COMPARISON OF NUTRITIONAL ADEQUACY AND OTHER VARIABLES WITH CHROMIUM, IRON, COPPER, ZINC, MAGNESIUM, AND MANGANESE CONCENTRATIONS IN HUMAN HAIR

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INTRODUCTION

It has been difficult to find a human tissue that can be used for determination of nutrient status of the minerals within the human body. Such a tissue must not only reflect body nutriture, it must also be readily accessible. For wide use as an analytical tissue, sampling procedures should be relatively painless and inexpensive for the donor and should not involve elaborate clinical procedures or hospitalization, particularly if data are to be collected on large segments of the population. Blood and urine have been used as readily accessible sampling sources; however, many nutrients especially the minerals in urine and blood do not equilibrate with the other tissues. A high level of a nutrient in these fluids may only indicate that body stores are being mobilized, but that body stores are adequate.

Some investigators feel that hair is a satisfactory indicator of the mineral status of the rest of the body and meets the requirements of being a painless, inexpensive biopsy tissue.

There are 25 macro and micro elements that are known to have nutritional applications in man. Some but not all of the metabolic roles of these minerals have been elucidated. Each has been associated with an overt deficiency symptom in man or has been established to perform a specific biochemical function in man.

Six minerals were chosen for this study. These six minerals were chromium (Cr), iron (Fe), copper (Cu), zinc (Zn), magnesium (Mg), and manganese (Mn). Each of the six has had a deficiency symptom or biochemical function either proved or postulated, and each has been shown to have nutritional applications in man.

The purpose of this research was to estimate the content of these six elements in the hair of a group of Oklahoma State University students, to accumulate data that would help establish normal ranges of these elements in hair, and to determine whether the variables of body size, gender, or adequacy of diet tended to affect the levels of these elements in the hair of the students tested. This study, through analysis of hair samples for Cr, Fe, Cu, Zn, Mg, and Mn, was designed to investigate the following objectives:

1. Determine whether hair content of any of these elements varies as a result of sex, weight, or dietary sufficiency in a group of university students 18 to 20 years old.
2. Test for intercorrelations among any of the analyzed elements.
3. Accumulate data in order to help establish normal values of these elements in hair.
4. Compare these data on the 18 to 20 year-old age group with data accumulated by other workers using other age groups.

The design of this study was based on the following hypotheses:

1. There are no differences in the Cr, Fe, Zn, Cu, Mg, or Mn content of hair associated with the sex, body size, or dietary sufficiency of this group of university students.
2. There are no intercorrelations among any of the analyzed elements.

CHAPTER II

REVIEW OF LITERATURE

Some of the 25 minerals that are known to have nutritional application in man are known to have biochemical functions, but these functions have generally not been associated with overt deficiency symptoms in man. Likewise, some mineral deficiency symptoms are well known, but the actual biochemical role of the mineral in causing the symptom has not been documented. Many of these elements are transition metals that serve in a protein molecule complex, such as Fe in the heme molecule, metalloenzymes or as enzyme activators. Some serve in ionic form as neurotransmitter substances such as calcium (Ca) in muscular contractions.

The six minerals involved in this study were each reviewed to determine what is known about their functions in metabolism, how body nutritional status is determined, and the dietary requirements for them. Interactions between and among these minerals were reviewed as was the use of hair as a biopsy material. In order to determine the dietary adequacy of subjects, methods of evaluting dietary standards and conducting nutrition surveys were examined.

Chromium

Chromium has been known to play a role in nutrition for a number of years, but its exact function is still a target of active research. It

has been estimated that there are only 6 to 12 mg of Cr in the human body (1).

Functions of Chromium

In 1959, Swartz and Mertz (2) noted that laboratory rats being fed a purified rat chow developed glucose intolerance symptoms similar to those in early human diabetes (3). They further established that such impairment could be prevented by adding Cr III supplements to the diets, and depleted stores could be repleted by oral doses of 20 to 50 µg of Cr. Continued work with mice and rats indicated a role for Cr in fatty acid synthesis, cholesterol homeostasis, and amino acid synthesis (1, 4).

In autopsy studies, American adults were found to have considerably less Cr in body tissues than persons from less developed countries (5, 6). Newborn infants had higher tissue Cr than adults (1). These facts could be explained by supposing a dietary deficiency that allowed gradual depletion of Cr stores. Such a deficiency could be caused by wide use of highly refined foods in this country (1). In support of this theory, foods such as whole wheat flour and raw sugar contain more Cr than the refined counterparts such as refined flour and sugar (1, 7, 8).

In the mid-sixties, improvement in glucose tolerance was demonstrated in adults and elderly people following supplementation with 150 to 250 µg Cr/day (as chromic chloride). Marked improvement in glucose tolerance was seen in young, malnourished children following Cr supplementation (9). However, supplementation results were inconsistent, and there was usually a lengthy lag period between beginning of supplementation and any improved tolerance. Further, inorganic Cr salts were shown

to be poorly absorbed (9).

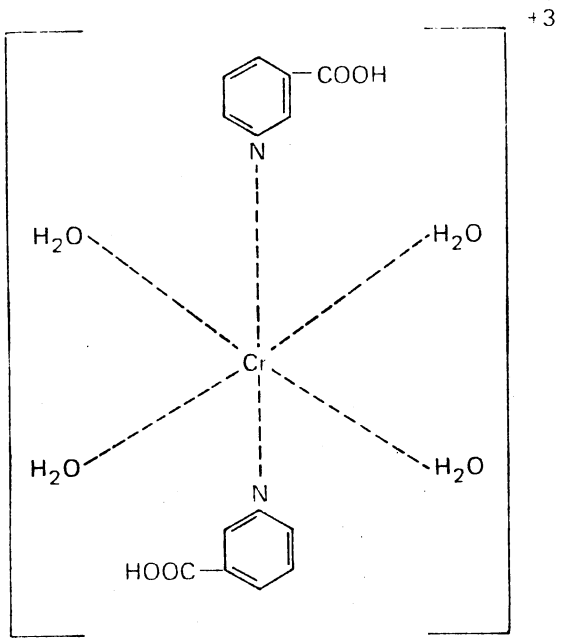
It was believed that the efficacy of Cr might be dependent on the valence state of the Cr used for supplementation. Chromium is present in nature in several valence states with Cr III and Cr VI the predominant forms. Chromium III salts were normally used for supplementation; but since foods contained both Cr III and Cr VI, the Cr VI might be more effective (1). However, animal experiments suggested that inorganic Cr salts, irrespective of valence state, were relatively ineffective compared with Cr compounds found in foods (9).

The most effective form of Cr has proven to be part of a glucose tolerance factor (GTF) molecule (3). Such a biologically active compound extracted from brewers yeast was a tetra-aquo-dinicotinate Cr complex (3, 10). The hypothesized structure is shown in Figure 1. In the living system the complex may be stabilized by replacing the coordinated water molecules with ligands, the amino acids: glycine, glutamic acid, and a sulfur-containing amino acid. Attempts to synthesize such a molecule have met with some success. The synthetic complex shows some of the same biological activity as the yeast complex in accelerating insulin-induced uptake of glucose by cells. The exact structure of a GTF molecule has not yet been determined (3, 9). Evidence indicates that GTF acts in the body as an insulin potentiator, aiding the uptake of glucose by the cells, but not as a substitute for insulin (3).

Determination of Chromium Status

Hair has been found to be useful for determining body Cr stores (11, 12, 13, 14). The Cr content of hair does not appear to be

affected by environmental contamination or distance from the scalp (15). The Cr level in human hair may be a good indicator of Cr status at the time the hair cells were formed; therefore, it reflects long-term nutritional habits rather than the intake for the previous day or meal (16, 17).



Source: W. Mertz, Effects and Metabolism of Glucose Tolerance Factor, Present Knowledge in Nutrition (1976).

Figure 1. Proposed Structure of GTF Complex.

Blood and urine Cr values fluctuate, apparently in response to an oral or intravenous glucose load or in response to blood insulin levels. Thus, a lack of urinary Cr rise when challenged with insulin (or glucose) could indicate depleted Cr stores (1, 3). Consequently, analyses of

blood or urine to determine Cr nutritive holds great promise but only as part of an insulin or glucose challenge test.

Chromium Dietary Requirements

Chromium research has been hampered by analytical difficulties (3). Chromium is present in most biological materials in only trace amounts, parts per billion (ng/g). Sample preparation methods developed for detecting inorganic Cr do not react similarly with all biological materials, and some is lost during preparation (18, 19). Dust, or metallic instruments add Cr to samples. Such contamination has been difficult to control. Furthermore, knowing how much Cr is present appears now to be less important than knowing how much is present in the biologically active form (3). For these reasons the dietary Cr requirement for man has not been quantitated (3, 20). However, Hambidge and O'Brien (21) have suggested a daily dietary requirement for adults of 0.1 mg. The average diet provides 50 to 100 μg/day. But Cr is poorly absorbed, with absorption of only about 0.5 per cent for inorganic Cr and 10 to 25 per cent for GTF Cr (1, 9). Since daily urinary losses are approximately 7 to 10 μg, a negative Cr balance is likely unless some of the dietary Cr is of high biological value (9). Chromium sources of high biological value include black pepper, meat products, whole grains, and particularly brewers yeast (10).

Iron

The need for Fe for transport of oxygen by erythrocytes has been known for many years. In 1933 when the U. S. Department of Agriculture first attempted to recommend dietary standards for vitamins and minerals,

Fe was one of the nutrients included (22). There are approximately 3 g of Fe in the human body (23).

Functions of Iron

Iron is present in the body in hemoglobin in erythrocytes, myoglobin in muscle, the cytochromes of the electron transport system, and in reductase, peroxidase, and other enzyme systems. Iron functions in these proteins and metalloenzymes in oxygen transport throughout the body and in cellular respiration (24). There is practically no ionic Fe in the body (22). Iron is in the metalloflavoprotein enzymes succinic dehydrogenase, DPNH-cytochrome reductase, and xanthine oxidase. It acts as a co-factor in aconitase in the TCA cycle. Iron has a role in the synthesis of collagen in the hydroxylation step of converting proline and lysine to protocollagen (22). The symptoms of fatigue and apathy associated with Fe deficiency anemia are associated primarily with the oxygen transport functions of iron (22). Iron is stored intracellularly in the proteins ferritin and hemosiderin and can be mobilized for hemoglobin synthesis as needed (24).

Determination of Iron Status

Several tests have been used to establish the nutritional adequacy of Fe in the human (17). The norms for these tests vary with age, sex, and altitude; but tables have been prepared which show the effect of these variables on test results. A suggested guide to the interpretation of hemoglobin and hematocrit tests taken at various altitudes shows the ranges of results to be expected from various age and sex groupings (17). One is a measure of the hemoglobin in whole blood, and the other, the

hematocrit, measures the packed cell volume of centrifuged whole blood. Another test often used is an erythrocyte count. These tests can detect an Fe deficiency that has progressed to the point that erythropoiesis and hemoglobin synthesis have decreased. They will not detect any low body stores. A test of serum iron binding capacity (IBC), where cells from the erythroid marrow are aspirated and stained, was found to be a better indicator of body stores (17, 25). However, a more recent method that estimates porphyrins in whole blood is believed to be the most sensitive test for detecting early deficiencies (17). Few studies have been found that reported Fe levels in hair and the materials and methods used vary widely (26, 27).

Iron Dietary Requirements

The average adult body has been estimated to contain about 3 g of Fe (23). Dietary recommendations for daily Fe intakes range from 10 mg for infants through 18 mg for women in the reproductive years with a 30 to 60 mg supplement recommended during pregnancy (20). Iron is poorly absorbed by the body. Heme Fe is absorbed more readily than is Fe in other forms (28). The presence of Zn, phytates, and phosphates in the gut inhibit Fe absorption while the presence of alcohol, valine, histidine, and ascorbic acid enhance absorption (20, 28, 29, 30). Food in the average adult diet contains about 6 mg Fe/1000 kcal (28). Women would have to eat about 3000 kcal/day to meet the 18 mg/day RDA. It has been estimated that 30 per cent of women have deficient Fe stores (28). Infants, because of the low Fe content of milk, are subject to Fe deficiency (20). It has been proposed that Fe enrichment of cereal

products be increased to help alleviate these deficiencies. Such enrichment might result in some males ingesting up to 30 mg/day; however, this amount is considered to be no threat to those individuals (20). Control of Fe levels in the body is primarily based on absorption, since there is no way for the body to excrete excess amounts of absorbed Fe. However, iron toxicity is usually seen only in cases of a genetically caused failure of the absorption control mechanism (hemochromatosis) or after prolonged, excessively high oral intakes of iron (siderosis) (24).

Iron is widespread in nature, but the primary sources of dietary Fe are animal products (except milk), whole grains, and enriched cereal products (22).

Copper

Copper is essential to all mammals although primary Cu deficiency in man is rare. Copper plays important roles in such vital tissues as blood, bone, and nerves. There are 100 to 150 mg Cu in the adult human (20, 23, 31).

Functions of Copper

Ceruloplasmin (ferridoxase) is a protein in plasma which contains Cu. Ceruloplasmin is necessary for the conversion of ferrous to ferric Fe. Copper is therefore vital for heme synthesis and for absorption, transport, and mobilization of Fe. Additionally, the erythrocytes themselves contain a cuproprotein, erythrocuprein (22). Erythrocuprein has superoxide dismutase activity which seems to be necessary to prevent damage by oxygen free-radicals in aerobic organisms (31, 32).

Cuproenzymes are necessary for the development of cross linkages in collagen and elastin. Bones fracture easily and experimental animals suffer osteoporosis and aortic hemorrhages because Cu deficiency alters these connective tissues (31, 32).

The function of Cu in the central nervous system is not clear. Lack of myelination and locomotor dysfunction are seen in Cu deficient animals, although there has been no role demonstrated for Cu in myelin synthesis. The cuproenzyme, dopamine beta-hydrolase, is required for the conversion of dopamine to the neurotransmitter substance norepinephrine (31, 32).

Tyrosinase, necessary for melanin formation, contains Cu. Thus a Cu deficiency can cause hypopigmentation. Cytochrome oxidase, the terminal oxidase in the electron transport system reducing O_2 to H_2O, also contains Cu (21).

Determination of Copper Status

Serum Cu concentration is constant and is independent of age, sex, food intake, or body stores (31). Many disease conditions such as infections, rheumatoid arthritis, or leukemia increase serum Cu concentrations, as do elevated estrogen levels in the blood in pregnancy or in women taking birth control pills (20, 31). For these reasons serum is imprecise for detecting Cu body stores. However, Cu in the plasma ceruloplasmin does reflect body stores. Since ceruloplasmin (ferridoxase) is a true oxidase, it can be measured accurately by enzyme reaction methods. Such a test for Cu body stores is relatively simple since the specificity of this enzyme reaction is high and minimizes problems associated with environmental sample contamination (33).

Urinary Cu concentration, although quite low, can be measured using atomic absorption spectroscopy (AAS); however, no data for use of urine as an index of Cu status have been reported.

Several researchers (27, 34, 35, 36, 37) have used human head hair as a test for tissue for Cu using AAS. Some found hair Cu concentration to be a more precise indicator of status than whole blood or plasma; and, with careful laboratory procedures, environmental contamination of the sample was minimized. Others have reported that Cu content of hair varied with age and gender of the individual but not with hair color so that hair Cu could be used as a nutrition index but only on a sex-and-age matched basis (17, 34, 35). Of further consideration in the interpretation of hair analyses is the report of Hambidge (37) that hair Cu values increase with increasing distance from the scalp, suggesting that environmental Cu contributes to the hair Cu content.

Copper Dietary Requirements

True primary Cu deficiency in man is debatable; however, deficiencies have been reported in infants fed only cows' milk with symptoms of diarrhea and neutropenia (17, 20, 21). Other symptoms that have been reported in human Cu deficiency are anemia, skeletal lesions, and achromotricia (17, 38).

The average diet provides 2 to 5 mg of Cu daily. Of this, 0.6 to 1.6 mg are absorbed (32). Less than five per cent of the absorbed Cu will be retained, with the excess excreted through bile (31). The 2 to 5 mg intake appears to be adequate, since an intake of 2 mg/day maintains Cu balance (20, 21). Copper deficiency has not been reported in infants fed human milk. Human milk contains up to 1.05 mg/ℓ of Cu. However,

cows' milk is a poor source containing only 0.015 to 0.18 mg of cu/ℓ.

Menkes' Kinky Hair Syndrome is a Cu deficiency disease of male infants that leads to early death. It is not a nutritional deficiency disease, however, since it results from a genetic-related failure in Cu absorption and metabolism (21). Similarly, Wilson's Disease is caused by a genetic failure that results in abnormally high retention of absorbed Cu (31).

Nuts, liver, shellfish, kidney, raisins, and dried legumes are good dietary sources of Cu (20).

Zinc

Zinc was shown to be necessary for animal life over 40 years ago. In 1963 Zn was also found to be necessary for human health and development, and its essential role in growth and sexual maturation were shown (39). It is estimated that there are from 2 to 3 g of Zn in the human body.

Functions of Zinc

Zinc is part of or activator for at least 20 enzymes (29). Zinc is a constituent of carbonic anhydrase, the carbondioxide carrier in erythrocytes which also helps maintain acid-base balance in the renal tubules (20). Zinc functions in protein metabolism as a cofactor in carboxy peptidase, in carbohydrate metabolism as part of lactic dehydrogenase, in DNA and RNA metabolism apparently in connection with DNA and RNA polymerases, and in alcohol metabolism as a part of alcohol dehydrogenase (29, 40). Alkaline phosphatases are Zn metalloenzymes and have been implicated in mucopolysaccharide synthesis (22). Zinc is related in an as

yet unknown way to insulin (31). It has been suggested that Zn may participate in some way with insulin in glucose uptake by the cells (29). A relatively large amount of Zn is used in metabolism of alcohol. Harper (40) has speculated that the low Zn levels in the livers of victims of alcoholic cirrhosis may be related to the metabolic demands of alcohol.

Zinc deficiencies have been associated with growth retardation, hypogonadism, delayed wound healing, skin lesions, alopecia, skeletal abnormalities, impaired learning ability, anorexia, and abnormalities of taste and smell (20, 21, 29, 39). Birth defects similar to those seen in human infants have been caused in rats by experimental Zn deficiencies (21, 41). It is postulated that these birth defects in rats were caused by the effect of Zn on DNA synthesis (21). In human subjects it has been shown that fetal size has been positively correlated with Zn concentration in amniotic fluid (29).

Determination of Zinc Status

The methods for determination of Zn nutritional status have not been standardized, nor have data been compiled for normal ranges of Zn in various body tissues. There is disagreement over which tissues accurately reflect body stores (17, 42). Zinc concentration in urine has been shown to be more reflective of protein catabolism, being elevated with starvation or injury, than an indicator of body stores (43, 44). However, in cases of overt Zn deficiency, urine levels were lower than in asymptomatic subjects (39). Zinc concentrations in human blood plasma have been reported to vary widely in apparently normal individuals (33, 44). Hurley (41) has reported that plasma Zn levels did fail with increasing Zn deficiency.

Some researchers found hair to be a better indicator of Zn status than either blood serum or urine. In one study, persons experiencing overt Zn deficiency symptoms had much less hair Zn than did asymtomatic subjects. After a period of supplementation and remission of symptoms, hair Zn concentration was found to equal or exceed that in the hair of normal subjects (16). Working with animals, these same researchers could find little change in serum levels during periods of deficiency and repletion. In other work, Zn concentrations in human hair were reported to vary with the age of subjects but not with sex (17, 20). A hair Zn concentration of less than 70 to 100 μg/g is considered subnormal (20, 45).

Zinc Dietary Requirements

The RDA for Zn is 15 mg/day for the adult but ranges from 3 mg/day for infants to 20 to 25 mg/day for pregnant or lactating women (20). A daily source of dietary Zn is necessary for the pregnant women since maternal body stores of Zn, particularly Zn in bone, do not appear to be mobilized to meet fetal needs in periods of dietary deficiency (20, 41).

The average Zn content of a mixed diet of adult Americans is between 10 and 15 mg/day. Metabolic studies have shown 8 to 10 mg/day are sufficient to maintain equilibrium in healthy adults. Therefore the RDA should meet the needs of most people. However, the Zn in foods is not always available since certain factors such as phytates and fiber in some high cereal diets reduce Zn absorption (29, 41).

Animal products such as meat, liver, eggs, and seafood (particularly oysters) are good dietary sources of Zn (20).

Magnesium

The human body contains about 25 mg of Mg, 70 per cent in bone and 30 per cent in soft tissue and fluids. It is a major cation of intracellular fluids, second only to potassium (K) (23).

Functions of Magnesium

As an intracellular cation, Mg is needed for conduction of nerve impulses. A Mg deficiency causes irritability through increased nerve conduction and increased transmission at the myoneural junction with muscular contractility and rigidity (46).

Magnesium, as a prosthetic group in various phosphokinases, activates the hydrolysis and transfer of phosphate groups; thus, Mg is involved in nearly every step of both anaerobic and aerobic glucose metabolism (47, 48). Magnesium also is required in various protein and lipid metabolic reactions such as in the transfer of CoA to form cholyl CoA or acetyl CoA (22). Magnesium is essential for protein synthesis through participation in ribosomal aggregation, binding of mRNA to ribosomes, and in synthesis and degradation of DNA (48). Symptoms of Mg deficiency include anorexia, wasting of muscles, edema, cheilosis, glossitis, loss of teeth, vasodilation, and soft tissue mineralization (38, 49). Magnesium deficiency is accompanied by hypocalcemia and hypokalemia because of not fully understood interactions among these three minerals (22, 48).

Hypomagnesemia has been related to the central nervous system hyperirritability seen in chronic alcoholism and alcoholic withdrawal (50, 51). It can also be caused by abnormal loss of body fluids as in

diarrhea or vomiting (22, 49). Magnesium stores in bone are not available to the body during periods of dietary deficiency (46, 48).

Determination of Magnesium Status

Nutritional status has usually been determined by analysis of either plasma, with 1.4 to 2.5 mg/100 ml considered normal, or serum, with 1.3 to 2.0 mEq/ℓ considered normal (1 mEq Mg = 12 mg) (17, 23). Plasma, fecal, and urinary values fall quickly in response to Mg deficiency (48). In cattle plasma Mg levels are affected by the Ca:P ratio and K level in the diet. These considerations could affect the reliability of using plasma, urine, or feces to determine long-term Mg nutritional status (52). Few data were found citing hair Mg values. Results of only two studies covering Mg levels in hair were mentioned. In those studies the method and amount found varied appreciably (27).

Magnesium Dietary Requirements

The recommended daily allowance of Mg is 350 mg for men and 300 mg for women (20). Magnesium, as a part of chlorophyll, is widespread in nature, and it is postulated that a natural human deficiency is unlikely. However, it is estimated that the average adult diet provides 120 mg/1000 kcal (46). Accordingly, women would have to consume 2500 kcal/day and men 2900 kcal/day in order to reach the recommended level of intake.

Cereal grains, vegetables, and nuts are rich in Mg; meat products are intermediate sources; and eggs and dairy products are poor. An habitual diet low in cereal or vegetables but high in milk, meat, and

eggs could provide insufficient Mg (46). Ingestion of large amounts of Mg in anti-acids or laxatives does not appear to be harmful (20).

Manganese

Manganese deficiency has not been identified in man, but it has been the cause of birth defects in animal studies and is known to be involved in several metabolic steps in the body. There are about 10 mg of Mn in the human adult (23).

Functions of Manganese

Manganese has been found in numerous enzymes, but in many of these other metals, particularly Mg, can replace Mn with no decrease in activity (31). However, Mn is clearly associated with synthesis of mucopolysaccharides as a cofactor in glycosyltransferases (53). Manganese deficiencies in animals have led to skeletal and connective tissue abnormalities such as "slipped tendon" in chickens and defective bone formation (22, 53). Ataxia, caused by malformation of the bones in the inner ear, has been observed in the offspring of Mn deficient mice.

A Mn containing metalloenzyme taken from mitochondria has shown superoxide dismutase activity. This implies a role for Mn in protecting mitochondria from oxygen free radicals since Mn deficiencies have caused changes in mitochondrial membranes similar to those caused by excessive amounts of oxygen (31, 53).

Manganese deficiencies in animals, or dams of animals, have also been associated with abnormal glucose tolerance, fatty livers, and improper brain function (22, 31, 53). There is also evidence that Mn is necessary for biosynthesis of prothombin (53). Manganese may be involved

in reproduction, protein and cholesterol synthesis, and fatty acid metabolism. However, the exact role of Mn in these conditions and functions has not been elucidated (20, 31).

Determination of Manganese Status

In normal adults plasma Mn concentrations are about 2.5 μg/100 ml (31). Animal studies do not show Mn levels in blood or urine to be good determinants of Mn status. Animal hair was shown to have great potential as a determinant, but there was some indication that Mn concentration might be influenced by hair color (33, 52). Apparently, human hair has not been used to determine Mn status.

Manganese Dietary Requirements

The estimated daily intake of Mn is about 3 to 9 mg, and about 40 per cent of the Mn is absorbed (31). This amount appears to meet body needs, but no RDA have been established. However, based on balance studies, a requirement of 2 mg/day has been suggested (20, 21, 53).

Manganese is widely distributed in foods with plant products tending to be good sources and animal products poor sources (53).

Interrelationships

Inorganic elements rarely stand alone in a nutritional sense (17). Many interrelationships are known to exist among and between body minerals, and many no doubt exist that are as yet unknown or unproven.

Many interactions have been noted among Fe, Cu and Zn. Zinc toxicity can be caused by inadequate Fe and Cu competing for absorption sites, and a high Zn intake can cause Fe and Cu deficiencies. Copper may be

deficient or toxic depending upon the relative proportion of Zn and Fe in the diet. In Fe deficiency, Cu accumulates in the liver, and with Cu deficiency Fe accumulates in the liver. In human hemochromatosis, both Fe and Cu plasma values are elevated. In most disease conditions, plasma Zn will be low and plasma Cr high (31, 32, 33, 48, 41). Cytochrome oxidase, a cuproenzyme, is the terminal oxidase of the electron transport system. This enzyme also has heme as a prosthetic group (31). Several cuproproteins are known; all also contain Zn and all have superoxide dismutase activity (32). Manganese, too, has been associated with superoxide dismutase activity (53).

High intakes of Mn interfere with Fe utilization and lower serum Mg (38). The interference among Fe, Cu, and Mn may be because all three, as well as Cr, are transported by transferrin. There may be competition for binding sites (1, 48). Magnesium can replace Mn in some enzyme reactions (53). Magnesium, Mn, and Zn are all involved in mucopolysaccharide synthesis (22, 29, 53). Chromium, Mn, and Zn are all implicated in some way with glucose intolerance (1, 29, 41).

These examples are presented as some of the interactions known among the elements considered in this study. There are also many interactions among the six minerals reviewed and other nutrients.

Hair as a Biopsy Material

Hair is used as a biopsy material because it is a simple, atraumatic, reliable method of assessing body stores of minerals (12, 34, 45). Hair reflects long-term nutritional status and is not as subject to day-to-day or meal-to-meal fluctuations as are some other biopsy materials (17).

Since trace elements are found in dust, sweat, and on laboratory tools, environmental contamination of hair samples was believed to be a possibility. While this point has not been clearly proved or disproved, several researchers have published data showing that with proper washing and sample preparation, contamination can be controlled (12, 17, 54). One advantage of the use of hair as a test tissue is that one hair sample can be prepared and read for a number of different minerals (11).

A problem when using hair as a biopsy material is the lack of clearly established normal values for content of the different minerals in hair and the effect of other factors (17, 45). As examples, Zn values are reported to vary with age but not sex, Cu varies with both age and sex, and Cr varies with age (17, 34, 45, 54, 55). Data should be amassed showing normal hair concentration ranges under all such conditions for minerals known to have a role in human nutrition.

Assessment of Nutritional Status

Assessment of nutritional status of a large group or an individual is based on dietary studies, laboratory or biochemical investigations, and clinical studies. Dietary studies furnish information about current food intakes. Laboratory or biochemical investigations, such as blood or urine analyses, reflect nutrient levels that are indicative of recent food intake. Clinical observations indicate results of nutrient intakes over long periods of time. Methods vary depending on whether the study covers population groups or individuals (22, 56).

Dietary Methodology

To determine individual food consumption, a variety of methods have been used. These include a 24-hour recall of food eaten; a 3 to 7 day record of food eaten kept by weight, household measures, or by estimated quantities; a questionnaire or interview to obtain general dietary data; a diet history taken by a trained interviewer to determine long-term food intake patterns; and laboratory studies where all food eaten is carefully weighed with duplicate aliquots analyzed. The method used would depend upon the type of information needed, the subject, and the cost. The most widely used is the 24-hour recall. When working with groups of individuals, one-day records for a large number of people are better than lengthy records for only a few people. Further, the 24-hour recall plan does not require the workers to be as highly trained as do the other mentioned methods (22, 56).

Laboratory or Biochemical Evaluations

Laboratory or biochemical evaluations usually are based on results obtained from blood, urine, or excreta analysis. Charts and other data are available allowing comparison of the test results of the subject population with standard norms.

Clinical Studies

Clinical studies are based on observations of various body areas such as lips, hair, tongue, teeth, gums, and nails for cheilosis, glossitis, or other conditions known to be associated with nutrient deficiency. Anthropometric measurements such as height and weight are

and compared with existing data on an age and sex basis. Clinical studies require a high degree of professional training on the part of the workers but provide the best information available for determining long-term nutritional status (22, 56).

Dietary Standards

Dietary standards covering intakes of specific nutrients are based on research designed to determine human nutrient requirements. Dietary standards estimate the reasonable levels of nutrients that should support normal function in most people and are designed for planning and evaluating diets (22).

The Recommended Dietary Allowances, the standards developed by the Food and Nutrition Board, National Research Council, National Academy of Sciences (20), are the standards most widely accepted in this country. These recommendations are based on amounts of nutrients necessary to prevent deficiency symptoms and support health in most healthy persons with an added margin of safety (except for energy requirements) to allow for individual variations and imprecision in estimating requirements. The recommendations are not intended to meet unusual needs caused by injury, illness, or genetic variation. They are revised every five years to incorporate changes indicated by continuing research. Specific nutrient recommendations are expressed in terms of age, weight, and sex. The safety margin included in the recommended allowance is designed to compensate for analytical imprecision and to cover normal variation in the needs of individuals (20).

CHAPTER III

PROCEDURES

In order to fulfill the objectives of this study, 88 college students were selected and their heights, weights, and sex recorded. An evaluation of their dietary intakes was made, and hair samples were collected and analyzed for mineral content. Following dietary evaluation and chemical analyses, the following data were available for each subject:

1. Body size as measured by a height-weight comparison.
2. An evaluation of dietary intakes.
3. Chromium, Fe, Cu, Zn, Mg, and Mn content of the head hair.

The procedures followed in the collection of these data included the collection of dietaries and hair samples, a dietary evaluation, and the chemical analysis of the subjects' hair samples. These data were then analyzed statistically. These procedures are described in more detail in this chapter.

Dietaries and Evaluation

Collection of Dietaries and Hair Samples

University students (26 males and 62 females) enrolled in Basic Human Nutrition were the subjects in this study. They were asked to record dietary information and contribute a hair sample. Four days,

one day each month for four months, were selected during the semester for recording food intake. Use of the cycle menus followed by the university cafeterias made it possible to select days without repeated meals and to prepare menu sheets in advance so that the students who ate in the residence halls had only to indicate the amount eaten on their menu sheet. An example of one of the menu sheets and the accompanying cover letter are presented in Appendix A. Snack foods, vitamin supplements, and all other intakes were to be noted. Students who did not eat in the residence halls were instructed in estimating serving size and were asked to maintain records of food intake on the same four days.

All students were provided with campus mail envelopes and returned their dietaries to the laboratory on the following day. On the selected days, a serving-size sample of each food offered was obtained in the cafeteria line and recorded, weighed, and measured. Students also were given a 200-dram pharmaceutical vial in which to collect a hair sample. All students were further asked to complete a data sheet showing age, sex, weight, height, whether or not they were diabetic, and whether they had dyed, bleached, or permanent-waved hair.

By taking dietaries on four days a better estimate of each student's eating habits could be made than if the study were limited to a single day. By limiting the diet recording to four days, it was possible to have more subjects than if only a few students had kept records for several days. Thus, the study was planned to have the advantages of the 24-hour recall dietary plan and yet to minimize the primary disadvantage of the one-day plan of inadvertently choosing an atypical day. Upon return of the dietaries, each was evaluated for adequacy of protein and calorie intakes. The individual's weight relative to the normal range

was determined. These evaluations were made by the following methods described as Body Size Evaluation, Protein Evaluation, and Calorie Evaluation.

Body Size Evaluation

Each individual included actual weight and height on the dietaries. These data were compared with a height-weight table (20) which showed a recommended weight range for each height (recommended weight for height $\pm$ about eight per cent for females and $\pm$ about nine per cent for males). Each person was rated as being under, normal, or overweight depending on whether the weight fell within the recommended weight range.

Protein Evaluation

Mean protein intake for each individual for the four days was calculated. This figure was then compared with the person's recommended daily protein allowance (0.8 g/kg of body weight) (20). If the mean protein consumption was below the recommended daily protein allowance, the subject was classified as having low protein intake.

Calorie Evaluation

Caloric adequacy was determined by whether the individual consumed sufficient calories to maintain his recommended body weight, based on anticipated activity, adjusted for body size and sex. By this criterion, students were rated as having low, normal, or high caloric intake. The methods for calculating caloric intakes and requirements are described below. The total caloric intake for each individual was calculated by

methods described in Meal Planning with Exchange Lists (57) and compared with food values listed in U. S. Department of Agriculture Handbook 8 (58).

Caloric Requirement. Actual caloric requirements were estimated according to energy demands. The following activity pattern was determined to fit the average college student:

6.0 hours of sleep
12.0 hours of very light activity
3.5 hours of light activity
1.0 hours of moderate activity
0.5 hours of heavy activity

Using this activity pattern and the caloric demand of each activity level (20), daily caloric requirements for each height, to the nearest inch, were determined within a range of $\pm$ 200 kcal. It was then determined whether the actual caloric intake for each student fell within, above, or below this range.

Preparation and Analysis of Hair Samples

The students were given several weeks to collect a hair sample. They were given a pharmaceutical vial and asked to fill it with their own head hair (about 2 g) obtained by whatever means they chose. The sample vials were returned during or before the final week of the semester. All students turned in hair samples, but those who had dyed, bleached, or permanently waved hair were not included in the study (54).

Preparation of Hair Sample

Each hair sample was visually inspected, and obvious foreign substances such as lint were removed. The sample was then washed in hexane, 95 per cent ethanol, and distilled water. The hair was dried

overnight in a 52° C drying oven and cooled in a desiccator. Samples of approximately 1 g were weighed to the nearest mg.

These washing procedures to remove environmental contaminants have been described in detail (12, 54). After being washed, hair samples were kept free from dust, and no metallic instruments or equipment were allowed to come into contact with the samples.

Analysis of Hair Sample

Analysis for trace minerals requires avoiding environmental contaminants such as dust. Laboratory equipment, glassware, and reagents also can contaminate samples. Only double distilled, deionized water was used throughout the analyses. The nitric acid used was glass distilled in the laboratory. All glassware was washed and rinsed in tap water, then rinsed three times in distilled water, allowed to stand in an acid bath prepared with distilled water, and then re-rinsed three times in distilled water. The glassware was dried in an enclosed drying oven and transferred to a sealed cabinet for storage. Samples and reagents were kept tightly covered at all times except when samples were being heated. Raised cover slips were used during heating. Only plastic or teflon-coated tongs, forceps, and tubing were used. Chrome-plated faucets and other metallic items were covered with plastic.

Digestion. The washed, dried, and weighed hair samples were covered with 60 ml concentrated 3:1 70 per cent HNO_3: 70 per cent $HClO_4$ in 200 ml beakers, capped with tight coverslips, and allowed to stand overnight. The tight coverslips were then replaced with raised coverslips, and the samples were heated to insure complete oxidation of organic matter. To

maintain a uniform, moderate temperature, the beakers were placed in a pan of washed sand, a thermometer inserted into the sand, and the pan placed on a hotplate. The digestion temperature was between 125 and 140° C, although occasionally a higher temperature was required to completely digest the sample to yield a clear digestate. Chromium was lost if the temperature exceeded 175° C.

The samples were allowed to evaporate to about 2 ml, usually requiring 15 to 19 hours. If at this point any of the samples were not clear, 5 ml 3:1 digestion acid mixture were added and heating continued. If still not clear (colorless), the procedure was repeated.

Reconstitution. When the samples were clear and colorless, 5 ml of 1:1 37 per cent HCl:H_2O were added to the beakers and evaporated to near dryness (about 1 ml). The sides of the beakers were rinsed using a squeeze bottle of distilled water and evaporated to about 1 to 2 ml. This rinsing was repeated three times. The beakers were removed from the heat and the sample carefully poured into 10 ml volumetric flasks. The sides and bottoms of each beaker were carefully rinsed with small amounts of distilled water and the rinse water added to the 10 ml flask. After a cooling period, the flasks were brought to volume with distilled water, and the samples filtered into plastic sample bottles and refrigerated.

Reading of Samples. Standard solutions in μg/g for each element were prepared which bracketed the expected concentrations of the hair samples. Standards and samples were then read on a Perkin-Elmer 303 Atomic Absorption Spectrophotometer using the appropriate lamp for each element. The standards' readings were used to plot standard curves, and the sample concentrations were then read from the standard curves. A

new standard curve was prepared each day. The concentration of an element in the hair was then calculated by the following formula:

$$\frac{\text{concentration of sample as taken from standard curve x 10 (dilution factor)}}{\text{weight of sample (grams)}} = \text{sample value } (\mu g/g).$$

Statistical Analysis

Means, ranges, standard deviations, and coefficients of variation were calculated for each mineral. Analyses of variance were done by sex for body size, category of protein intake, and category of caloric intake for each mineral. Cross products analyses of the data from body size and dietary adequacy studies were done by sex, and scatter diagrams were made showing the plots of hair content by category for each mineral against the other.

Correlation matrices were computed to determine interrelationships among the six minerals based on data for males, for females, and for all subjects pooled. Analysis of variance based on pooled data from all subjects was computed for each mineral. When F-values justified further comparisons, mean differences of effects were compared with calculated least significance difference (LSD) values.

CHAPTER IV

RESULTS AND DISCUSSION

Data were examined to determine relationships of body size, protein adequacy and caloric adequacy with levels of each of the six minerals in hair and to determine intercorrelations between levels of minerals in hair. The mean value for each mineral in the hair of the subjects was determined for comparison with values reported by other researchers.

Table I summarizes the mean values for each mineral for the females, the males, and for both sexes. The standard deviations from the means (S.D.) and the highest and lowest values found for each mineral are also shown. Appendix B contains raw data showing observed mineral levels for each subject.

Results are discussed first in terms of the relationships of body size and dietary adequacy with the mineral content of the hair. Then results are discussed in terms of each mineral individually. The intercorrelations between levels of minerals in hair are presented. A comparison of the concentrations of the minerals found in the hair is made with the amount of minerals known to be in the body.

Association of Minerals in Hair with Body Size, Caloric and Protein Intakes

Relationships between mineral concentrations in hair and body size, caloric adequacy, and protein sufficiency proved to be nonsignificant

TABLE I

MINERAL CONCENTRATIONS IN HUMAN HAIR (μg/g)[a]

Mineral	n	Mean	S.D.	Low	High
		Females			
Cr	62	.90	0.47	0.0	2.4
Fe	52	9.0	5.1	2.0	35.
Cu	52	42.	30.	8.4	130.
Zn	52	150.	69.	63.	490.
Mg	36	220.	84.	89.	400.
Mn	52	0.87	0.74	0.0	4.
		Males			
Cr	26	1.0	0.98	0.0	3.8
Fe	26	7.5	3.3	3.5	18.
Cu	26	42.	35.	7.5	150.
Zn	26	180.	100.	79.	480.
Mg	26	250.	96.	71.	390.
Mn	26	0.65	0.48	0.0	2.2
		Males and Females			
Cr	88	0.93	0.66	0.0	3.8
Fe	78	8.40	4.6	2.0	35.
Cu	78	42.	31.	7.5	150.
Zn	78	160.	81.	63.	490.
Mg	62	230.	89.	71.	400.
Mn	78	0.79	0.67	0.00	4.0

[a]In this and subsequent tables data are rounded to two significant digits.

for males, although some findings of interest were noted. However, analyses of variance for data on females and on the total sample did show significance in some categories.

Body Size-Mineral Relationships

Table II presents the mean mineral content of the hair according to the sex and category of body weight of the subjects. The body-size categories were determined by taking the student's ideal weight for height plus or minus about eight per cent for females and about nine per cent for males.

Data in Table II indicate that the Cr content of the hair varied only 0.04 μg/gm between normal weight males and females; however, in both sexes, the underweight individuals had higher mean Cr values. In regard to Fe and Cu, females at normal weight had higher levels of these two minerals than the males. Among females, those of normal weight had highest levels. Among males, those of normal weight had the lowest levels. The underweight males had a higher mean for Zn than the males of other sizes; but for the famales the overweight size had the highest mean. The Mg content of hair tended to be higher with higher weight for the females but not for the males. Magnanese differences were slight within sex but females had higher levels than males.

When the observed differences reflected in Table II were tested for significance, the analyses of variance did not indicate any significant sources of variance. However, when data from all subjects were pooled, the Cr content of the hair of the underweight individuals, sex effects removed, was significantly higher ($P < .05$) than for the normal and overweight categories. Table III summarizes results of all analyses of

TABLE II

MINERAL CONTENT OF HAIR (IN μg/g) ACCORDING TO BODY SIZE

Category of Body Size	n[a]	Cr	Fe	Cu	Zn	Mg	Mn
			Females				
Underweight		1.1 (20)	8.9 (17)	34. (17)	150. (17)	190. (9)	.81 (17)
Normal Weight (± 8%)		.82 (26)	10. (20)	49. (20)	140. (20)	210. (16)	.90 (20)
Overweight		.84 (16)	7.3 (15)	41. (15)	180. (15)	260. (11)	.88 (15)
			Males				
Underweight	5	1.8	9.8	47.	260.	290.	.66
Normal Weight (± 9%)	14	.86	6.5	35.	150.	210.	.61
Overweight	4	.47	7.2	45.	120.	260.	.63

[a]Numbers of female subjects are reported in parentheses.

variance. There were also indications of possible significant interaction of sex and body size in regard to the Zn content of subjects' hair.

TABLE III

SUMMARY OF ANALYSES OF VARIANCE USING POOLED DATA

Basis for Categories	Mineral	F	Level of Significance
Body Size	Cr	2.62	<.05
	Fe	1.24	NS
	Cu	.64	NS
	Zn	2.68	<.05
	Mg	1.75	NS
	Mn	.65	NS
Caloric Intake	Cr	.21	NS
	Fe	.62	NS
	Cu	1.15	NS
	Zn	2.29	NS
	Mg	1.41	NS
	Mn	3.26	<.05
Protein Intake	Cr	2.24	<.05
	Fe	1.05	NS
	Cu	1.07	NS
	Zn	0.00	NS
	Mg	.89	NS
	Mn	1.27	NS

Calorie-Mineral Relationships

Table IV shows for females and males the mineral content of the hair according to the caloric adequacy or sufficiency of calories to maintain body weight. Caloric sufficiency was determined by calculating energy requirements based on body size and activity. Based on the

criterion described in Chapter III for determining energy requirements, 40 females and 17 males had intakes below recommended levels. Only three (two females and one male) had intakes above recommended levels. The body size comparison (Table II) showed fewer subjects to be underweight and more overweight than would be predicted by their daily calorie intake. This finding may be due to a lower daily activity level than estimated in calculating daily caloric intake.

TABLE IV

MINERAL CONTENT OF HAIR (IN μg/g) ACCORDING TO CALORIC ADEQUACY OF DIET

Category of Caloric Adequacy	n[a]	Cr	Fe	Cu	Zn	Mg	Mn
			Females				
Low		0.94 (40)	9.5 (35)	49. (35)	150. (35)	240. (35)	0.82 (35)
Normal (± 200 kcal)		0.84 (20)	7.5 (15)	28. (15)	150. (15)	190. (11)	0.76 (15)
High		0.73 (2)	7.7 (2)	21. (2)	190. (2)	(0)	2.40 (2)
			Males				
Low	17	0.70	7.5	29.	140.	250.	0.66
Normal (± 200 kcal)	3	0.62	8.3	43.	260.	210.	0.42
High	1	0.60	5.5	50.	130.	390.	0.30

[a]Number of female subjects is shown in parentheses.

As with the underweight subjects (Table III), the individuals with low caloric intake showed a somewhat higher mean level of hair Cr. The females with low caloric intake had higher mean levels of Fe and Cu but the males with normal and high intakes had the higher Fe and Cu levels. The mean levels of Zn did not vary widely except for males with normal intake. If Mg data for the one male for high caloric intake are excluded, females and males with low caloric intake had higher Mg levels than those with normal intake. For the subgroups of females and males with at least 15 subjects, the range in level of Mn is from 0.66 to 0.82.

When F-tests indicated a basis for further comparison of data, tests of least significant difference (LSD) were performed. Females in the low calorie category had Cu values significantly different ($P < .05$) from normal individuals. The females with a high intake of calories had hair Mn levels significantly higher ($P < .05$) than the low and normal calorie intake categories. The high calorie intake group in the pooled data comparisons (males and females) for Mn ($P < .05$) were also significantly higher than the other two subgroups (Tables III and IV). The hair Zn levels were not significantly different from each other in either single sex or pooled data analyses. F values for Cr and Fe were not significant as reported in Table III.

Protein-Mineral Relationships

Adequacy of protein intake was determined by whether the subject consumed at least 0.8 g of protein per kg body weight, or the Recommended Dietary Allowance. The protein-mineral intakes are shown in Table V. These data indicate that for all the minerals except Cr, the females who

ate less than the recommended intakes had higher values than the other females. Among the males, the group consuming less than RDA had higher means for Fe, Mg, and Mn, but lower means for Cr, Cu, and Zn than the other males.

TABLE V

MINERAL CONTENT OF HAIR (IN μg/g) ACCORDING TO PROTEIN INTAKES

Category	n[a]	Cr	Fe	Cu	Zn	Mg	Mn
			Females				
Less than RDA[b]		0.83 (5)	9.1 (4)	62. (4)	160. (4)	320. (2)	0.91 (4)
Equal to RDA		0.90 (57)	8.8 (48)	40. (48)	150. (48)	220. (34)	0.86 (48)
			Males				
Less than RDA	4	0.52	9.2	20.	110.	270.	0.55
Equal to RDA	16	0.62	6.4	43.	160.	240.	0.50

[a]Numbers of female subjects are shown in parentheses.

[b]RDA--Recommended Dietary Allowance.

Analyses of variance indicated no significant differences, associated with protein intake in hair mineral content of either sex when considered separately. In analyses of variance in pooled data (Table III) protein intake was not a significant source of variance except for

Cr. Females had significantly ($P < .05$) higher levels of Cr than males. There was also a trend toward those with higher protein intakes having higher Cr levels.

Mineral Values in Hair

Each mineral is discussed separately with the means and ranges reported. (A summary of the means and ranges is presented in Table II.) Correlations that were found with the other minerals are also reported. These findings are further compared with previously reported levels of these minerals in human hair. Previous data are reported from studies using a wide variety of analytical methods and reporting measures. For this reason, exact comparisons are difficult.

Chromium

Means and Comparisons. The mean values for Cr in the hair of the subjects are presented in Table VI. Hair Cr values have been reported that range from 0.2 to 20.0 ppm (µg/g) with a mean value of 0.5 µg/g (12, 33). Using methods similar to those in this study, Blalock (12) reported a mean Cr value of 1.056 µg/g with a range of 0.06 to 3.0 for older females. Chan (26), who was working with college students, reported a mean of 0.660 µg/g and a range of 0.382 to 1.813. As in this study, Chan found a slightly lower mean for females (0.638 µg/g) than for males (0.684 µg/g). Hambidge (59) found normal children have a mean hair Cr value of 0.850 µg/g. Thus the means reported in this study, although slightly higher, appear to be comparable to those reported by others.

TABLE VI

MEAN CHROMIUM VALUES (IN μg/g)

Group	n	Means	Ranges
Females	62	0.90	0 - 2.4
Males	26	1.0	0 - 3.8
Males and Females	88	0.93	0 - 3.8

An interesting point raised in this research is that all subjects who had low caloric intakes had higher mean hair Cr values (Table IV). It has been determined that glucose and/or insulin mobilize Cr from body stores (1, 9). It could be postulated that persons with high caloric intake use more Cr and over time deplete their Cr stores. In contrast with subjects of low caloric intake, those with low protein intake (Table V) had less hair Cr.

Recent studies have shown meat products which are good protein sources, are also good sources of biologically active Cr (3, 10). However, only five females and four males fell in the low protein intake category. Further, mean differences amounted to only 0.10 μg/g or less.

Correlations. Tables VII, VIII, and IX present the intercorrelations among the six minerals for the total sample, the females, and the males. In this study there was a positive correlation between Cr and Fe for the males ($P = .01$) and for all subjects combined ($P = .02$). Correlations found between Cr and the other four minerals were nog significant statistically.

TABLE VII

CORRELATION MATRIX FOR MINERALS IN HAIR OF MALES AND FEMALES

	Cr	Fe	Cu	Zn	Mg	Mn
Cr		.25*	-.00	.11	.06	.08
Fe			.17	.11	.16	.20
Cu				.07	.10	.10
Zn					.43**	.05
Mg						.27*
Mn						

*P $\leq$ .05.

**P $\leq$ .01.

TABLE VIII

CORRELATION MATRIX FOR MINERALS IN HAIR OF FEMALES

	Cr	Fe	Cu	Zn	Mg	Mn
Cr		.21	.02	-.19	-.23	-.04
Fe			.22	.02	.12	.09
Cu				-.11	.16	.14
Zn					.60**	.03
Mg						.29
Mn						

**P $\leq$ .01.

TABLE IX

CORRELATION MATRIX FOR MINERALS IN HAIR OF MALES

	Cr	Fe	Cu	Zn	Mg	Mn
Cr		.47**	-.03	.30	.23	.32
Fe			.04	.45*	.41*	.63**
Cu				.30	.05	-.01
Zn					.22	.20
Mg						.34
Mn						

*P $\leq$ .05.

**P $\leq$ .01.

Iron

Means and Comparison. The means and ranges for the Fe content in the hair of the subjects are shown in Table X. Chan (26) has reported a mean Fe content of 14.777 µg/g in hair of 10 college students. Although her mean is higher than the combined mean reported in this study, Chan, too, found males to have somewhat lower mean levels of hair Fe (14.35 µg/g) than females (15.41 µg/g).

Harrison (27) reported a mean value for iron in males of 15.3 with a range of 9.2 to 38.6 µg/g. Values ranging from 0.8 through 141 have been published, but the methods used varied widely. The results regarding Fe, considering sex, body size, caloric and protein intakes (Tables I, II, IV, and V), were not expected. Those with low intakes

of protein (usually a good Fe source) were found to have more Fe in hair. Also, students who had a low intake of calories and those who were underweight had higher mean levels of Fe than those who ate more and those who were overweight. Females had a higher Fe value than males (8.9 vs. 7.5 μg/g). Since females had a higher Fe requirement, lower hemoglobin and erythrocyte values, and more prevalent iron deficiency, finding a higher value for Fe in the hair of females was not anticipated.

TABLE X

MEAN IRON VALUES (IN μg/g)

Group	n	Means	Ranges
Females	52	8.9	2.0 - 35
Males	26	7.5	3.5 - 18
Males and Females	78	8.4	2.0 - 35

Correlations. The Fe content in the hair of the male subjects was significantly correlated with all of the other minerals in the study except Cu as reported in Table IX. In contrast, levels of Fe were not significantly correlated with any other minerals for the females (Table VIII). In the combined male and female sample, there was a significant correlation between Fe and Cr (Table VII).

Copper

Means and Comparisons. The means and ranges of the hair Cu content in the subjects are presented in Table XI.

TABLE XI

MEAN COPPER VALUES (IN μg/g)

Group	n	Means	Ranges
Females	52	42	8.4 - 130
Males	26	42	7.5 - 150
Males and Females	78	42	7.5 - 150

Although individuals differed, the means for both males and females were identical. Mean Cu values in hair reported by other workers were generally lower than the values reported in this study, varying from 11 μg/g through 30 μg/g (26, 27, 34, 35, 36, 37). Copper levels in hair have been reported to range from 8 to 250 μg/g (35). Copper values in hair have further been reported to vary with age, sex, and distance from the scalp. Previous studies have not generally reported the sex or age of subjects, and sample preparation and analytical methods vary greatly. Therefore, comparisons of results are difficult.

Correlations. No correlations between Cu and the other minerals proved to be statistically significant. See Tables VII, VIII, and IX.

Zinc

Means and Comparisons. The means and ranges of the Zn concentration in the hair of the subjects are presented in Table XII.

TABLE XII

MEAN ZINC VALUES (IN μg/g)

Group	n	Means	Ranges
Females	52	150	63 - 490
Males	26	180	79 - 480
Males and Females	78	160	63 - 490

A normal range of 100 to 200 ppm (μg/g) of Zn has been reported in human hair (21). Denver school children, as reported in a study by Kelvey (45), were considered deficient if hair values were below 70 ppm. Strain et al. (16) reported that individuals with about 45 ppm had Zn deficiency symptoms, while normal and repleted subjects had mean values of 103 and 121 ppm. They reported a mean hair Zn value for American males of about 119 ppm. Others using a variety of methods found "normal" means of from 163 to 181 with ranges from 24 to 562 ppm (27, 33).

Although sex differences have not been reported in regard to Zn levels in hair, males in this study had higher mean values than females. In regard to body size and protein consumption comparisons, Zn values were different for males and females (Tables II and V).

In this survey, 11 of the students including nine of the females, had hair Zn levels below 100 µg/g, and two of the females had levels below 70 µg/g. These subjects may have had insufficient Zn stores since values below 100 µg/g indicate low reserves. The 11 observations of low Zn concentrations are presented in Table XIII.

TABLE XIII

OBSERVATIONS OF ZINC LEVELS BELOW 100 µg/g

Sex	Zn in Hair (µg/g)
M	94
F	94
F	80
M	79
F	75
F	74
F	73
F	71
F	71
F	63
F	63

Correlations. Correlations between Zn and Fe in the male subjects were significant (P = .02) as reported in Table IX. Zinc and Mg were

significantly correlated in both the female group ($P < .01$) and the total sample ($P < .01$) as reported in Tables VII and VIII.

Magnesium

Means and Comparisons. The mean values and ranges of individual values found for Mg in the hair of the subjects are given in Table XIV. Hair Mg was found in larger amounts than any other mineral tested in this study. Magnesium is also the most plentiful in the human body of the minerals studied.

TABLE XIV

MEAN MAGNESIUM VALUES (IN µg/g)

Group	n	Means	Ranges
Females	36	220	89 - 400
Males	26	250	71 - 390
Males and Females	62	230	71 - 400

The mean hair Mg content was lower for females than males. In the body size comparison, the overweight females showed the highest hair Mg and at 260 µg/g, this was the same as the overweight males but less than underweight males (Table II).

Based on this relationship between caloric intake and estimated Mg content of food (120 mg/1000 kcal), only 11 of the subjects would have

received the Recommended Dietary Allowance for Mg. However, except for one high value for one overweight male, there seemed to be no particular relationship between calorie intake or body size and Mg level in hair.

Correlations. Magnesium levels in hair were correlated with Fe in the males ($P < .04$), with Zn in the females ($P < .01$), and with Zn in the pooled data ($P < .01$). See Tables VI, VIII, and IX.

Manganese

Means and Comparisons. The mean Mn values in hair and the high and low values found are presented in Table XV.

TABLE XV

MEAN MANGANESE VALUES (IN μg/g)

Group	n	Means	Ranges
Females	52	0.87	0 - 4.0
Males	26	0.65	0 - 2.2
Males and Females	78	0.79	0 - 4.0

Chan (26) found a mean Mn concentration of 0.855 μg/g in the hair of college students with little difference between males and females. Her values are similar to the values in this study, but females tended to have higher mean Mn values than the males.

Correlations. Positive correlations were found between Mn and Fe in males ($P < .01$) and between Mn and Mg in the pooled data ($P = .04$). See Tables VII and IX.

Correlations Among the Minerals

Tables VII, VIII, and IX present the intercorrelations between the six minerals involved in this study based on the data for the total sample. Causes and practical importance of these correlations are not apparent and would indicate the need for further study. It is noteworthy that significant Fe correlations in the males were found with all but one of the other minerals, and Fe was involved in every correlation for the males. However, there were no significant correlations between Fe and other minerals in hair of females. There were no significant correlations found with Cu in any of the analyses, although one reference cited a correlation between the levels of Zn and Cu in hair (35).

CHAPTER V

SUMMARY AND RECOMMENDATIONS

Summary

College students in a basic nutrition class, 26 males and 62 females, participated in the study. An evaluation of their dietary intakes on four pre-selected days approximately one month apart was made. The protein and calorie intakes were determined for each subject on each of the four days. Data on the height and weight of each student were also collected.

Each subject was asked to submit an approximate two gram hair sample on which analyses for six minerals were made. Relationships between body size, protein intake and calorie intake with each of the six minerals in the hair were determined. The mean values for each mineral in the hair of the subjects were compared with values reported by other researchers. Statistical analyses were further calculated and reported.

Minerals determined were chromium, iron, copper, zinc, magnesium and manganese. Correlations between the minerals were further determined and reported.

In regard to body size-mineral relationships, the Cr content of hair was found to be highest in underweight subjects, both male and female. This was significantly higher ($P < .05$) than the Cr levels of

the normal and overweight subjects. Females of normal weight had higher hair levels of Fe and Cu than did males at any weight. Underweight males had the highest mean level of Zn while females overweight showed the highest. Hair Mg tended to be higher with higher weight for females but not males. Females had higher levels of Mn than males but differences were slight within groups.

In calorie intake-mineral relationships, subjects with low calorie intake had somewhat higher mean levels of hair Cr. Females with low calorie intake had higher levels of Fe and Cu but males with normal and high intakes had the higher Fe and Cu levels. Females of low caloric intake had higher hair Cu concentrations ($P < .05$) than females in the other categories. The Zn levels did not vary widely for males with normal intakes. When Mg data for one male is excluded, both females and males with low calorie intake had higher Mg levels than those of normal intake. Females with a high intake of calories had hair Mn levels significantly higher ($P < .05$) than the other two categories as did all high calorie intake subjects pooled ($P < .05$).

In regard to protein-mineral relationships, with the exception of Cr. females with protein intake at less than RDA level had higher values of all minerals. Among males, the group consuming less than RDA amounts of protein had higher means for Fe, Mg and Mn but lower means for Cr, Cu and Zn than the other males. Females had significantly higher ($P < .05$) levels of Cr than did males. There was also a trend toward those with higher protein intakes having higher Cr levels.

The mean hair Cr for the combined groups was 0.93 µg/g with a range of 0 to 3.8 µg/g. The hair Fe levels were 8.4 µg/g combined with a range of 2.0 to 35 µg/g. Copper levels were determined to range between 7.5

and 150 μg/g with a mean of 42 μg/g. Zinc hair levels ranged from 63 to 490 μg/g with a mean of 160 μg/g. For Mg, a mean of 230 μg/g was determined with a range of 7 to 400 μg/g while Mn ranged from 0 to 4.0 with a mean of 0.79 μg/g.

Among females, there was a significant correlation only between mean hair content of Zn and Mg ($P < .01$). However, there were correlations in the mean content of males' hair between Mn and Fe ($P < .01$), Cr and Fe ($P < .02$), Fe and Zn ($P < .03$), and Fe and Mg ($P < .04$). When data for both sexes were combined, there were correlations between Mg and Zn ($P < .01$), Cr and Fe ($P < .03$), and Mg and Mn ($P < .04$).

Recommendations for Further Study

Several important findings were developed during this study that merit further investigation.

1. Particularly recommended for further investigation is the indication that some of the female subjects, all of childbearing age, had deficient Zn stores. Normal values of hair Zn are between 100 and 200 μg/g, with below 70 μg/g considered deficient. There were 11 individuals in the study with subnormal hair Zn values, and of these, nine were females (17 per cent of the women of the study). Two of the females had values below 70 μg/g. In view of the research linking Zn deficiency during pregnancy with a high incidence of birth defects in animals, and its known role in RNA and DNA synthesis, further study of Zn levels among women in this age group is indicated. The possible effect of Zn deficiency on both male and female gamete development would seem to indicate the need for further study.

2. In this study subjects were asked to keep food records for one day a month for four months on the assumption that a four-day study would yield a better estimate of the individual's food habits over a longer period of time than would a single-day record. The dietaries kept by the students could be compared on a day-by-day basis with the mean intake for the four days to determine whether the four-day study is a more precise indicator of food habits. Time savings might be shown if the precision of a one-day study were defined and shown to yield comparable results as a study covering several days.

3. A comparison of minerals in the dry weight versus ashed weight of hair, with the protein and lipid content of the hair, would determine whether the protein and lipids in hair increase or dilute the mineral content.

4. Human hair should continue to be analyzed and data reported leading to the establishment of normal values of minerals usually found in hair and to the use of hair as a biopsy material. Methods of analysis and reporting of data should be standardized.

5. Analytical methods for minerals, such as Cr, found in foods in very small amounts (ng/g), should be improved. Such methods would allow comparison of actual levels of trace minerals in foods eaten with the actual content of minerals in hair. Tables showing trace mineral content of foods could also be used to compare intakes over a period of time to stored levels in hair.

6. A comparison of hair levels of minerals to levels in other body tissues such as blood would be useful in evaluating the meaning of the findings.

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APPENDIXES

APPENDIX A

LETTER AND MENU PRINTOUT

OKLAHOMA STATE UNIVERSITY

MEMORANDUM

DATE: October 7, 1974

TO: FNIA 1112 Students

FROM: Esther Winterfeldt, FNIA Dept. Head Esther Winterfeldt

SUBJECT: Chromium Research

Thank you very much for your participation in our dietary chromium study. We hope you are making progress in collecting your hair sample. If you have any questions about this research or your part in it, feel free to call the Department Office (6007) or Mrs. Knight (7113). The next day that we want you to keep tabs of your food intake is this Wednesday, October 9. We are attaching a menu printout sheet for you to use. Please return it Thursday morning via campus mail in the envelope we have provided.

For those of you who eat in the dorms, it is not necessary for you to estimate the size of serving of the foods served on the line since we will pick up and measure a "serving" of each of these items - just an indication of the number of servings is sufficient. It would be helpful if you indicate serving sizes on self-serve items such as peanut butter. Also, an estimate of serving size needs to be made for foods eaten away from the dorm cafeteria; and, somewhere, on the menu printout sheet, write in an estimate of your water intake.

For you who do not eat at the dorms, please fill out the data on the top of the menu printout sheet; then just use the back side of the sheet to record the food eaten and the amount. Also, estimate your daily water intake.

We hope that our research will add needed information to the national pool of knowledge concerning dietary chromium, and you are a significant part of this research.

NAME_____ STUDENT COMPUTER NO._____ STILLWATER ADDRESS_____

SECTION_____ DATE_____ HEIGHT_____,WT._____,AGE____ SEX_____ DIABETIC?_____

6 AM TO 10 AM

AMT ITEM

___ TOMATO JUICE
___ ORANGE JUICE
___ APRICOT NECTAR
___ BANANA
___ STEWED PRUNES

___ HARD OR SOFT COOKED EGG
___ SCRAMBLED EGGS
___ BACON (HOW MANY SLICES?)

COLD CEREAL (WHICH ONES?)
___ _____
___ _____

___ WHITE TOAST
___ WHOLE WHEAT TOAST
___ JELLY
___ MARGARINE
___ PEANUT BUTTER

___ FRIED CINNAMON ROLLS
___ APPLESAUCE CAKE DONUT

___ HOMO MILK ___ SKIM MILK
___ BUTTERMILK ___ CHOC MILK
___ COFFEE ___ TEA
___ SUGAR ___ CREAM
___ EXTRA SALT ___ PEPPER
___ HOT CHOCOLATE

VITAMIN SUPPLEMENT (BRAND NAMES)
___ _____
___ _____

OTHER THINGS
___ _____
___ _____
___ _____
___ _____

10 AM TO 4 PM

AMT ITEM

___ HOT TURKEY SANDWICH/GRAVY
___ HAM & BEANS

___ MASHED POTATOES
___ GRAVY
___ GLAZED APRICOTS
___ 7 MINUTE CABBAGE

___ TOSSED SALAD
MAKE YOUR OWN SALAD
___ TOSSED SALAD
___ TOASTED ROLL SLICES
___ CARROT STICKS
___ RADISHES
___ CUCUMBERS
___ JULIENNE MEAT
___ PARMESAN CHEESE
___ SALAD DRESSING (CHEESE)
___ SALAD DRESSING (FRENCH)
___ SALAD DRESSING (OIL & VINEGAR)
___ SALAD DRESSING (THOUSAND ISLAND)
___ COTTAGE CHEESE
___ RED PEPPERS
___ ORANGE GELATIN/ORANGE & GRAPEFRT

CONDIMENT CART
___ TOMATOES
___ ONIONS
___ PICKLES
___ TOMATO & RICE SOUP

___ WHITE BREAD
___ WHOLE WHEAT BREAD
___ CORNBREAD
___ JELLY
___ MARGARINE
___ PEANUT BUTTER

10 AM TO 4 PM (CONTINUED)

AMT ITEM

___ CHOC. CAKE/WHITE ICING
___ OATMEAL COOKIES
___ APPLE DUMPLINGS
___ ICE CREAM (CHOC.)
___ ICE CREAM (VANILLA)
___ FRUIT

___ HOMO MILK ___ SKIM MILK
___ BUTTERMILK ___ CHOC MILK
___ COFFEE ___ TEA
___ SUGAR ___ CREAM
___ EXTRA SALT ___ PEPPER
___ FRUIT DRINK ___ COKE
___ MR. PIP ___ SPRITE

___ HAMBURGER
___ BUN
___ POTATO CHIPS

OTHER THINGS
___ _____
___ _____
___ _____
___ _____
___ _____
___ _____
___ _____

FROM 4 PM TO 3 AM

AMT ITEM

___ LIVER & ONIONS
___ ROAST BEEF
___ DRESSING & GRAVY

___ PARSLIED POTATOES
___ GREEN BEANS
___ MIXED VEG. IN CREAM

___ TOSSED SALAD
___ SALAD DRESSING (FRENCH)
___ SALAD DRESSING (CHEESE)
___ SALAD DRESSING (OIL & VINEGAR)
___ SALAD DRESSING (THOUSAND ISLAND

___ COTTAGE CHEESE
___ BACON (HOW MANY SLICES?)

___ CHERRY GELATIN /ICE CREAM

___ CARROT STICKS
___ CELERY STICK
___ RADISHES

___ WHITE BREAD
___ RYE BREAD
___ WHOLE WHEAT ROLL
___ MARGARINE

___ CHOC. CAKE/WHITE ICING
___ COCONUT CREAM PIE
___ ICE CREAM (CHOC.)
___ ICE CREAM (VANILLA)
___ FRUIT

___ HOMO MILK ___ SKIM MILK
___ BUTTERMILK ___ CHOC MILK
___ COFFEE ___ TEA
___ SUGAR ___ CREAM
___ EXTRA SALT ___ PEPPER
___ FRUIT DRINK ___ COKE
___ MR. PIP ___ SPRITE

OTHER THINGS
___ _____
___ _____
___ _____
___ _____
___ _____

APPENDIX B

MINERALS IN HUMAN HAIR, AND SIZE, CALORIE, AND PROTEIN EVALUATIONS

TABLE XVI

INDIVIDUAL DATA FOR MINERALS IN HUMAN HAIR, BODY SIZE, CALORIC AND PROTEIN ADEQUACY BY SEX

Cr	Fe	Cu	Zn	Mg	Mn	Body Size[1]	Caloric Adequacy[2]	Protein Adequacy[3]
				Females				
0.40	6.50	27.49	262.45	359.93	0.50	3	1	2
0.60	4.50	16.95	146.11	264.29	0.30	3	1	2
0.99	4.96	52.56	126.44	309.90	3.47	3	2	2
0.80	6.48	27.92	70.80	119.66	0.50	2	1	2
0.74	6.80	41.47	168.84	313.98	0.44	1	1	2
0.80	5.99	26.97	232.24	399.96	1.00	3	1	2
0.40	7.41	13.82	493.73	335.74	0.99	3	2	2
1.53	5.43	27.99	157.99		0.85	2	1	2
1.57	9.35	39.37	262.82		0.79	1	2	2
0.70	2.00	10.00	134.03	144.70	0.50	2	2	2
0.80	7.97	127.99	152.39		1.49	2	1	2
1.48	8.88	49.84	201.34		1.23	3	1	1
1.69	7.45	34.25	134.03	233.32	0.99	2	1	2
0.47	9.39	11.74	152.20		4.04	1	3	2
0.90	10.47	42.86	239.23		1.50	1	1	2
1.00	5.49	24.94	161.63		0.50	3	1	2
1.48	5.25	18.95	124.80		0.74	1	2	2
0.46	5.95	35.26	178.57		0.18	1	2	2
0.99	9.41	8.42	178.27		0.50	2	1	1
0.50	6.99	16.47	152.68		1.00	3	1	2
1.00	12.50	23.50	131.50	154.98	0.10	2	1	2
0.70	5.50	12.99	79.96	127.44	1.50	2	1	2
1.08	11.32	32.35	152.59	242.64	1.08	1	1	2
0.70	10.48	69.36	118.26	251.50	0.20	3	1	1

TABLE XVI (Continued)

Cr	Fe	Cu	Zn	Mg	Mn	Body Size[1]	Caloric Adequacy[2]	Protein Adequacy[3]
0.60	3.50	59.93	74.91	117.36	0.50	2	2	2
0.00	8.50	12.50	73.00	130.00	0.50	1	2	2
1.80	10.49	30.47	73.93	133.37	0.20	1	2	2
0.80	15.44	52.29	125.98	196.69	1.00	1	2	2
1.00	9.50	13.50	106.48	178.46	0.30	3	2	2
0.99	6.47	17.90	62.66	161.63	0.99	3	2	2
0.50	11.99	96.40	126.37	259.74	0.20	1	1	2
1.03						1	1	2
0.79	13.38	70.86	141.72	252.73	1.98	2	1	2
0.49						1	2	2
1.59						1	1	2
0.00						3	1	2
0.48						3	2	1
1.39	25.87	57.21	127.35	353.20	0.90	2	1	2
0.51	7.60	119.81	139.22	379.62	1.69	2	1	1
1.32	5.88	40.39	169.65	279.08	0.59	3	1	2
0.00	7.00	26.76	177.40	166.32	0.00	2	1	2
0.80	7.50	16.49	129.45	217.91	0.50	1	2	2
2.40	6.49	24.96	93.84	88.85	0.50	1	1	2
0.89	7.45	24.83	119.67	150.46	0.30	1	2	2
0.00	5.82	27.17	148.50	281.44	0.97	2	1	2
0.90	6.00	76.92	182.30	219.76	0.50	3	1	2
0.40	8.00	98.98	153.97	211.96	1.00	2	1	2
1.66	10.39	24.95	63.40		1.04	1	1	2
1.36	8.23	43.07	153.91		0.00	1	1	2
0.98	5.90	29.51	230.20		0.79	1	3	2
1.16	11.60	76.39	70.59	103.47	1.45	2	1	2
0.88	9.32	39.24	138.33		0.78	3	1	2
0.60	8.97	24.41	206.24		0.50	2	2	2

TABLE XVI (Continued)

Cr	Fe	Cu	Zn	Mg	Mn	Body Size[1]	Caloric Adequacy[2]	Protein Adequacy[3]
1.10	11.00	68.50	110.00	232.00	0.50	2	1	2
1.40	10.50	124.44	103.95	137.81	0.90	3	1	2
1.05						2	2	2
0.98						2	2	2
1.19						2	1	2
1.26	34.72	52.08	209.91	236.74	1.26	2	1	2
0.47						2	1	2
0.78						2	2	2
0.40	6.50	22.99	127.95	209.91	0.80	2	1	2
				Males				
0.80	9.50	152.42	334.83	329.44	1.00			
1.50	8.49	7.49	335.20	224.80	1.00			
0.60	7.49	17.96	261.98	194.61	1.00	2	1	2
0.90	9.50	30.99	249.90	249.90	0.50			
0.30	6.50	27.50	262.45	359.93	0.50	2	1	2
0.20	4.50	8.50	143.43	84.96	0.40	2		1
3.24	10.36	20.08	187.82	272.02	0.65	2	1	
0.30	4.50	13.49	94.42	274.78	0.20	2	1	2
0.80	13.50	85.00	482.00	285.00	0.50	1	2	2
0.77	6.40	20.49	125.48	140.85	0.26	1	2	2
0.80	8.49	43.96	101.92	319.74	0.50	2	1	2
0.80	5.00	20.99	102.44	249.85	1.00	2	1	2
0.80	12.97	11.47	78.81	364.13	0.50	3	1	1
0.85	5.86	95.80	105.40	90.50	0.53	2	1	2
1.10	4.50	84.90	115.48	89.85	0.10	2	1	2
0.30	5.00	22.98	187.31	204.80	0.50	2	2	2
2.12	9.80	30.21	165.77	383.80	1.60	2	1	

TABLE XVI (Continued)

Cr	Fe	Cu	Zn	Mg	Mn	Body Size[1]	Caloric Adequacy[2]	Protein Adequacy[3]
0.30	6.48	49.91	131.75	254.52	0.80	2	1	1
0.60	5.50	49.96	131.39	394.68	0.30	1	3	2
3.77	5.66	58.49	309.43	301.89	0.00	1	2	
0.60	7.00	96.47	151.95	294.91	1.00	3	1	2
0.85	7.11	21.33	102.36	71.08	0.28	2	1	2
0.30	6.00	34.49	137.97	333.93	0.50	2	1	2
3.14	17.95	22.44	240.13	336.63	2.24	1	1	
0.00	4.48	36.35	126.48	189.22	0.50	3	1	
0.70	3.50	17.98	109.90	259.77	0.40			2

[1]Size: 1 = underweight, 2 = normal weight $\pm$ 8 to 9 per cent, 3 = overweight.

[2]Calories: 1 = low, 2 = normal $\pm$ 200 kcal, 3 = high.

[3]Protein: 1 = less than RDA, 2 = equal to RDA.

VITA

Norma Sue Knight

Candidate for the Degree of

Doctor of Philosophy

Thesis: COMPARISON OF NUTRITIONAL ADEQUACY AND OTHER VARIABLES WITH CHROMIUM, IRON, COPPER, ZINC, MAGNESIUM, AND MANGANESE CONCENTRATIONS IN HUMAN HAIR

Major Field: Food Science

Biographical:

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