described in Table I. The chromium level ranged from 2.1427 ug/g in a male diabetic to 7.9098 ug/g in a female control. The spike analysis of each sample is recorded in Appendix G.

The mean hair chromium of the diabetic, obese, obese diabetic and control groups are given in Table II. Even though there was great individual variation within the groups, the means are relatively close. The obese group, when compared to the control and diabetic groups, is lower by .7002 and .7103 ug/g, respectively. An analysis of variance (Table III) indicated no significant difference between groups. It also indicated that there was no significant difference between sexes and between both sex and group.

When the groups are divided by sex (Table IV) the means indicate some difference due to sex. The means of the three male groups are very close: Control = 3.112, Diabetic = 3.447 and Obese = 3.346. The means of the four female groups are more varied: Control = 4.225, Diabetic 4.075, Obese = 2.846 and the two Obese Diabetic = 3.935. Figures 1 and 2 graphically demonstrate the difference in chromium levels of the male and female. Figure 1 demonstrates the small variation between the chromium means of the male groups. For females it showed the closeness

TABLE I

GENERAL DESCRIPTION AND INFORMATION
ABOUT INDIVIDUAL SUBJECTS

Group	No. in Group	Hair Color	Sex	ug Cr/g of Hair	Run	Curve	% Body Fat	Weight in lbs.
C	01	Dark Brown	F	2.8508	6	3	18.81	125
C	02	Dark Brown	F	2.9324	1	1	15.48	111
C	03	Light Brown	M	2.6352	3	2	2.40	123
C	04	Dark Brown	M	4.3952	3	2	11.33	151
C	05	Blond	M	3.1046	4	2	11.37	160
C	06	Blond	F	2.7437	3	2	15.26	124
C	07	Blond	F	5.5619	3	2	12.00	110
C	08	Light Brown	M	3.6762	7	3	5.78	156
C	09	Red	F	3.2782	2	1	19.17	135
C	10	Dark Brown	F	5.1640	3	2	15.84	101
C	11	Light Brown	M	2.6014	5	3	3.37	166
C	12	Dark Brown	M	2.2595	4	2	3.93	164
C	13	Light Brown	F	4.0376	2	1	14.25	103
C	14	Dark Brown	F	3.5471	1	1	16.47	120
C	15	Light Brown	F	7.9098	4	2	19.11	134
D	01	Dark Brown	F	2.9732	1	1	17.93	129
D	02	Dark Brown	M	2.1427	2	1	7.58	147
D	03	Light Brown	F	3.7801	4	2	16.28	106
D	04	Dark Brown	F	5.6319	7	3	20.69	132
D	05	Dark Brown	M	3.6676	6	3	6.26	140
D	06	Dark Brown	F	4.7204	7	3	20.31	127
D	07	Blond	M	2.2722	2	1	11.15	158
D	08	Light Brown	M	6.6998	4	2	3.26	136
D	09	Dark Brown	F	3.2046	2	1	17.14	122
D	10	Dark Brown	M	2.4556	3	2	8.87	126
D	11	Dark Brown	F	4.1405	2	1	19.09	107
O	01	Dark Brown	M	2.8054	5	33	25.01	251
O	02	Light Brown	M	3.6030	7	3	29.53	248
O	03	Light Brown	M	3.5028	1	1	30.84	296
O	04	Dark Brown	M	4.2740	4	2	33.08	233
O	05	Dark Brown	M	2.4870	3	22	32.68	187
O	06	Dark Brown	F	3.7294	5	3	38.82	222
O	07	Dark Brown	M	2.9401	4	2	33.21	293
O	08	Dark Brown	F	3.1404	6	3	38.65	146
O	09	Red	F	2.7743	4	2	34.90	182
O	10	Dark Brown	F	3.1200	1	1	49.51	263
O	11	Dark Brown	M	3.8122	6	3	40.27	320
O	12	Dark Brown	F	2.4636	3	2	47.54	281
O	13	Dark Brown	F	2.3945	2	1	51.82	327
O	14	Dark Brown	F	2.7664	4	2	41.77	257
O	15	Dark Brown	F	2.3817	1	1	45.75	288

TABLE I (Continued)

Group	No. in Group	Hair Color	Sex	ug Cr/g of Hair	Run	Curve	% Body Fat	Weight in lbs.
OD	01	Light Brown	F	3.7673	1	1	29.93	162
OD	02	Dark Brown	F	4.1031	6	3	30.82	166

C = Control; D = Diabetic; O = Obese; OD = Obese-diabetic

TABLE II

A COMPARISON OF THE MEAN HAIR CHROMIUM LEVELS OF CONTROL, DIABETIC, OBESE AND OBESE-DIABETIC SUBJECTS

Group	Number	Cr
Controls	15	3.7798
Diabetic	11	3.7899
Obese	15	3.0796
Obese-Diabetic	2	3.9352

of the diabetic, normal, and obese diabetic and a drop to the obese group. Figure 2 compares the means of the females and males in each group. The means of the females are higher in the control and diabetic group and lower in the obese group. Figure 2 shows the almost linear mean chromium level of the males in all these groups.

TABLE III

MULTIPLE AOV FOR CR DIFFERENCES DUE TO SEX, GROUP, AND SEX AND GROUP

Source	DF	SS+	MS	F	OSL*
Total	42	61.615			
Sex	1	.933	.933	.671	.42
Group	3	7.382	2.461	1.768	.17
Sex and Group	2	4.993	2.496	1.794	.18
Error	36	50.108	1.392		

+Some of squares do not add to total since the design was unbalanced. These values were obtained as the partial SS from a regression analysis.

*(Observed Significance Level.) Probability of obtaining a value larger than the F value observed of the null hypothesis.

TABLE IV

A COMPARISON OF MALE AND FEMALE MEAN HAIR CHROMIUM LEVELS OF CONTROL, DIABETIC, OBESE AND OBESE-DIABETIC SUBJECTS

Group	Sex	Number	Cr
Control	F	9	4.225
Control	M	6	3.112
Diabetic	F	6	4.075
Diabetic	M	5	3.447
Obese	F	8	2.846
Obese	M	7	3.346
Obese-Diabetic	F	2	3.935
Overall Means		43	3.545

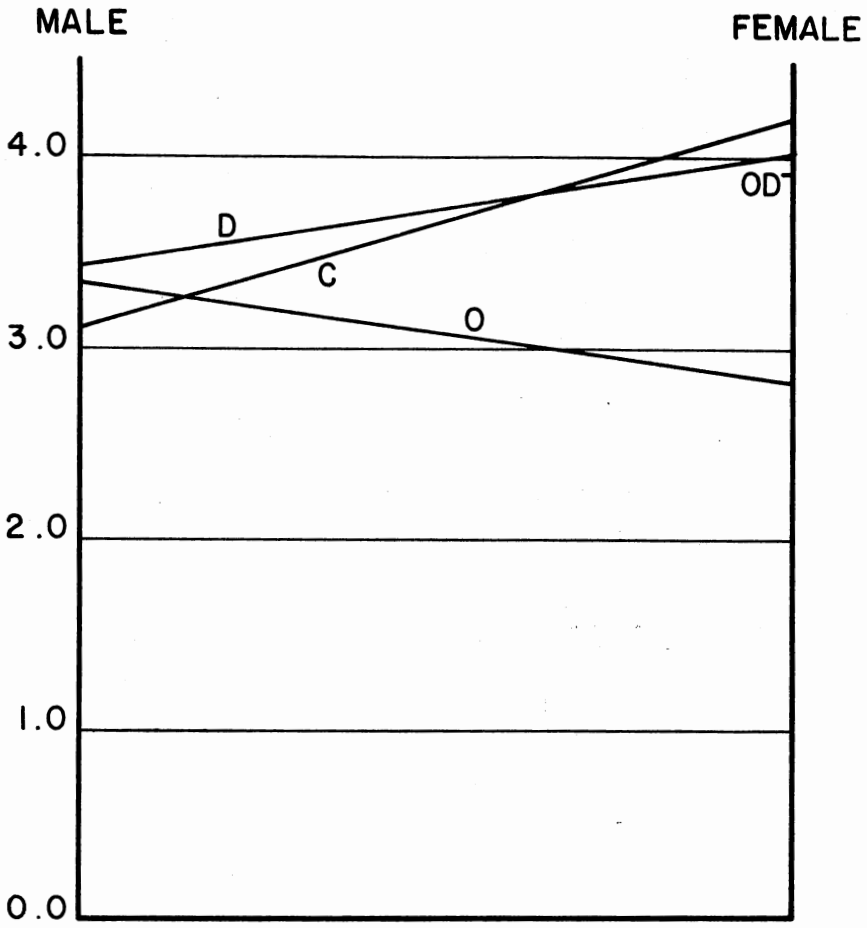

Figure 1. Mean Hair Chromium Concentration of the Major Groups Differentiated by Sex

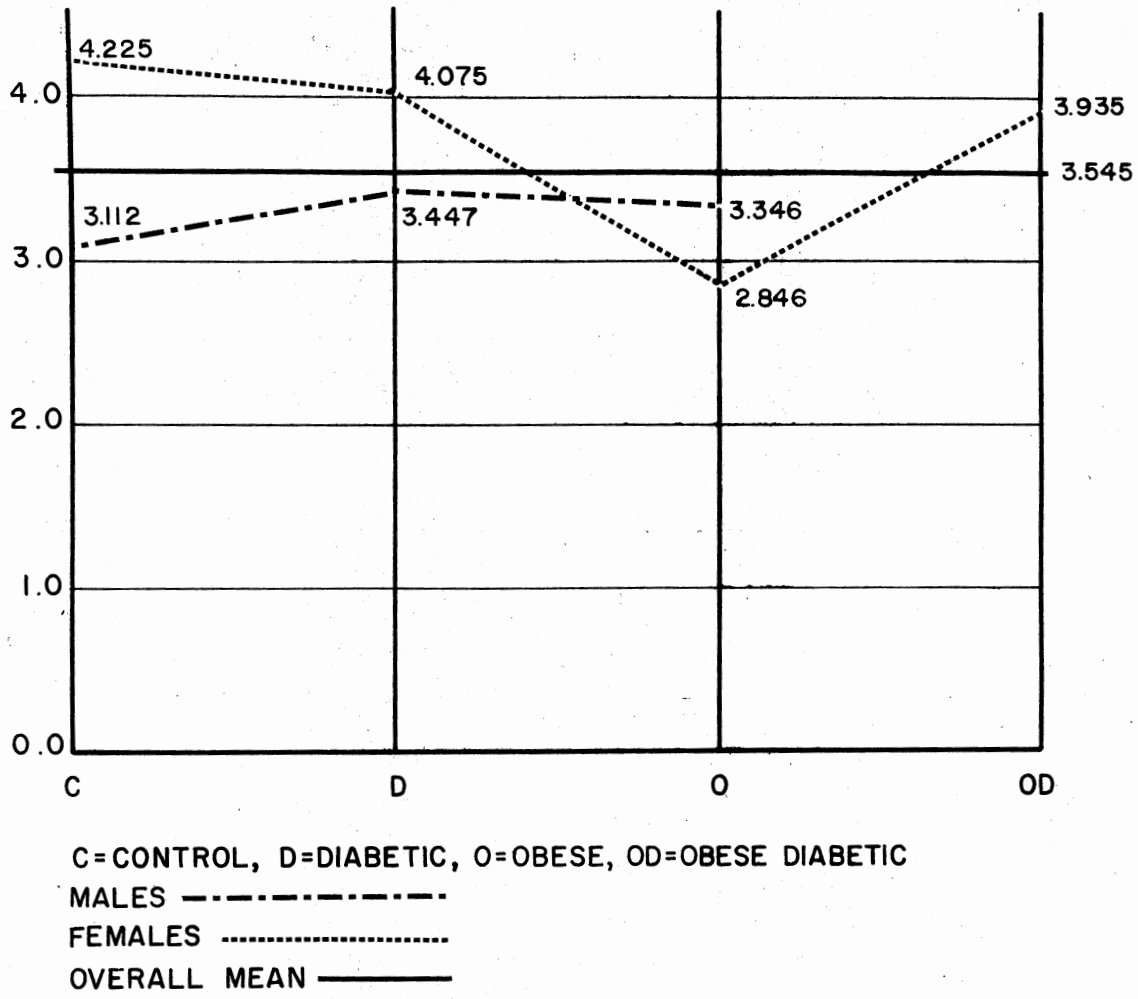

Figure 2. Mean Hair Chromium Concentration of Each Sex Differentiated by the Major Groups

An analysis of variance was used to determine if the curve or the run made any difference in chromium level. The two AOV's (Tables V and VI), showed no statistical differences between runs or curves.

An AOV (Table VII) was used to determine whether body fat percentages of subjects could be used to determine the chromium content of hair. Three tests were run. In the first test the fat percent was used without any allowances made for differences in body fat due to sex. The other two tests made allowances for the sex differences in body fat. In the second test the normal body fat (15 percent for men and 21 percent for women) was subtracted from the individual body fat. This method was the best statistically and indicates that some allowance must be made for the differences between sexes when working with body fat and body chromium levels. On the third test even greater allowances for sexual differences in normal body fat were made. In this test the normal body fat for that particular sex was divided into the individual body fat of the subject. This last method seemed to make too great an allowance for the sexual differences in normal body fat. None of the three models attempted indicated that chromium content of the hair could be predicted adequately from fat percentage.

TABLE V

AOV FOR CR DIFFERENCES DUE TO RUN

Source	DF	SS	MS	F*
Total	42	61.615	1.467	
Run	6	8.669	1.445	.982
Residual	36	52.946	1.471	

*Not significant. (F would have to be 2.37 to be significant at the 5 percent level.)

TABLE VI

AOV FOR CR DIFFERENCES DUE TO CURVE

Source	DF	SS	MS	F*
Total	42	61.615	1.467	
Curve	2	3.800	1.900	1.3144
Residual	40	57.815	1.445	

*Not significant. (F would have to be 3.23 to be significant at the 5 percent level.)

TABLE VII

AOV FOR FAT PERCENTAGES AS A FACTOR IN DETERMINING CHROMIUM CONTENT OF HAIR

Source	df	SS	MS	F	
Fat	1	2.069	2.067	1.424	.24
Error	41	59.546	1.452		
Fat Differences*	1	3.379	3.379	2.379	.13
Error	41	58.235	1.420		
Fat PG**	1	2.402	2.402	1.663	.20
Error	41	59.213	1.444		

*Fat Differences = Fat--21 for females
Fat--15 for males

**Fat Percent = Fat--21 for females
Fat--21 for males

CHAPTER V

DISCUSSION

This study was developed to investigate and compare chromium levels in the hair of diabetics of normal weight, obese non-diabetics and normal weight non-diabetics. Since research suggests that human chromium deficiency contributes to some forms of diabetes, there has been further research comparing chromium levels of diabetics to non-diabetics. Chromium research comparing diabetics and overweight subjects is minimal, yet there is the possibility that a relationship exists. The methods, materials and results of this study are discussed in this chapter.

None of the statistical tests used gave any significant results in this study. Yet there were several observations that can be made from the information supplied.

Discussion of Methods and Materials

The possibility of seasonal variation in hair chromium was considered. There is the possibility that chromium absorption varies with the seasons, but little proof of this. If seasonal variation is used as a limiting factor in a research project, all hair must be collected during a certain limited time period and all hair used must be the same length measured from the scalp. Since a long strand of hair can represent several years' growth, if seasonal variation is to be a

limiting factor then hair length is as important as collection time. Seasonal variation was not used as a limiting factor in the project because little actual research has been published to prove or disprove seasonal variation for chromium and because such large amounts of hair were needed and the hair length varied greatly from each subject.

Studies have shown that children have high chromium levels and elderly people have low chromium levels. All subjects used in this study were between the ages of 18 and 27. The age restriction on subjects used was to control differences in hair chromium concentration due to age.

A relatively large amount of hair, approximately two grams, was used in each sample. Because chromium is found in such minute amounts, this large quantity of hair was used in hopes of increasing the chromium found in the sample, thereby making the Atomic Absorption Spectrophotometer readings more accurate. When the digestion temperature was lowered to prevent volatilization of chromium, the two grams became more difficult to digest and extra acid had to be added to complete the digestion process. One gram hair samples were easier to digest at this lower temperature and appeared to give just as accurate results as two gram samples. Using one gram of hair per sample at this lower temperature would be recommended for future research.

Preventing the contamination of the samples with chromium during the washing and wet ashing process was of the utmost importance. The hair was washed in a manner in which extraneous chromium found in the dirt and grease would be removed, leaving only the endogenous chromium. All materials touching the sample from washing the hair until the Atomic

Absorption analysis were cleaned to prevent sample contamination. To prevent chromium contamination from water, distilled de-ionized water was used. Possible contamination from dust or air was kept small by keeping the sample's exposure to the atmosphere at a minimum.

Chromium is volatile to heat (46, 83); therefore, controlling the temperature is important. For uniform results all samples must be heated to the same temperature. Even if a low temperature is used during this phase, it is possible that some volatilization may occur. As long as each sample is subjected to the same temperature, it can be assumed that the chromium lost is equal in all samples. Hot spots on the hot-plate and the varying thicknesses of glass on the bottom of the pyrex beakers used during digestion may have caused a slight individual variation in the heat each sample received. Due to the low temperature at which the sample was digested, it is believed that this had little effect on changing the level of chromium found in the samples. Using a hot water bath instead of a hotplate may alleviate possible chromium loss due to the hot spots found on a hotplate.

After the hair samples were digested they were stored in polyethylene bottles until analysis. There was the possibility that during the storage time chromium could be lost from the solution due to absorption on the container walls. A study (81) has shown that unlike trivalent chromium, losses of hexavalent chromium due to absorption on polyethylene containers was negligible. Since all trivalent chromium was changed to hexavalent chromium during the digestion process, losses of chromium due to absorption on the sample storage containers should be negligible.

During the chromium analysis of the hair samples the spectrophotometer was operated at 14 milliamps instead of the recommended 25 milliamps. The lower voltage gave good results during the analysis of the samples and the standard solutions. It appears that the higher voltage is not necessary for accurate measurement of the chromium in the samples.

Discussion of Results

The chromium levels found in the hair of all groups were much higher than the levels previously reported. In this study the mean chromium hair levels of the major groups were 3.79, 3.08, and 3.78 ug/g in the diabetic, obese, and control group. Hambidge found mean hair chromium levels of .56 and .85 ug/g in diabetic and normal children and Chan (4) found mean hair chromium levels of .97 and .63 ug/g in diabetic and normal students. Theories for this difference in chromium levels are listed and discussed below.

1. The water and soil from the area in which this study was made contained higher concentrations of chromium than that found in the areas where other studies took place. This was not likely since Chan's study (4) and this study took place in the same area with the same age group but her chromium levels were much lower.

2. The samples were contaminated in some manner. This theory was also unlikely since the acid blanks used in this study contained negligible amounts of chromium which would add little to the total amount of chromium found in the sample (Appendix D, Table VIII).

3. The lower level of heat used in the digestion of the hair samples may have reduced the amount of chromium volatilized, giving a

more accurate reading of the chromium levels found in the hair samples. For example, before this study began, a test was used to determine approximate recovery of chromium from this procedure. Instead of hair, 5 ml of a chromium standard containing 1 ug of chromium/ml was pipetted into four beakers. The procedure was the same as used in this study. When the test was completed and the solution was reconstituted to 5 ml, the chromium concentrations were graphed at .91, .93, 1.04 and 1.10 ug/ml. This test indicated that the low level of heat used during the wet-ashing procedure gives an accurate reading. Older studies used high levels of heat when ashing the samples for chromium studies. Recent studies show that heat volatilizes chromium (46, 83); this last theory seems the most reasonable.

Table I shows the large variation of chromium levels within each group. It is possible that individual dietary habits cause the variation in body chromium levels. Little is known about the dietary habits of most of the subjects in this study, but information is known about a limited number of them. The female control with the highest body chromium level (7.9098 ug/g of hair) has a diet high in beef. Another female control with a high body chromium level (5.5619 ug/g) of hair has a diet high in whole grain bread and meat.

There is no significant difference in hair chromium levels between the males and females. However, Table III and Figure 1 show that except for the Female Obese Group the women have slightly higher chromium levels then the men. The Obese Female Group has the lowest mean hair chromium level (2.846 ug/g) and is lower in chromium than the mean of the lowest male group by .266 ug/g and the mean of the lowest female group by 1.089 ug/g. The means of the three male groups are

almost linear while the four female groups show more variation (Figure 2). From the information obtained (Figures 1 and 2), it is considered a possibility that sex is a factor in the absorption of chromium. It is also possible that individual diet could have caused this difference in chromium levels. It is possible that the low chromium level found in the Female Obese group may be the result of a poor diet exceptionally high in refined carbohydrates.

The means of the diabetic and control groups were very close. No difference was found between these two groups. This finding is in agreement with Blalock (1). In her study a comparison of hair chromium was made of maturity onset diabetic and non-diabetic females and no significant differences were found between them. Yet other studies found diabetics with lower chromium concentrations than the controls (4, 31).

An analysis of variance (Table VII) was used to determine the chromium content of hair. These three tests showed that fat percentages are not related to the chromium content of hair. The second test indicated that possibly some allowance should be made for the differences between sexes when working with body fat and body chromium. It is possible that there would be no difference in male and female chromium levels (except for the obese females) if an allowance was made for the sexually different normal body fat levels (Figures 1 and 2).

An analysis of variance (Tables V and VI) was used to determine if the curve or the run made any difference in chromium level. Three different curves and seven different runs (a run is a group of samples on the hotplate) were used in this study. The curves and runs were

initiated at different times so this test was used to determine if some undetermined conditions caused differences in the chromium level of the samples. No statistical differences in chromium levels was found to be the result of the runs or the curves.

CHAPTER VI

SUMMARY, CONCLUSIONS AND SUGGESTIONS

Summary and Conclusions

Hair samples weighing approximately 2 g were collected from 11 diabetic subjects of normal weight, 15 obese non-diabetics and 15 normal college students as controls. These students were selected to study and compare the differences in the chromium levels in hair as an indication of body chromium levels. Each subject was interviewed for information about age, hair color, and sex. The percentage of body fat was then calculated by the Brozch and Keys method for men and for women the Sloan, Burt and Blyth method. Each subject collected their hair sample by saving the hair from haircuts or removing it from hair brushes. After all hair samples were collected, the samples were washed, dried, wet-ashed and analyzed by atomic absorption spectrometry. Results from this study can be summarized as follows:

1. No significant difference was found in the hair chromium concentrations between the diabetic and obese groups.

2. There was no significant difference found between the hair chromium concentration of the diabetic and control groups.

3. There was no significant difference found between the hair chromium concentration of the obese and control groups.

4. There was a slight difference in hair chromium concentration between the male and female subjects. This difference was not

significant.

5. Due to the great variety of chromium levels within each group, percentages of body fat of subjects could not be used as a method to determine the body chromium levels.

6. There was little difference in hair chromium concentrations when the atomic absorption spectrometry curves were compared.

7. There was little difference in hair chromium concentrations when the runs were compared.

Suggestions

In the field of nutrition, chromium research is a relatively new and open field. Not only is the amount of research in this area small, but many of the conclusions based on that research contradict themselves. In this research project the need for further study of chromium in certain areas was highlighted. Suggested research projects are listed below:

1. Many researchers feel that adult onset diabetes are caused by a chromium deficiency. Yet research has produced many conflicting studies in this area. It appears that further research is needed--perhaps with a larger number of subjects.

2. Research needs to determine if chromium levels are affected by different types or amounts of insulin.

3. Further research is needed to determine if body chromium levels are influenced by the sex of the subject.

4. Research is needed to determine if a relationship exists between diet and body chromium levels.

5. There needs to be still more research to determine if obesity influences body chromium levels.

SELECTED BIBLIOGRAPHY

1. Blalock, Beta J.: A study of elderly female maturity-onset diabetic and elderly non-diabetic women's hair chromium. Unpub. M.S. thesis, Oklahoma State University, 1975.

2. Campbell, W. J. and Mertz, W.: Interaction of insulin and chromium (III) on mitochondrial swelling. Am. J. Physiol. 204:6, 1963.

3. Carter, J. P., Kattab, A., Abd-El-Hadi, K., Davis, J. T., Cholmy, A. E., and Patwardhand, U. N. Chromium (III) in hypoglycemia and in impaired glucose utilization in Kwashiorkor. Am. J. Clin. Nutr. 21:3, 1968.

4. Chan, Jacinta, N.: A comparison of hair chromium levels in college students. Unpub. M.S. thesis, Oklahoma State University, 1973.

5. Chatterjee, G. C., Roy, R. K., Sasmal, N., Panerjee, S. K., and Majumder, P. K.: Effect of chromium and tungsten on L-ascorbic acid metabolism in rats and chicks. J. Nutr. 103:78, 1973.

6. Christian, G. D., Knoblock, E. C., Purdy, W. C., and Mertz, W.: Polarographic study of chromium-insulin-mitochondrial interaction. Biochem. Biophys. Acta. 66:420, 1963.

7. Collins, R. J., Fromm, P. O., and Collings, W. D.: Chromium Excretion in the dog. Am. J. Physiol. 201(5):795, 1961.

8. Curran, G. L., and Azarnoff, D. L.: Effect of certain transition elements on cholesterol biosynthesis. Fed. Proc. 20 (supplement No. 10):109, 1961.

9. Curran, G. L.: Effect of certain transition group elements on hepatic synthesis of cholesterol in the rat. J. Biol. Chem. 210:275, 1954.

10. Davidson, I. W. F., and Blackwell, W. L.: Changes in carbohydrate metabolism of squirrel monkeys with chromium dietary supplementation. Proc. Soc. Exp. Bio. Med. 127:66, 1968.

11. Davidson, I. W. F., and Burt, R. L.: Physiologic changes in plasma chromium of normal and pregnant women: effect of a glucose load. Am. J. Obstet. Gynecol. 116:601, 1973.

12. de Vries, Herbert A.: Estimation of body composition from skinfold measurements in man. Physiol. of Exer. Dubuque: Wm. C. Brown Company Publishers., 1971, pp. 107-110.

13. de Vries, Herbert A.: Estimation of body composition from skinfold measurements in man. Physiol. of Exer. Dubuque: Wm. C. Brown Company Publishers, 1971, pp. 113-114.

14. Doisy, R. J., Streeten, D. H. P., Souma, M. L., Kalafer, M. E., Rekant, S. I., and Dalakos, T. G.: Metabolism of Chromium in Human Subjects, Normal, Elderly and Diabetic Subjects. Ed. W. Mertz and W. E.Cornatzer. New York: Dekker, Inc., 1971.

15. Donaldson, R. M., Jr., and Barreras, R. F.: Intestinal absorption of trace quantities of chromium. J. Lab. and Chem. Med. 68(No. 3):448, 1966.

16. Dolger, H. and Seeman, B.: How to Live With Diabetes. New York: Pyramid Publications, Inc., 1965.

17. Feldman, F. J., and Purdy, W. C.: The atomic absorption spectroscopy of chromium. Anal. Chim. Acta. 33:273, 1965.

18. Feldman, F. J., Knoblock, E. L., and Purdy, W. C.: The determination of chromium in biological materials by atomic absorption spectroscopy. Anal. Chim. Acta. 38:489, 1967.

19. Flynn, A., Fratianne, R. B., Hill, O. A., Pories, W. J., and Strain, W. H. Malversation in hair analysis. Am. J. of Clin. Nutr. 24:893, 1971.

20. Friedman, G. J. Diet in the treatment of diabetes mellitus. In Goodhart, R. S. and Shils, M. E., eds., Modern Nutrition in Health and Disease, Dietotherapy. Philadelphia: Lea and Febiger, 1973.

21. Clinsman, W. H., Feldman, F. J., and Mertz, W.: Plasma chromium after glucose administration. Science. 152:1243, 1966.

22. Grande, F., and Keys, A.: Body weight, body composition and caloric status. In Goodhart, R. S., and Shils, M. E., eds., Modern Nutrition in Health and Disease, Dietotherapy. Philadelphia: Lea and Febiger, 1973.

23. Gurson, C. T., and Saner, G.: Effect of chromium on glucose utilization in marasmic protein-calorie malnutrition. Am. J. Clin. Nutr. 24:1313, 1971.

24. Gurson, C. T., and Saner, G.: Effects of chromium supplementation on growth in marasmic protein-calorie malnutrition. Am. J. Clin. Nutr. 26:988, 1973.

25. Hambidge, K. M.: Chromium nutrition in the mother and the growing child. In Mertz, W., and Cornatzer, W. C., eds., Newer Trace Elements in Nutrition. New York: Dekker, Inc., 1971.

26. Hambidge, K. M.: Chromium nutrition in man. Am. J. Clin. Nutr. 27:505, 1974.

27. Hambidge, K. M., and Baum, J. D.: Hair chromium concentration of human newborn and changes during infancy. Am. J. Clin. Nutr. 25:376, 1972.

28. Hambidge, K. M., Franklin, M. L., and Jacobs, M. A.: Changes in hair chromium concentrations with increasing distances from hair roots. Am. J. Clin. Nutr. 25:380, 1972.

29. Hambidge, K. M., Franklin, M. L., and Jacobs, M. A.: Hair chromium concentrations: effects of sample washing and external environment. Am. J. Clin. Nutr. 25:384, 1972.

30. Hambidge, K. M., and Rogerson, D. O.: Comparison of hair chromium levels of nulliparous and parous women. Am. J. Obstet. Gynecol. 103:320, 1969.

31. Hambidge, K. M., Rogerson, D. O., and O'Brien, D.: The concentrations of chromium in the hair of normal children and children with juvenile diabetes mellitus. Diabetes. 17:517, 1968.

32. Hopkins, L. L.: Distribution in the rat of physiological amounts of injected Cr^{51}(III) with tine. Am. J. Physiol. 209(4): 731, 1965.

33. Hopkins, L. L., Ransome-Kuti, O., and Majaj, A. S.: Improvement of impaired carbohydrate metabolism by chromium (III) in malnourished infants. Am. J. Clin. Nutr. 21(3):203, 1968.

34. Hopkins, L. L., and Schwarz, K.: Chromium (III) binding to serum proteins, specifically siderophilin. Biochim. Biophys. Acta. 90:484, 1964.

35. Imbus, H. R., Cholak, J., Miller, L. H., and Sterling, T.: Boron, chromium, cadmium and nickel in blood and urine. Arch. Environ. Health. 6:286, 1963.

36. Isaac, R. A., and Kerber, J. D.: Atomic absorption and Flame photometry: techniques and uses in soil, plant and water analysis. In Walsh, L. M., ed., Instrumental Methods for Analysis of Soil and Plant Tissues. Madison: Soil Science Society of America, Inc., 1971.

37. Klevay, L. M.: Hair as a biopsy material. Am. J. Clin. Nutr. 23(3):284, 1970.

38. Klevay, L. M.: Hair as a biopsy material. Am. J. Clin. Nutr. 23(4):377, 1970.

39. Klevay, L. M.: Hair as a biopsy material (II) assessment of copper nutrition. Am. J. Clin. Nutr. 23(9):1203, 1970.

40. Klevay, L. M.: Hair as a biopsy material. Am. J. Clin. Nutr. 25:263, 1972.

41. Kraintz, L., and Talmoge, R. V.: Distribution of radioactivity following intravenous administration of trivalent chromium 51 in rat and rabbit. Proc. Soc. Exp. Biol. Med. 81:490, 1952.

42. Masironi, R., Wolf, W., and Mertz, W.: Chromium in refined and unrefined sugars--possible nutritional implications in the etiology of cardiovascular diseases. Bulletin, World Health Organization. 49:322. 1973.

43. Martin, G. D., Stanley, J. A., and Davidson, W. F.: Corneal lesions in squirrel monkeys maintained on a low-chromium diet. Investigative Opthalmology, 11:153, 1972.

44. Mathur, R. K., and Doisy, R. J.: Effect of diabetes and diet on the distribution of tracer doses of chromium in rats. Proc. Soc. Exp. Bio. Med. 139:836, 1972.

45. Menis, O., and Rains, T. C.: Sensitivity, detection limit, precision and accuracy in flame emission and atomic absorption spectrometry. In Mavrodineanu, R., ed., Analytical Flame Spectrometry. New York: Springer-Verlag, 1970.

46. Mertz, W.: Biological role of chromium. Fed. Proc. 26:186, 1967.

47. Mertz, W.: Chromium occurrence and function in biological systems. Physiological Review. 49(2):163, 1969.

48. Mertz, W.: Aspects of nutritional trace element research. Fed. Proc. 29(4):1482, 1970.

49. Mertz, W., and Roginski, E. E.: The effect of trivalent chromium on galactose entry in rat epididymal fat tissue. J. Biol. Chem. 238(3):868, 1963.

50. Mertz, W., and Roginski, E. E.: Effects of chromium (III) supplementation on growth and survivial under stress in rats fed low protein diets. J. Nutr. 97:531, 1969.

51. Mertz, W., and Roginski, E. E.: Chromium metabolism: the glucose tolerance factors. In Mertz, W. and Cornatzer, W. E., eds., Newer Tracer Elements in Nutrition. New York: Dekker, Inc., 1971.

52. Mertz, W., Roginski, E. E., Feldman, F. J., and Thurman, D. E.: Dependence of chromium transfer into the rat embryo on the chemical form. J. Nutr. 99:363, 1969.

53. Mertz, W., Roginski, E. E., and Reba, R. C.: Biological activity and fate of trace quantities of intravenous chromium (III) in the rat. Am. J. Physiol. 209(3):489, 1965.

54. Mertz, W., Roginski, E. E., and Schroeder, H. A.: Some aspects of glucose metabolism of chromium-deficient rats raised in a strictly-controlled environment. J. Nutr. 86:107, 1965.

55. Mertz, W. E., Roginski, E. E., and Schwartz, K.: Effect of trivalent chromium complexes on glucose uptake by epididymal fat tissue of rats. J. Biol. Chem. 236(2):318, 1961.

56. Mertz, W., and Schwarz, K.: Relations of glucose tolerance factor to impaired intravenous glucose tolerance of rats on stock diets. Am. J. Physiol. 196(3):614, 1959.

57. Murthy, C. K., Rhea, U., and Peeler, J. T.: Levels of antimony, cadmium, chromium, cobalt, manganese and zinc in institutional total diets. Envir. Sci. Tech. 5(5):436, 1971.

58. Roginski, E. E., and Mertz, W.: Dietary chromium and amino acids incorporated in rats on a low-protein ration. Fed. Proc. 26:301, 1967.

59. Roginski, E. E., and Mertz, W.: An eye lesion in rats fed low-chromium rations. J. Nutr. 93:249, 1967.

60. Roginski, E. E., and Mertz, W.: Effects of chromium (III) supplementation on glucose and amino acid metabolism in rats fed a low-protein diet. J. Nutr. 97:525, 1969.

61. Sandstead, H. H., Burk, R. F., Booth, C. H., and Darby, W. J.: Current concepts on trace minerals. Med. Clin. of N. Am. 54(6):1509, 1970.

62. Savory, J., Mushak, P., and Sunderman, F. W.: Gas chromatographic determination of chromium in serum. J. Chrom. Sci. 7:674, 1969.

63. Schroeder, H. A.: Serum cholesterol levels in rats fed thirteen trace elements. J. Nutr. 94:475, 1963.

64. Schroeder, H. A.: Diabetic-like glucose levels in chromium deficient rats. Life Sci. 4:2057, 1965.

65. Schroeder, H. A.: Chromium deficiency in rats: a syndrome stimulating diabetes mellitus with retarded growth. J. Nutr. 88:439, 1966.

66. Schroeder, H. A.: Cadmium, chromium and cardiovascular disease. Circulation. 35:570, 1967.

67. Schroeder, H. A.: The role of chromium in mammalian nutrition. Am. J. Clin. Nutr. 21(3):230, 1968.

68. Schroeder, H. A.: Losses of vitamins and trace minerals resulting from processing and preservation of foods. Am. J. Clin. Nutr. 24:562, 1971.

69. Schroeder, H. A.: The role of trace elements in cardiovascular disease. Med. Clin. N. Am. 58:381, 1974.

70. Schroeder, H. A., Balassa, J. J., and Tipton, I. H.: Abnormal trace metals in man--chromium. J. Chron. Dis. 15:941, 1962.

71. Schroeder, H. A., and Balassa, J. J.: Influence of chromium, cadmium and lead on rat aortic lipids and circulating cholesterol. Am. J. Physiol. 209(2):433, 1965.

72. Schroeder, H. A., Balassa, J. J., and Venton, W. H.: Chromium, lead, cadmium, nickel and titanium in mice: effect on mortality, tumors and tissue levels. J. Nutr. 83:239, 1964.

73. Schroeder, H. A., Balassa, J. J., and Venton, W. H.: Chromium, cadmium and lead in rats: effects on life span, tumors and tissue levels. J. Nutr. 86:51, 1965.

74. Schroeder, H. A., and Nason, A. P.: Interaction of trace metals in rat tissues--cadmium and nickel with zinc, chromium, copper, manganese. J. Nutr. 104:167, 1974.

75. Schroeder, H. A., Mitchener, M., and Nason, A. P.: Influence of various sugars, chromium and other trace minerals on serum cholesterol and glucose of rats. J. Nutr. 101:247, 1971.

76. Schroeder, H. A., Venton, W. H., and Balassa, J. J.: Effect of chromium, cadmium and other trace elements on the growth and survival of mice. J. Nutr. 80:39, 1963.

77. Schroeder, H. A., Venton, W. H., and Balassa, J. J.: Effects of chromium, cadmium and lead on the growth and survival of rats. J. Nutr. 80:48, 1963.

78. Schwarz, K., and Mertz, W.: A glucose tolerance factor and its differentiation from factor 3. Arch. Biochem. Biophy. 72:515, 1957.

79. Schwarz, K., and Mertz, W.: Chromium (III) and the glucose tolerance factor. Arch. Biochem. Biophy. 85:292, 1959.

80. Schwarz, K., and Mertz, W.: A physiological role of chromium (III) in glucose utilization (a glucose tolerance factor). Fed. Proc. 20 (Supplement No. 10):111, 1961.

81. Shendrikar, A. D., and West, P. W.: A study of absorption characteristics of traces of chromium (III) and (IV) on selected surfaces. Anal. Chim. Acta. 74:91, 1974.

82. Tipton, I. H., Cook, M. J., Stainer, R. L., Boye, C. A., Perry, H. M., and Schroeder, H. A.: Trace elements in human tissue. Health Physics. 9:89, 1963.

83. Toepker, E. W., Mertz, W., Roginski, E. E., and Polansky, M. M.: Chromium in foods in relation to biological activity. J. Agr. Food Chem. 21(1):69, 1973.

84. Underwood, E. J.: Chromium in Trace Elements in Human and Animal Nutrition. London: Academic Press, Inc., 1971.

85. Willis, J. B.: Atomic absorption spectrometry. In Mavrodiveonu, R., ed., Analytical Flame Spectroscopy. New York: Verlag, 1970.

86. Wolf, W., Mertz, W., and Masironi, R.: Determination of chromium in refined and unrefined sugars by oxygen plasma ashing flameless atomic absorption. J. Agr. Food Chem. 20:1037, 1974.

87. Trivalent chromium in human nutrition. Nutr. Rev. 25(2):49, 1967.

88. Intestinal absorption of chromium. Nutr. Rev. 25(3):76, 1967.

89. Dietary chromium and eye lesions. Nutr. Rev. 26(7):223, 1968.

90. Chromium and carbohydrate metabolism in infantile malnutrition. Nutr. Rev. 26(8):235, 1968.

91. Chromium and glucose tolerance. Nutr. Rev. 26(9):281, 1968.

92. Sugar, chromium and serum cholesterol. Nutr. Rev. 27(6):185, 1969.

93. Nutritional trace element research. Nutr. Rev. 29(4):90, 1971.

94. Hair changes in human malnutrition. Nutr. Rev. 29(6):143, 1971.

95. Trace elements in human nutrition: report of a WHO expert committee. WHO Technical Report, Series #532, 1973.

96. Chromium enrichment of foods urged. Med. World News. October 11, 1974, pp. 33-34.

APPENDIX A

PERSONAL DATA SHEET

PERSONAL DATA SHEET

Please answer each question.

1. Sex _____

2. Age _____ years

3. Hair color _____ Black

   _____ Dark Brown

   _____ Light Brown

   _____ Blond

   _____ Red

4. Do you have any metabolic disorder (other than diabetes)?

   _____ Yes _____ No

   A. If yes, what? _____

   B. What drugs, if any, do you take for this disorder? _____

   _____

   C. How long have you had this disorder? _____

For diabetics only:

1. How long have you been diabetic? _____ years

2.

Medication you are on or have been on:	Type	Years	Take Now Yes	Take Now No
Diet				
Oral Drugs				
Insulin				

To be filled out by researcher:

1. _____ Height

2. _____ Weight

3. Skinfold measurements

Female _____ Arm

_____ Iliac

Male _____ Chest

_____ Abdominal

_____ Arm

APPENDIX B

METHODS USED TO DETERMINE PERCENT BODY FAT IN MEN AND WOMEN

METHODS USED TO DETERMINE PERCENT BODY FAT IN MEN AND WOMEN

Normal weight is considered less than 21 percent body fat for women and 15 percent body fat for men.

Obese is considered more than 30 percent body fat for women and 25 percent body fat for men.

Because men and women store fat in different places, two different methods were used to determine percent of body fat. For men the method developed by Brozch and Keys (12) was used, and the Sloan, Burt and Blyth method (13) was used for the women. The skinfold measurements used in both methods were made with a Lange Skinfold Caliper.

For men three skinfold measurements were taken, the abdomen and the triceps. The percent of body fat was calculated from the formulas (12).

I. $S.G. = 1.1017 - 9.000282A = 0.000736B - 0.000883D$

where S.G. = Specific Gravity
A = Abdominal Skinfold thickness in mm
B = Chest Skinfold thickness in mm
D = Arm Skinfold thickness in mm

II. $\text{Percent Body Fat} = 100 \left(\frac{5.548}{S.G.} - 5.044\right)$

For women two skinfold measurements were taken: the iliac and the triceps. The percent of body fat was calculated from these two formulas (13).

$$X_1 = 1.0764 - 0.0081 X_2 - 0.0088 X_3$$

where X_1 = density in gm/ml
X_2 = Iliac Skinfold thickness in mm
X_3 = Triceps Skinfold thickness in mm

APPENDIX C

SPIKE INFORMATION PER SAMPLE

SPIKE INFORMATION PER SAMPLE

Subject Number	Sample + Spike Cr in ug	Sample Cr in ug/g	Cr/gm of Hair in ug
	CONTROLS		
1	7.56	4.56	2.5999
2	8.64	5.64	2.8515
3	8.64	5.64	3.2310
4	11.04	8.04	4.5893
5	6.96	3.96	2.5613
6	8.28	5.28	2.9565
7	12.96	9.96	5.7110
8	9.84	6.84	3.9290
9	11.76	8.76	4.7076
10	12.00	9.00	5.4678
11	7.56	4.56	2.5239
12	7.32	4.32	2.4403
13	13.08	10.08	5.5752
14	11.76	8.76	4.8551
15	16.20	13.20	8.2212
	DIABETICS		
1	8.88	5.88	3.2375
2	9.24	6.24	3.4283
3	9.60	6.60	3.9602
4	11.40	8.40	6.3077
5	10.08	7.08	4.0573
6	11.64	8.64	4.8553
7	6.84	3.84	2.4236
8	9.96	6.96	7.0653
9	10.56	7.56	4.3262
10	7.56	4.56	2.6041
11	11.28	8.28	5.5296
	OBESE		
1	7.92	4.92	2.7605
2	9.48	6.48	3.6480
3	11.28	8.28	4.1433
4	10.80	7.80	4.4450
5	7.56	4.56	2.5774
6	8.16	5.16	3.7733
7	7.68	4.68	2.7520
8	9.12	6.12	5.0898
9	7.92	4.92	2.7856
10	8.88	5.88	2.7796
11	8.16	5.16	2.8937
12	7.56	4.56	2.6126
13	8.40	5.40	3.3155
14	8.28	5.28	2.9213
15	9.24	6.24	3.0961
	OBESE-DIABETIC		
1	12.84	9.84	4.5765
2	10.08	7.08	4.3358

APPENDIX D

AMOUNT OF CHROMIUM FOUND

IN THE ACID BLANKS

TABLE VIII

AMOUNT OF CHROMIUM FOUND
IN THE ACID BLANKS

Run	Curve	Atomic Absorption Spectrophotometer Readings	ug of Cr/Sample
1	1		Ruined
2	1	1	.05
3	2	6	.5
4	2	4	.28
5	3		Spilled
6	3	4	.30
7	3	3	.25

VITA

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