A STUDY OF ELDERLY FEMALE MATURITY-ONSET DIABETIC AND ELDERLY NON-DIABETIC WOMEN'S HAIR CHROMIUM

By

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1970

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1975

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ACKNOWLEDGMENTS

I would like to acknowledge and express my sincere appreciation to Dr. Esther A. Winterfeldt, my major adviser, for her academic guidance, assistance in solving problems encountered during the course of this study, and aid in the preparation of the manuscript.

To Dr. Lester Reed, professor of Agronomy, I am expecially indebted for his generous allowance of time, guidance and assistance in the laboratory aspects of the study, and service as a member of my advisory committee.

The other members of my advisory committee, Dr. Donna Bose and Dr. Bernice Kopel, I would like to also note for their kind encouragement and interest in my study and to Dr. John L. Folks I am appreciative for the statistical analysis of the data.

I would like to thank the subjects who willingly contributed their time to participate in this study because without their cooperation this study would not have been possible. Also, I am grateful to Mrs. Joyce Gazaway for typing this thesis and my fiance, Mr. Brad Hamon, for his encouragement and assistance in the manuscript preparation and publication.

I am indebted to all my professors and instructors at Oklahoma State University, especially the ladies of the Department of Food, Nutrition and Institution Administration, for the knowledge I have received during the period of my graduate study and finally I would

like to express my appreciation to the Department of Food, Nutrition and Institution Administration for a teaching assistantship for the financial support of my graduate study.

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CHAPTER I

INTRODUCTION

Evidence accumulated in the past two decades indicates chromium is essential in human nutrition. Chromium has been found to be present in human foods and water and to be widely distributed in the tissues of the human body. The presence of chromium in the human body appears to be important. Evidence indicates chromium acts as a co-factor for insulin in the reactions by which sugars are translocated across the cell membranes. Thus, chromium appears to be essential in human nutrition for normal human carbohydrate metabolism.

To study human chromium nutrition, hair is a valuable biopsy tissue. This tissue may be collected atraumatically from human subjects, stored indefinitely until analysis, and the quantity present in this tissue is adequate for accurate quantitative chromium analysis. Also, research has suggested the chromium in adequately washed hair is endogenous in origin, a reflection of total body chromium, and that comparisons of population groups' hair chromium may be used to indicate human chromium deficiency or low tissue chromium stores.

As a result of previous human chromium nutrition studies, it has been speculated that los tissue chromium stores, particularly in elderly Americans, may be involved in the etiology of or related to maturity-onset diabetes. The primary purpose of this study was to investigate

this possible relationship by studying and comparing the hair chromium of elderly female maturity-onset diabetes and normal elderly women.

CHAPTER II

REVIEW OF LITERATURE

Chromium is present in the human body and appears to be a metabolized and functional nutrient. To the extent that chromium participates in the cellular metabolism of carbohydrates, fats, and proteins, chromium deficiency may be indicated in disorders of metabolism, particularly that of carbohydrates. To further explore possible relationships between chromium deficiency and disease states, methods have been developed to evaluate a person's body chromium status.

Chromium

Occurrence

Chromium is ubiquitous in the universe. When sensitive methods of analysis are used, it can be detected in air, soil, plants, animals, water, food, and the body of man (55).

Man. Estimates of the total amount present in man range from 1.72 to 9.63 ug/g. The chromium is widely distributed among body tissues but appears to concentrate in some tissues more than others. Bone tissue contains the least amount of chromium and has a concentration of less than 0.02 ug/gm. Lung tissue contains a fairly large amount, due to air-borne metal contamination (29, 53, 55). Hair tissue is also high in chromium and has been used as a biopsy material in several studies of

human nutrition (14, 15, 16, 29). Values ranging from 0.2 to 20 ppm have been reported (29). The largest portion of body chromium concentrates in skin, muscle, and fat tissue and these tissues are thought to represent a storage depot for this trace nutrient (30, 53). Tissue levels of chromium can be affected by age, environment, parity, and diabetes mellitus and these variations in tissue chromium concentrations will be discussed.

Chromium, in human tissue, is thought to exist in the trivalent state because, although chromium can exist in matter in any one of six oxidative states from -2 to +6, only the divalent, the trivalent, and the hexavalent states are common. As divalent chromium is easily oxidized by air and hexavalent chromium is readily reduced by organic material, these forms are not thought to exist in human tissue (29).

Water. The valence state of chromium in water with little particulate matter (i.e., potable water) is thought to be hexavalent and the quantity present in municipal water supplies ranges from a non-detectable amount to a maximum of 35 ppb (29, 55). It was indicated in a 1964 study that the median chromium concentration of municipal water supplies is approximately 9.43 ug/liter (29).

Food. The chromium concentration of human foods ranges from non-detectable levels to 10 ug/gm. However, as shown in Tables I and II, only a few foods have a large concentration or greater than 0.5 ug/gm and for the majority of human foods the chromium content is extremely small (49, 53, 55). Schroeder et al. (53, 55) analyzed approximately 160 different foods including a number of varieties of spices, condiments, dairy products, meats, shellfish, vegetables, fruits, grains, cereals, sugars, syrups, animal fats, vegetable oils, and nuts. Of

TABLE I

CONDIMENTS, SPICES, AND FOODS KNOWN TO CONTAIN NO DETECTABLE CHROMIUM

Blackberries, wild	Raw sugar
Cranberry jelly	From the American Sugar Company
Final molasses	From Lion
Flour, wheat (all purpose)	Sucaryl
Lobster	Superfine domino sugar
Mild cider jelly	Sweet-n-Low sugar
Potato, white	Thyme, grape herb jelly
Radishes	Wild cherry jelly

Source: Schroeder, Henry A., Alexis P. Nason, B. S. Tipton, and Isabel H. Tipton. "Chromium Deficiency as a Factor in Atherosclerosis." J. Chron. Dis., Vol. 23 (1970), 123-142.
Schroeder, Henry A., Joseph J. Balassa, and Isabel H. Tipton. "Abnormal Trace Metals in Man-Chromium." J. Chron. Dis., Vol. 15 (1962), 941-964.

TABLE II

CONDIMENTS, SPICES, AND FOODS KNOWN TO CONTAIN GREATER THAN 0.5 UG/G CHROMIUM

Amber sucrose corn syrup	Pancake syrup, inverted type
Brown sugar, domino	Peas
Chili powder	Pepper, black
Cloves	Puffed rice
Millet	Thyme

Source: Schroeder, Henry A., Alexis P. Nason, B. S. Tipton, and Isabel H. Tipton. "Chromium Deficiency as a Factor in Atherosclerosis." J. Chron. Dis., Vol. 23 (1970), 123-142. Schroeder, Henry A., Joseph J. Balassa, and Isabel H. Tipton. "Abnormal Trace Metals in Man-Chromium." J. Chron. Dis., Vol. 15 (1962), 941-964.

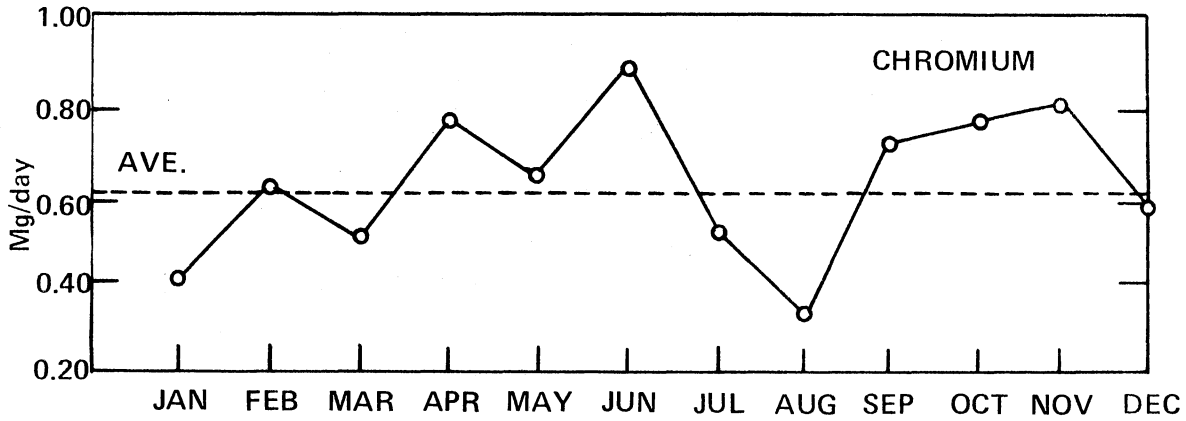
these, almost 78% had a chromium concentration of 0.2 ug/gm or less.

In part, nature is responsible for the small chromium concentration of human food. It has provided control mechanisms that prevent excessive deposition of chromium in plant and animal tissue (53). Man, too, is responsible because he refines and processes most of his food and these procedures can also reduce the chromium content (46, 49, 50, 53, 55).

Diet. The average quantity of chromium Americans ingest daily covers a wide range. The lowest figure was reported by Mertz (29). Only five micrograms were found in the diet of a group whose primary source of animal protein was seafood. Levine et al. (25) determined the daily chromium intake for seven days of elderly subjects eating institutional diets and of young people eating ad libitum. The institutional diet had an average of 52 ug per day and the ad libitum diet had an average of 65 ug per day. Schroeder (55) analyzed an institutional diet for one day. This diet provided 78 ug of chromium of which 8 ug appeared in the breakfast, 70 ug in the dinner, and none in the supper. Finally, at three time intervals, Tipton et al. (64, 65) analyzed for one week the normal diet of two adults. The values reported were: 1965, 7 to 33 ug/day; 1966, 330 to 440 ug/day; 1969, 220 to 290 ug/day.

Subjects' dietary preferences have been suggested as one explanation for variation in the reported dietary intakes. Most foods such as refined foods, seafoods, and several vegetables are low in chromium and habitual preferences for these foods will result in a low chromium intake (29, 30, 49, 55). Other explanations for the observed variability may be geographical location and the time of year of the study. While surveying the chromium intake of children for one year at 28

locations, Murthy (39) found the average chromium intake of subjects from eastern United States cities was significantly greater than the chromium intake of subjects from western United States cities. Furthermore, the average chromium intake varied significantly from one season to another. Usually, chromium intake tended to peak in the spring, to be lowest in the summer, and to be approximately constant in the fall and the winter. A graph of these seasonal variations in chromium intake found by Murthy (39) may be seen in Figure 1.



Source: Murthy, Gopala A., Ulyses Rhea and James T. Peeler. "Levels or Antimony, Cadmium, Chromium, Cobalt, Manganese and Zinc in Institutional Diets." Envir. Sci. and Tech. Vol. 5 (1971), 436-442.

Figure 1. Seasonal Variations in Human Subjects' Chromium Intake Found by Murthy in 1967

Metabolism

The site of the absorption of ingested chromium is thought to be either the jejunum or upper ileum (6, 53). The mode of chromium

absorption is not known but in vitro studies suggest it is either passive or facilitated diffusion (31). The efficiency of chromium absorption appears to be primarily dependent on the form of the chromium and the nature of the intestinal milieu. Natural organic chromium complexes, such as those found in food, are better absorbed than inorganic complexes and hexavalent chromium is better absorbed than trivalent (26, 31, 35, 67). An excessively low intestinal pH hinders chromium absorption by reducing the chromate and an excessively high pH hinders absorption by affecting olation (6, 29). The presence of suitable ligands in intestinal juices will facilitate chromium absorption, as these ligands protect chromium from olation, but neither a large intake nor chromium deficiency appears to increase the amount absorbed (29, 35).

Once absorbed, chromium appears in the beta-globulin fraction of blood, bound primarily to siderophilin. In this bound state, chromium is transported through the circulatory system to sites of excretion and tissue storage (29, 35).

Chromium stored in tissue can be reabsorbed into the blood and, although the tissue or tissues from which reabsorption occurs are not known, it is known this process occurs in response to an increase in blood glucose or circulating insulin (11). For example, Glinsmann et al. (11) found one young healthy subject to respond to a 100 gram oral glucose load with a distinct rise in plasma chromium. The plasma chromium rose from a control level of 27 ng per ml to a level of 83 ng per ml in 30 minutes. Also, after a 100 gm oral glucose load, Mertz (29) found five subjects' average increase in plasma chromium to be 28

ppb fasting, after 30 minutes 51 ppb, 60 minutes 59 ppb, and 41 ppb after 120 minutes.

The fate of mobilized tissue stored chromium following glucose stimulation is urinary excretion. In some cases, the chromium concentration of a person's urine doubles following the ingestion of 100 gm of glucose (50). Urinary excretion is also the major means of eliminating excess absorbed chromium, as the chromium excreted in feces probably represents unabsorbed chromium (21, 24, 66).

Functions

In early animal studies designed to investigate the functions of the chromium occurring in and metabolized by biological systems, trivalent chromium was established as essential to the rat for normal glucose tolerance (33, 35, 36, 50, 54, 57, 59).

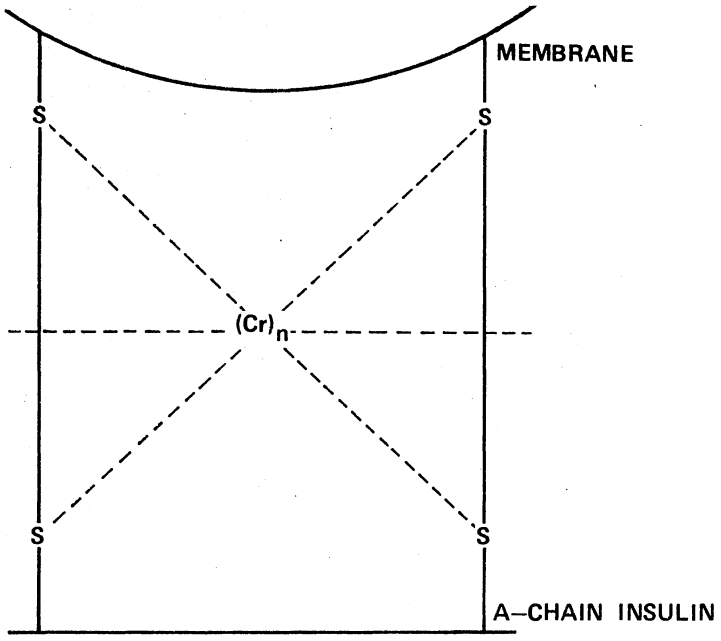
Rats raised on chromium deficient diets developed progressively impaired glucose tolerance and diminished response of isolated tissues to insulin in vitro (33, 35, 36). Further, rats raised on chromium deficient diets and in quarters designed to eliminate all trace element contamination develop symptoms of mild human diabetes mellitus (33, 47, 56, 57). These physiological abnormalities resulting from chromium deficiency were found to be prevented or reversed by supplementing the animals with absorbable chromium complexes (33, 37, 50).

As in the rat, chromium appears to be essential to humans for normal glucose tolerance. Humans with normal glucose tolerance mobilize chromium into the blood in response to glucose stimulation. Conversely, glucose stimulation will not result in the mobilization of chromium into the blood of subjects showing abnormal glucose tolerance (11). Chromium

supplements given in low doses for long periods of time have been shown to markedly improve or restore to normal the glucose tolerance of some diabetic subjects and some glucose intolerant subjects (12, 25).

The role of chromium in the maintenance of normal glucose tolerance is not precisely known. However, it is known chromium does not act as hypoglycemic agent (11) and in vitro studies suggest it acts as a cofactor for insulin. It is thought to facilitate the action of the hormone by participating in a ternary complex in reactions resulting in the translocation of sugars across cell membranes. This ternary complex is between chromium, the A chain disulfide of insulin, and cell membrane sulfhydrl groups (1, 3, 4, 28, 39, 46). A hypothetical model as depicted by Mertz (28) of this ternary complex may be seen in Figure 2.

As a co-factor for insulin, chromium also appears to be functional in protein synthesis and lipogensis. Rogenski and Mertz (43, 44) raised rats from weaning on chromium deficient diets with and without a supplement of two ppm chromium. In the supplemented animals, insulin stimulated the cellular transport of AIB (an amino acid analog) into heart tissue and the incorporation of three amino acids into heart protein more effectively than in chromium deficient controls. Mertz et al. (34) also showed that the addition of physiological quantities of chromium to chromium deficient epididymal fat tissue increased the rate of incorporation of glucose carbons into this fat tissue. The presence of physiological amounts of insulin in the reaction mixture were required for this effect to occur.



*$(Cr)_n$ indicates that chromium may be present in this complex in the form of a polynuclear species.

Source: Mertz, Walter. "Biological Role of Chromium." Fed. Proc., Vol. 26 (1967), 186-192.

Figure 2. Hypothetical Model of Membrane-Insulin-Chromium Complex as Depicted by Mertz

Chromium Deficiency

Due to the involvement of chromium in carbohydrate, protein, and fat metabolism, experimental chromium deficiency[1] in animals is associated with impaired growth (47, 57, 59). In a one-year study, Schroeder

[1]The term chromium deficiency is not used to describe a state of complete absence of this trace element in tissue but is used to describe a state of sub-optimal available concentrations of the nutrient in the living organism (28).

(47) fed rats from weaning chromium deficient rations (less than 0.17 ug/gm Cr) and compared the growth of the deficient animals to that of animals raised on the same chromium deficient rations but with two ppm chromic acetate in drinking water. The chromium deficient animals weighed 25% less at 60 days, 14% less at 90 days, and 12% less at one year than the chromium sufficient controls. White mice raised from weaning on chromium deficient rations weighed 12% less at six months and 22% less at 12 months than chromium sufficient animals (59).

Other implications of experimental chromium deficiency in animals include:

1. lack of survival under stress (32),
2. reduced longevity (48, 56, 59),
3. tissue lesions, particularly of the eye (27, 42),
4. symptoms of mild atherosclerosis including hypercholesteremia, hyperlipidemia, increased amounts of lipids in the aorta, and atheroschlerotic plaques in vessels (51, 52, 53, 54, 58),
5. abnormal glucose tolerance (33, 34, 37), and
6. other symptoms of mild human diabetes mellitus (47, 48).

In man, experimental chromium deficiency has not yet been induced but there is evidence suggesting some Americans may be chromium deficient. Tipton et al. (62, 63, 64, 65) and Schroeder et al. (53, 55) made an extensive study of tissue levels of chromium in individuals from four parts of the world. Using emission spectroscopy, which can detect as little chromium as 0.1 ug/gm, these workers found that the concentration of chromium in major tissues and organs of Americans, both young and old, was lower than the chromium concentration of the same tissues and organs of foreigners. For example, in nine major organs, it was found that Africans had 1.4 times, Near Easteners, 4.4 times and Far

Easteners 5 times as much chromium as did Americans. Also, it was found that in Americans there was a geographical difference in tissue levels of chromium. Chromium was detected in only 17% of the liver samples from Denver but was detected in 100% of the liver samples from Chicago and New York. Finally, it was found that in both foreigners and Americans the chromium concentration of tissue declines with age, but in Americans the decline is more predominate and more intense. Chromium was present in all tissues of young subjects studied--both foreign and American. In foreign adults, chromium was undetected in only 1.5% of the samples studied but, in American adults, chromium was undetected in 17.6% of the samples studied.

Chromium Deficiency and Carbohydrate Metabolism

What the implications might be of chromium deficiency in man are not fully known. However, due to the importance of chromium in the maintenance of insulin function, it is suggested one implication is impaired carbohydrate metabolism (55).

In the past few years, several studies have been made to investigate the possible relationship between human chromium deficiency and impairments of carbohydrate metabolism (i.e., juvenile diabetes, glucose intolerance, and maturity-onset diabetes). For example, Hambidge et al. (16) investigated the possible relationship between juvenile diabetes and chromium deficiency. It was found that the chromium concentration of a group of diabetic children was significantly less than that of a group of normal children. Glinsmann et al. (11) investigated the response of plasma chromium to glucose in normal healthy adults and

three adult-onset diabetics with impaired glucose tolerance. The plasma chromium of the diabetic subjects was shown not to exhibit a normal rise in response to the glucose. Levine et al. (25) and Glinsmann and Mertz (13) investigated the effects of orally supplementing maturity-onset diabetics and elderly glucose intolerant subjects with chromium. In 40 and 50% of the subjects, respectively, glucose tolerance was either markedly improved or restored to normal. Schroeder (50) also studied the effects of oral chromium supplements on the glucose tolerance of maturity-onset diabetics. In the 12 treated diabetics, given up to 1.0 mg chromium chloride, improvement in glucose tolerance was observable in four. Finally, Morgan (38) measured the hepatic chromium concentration of normal elderly subjects and elderly maturity-onset diabetics. The hepatic chromium concentration of the maturity-onset diabetics was found to be significantly less than that of the normal subjects.

Assessment of Nutritional Status

Several methods have been used to attempt to evaluate individual's and population's nutritional status with regard to chromium. Several investigators have suggested of these methods that an analysis of human hair chormium concentration may be the safest, the most convenient, yet equally reliable procedure (19, 29).

The reasons are:

1. Hair can be obtained without threat of physiological damage to the subject and may be stored indefinitely until analysis (29).

2. Hair is high in chromium (from 0.2 to 20 ppm) which reduces problems of measurement due to low analytical sensitivity (29, 41).

3. Unlike plasma chromium, the chromium in hair appears not to be subject to temporary fluctuations (29).

4. The chromium in adequately washed hair appears to be endogenous in origin (19).

5. There appears to be a correlation between the chromium concentration of human hair and the chromium concentration in other body tissues (17).

In the quantitation of chromium in hair and other biological materials, an atomic absorption spectrophotometer is perhaps the most widely used analytical instrument (10, 19). Although other instruments are available and the procedures for their use have been developed (40, 41, 45), the frequent choice of this instrument is due to its high sensitivity, to the ease of sample preparation, and to the reduction of interference from associated cations (9, 10).

CHAPTER III

METHODS AND MATERIALS

Evidence has suggested low tissue chromium levels may be associated with maturity-onset diabetes in elderly humans. The design of this study was to investigate this possible relationship by studying and comparing elderly, female diabetics' and non-diabetics' hair chromium. The methods and materials employed in implementing this design will be discussed in this chapter.

Subject Selection and Characteristics

From three Stillwater nursing homes, 28 elderly females were non-randomly selected. Twelve were maturity-onset diabetics and constituted the diabetic group. Sixteen were apparently normal women and constituted a control group. Men were not selected for this study because of inadequate amounts of hair for sampling. Females were not selected if they had recently bleached, dyed, or permanented hair or were taking vitamin mineral supplements containing chromium (19). Females, allowed to participate as control subjects, had no family history of diabetes or other health condition that might affect results (53). Diabetic subjects were selected on the basis of a diagnosis by a doctor and by the age of onset of diabetes. Juvenile diabetics or subjects becoming diabetic before the age of 20 were not included in this study.

A comparison and summary of control and diabetic groups' general characteristics is presented in Table III.

TABLE III

GENERAL CHARACTERISTICS OF CONTROL GROUP AND MATURITY-ONSET DIABETIC GROUP

Characteristics	Control Group	Diabetic Group
Number of Subjects in Group	16	12
Age		
Mean	73	76
Range	41-94	60-87
Number of Years Resident of Nursing Home		
Mean	3	2.58
Range	1 week-8 years	1 month-6 years
Number of Years Resident of Stillwater Area	majority-life	majority-life
Number of Children		
Mean	2.44	3.33
Range	0-8	0-7
Number of Years Diabetic		
Mean		12
Range		1-30
Age of Onset of Diabetes		
Mean		61
Range		27-80

Subject Interview and Hair Sample Collection

Subjects selected were interviewed to obtain information about age, parity, residence, health and medication. At the time of the interview, a hair sample weighing about 1.5 g was collected. The time of sample collection was six weeks to avoid possible seasonal variations in hair chromium (18). The hair was cut with thinning scissors. A particular hair length or site was not chosen for sampling, as it was assumed the size of the sample would negate any variation between individual hairs (16). The collected sample was stored in a twist top plastic pill vial.

Sample Preparation

In preparation for analysis, hair samples were removed from storage vials and washed according to the hexane-ethanol-distilled water procedure of Hambidge (19). The washed hair was transferred to a clean 100 ml glass beaker, covered with a watch glass, and dried at 78° C overnight in a drying oven. The procedures used in this study to wash hair and to obtain clean beakers and other apparatus may be seen in Appendix A.

After drying, the samples were allowed to cool for two hours in a desiccator and then the weight of the sample was determined. This weight was determined by weighing the beaker for sample digestion (a 300 ml tallform pyrex beaker), adding the hair sample to the tall form beaker, weighing the tall form and hair sample, and calculating the hair weight.

Sample Digestion and Preparation for Analysis

To digest hair and other biological materials for spectrophotometric analysis for chromium, both wet ashing and dry ashing have been used with various combinations of the ashing aids nitric, perchloric, and sulfuric acid (5, 9, 10, 15, 19, 38). The selection of the method of ashing and the ashing aids to be used in this study was based on the work of Chan (2) and a pre-experimental study.

Chan (2) found wet ashing with a 3:1 nitric perchloric acid mixture to provide statistically significant better chromium recovery than dry ashing and dissolving the ash in a 3 ml of dilute hydrochloric acid.

The results of a pre-experimental study showed wet ashing with a 3:1 nitric perchloric acid mixture to provide better chromium recovery than wet ashing with a 3:1 nitric sulfuric acid mixture. Thus, in this study, all hair samples were wet ashed with a 3:1 nitric[1] perchloric[2] acid mixture.

Initially, 75 ml of the 3:1 nitric perchloric digestion mixture was added to each hair sample and acid blank. The samples were covered with a watch glass and then heated at approximately 75° C for about two hours. After two hours the temperature of the hot plate was increased slightly and heating was continued until the samples were near dryness.

If at this time, white fumes of perchloric acid had not formed in the reaction beaker and the solution was not clear, an additional 25 ml

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[1]Baker's Analyzed Reagent-Reagent Grade.

[2]Baker's Analyzed Reagent-Reagent Grade.

aliquot of digestion acid mixture was added to all samples of the set and blanks. Heating was then continued.

When the sample solution cleared, the white dense fumes of perchloric acid formed, and the reaction beaker was near dryness, a 1.5 ml aliquot of 6 N hydrochloric acid was added to aid in driving off excess perchloric acid that might interfere with atomic absorption analysis. Heat was also applied to the sample for a few minutes or until the samples were near dryness. Sides of beakers and watch glasses were then rinsed with de-ionized distilled water and evaporated to almost dryness three times.

After the third time, the beaker was rinsed again with de-ionized distilled water and the contents of the beaker funneled into a 10 ml volumetric flask. The volumetric was brought to volume with de-ionized distilled water and the sample was then transferred to a 2 oz. polyethylene bottle. The sample was stored in this bottle under refrigeration until time of analysis.

Atomic Absorption Analysis for Chromium

When all samples were ready for analysis, standard solutions with concentrations of 0, 0.05, 0.1, 0.255, 0.5, 1, 2, and 5 ppm were prepared from a 1,000 ppm stock standard solution.[3] Both samples and standards were analyzed in triplicate at room temperature using a Perkin Elmer 303 atomic absorption spectrophotometer with a Boling burner and an air-acetylene flame. The instrumental settings used for chromium analysis were:

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

Meter Response	1
Scale Expansion	1
Gain	4
Slit	3
Range	UV
Air Flow	6.5
Fuel Flow	7
Wave Length	3574A

Using the atomic absorption measurements of standard solutions, a standard curve was prepared. From this curve, the concentration of the samples was determined and microgram of chromium per gram of hair calculated.

Statistical Analysis of Data

For each subject a Fortran computer card was prepared. The information coded on each card included subject number, group number (1 = diabetic, 2 = non-diabetic), age, parity, years diabetic, and hair chromium concentration.

The degree of equivalence of diabetics' and controls' hair chromium was resolved by analysis of variance and the similarity of insulin dependent, noninsulin dependent, and controls' hair chromium discerned by a t-test. A t-test was also employed to compare hair chromium of recent diabetics, long-term diabetics, and control subjects. In addition, correlation coefficients were calculated for hair chromium versus parity and hair chromium versus age for both the control and diabetic groups. The significance of the correlation coefficients was tested.

Details of all statistical tests may be seen in Appendix B.

CHAPTER IV

RESULTS

Hair chromium analysis results and a comparison of diabetics' and controls' hair chromium are represented in Table IV. As illustrated by the table, chromium was found to be present in all hair samples analyzed and the quantity present ranged from approximately 0.06 ug/g to 3.7 ug/g. The diabetic group had a slightly larger hair chromium concentration than the control group. The mean hair chromium of 12 diabetics was 1.0759 ug/g and 1.0563 ug/g was the mean hair chromium of 16 control subjects. The variation of individual subject's hair chromium from the group mean was large but similar for both groups and a two-way analysis of variance indicated the two groups' hair chromium did not differ significantly. (For details of the two-way analysis of variance test see Appendis B, Table VIII).

Figures 3 and 4 depict hair chromium concentration in relation to age and in relation to subject parity. Diabetics are represented by light circles; control subjects are represented by dark circles. As illustrated by these scatter diagrams, no significant positive or negative correlation between hair chromium level and age or hair chromium level and parity existed for either group.

Non-insulin diabetics', insulin diabetics', and control subjects' hair chromium means are compared in Table V. The means show an increase in the order of non-insulin diabetics, control subjects, and insulin

TABLE IV

A COMPARISON OF INDIVIDUAL AND GROUP
HAIR CHROMIUM CONCENTRATIONS

Control Group		Diabetic Group	
Subject Number	Hair Chromium (ug/g)	Subject Number	Hair Chromium (ug/g)
1	2.9897	17	0.3996
2	0.9520	18	0.3495
3	0.8483	19	0.8826
4	1.5656	20	1.0401
5	2.7307	21	0.4366
6	0.0595	22	0.8269
7	0.4116	23	3.6673
8	0.3531	24	1.9351
9	0.3781	25	0.4002
10	0.3333	26	1.2756
11	2.6247	27	0.4278
12	0.4762	28	1.2809
13	1.1620		
14	0.4031		
15	0.7914		
16	0.8221		
1.0563 ug/g	- MEAN -	1.0759 ug/g	
0.0595 - 2.9897 ug/g	- RANGE -	0.3495 - 3.6673 ug/g	
0.9346 ug/g	- STANDARD DEVIATION -	0.9492 ug/g	

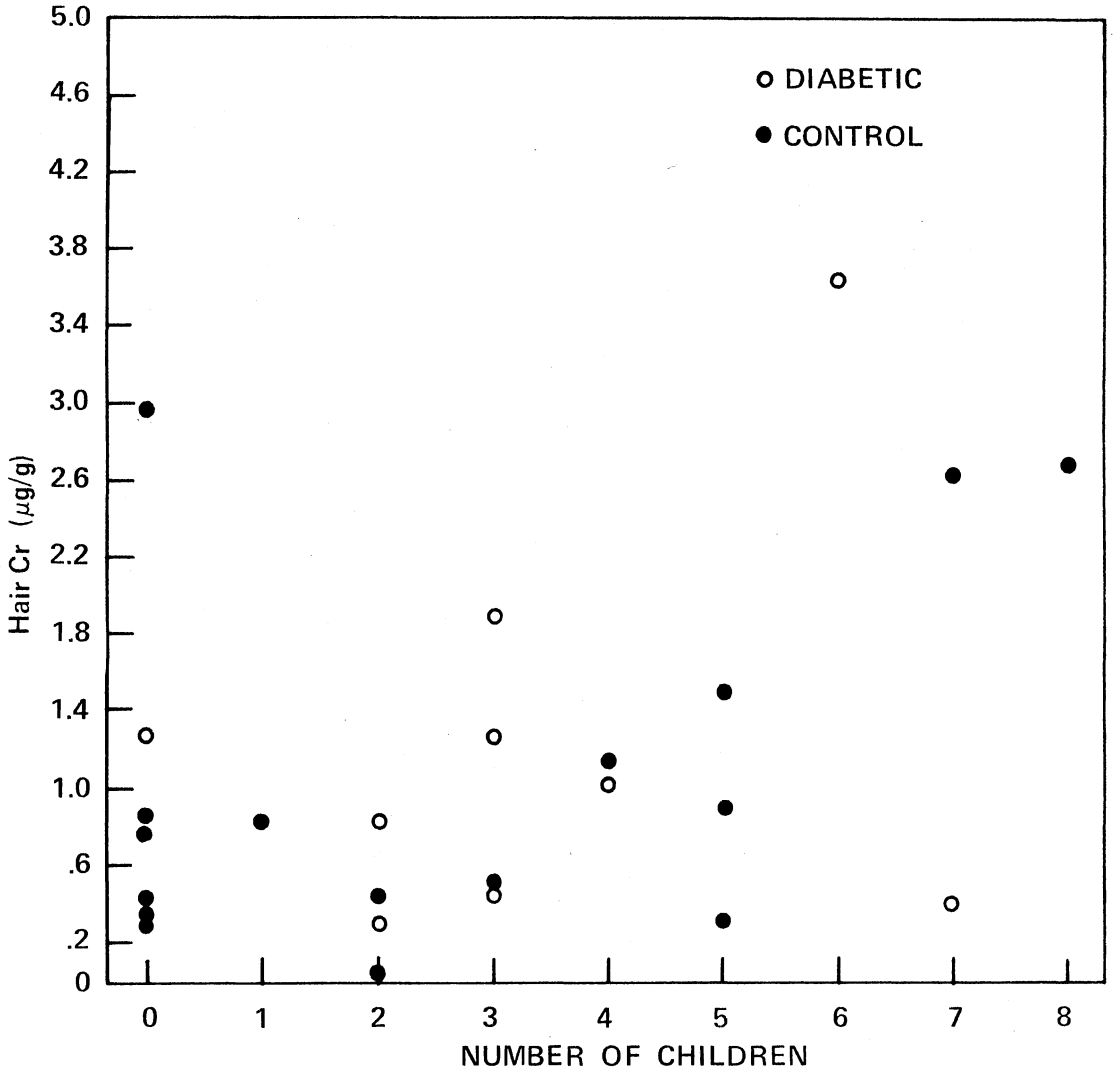


Figure 3. Hair Chromium Concentration in Relation to Parity For All 28 Subjects

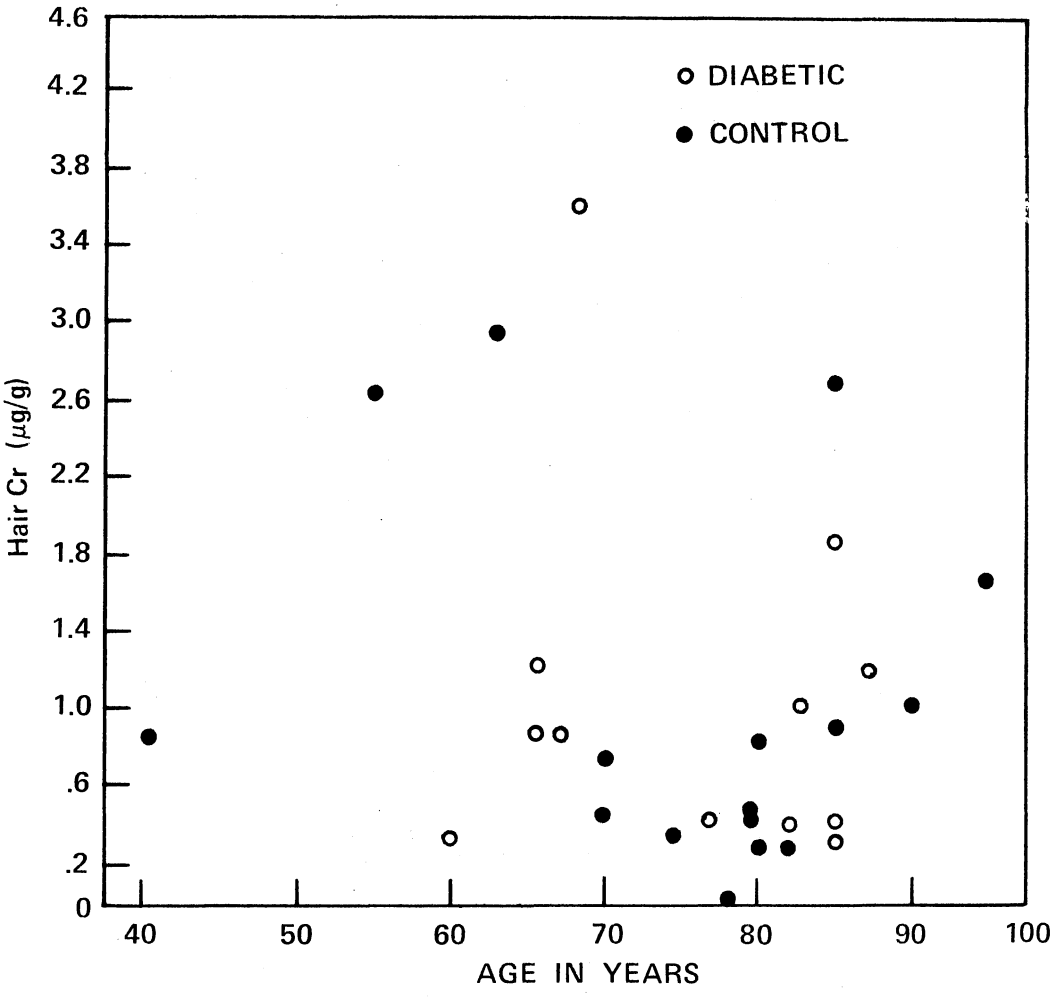


Figure 4. Hair Chromium Concentration in Relation to Age For All 28 Subjects

diabetics with the values of 0.5618 ug/g, 1.0563 ug/g, and 1.5914 ug/g, respectively. Neither insulin diabetics' and control subjects' nor non-insulin diabetics' and control subjects' hair chromium differed significantly. However, a significant difference did exist between the hair chromium of non-insulin receiving diabetics and insulin receiving diabetics.

TABLE V

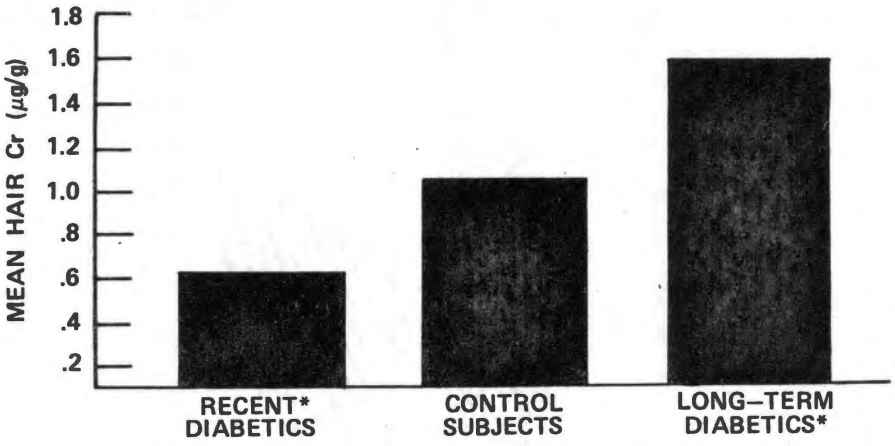
A COMPARISON OF INSULIN DIABETICS', CONTROLS', AND AND NON-INSULIN DIABETICS' MEAN HAIR CHROMIUM LEVELS

Group	Number of Subjects	Hair Chromium* (ug/g)
Insulin Diabetics	6	1.5914
Controls	16	1.0563
Non-Insulin Diabetics	6	0.5618

*Difference between insulin diabetics' and non-insulin diabetics' hair chromium significant by a t-test ($0.05 > p > 0.10$). (For details of t-tests see Appendix B, Tables IX, X, XI).

The diabetic group consisted of six subjects becoming diabetic in the past five years and six subjects diabetic for more than ten years. These recent and long-term diabetics' hair chromium and the hair chromium of controls are compared in Figure 5. As the figure illustrates, the six long-term diabetics had the largest hair chromium concentration

(1.6059), the six recent diabetics the smallest concentration (0.5472), and the 16 control subjects an intermediate concentration (1.0563). Although both long-term and recent diabetics' hair chromium differed from that of controls by approximately 0.5 ug/g, these differences were not statistically significant. However, a statistically significant difference between the hair chromium of recent and long-term diabetics was indicated by a t-test. This difference was significant at the 0.05 level. (For details of t-tests see Appendix B, Tables VII, VIII, XIV.)



*Difference in hair chromium of recent and long-term diabetics significant ($p \geq 0.05$) according to a t-test.

Figure 5. Bar Graph Comparing Recent Diabetics', Control Subjects' and Long-Term Diabetics' Mean Hair Chromium

CHAPTER V

DISCUSSION

As early as 1957, Schwarz and Mertz (61) found chromium deficiency caused experimental animals to be glucose intolerant and to exhibit symptoms of mild human diabetes mellitus. Later, it was observed, elderly maturity-onset diabetics' plasma chromium did not respond normally to glucose stimulation and the glucose tolerance of some elderly maturity-onset diabetics could be improved by chromium therapy (14, 25). More recently, elderly American's tissue chromium concentrations were found to be less than those of foreign elderly and younger Americans (50). Also, maturity-onset diabetics' hepatic chromium levels were observed to be less than those of normal elderly subjects (38). Collectively these observations have suggested human chromium deficiency may be involved in the etiology or related to maturity-onset diabetes in elderly humans.

Methods and Materials

Previous experimental designs employed to investigate this possible relationship include measuring diabetic's plasma chromium response to glucose stimulation, assessing diabetic's glucose tolerance improvement following chromium supplementation, and comparing post morten diabetic's hepatic chromium levels to those of control subjects (14, 25, 38). In lieu of one of these, the experimental design chosen for this

investigation was to study and compare elderly maturity-onset diabetics' and non-diabetics' hair chromium. There were several reasons for this choice. For one, previous research has indicated hair chromium levels are an index of body chromium levels (17, 18, 29). Also, in comparison to previous designs used, a study and comparison of subject's hair chromium is safer for the subjects involved, lends itself to greater subject participation, and appears to be an equally reliable procedure for investigating this possible relationship (29).

From the 28 subjects of this study, hair samples were collected between the last week in January and the first week in March. Hambidge (18) has found a single subject's hair chromium concentration may vary by as much as 100% over a long period of time (i.e., a year) but will remain relatively constant over a short period of time. Thus, by using a short six-week period for hair sample collection, the necessity of considering the effect of the variable, time of hair sample collection, upon the data was eliminated.

Collected hair samples were stored in plastic pill vials, and this procedure proved satisfactory. Storage in the vials provided a dust-free environment for samples, thus excluding the possibility of sample contamination with extraneous chromium of laboratory dust and air. Also, this procedure proved satisfactory in that the samples did not cling to the vials; all hair of a sample was easily removed in preparation for sample washing.

To ensure the trace element concentration obtained upon analysis reflected only that present within the hair structure and was accurate, sample washing was important to remove non-endogenous loosely bound hair chromium, hair dirt, and hair grease. These foreign particles may be

removed from hair with organic solvents or detergents but not by only distilled water (20). The choice between organic solvents and detergents appeared to be relatively unimportant and was a matter of convenience (19). As the organic solvents, hexane and ethanol, have been most frequently used in other hair chromium analysis studies, they were also used in this analysis (19, 20).

In general, quantitative hair chromium analysis by atomic absorption spectrophotometry was a procedure of only a few steps. These steps were:

1. weighing the washed hair sample,
2. obtaining from the hair a mineral ash solution by wet ashing or dry ashing the hair and dissolving or extracting the ash in a suitable solvent (The rationale for chosing wet ashing for this study was discussed in Chapter III.),
3. measuring the chromium concentrationof the mineral ash solution by atomic absorption spectrophotometry, and
4. calculating the chromium concentration of the weighed hair sample.

This method for quantitative hair chromium analysis, in comparison to other methods for quantitative hair chromium analysis, was relatively uncomplicated and was also desireable in that it was extremely sensitive and easily adaptable to very small sized samples. However, the quality (precision and accuracy) of the analysis was especially dependent upon laboratory technique.

Chromium is freely present in most air, dust, water, and on any reaction vial previously used in chromium analysis (20, 29). Optimum precision and accuracy may be achieved only when chromium is not introduced into a sample from any of these sources. It is recommended that de-ionized water be employed in all steps of the procedure requiring

water; all glassware used should be acid rinsed, covered, and stored in a dust-free environment. Also, all samples must not be excessively exposed to air and dirt, and only chromium-free reagents should be used in all steps of the procedure. Reaction vials employed should be new, silicone coated, or vials not previously used in chromium analysis (5).

Results

Using the methods of this study, the hair chromium concentration of 28 elderly women (12 maturity-onset diabetics and 16 normal subjects) was determined. The hair chromium concentration means observed for non-diabetics and diabetics were 1.0563 ug/g and 1.0759 ug/g, respectively.

These mean hair chromium values observed for the elderly maturity-onset diabetics and the elderly non-diabetics are unique in two ways. For one, to date, either elderly humans' (diabetic and non-diabetic) hair chromium levels have not been studied or have not been reported. Therefore, the values observed and reported in this thesis for the two groups represent baseline hair chromium levels for the elderly, female, maturity-onset diabetic and non-diabetic populations. Also, the means observed for the two groups are unique in that they are slightly higher than any others previously reported. The hair chromium values observed for the elderly, female diabetics and the non-diabetics in this study were, on the average, approximately 0.3 ug/g higher than those reported by Chan (2) for Chinese young adults, Caucasian young adults, and diabetic young adults. They were, on the average, approximately 0.5 ug/g higher than those reported by Hambidge (16) for juvenile diabetics, normal children, young nulliparous women, and young parous women.

Previously, lower tissue chromium concentrations of elderly maturity-onset diabetics as compared to elderly non-diabetics had been observed by Morgan (38), but the same observation was not made in this study. A comparison of the 16 non-diabetics' and 12 maturity-onset diabetics' hair chromium concentrations did not indicate the two groups' hair chromium differed significantly. The reason the same observation was not made in this study, or the reason for the lack of agreement between this study and Morgan's study, may be a result of excluding and including juvenile diabetics. Juvenile diabetics were excluded as subjects in this study for two reasons: (1) juvenile diabetes was considered a disease of a different nature than maturity-onset diabetes (25); and (2) juvenile diabetics' tissue chromium levels are known to be less than normal (16). Diabetics were included in Morgan's study when a subject's blood sugar was greater than 120 mg% at two hours. By using this criteria for the selection of diabetics, Morgan may have included juvenile diabetics as subjects. This lack of precaution by Morgan to exclude juvenile diabetics may have resulted in the finding of diabetics' tissue chromium to be less than non-diabetic subjects. It may also account for the difference in the results of Morgan's study and this study.

While parity is known to affect the hair chromium level of young women (ages 20 to 40) (15), it does not seem to have an effect on the hair chromium of elderly women whether diabetic or non-diabetic. In this study, the correlation between diabetic and non-diabetic elderly women's hair chromium concentration and parity was studied and neither a significant negative or positive correlation was observable for either group. However, this observation that parity does not affect elderly

women's hair chromium levels is logical in view of the ages of the women studied. The age range for the diabetic group was 60 to 87 and the average age 76. The age range of the non-diabetic or control group was 41 to 94 and the average was 73. Thus, the majority of the subjects of both groups were far past the parturient period of life. Therefore, if previous pregnancies had depleted the women's body chromium stores, enough time had elapsed for the stores to be repleted.

In a large scale spectographic study, American infants, children, adolescents, and young adults' tissue and organ chromium concentrations were determined and compared. The comparison indicated that in most tissues and organs the chromium concentration declines with age (53, 55). However, the results of this and other studies indicate this decline with age is observable only when comparing tissue and organ chromium levels of all the various age groups, but is not observable when studying tissue chromium levels in relation to age for a single age group. Hambidge (16) studied the regression of children's hair chromium with age but found no significant regression. Morgan (38) evaluated the correlation between elderly adults' hepatic chromium levels and age but did not observe a negative correlation. Also, for the women of this investigation no evidence of a negative correlation was found between the women's hair chromium concentration and age.

In this study, a statistically significant difference was found between the hair chromium of recent diabetics and long-term diabetics, and between the hair chromium of non-insulin diabetics and insulin diabetics. The hair chromium of recent diabetics was significantly less than that of long-term diabetics. The hair chromium of non-insulin diabetics was significantly less than that of insulin diabetics.

Chan (2), studying college age diabetics, found similar results. Also, Glinsmann (12), studying elderly glucose intolerant subject's response to chromium supplementation, observed the subject's response to the chromium was dependent upon the nature of this impairment. Slightly glucose intolerant subjects were improved by chromium supplementation but severely glucose intolerant subjects were not.

The observations of Glinsmann and Chan and the observations made in this study concerning non-insulin diabetics, insulin diabetics, recent diabetics and long-term diabetics collectively may be viewed or explained in several ways. Four possible explanations are:

1. It may be that insulin therapy causes a change in chromium metabolism such that more is stored in the hair.

2. It is possible that only at the initial stages of maturity-onset diabetes is chromium deficiency a cause of the impairment in glucose tolerance.

3. It is conceivable that there are two forms of diabetes: a mild form not requiring insulin that is related to chromium deficiency, and a more severe form requiring insulin that is not related to chromium deficiency.

4. It may be that of the initial stages of diabetes (i.e., when the impairment is mild) chromium deficiency is related to, or is a possible cause of, the impairment. However, when the impairment is not corrected with chromium, it becomes more severe with time, and insulin therapy is initiated. Finally, the insulin therapy causes a change in chromium metabolism and more chromium is stored in the diabetic's hair.

Whether one or any of these explanations are correct will be determined only by further study.

CHAPTER VI

SUMMARY AND CONCLUSIONS

From Stillwater nursing homes, 28 females with an average age of 75 were non-randomly selected. Sixteen of the elderly selected were apparently healthy non-diabetic women. Twelve were maturity-onset diabetics. The women were all chosen for the purpose of studying and comparing elderly, female non-diabetics' and diabetics' hair chromium levels.

A hair sample weighing approximately 1.5 g was collected from each of the women and at the time of hair collection, the women were interviewed for information about age, parity, health and medication. The hair samples were washed, weighed, wet ashed, and analyzed by atomic absorption for chromium. The atomic absorption measurements of the chromium in the ash were used to calculate the diabetics' and non-diabetics' hair chromium concentrations.

Upon comparison, no significant difference was found between the diabetics' and non-diabetics' hair chromium concentration. This finding does not lend support to the concept that lower than normal tissue chromium levels are related to maturity-onset diabetes and elderly human subjects. Also, this finding suggests that additional studies need to be undertaken to compare elderly maturity-onset diabetics' and non-diabetics' tissue chromium levels. Many investigators have hypothesized that a relationship does exist between low tissue chromium levels and

maturity-onset diabetes. If this hypothesis is to be accepted as a truth, additional observations must be reported contradicting the lack of difference found in this investigation.

In this investigation, evidence was also found suggesting age and parity do not affect, or are not related to, elderly women's hair chromium levels. The value of this evidence is that it indicates human hair is a particularly valuable tissue for studying and comparing elderly, female maturity-onset diabetics' and non-diabetics' tissue chromium. The lack of effect of increasing age and parity on the women's hair chromium reduces the number of parameters to be considered. With this reduction of parameters, the effect of the diabetes per se on the human subject's hair chromium may be more readily deciphered.

Finally, for the diabetics of this study, a significant difference was discerned between the hair chromium level of insulin and non-insulin diabetics and between the hair chromium levels of recent (diabetic less than five years) and long-term diabetics (diabetic more than ten years). The importance of these findings is not known at this time. However, their verification with a larger, random sample may be of value in designing future studies concerned with maturity-onset diabetes, chromium nutrition, and elderly humans.

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APPENDIX A

APPARATUS AND HAIR WASHING PROCEDURES

TABLE VI

A PROCEDURE FOR WASHING APPARATUS TO BE USED IN QUANTITATIVE CHROMIUM ANALYSIS

All glassware and other equipment used in the procedure for hair chromium analysis were carefully washed to avoid contamination of samples with chromium present in the air, dust, and laboratory. The procedure for washing was:

1. The object was washed with hot tap water and Alconox (Alcono, Inc. - Scientific Products).
2. It was rinsed three times with de-ionized distilled water.
3. It was rinsed with 3N nitric acid (Reagent Grade.
4. Following acid rinsing, the object was rinsed again with distilled water.

TABLE VII

PROCEDURE FOR WASHING HAIR TO BE ANALYZED FOR CHROMIUM AS SUGGESTED BY HAMBIDGE

1. In a clean beaker, hair samples were immersed in hexane* (25 ml/100 mg hair), stirred vigorously, and allowed to soak 15 minutes. After soaking, the hexane was decanted and the hair was rinsed with de-ionized distilled water. The rinse water was decanted and the hair transferred to a clean beaker.
2. In the second beaker, the above procedure was repeated except that ethanol 95% (25 m/100 mg hair) was substituted for the hexane.
3. In the third clean beaker, the hair was rinsed three times with de-ionized distilled water.

*Baker's Analyzed Reagent - Reagent Grade.

Source: Hambidge, Michael K., Michael L. Franklin and Margaret A. Jacobs. "Hair Chromium Concentration: Effect of Sample Washing and External Environment." Am. J. Clin. Nutr., Vol. 25 (1969), 83-91.

APPENDIX B

STATISTICAL TEST USED IN THE THESIS RESEARCH

TABLE VIII

ANALYSIS OF THE VARIANCE OF DIABETICS' AND CONTROL SUBJECTS' HAIR CHROMIUM CONCENTRATIONS

Source	df	Sum of Squares	Mean Square	F*
Total	27	2286.3085		
Group (Between Groups)	1	52.9471	52.9470	0.6163902
Error (Within Group)	26	2233.3614	85.8985	

*Difference in hair chromium of two groups not significant.

TABLE IX

t-TEST FOR INSULIN DEPENDENT DIABETICS AND CONTROL SUBJECTS

Hypothesis tested:

H_o: There is no significant difference in hair chromium concentration of two groups.

H_a: There is a significant difference between mean hair chromium of two groups.

	Control Group	Insulin Dependent
Mean Cr. Concentration ug/g*	1.0563	1.5914
Number of Subjects	16	6

$$t,20 = \frac{1.0563 - 1.5914}{.9492\sqrt{\frac{1}{16} + \frac{1}{6}}}$$

$t,20 = -1.13030$

Critical Values = $t.025,20 = -2.0860$

*H_o - Accepted; Difference Not Significant

TABLE X

t-TEST FOR NON-INSULIN DEPENDENT DIABETICS
AND CONTROL SUBJECTS

Hypothesis tested:

H_o: There is no significant difference ($p \leq .05$) in hair chromium concentration of two groups.

H_a: There is a significant difference ($p \leq .05$) in hair chromium concentration of two groups.

	Control Group	Non-Insulin Dependent
Mean Cr. Concentration ug/g*	1.0563	.5618
Number of Subjects	16	6

$$t,20 = \frac{1.0563 - 0.5618}{.8173 \sqrt{\frac{1}{16} + \frac{1}{6}}}$$

$t,20 = +1.2642$

Critical Value = $t.975,20 = +2.0860$

*H_o - Accepted; Difference Not Significant

TABLE XI

t-TEST FOR INSULIN DEPENDENT DIABETICS AND NON-INSULIN DEPENDENT DIABETICS

Hypothesis tested:

H_o: There is no significant difference in hair chromium concentration of two groups.

H_a: There is a significant difference in hair chromium concentration of two groups.

	Insulin Dependent	Non-Insulin Dependent
Mean Cr. Concentration ug/g*	1.5914	1.0563
Number of Subjects	6	6

$$t,10 = \frac{1.5914 - 1.0563}{.8203\sqrt{\frac{1}{6} + \frac{1}{6}}}$$

$t,10 = 2.2187$

Critical Value - $t.95,10 = 1.8125$

*H_o - Rejected in Favor of Alternative; Difference is Significant $.05 > p > .10$

TABLE XII

t-TEST FOR LONG-TERM DIABETICS AND CONTROL SUBJECTS

Hypothesis tested:

H_o: There is no significant difference in hair chromium concentration of two groups.

H_a: There is a significant difference in hair chromium concentration of two groups.

	Control Group	Long-Term Diabetic
Mean Cr. Concentrations ug/g*	1.0563	1.6059
Number of Subjects	16	6

$$t,20 = \frac{1.60593 - 1.0561}{.9838\sqrt{\frac{1}{16} + \frac{1}{6}}}$$

$$= 1.18497$$

Critical Value - $t_{.975,20} = 2.0860$

*H_o - Accepted, Difference Not Significant

TABLE XIII

t-TEST FOR RECENT DIABETICS AND CONTROL SUBJECTS

Hypothesis tested:

H_o: There is no signficiant difference in hair chromium concentration of two groups.

H_a: There is a significant difference in hair chromium concentration of two groups.

	Control Group	Short-Term Diabetics
Mean Cr. Concentrations ug/g*	1.0563	0.5472
Number of Subjects	16	6

$$t,20 = \frac{1.0563 - 0.5472}{.8182\sqrt{\frac{1}{16} + \frac{1}{6}}}$$

$$= 1.3184$$

Critical Value = $t.974,20 = 2.0860$

*H_o - Accepted; Difference Not Significant

TABLE XIV

t-TEST FOR LONG-TERM DIABETICS AND RECENT DIABETICS

Hypothesis tested:

H_o: There is no significant difference in hair chromium concentration of two groups.

H_a: There is a significant difference in hair chromium concentration of two groups.

	Short-Term	Long-Term
Mean Cr. Concentration ug/g*	0.5472	1.6059
Number of Subjects	6	6

$$t,10 = \frac{1.6059 - 0.5472}{.8092\sqrt{\frac{1}{6} + \frac{1}{6}}}$$

$$= 2.3627$$

Critical Value = $t_{.975,10} = 2.2281$

*H_o - Rejected in Favor of Alternative; Difference is Significant ($p \geq .05$)

VITA

Beta Jo Blalock

Candidate for the Degree of

Master of Science

Thesis: A STUDY OF ELDERLY FEMALE MATURITY-ONSET DIABETIC AND ELDERLY NON-DIABETIC WOMEN'S HAIR CHROMIUM

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