RELATIONSHIP BETWEEN ZINC AND COPPER NUTRITIONAL STATUS AND RISK FACTORS ASSOCIATED WITH CARDIOVASCULAR DISEASE

by

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INTRODUCTION

Cardiovascular disease (CVD) presently stands as the leading cause of death in the United States and is most probably due to a multitude of factors. The multi-faceted etiology of atherosclerotic CVD has been repeatedly emphasized and amply documented.

The dietary characteristics which have most often been considered as possible risk factors are: a) an overconsumption of foods (1,2); b) an excess of dietary fat (3-5); c) a low ratio of polyunsaturated to saturated fatty acids (P/S ratio) in the diet (6,7); d) an excess of dietary cholesterol (8-10); e) an excess of dietary sucrose (11,12); f) a high consumption of common salt (13,14); g) a deficiency of dietary fiber (15-17); and h) the softness of the water supply (18-20). Other dietary factors which have been suggested as relevant include: a) an excess intake of coffee (21); b) an excess consumption of alcohol (22); c) an excess or deficiency of certain vitamins, specifically pyridoxine (23), thiamin (24), and ascorbic acid (25,26); d) a deficiency of chromium in foods (27); and e) an imbalance of certain trace elements (28-31). The possible importance of trace elements with reference to CVD requires further investigation.

The research study herein focused specifically on a postulated relationship between CVD and an altered balance of zinc and copper. The principal investigator in this area, Dr. L. M. Klevay, a researcher with the USDA Human Nutrition Laboratory, has proposed that an epidemiologic and metabolic imbalance of zinc and copper, as characterized by a high ratio of zinc to copper, is a major factor in the etiology of

CVD (32). Klevay has attempted to associate this ratio of zinc to copper not only with many aspects of CVD, but also with cholesterol metabolism. In reviewing the literature, it appeared that such a proposed alteration in the ratio of zinc to copper, as the primary cause of CVD, was a highly speculative one. Whether it is associated with the cause, however, remains to be established. Klevay's proposed relationship has not yet been satisfactorily proven experimentally.

This research study was designed to test the hypothesis that there is no statistically significant relationship between the Zn/Cu ratio, as characterized by a high ratio of zinc to copper, and the presence of risk factors associated with susceptibility to CVD. The objectives of this investigation were:

1. To determine the Zn/Cu ratio of hair samples from male subjects, ranging from 19 to 59 years of age.
2. To determine if an increasing or decreasing zinc and/or copper content of hair existed with increasing age.
3. To test for a statistically significant relationship between the Zn/Cu ratio and the presence of risk factors (hypertension, obesity, elevated serum cholesterol and triglycerides, cigarette smoking, and exercise-activity level) associated with susceptibility to CVD (33).

REVIEW OF LITERATURE

Cholesterol and saturated fat have been the dietary agents most closely related to coronary heart disease (CHD), but the ratio of zinc to copper may be the preponderant factor in the etiology of the disease. Attempts have been made to relate a high ratio of zinc to copper, as found in preliminary investigations (34), to a variety of epidemiological features of CHD, as well as to the metabolism of cholesterol. Such epidemiological parameters examined included: a) the increased consumption of fat; b) the increased consumption of sucrose; c) the decreased consumption of vegetable fiber; d) consumption of soft water; e) lack of exercise; and f) the zinc and copper content in human foods and/or diets, as well as various alterations in the physiologic status that produced adverse changes in the distribution of zinc and copper in certain important organs.

In a preliminary investigation, Klevay (34) produced hypercholesterolemia in rats by an increase in the ratio of zinc to copper ingested. Experimental rats received solutions containing concentrations of zinc and copper ranging from 10 to 20 and 0.25 to 0.5 ug/ml. respectively (Zn/Cu ratio-40). The concentrations of zinc and copper in the control solutions were 10 and 2 ug/ml, respectively (Zn/Cu ratio-5). Such a ratio was considered high for the rat but may or may not be necessarily high for a human. As nutritional requirements vary among species, so may optimal ratios between nutrients vary among species.

In an attempt to extend his hypothesis to include those changes in risk of CHD associated with changes in the amount of dietary fat

consumed, Klevay (35) tested the hypothesis that foods with a large amount of fat were also foods with a high ratio of zinc to copper. The median value of 1.1 g fat/100 g of food and a median zinc to copper ratio of 11.2 were found. Approximately two-thirds of the foods with Zn/Cu ratios above the median were of animal origin. Although the association of fat and the ratio of zinc to copper of foods was quite strong as measured by chi-square ($p < 0.001$), the association was not perfect. Certain foods were high in fat and had a low Zn/Cu ratio (e.g. beef liver); others were low in fat and had a high Zn/Cu ratio (e.g. cauliflower). Although, on the 'average', diets high in fat had a high ratio of zinc to copper, exceptions were evident. The data did not permit any statement regarding the relationship of the ratio of zinc to copper to the quality of the fat in the foods. If associations with risk of CHD or the metabolism of cholesterol are being sought, establishment of a relationship among zinc, copper, and the quality of fat could provide a possible important extension of this hypothesis.

In an attempt to summarize available data on the ratio of zinc to copper in human diets, Klevay (36) reviewed the work of several investigators who had measured both zinc and copper in meals and diets. The Zn/Cu ratio showed a twelve-fold variation (a range of 5 to 61) with a median of 27 reported. The amount of zinc in most of the diets considered in these studies was close to that required by adults. Those diets with a high ratio of zinc to copper contained less copper than the amount thought to be required. Thus, it was possible that an absolute deficiency of copper could have contributed significantly to

the increase in the risk of CHD as associated with a high ratio of zinc to copper. But whether such a metabolic imbalance was due to a relative or an absolute deficiency of copper remains to be more intensely studied.

From a study (37) of analyses of food in the United States in 1942 and 1966 it was concluded that the amount of copper in food decreased during that period. During that same period risk of death from CHD increased steadily in spite of a probable increase in the intake of polyunsaturated fat. Such a fall in the amount of copper in food was consonant with Klevay's Zn/Cu ratio hypothesis.

In a more recent study, Klevay (38) attempted to test the hypothesis that a positive association existed between the risk of death due to CHD in 47 cities in the United States and the availability of milk with a high ratio of zinc to copper. That is, the risk of mortality was proposed to increase as the ratio of zinc to copper in milk increased. Mortality rates were used directly; ratios of zinc to copper were calculated from the mean values of zinc and copper published for each city. Calculation of the correlation coefficient between these two measurements gave a $p < 0.02$ value. This correlation accounted for 12.5% of the variation in mortality. On this basis, at least some of the geographic variation in the risk of CHD may have been explained.

The ratio of zinc to copper of human milk calculated from the work of several authors was shown by Klevay (36) to be approximately 6 in contrast to about 38 for cow's milk. Klevay (36) postulated that

if breast feeding was considered as having an apparent protective effect in regard to coronary artery atherosclerosis, a ratio of 6 might be judged then as desirable for infants, with a ratio of 38 as being possibly too high.

In a more recent investigation concerning the interrelationships among zinc and copper, lipid metabolism and CHD, Klevay (39) demonstrated that dietary fat had less effect on serum cholesterol than the Zn/Cu ratio. Rats fed 55% lard and a low ratio of zinc to copper did not become hypercholesterolemic, whereas rats fed lard and a high ratio of zinc to copper exhibited very high serum cholesterol levels. Bustamante et al. (40) reported results which support Klevay's hypothesis in the sense that the reported increase in the Zn/Cu ratio in subjects with arteriosclerosis was associated with altered lipid metabolism. The arteriosclerotic subjects exhibited serum zinc and copper levels of 448 and 212 ug/100 ml, respectively. Other values reported included: ceruloplasmin 24, cholesterol 239, total lipids 1076 and triglycerides 105 mg/100 ml. Corresponding values for a group of healthy controls were 90 and 151 ug/100 ml 14, 174, 698, and 82 mg/100 ml.

As previously mentioned, large amounts of fat (3-5) and sucrose (11,12) have been among the dietary factors associated with an increased risk of CHD, whereas large amounts of dietary fiber (16) have been associated with a decreased risk. As a means of unifying and reconciling various and conflicting hypotheses of CHD, Klevay (33) attempted to relate such epidemiological and metabolic data to his

hypothesis of a metabolic imbalance of zinc and copper. Phytic acid, a naturally occurring chelating agent, has been shown to substantially decrease the absorption of zinc by man from the intestinal tract (41). Although no data on the absorption of copper were presented, at an alkaline pH in vitro a copper complex with sodium phytate was soluble whereas the zinc complex was insoluble. Thus in theory, Klevay (33) postulated that the phytic acid in diets low in fat and sucrose and high in fiber may have had the protective effect of decreasing the ratio of zinc to copper absorbed. On the other hand, it was likely that diets high in fat and sucrose and low in fiber contained zinc and copper in amounts that corresponded to a high ratio. Klevay (33) pointed out then that diets associated with a high risk of CHD were likely to contain zinc and copper in a high ratio and were low in phytic acid and fiber, chelating agents likely to have been protective against such a high ratio.

Hirst et al. (42) have shown that death due to CHD was less common among persons with cirrhosis than among control patients. Klevay (33) pointed out that patients with cirrhosis lost excessive zinc in urine, had less zinc and more copper in the liver, and had a lower ratio of zinc to copper in the liver. The lower ratio of zinc to copper in the liver was postulated to be singularly important as this organ is the major site of cholesterol synthesis and catabolism.

Men who exercised regularly were shown to have a lower risk of CHD than men who did not exercise (43). Prasad et al. (44) have reported that the concentration of zinc (0.93 mg/l) in sweat was approximately

sixteen times that of copper (0.058 mg/1). Klevay (33) thus claimed that an increase in physical activity leading to an increase in sweating may have had the protective effect of causing a relatively greater loss of zinc than of copper from amounts already absorbed and available for metabolic use, resulting in a lower Zn/Cu ratio.

McKenzie and Kay (45) showed that among women randomly selected and not under therapy, those with elevated blood pressures had a ratio of zinc to copper in urine less than half that of normotensive women. This difference in the ratio was primarily attributed to a significantly greater excretion of copper by the hypertensive women. Such findings are in contrast to those of Olantunbosun et al. (46) who measured serum copper and zinc in hypertensive subjects and normotensive controls. Serum copper and total cholesterol were significantly increased in the hypertensive patients, but their serum zinc levels did not differ from those of the controls. Whether hypertension led to a metabolic imbalance of zinc and copper that produced hypercholesterolemia and increased the risk of CHD or whether a metabolic imbalance led to both hypertension and hypercholesterolemia has not been determined at this stage.

Ascorbic acid has been shown to decrease the absorption of copper from the intestinal tract (47). Consequently, ingestion of large amounts of ascorbic acid, as has become fashionable, Klevay (33) claimed might be expected to increase the ratio of zinc to copper absorbed from the intestinal tract and produce hypercholesterolemia.

Several reports (18-20) have demonstrated that residence in areas

where hard water was drank was associated with a lower risk of CHD. Biork et al. (20) found a negative correlation between the hardness of drinking water and the mortality form heart disease. Such a close relationship exists between the hardness of water and the concentration of calcium in the water that the United States Geological Survey reports the hardness of water as calcium carbonate. Additionally, the concentration of copper in hard water has been reported as higher than that in soft water; zinc concentrations have been reported as similar (20). Klevay (33) postulated that the drinking of hard water may exert another protective effect in two ways: 1) an increased consumption of calcium may cause a change in the distribution of zinc within the body resulting in a decrease in the ratio of zinc to copper in the liver; and 2) the higher concentration of copper in hard water would tend to decrease this ratio and that of the zinc to copper directly absorbed from the intestinal tract.

Kime and Sifri (48) raised several objections to the Klevay hypothesis. These investigators pointed out that the high dietary zinc effect on liver zinc content was only a species specific phenomenon. Such species variability concerning liver zinc accumulation should behoove one to practice extreme caution in the advocation of lower dietary zinc to copper ratios for humans; very little information exists concerning the human metabolic responses towards such dietary modifications. An increased zinc content in the liver can be due to other factors besides an increased dietary zinc; either metabolic abnormalities (hypothyroidism or diabetes) or a deficiency of Vitamin A

would allow zinc to build up in the liver and result in an increased ratio of zinc to copper. Kime and Sifri (48) further pointed out that endurance type exercises were more beneficial in improving the cardiovascular system when compared with exercises that produced massive muscle bulk which Klevay (33) claimed utilized more zinc.

Variations in the plasma levels of copper and zinc have been reported by several workers (49,50). Versieck et al. (51) reported results which did not tend to support an increase in the Zn/Cu ratio. Analysis of blood from 16 patients, following myocardial infarction, showed a significant increase in serum copper with a concurrent significant decrease in serum zinc.

Some of the knowledge of the pathology of cardiovascular disease has been augmented by the work of Webster (52) who estimated 26 elements in heart tissue from normal subjects and from persons who had suffered coronary infarction. The comparison was made first between normal tissue and undamaged tissue from victims of coronary infarction and secondly between damaged and undamaged myocardial tissue from the latter group. In the first series, myocardial tissue from victims of coronary infarction showed a diminished concentration of copper and molybdenum and an enhanced concentration of arsenic and cerium. In the second series of comparisons, damaged tissue showed (among others) diminished levels of cobalt, potassium, and zinc, and enhanced levels of calcium and sodium. Berman et al. (53) reported copper levels of from 127 to 467 ug/100 g in autopsied heart tissue.

Wacker et al. (50) first reported that plasma zinc concentrations

collected randomly, following acute myocardial infarction, were decreased. These observations have since been confirmed by Halsted and Smith (55). The decrease in plasma zinc concentrations was postulated to be due to a mobilization of zinc (very likely as a metalloenzyme) to the area of tissue injury to participate in the reparative processes of the myocardium. Small decreases were also reported for copper plasma levels, though no theories were proposed for such findings.

Copper has been shown to be involved in the maintenance of vascular integrity. Apparently, copper is concerned with the cross-linking of collagen and elastin (largely dependent upon the quality and quantity of each), which leads to stability of the large arteries. The role of copper in the maturation of these connective tissue proteins has been studied extensively. Carnes (56) has published an excellent review of the histopathology of connective tissue resulting from a copper deficiency.

That copper deficiency can result in cardiac lesions was first reported by Bennetts (57) who described "falling disease" in cattle. A sudden death was believed to be due to heart failure; the lesions consisted of atrophy of the myocardium. More recently, Kelly et al. (58) observed heart failure among young rats whose dams were fed a copper-deficient diet. In contrast to the lesions described in cattle, the hearts were grossly enlarged and some displayed aneurysms at the apex. Variable areas of necrosis were noted in both the ventricular and atrial musculature; hemopericardium frequently occurred. Although

defective connective tissue has been associated with CVD in some species, the lesions described above may have implicated a more direct myocardial defect.

No doubt, further information is needed about each aspect of the homeostatic mechanisms that control the normal distribution of zinc and copper in the body. Though it has been difficult to quantitate the zinc finally usuable by the body, several factors that effect both its absorbability and metabolic efficiency have been reported. Some of these factors were of a nutritional or dietary origin; others were metabolic or therapeutic in origin. Data on the zinc content of a diet has not necessarily been a reliable index of the amount of zinc available to meet nutritional needs. Abdulla and Norden (59) demonstrated that the availability of zinc from foodstuffs appeared to vary greatly among individuals, as did the effect of food on zinc metabolism. Not only has the zinc content of the diet been shown to be highly dependent on the dietary protein content, but the protein content of the diet has also appeared to influence the absorption and retention of zinc (60). Phytate has been shown to inhibit the absorption of zinc (61,62), and the presence of a high calcium intake (61) accentuated this inhibition. The gastrointestinal tract appears to play the major role in regulating body content of zinc through its capabilities for absorption and excretion of this trace element (63). Nonetheless, it has been ascertained that considerable zinc is not absorbed, or if absorbed, is not efficiently utilized and is quickly excreted.

In an examination of copper, concentrations in the blood appeared

to be primarily reflective of dietary intake (64). As with zinc, other dietary factors influenced its absorbability and availability: acids appeared to enhance its absorption (64); calcium, on the other hand, appeared to depress its absorption (64). Furthermore, Van Campen (65) and Magee and Matrone (66) have reported that excess zinc in the diet tended to result in a deficiency of copper, apparently by producing liver cytochrome oxidase and catalase activity.

Many drugs have been shown to contain an organic tertiary structure that chelates trace elements (67), with the net effect of increasing their excretion. This is known to occur both with zinc and copper. More data are needed on the effects of drugs on absorption, in particular, whether or not the drug or its excreted metabolite binds one or more trace elements when excreted, thereby causing an excessively elevated excretion.

For these reasons, and because of shared chemical properties, it appeared reasonable to conclude that measurements of the zinc and copper content of the subjects' normal diets would be of little aid in the assessment of long-term zinc and copper nutriture. Along these same lines, medications taken by the subjects in this study were not considered as a separate parameter. Knowledge of their intake was examined only insofar as it may have influenced the Zn/Cu ratios found in such individuals.

As CVD has most often been considered a long-term (chronic) disorder, measurement of the Zn/Cu ratio should be one which was the most reflective indices of long-term nutriture. Enzyme activity

measurements of copper and zinc have not been firmly established as valid nutritional status assessment techniques for these nutrients. The enzyme activity measurements have most often been applied to deficiency states and, as of yet, relatively little is known about the metabolic defects responsible for the classic deficiency symptoms. Zinc has been shown to be both a structural and functional component in a number of enzymes, i.e., the zinc metalloenzymes. Because of such a relationship, numerous studies have been published which have attempted to relate the appearance of deficiency symptoms to reduced enzyme activities. Reported observations have by no means been unanimous. Discrepancies have been attributed to the many differences in experimental conditions, diet compositions, assay procedures, and the base on which the enzyme activities were expressed. According to Prasad (68,69), the many metabolic processes regulated by zinc metalloenzymes are dependent upon the tissue levels of zinc available to control their synthesis and activity. Thus, it seems a rather difficult task to try to account for all the different factors of influence, in the available studies, on the response of zinc metalloenzymes.

Various findings have been reported on the carbonic anhydrase activity of different tissues and organs in relation to zinc deficiency. Researchers have observed essentially little change in the activity of this zinc metalloenzyme as compared to pair-fed animals (70-72). Even in severe deficiency states, an appreciable decrease in the activity of carbonic anhydrase was not noted. It has been postulated (73) that those metalloenzymes that bind zinc with a very high affinity were

still fully active, even in the extreme stages of zinc deficiency.

Most body zinc, with the exception of bone and hair, has been shown to exchange fairly rapidly with the loosely bound zinc in the plasma. Beisel and coworker (74) and Lindeman et al. (75) reported that plasma zinc depression accompanied periods of acute stress. In addition, a slight but definite circadian rhythm for plasma zinc has recently been detected in man (76,77). The rapidity with which biochemical changes arise in response to zinc nutriture would have presented possible complications in this study.

Hair content of zinc and copper has been reported to be a useful clinical index of status (78). A slow turnover rate of zinc and copper in the hair has been reported (79,80) and as such, values obtained appeared to be indicative of a long-term state. Briggs and Briggs (81), Klevay (82,83), and Petering et al. (84) have reported that concentrations of zinc and copper in hair appeared to reflect their nutriture.

Several investigators (82,84,85) have reported on hair as a suitable biopsy material. Hair has been reported also to have the advantage of ranking comparatively high among tissues in its content of zinc. In this particular study, hair was considered ideal for use as an assessment technique for zinc and copper nutritional status. The sample was readily obtainable, easily collected, conveniently stored, and did not readily deteriorate.

Hair is formed in the hair follicle out of relatively small protein molecules present in the serum. During growth, keratinization

takes place; the molecules become larger and -Zn-S- bridges are formed. In an autoradiographic investigation, Mawson et al. (86) showed that after treatment of rats with Zn-65, this isotope was found only near the hair follicle. These results indicated that at those spots, exchange of non-active Zn with Zn-65 was possible, whereas farther away from the follicle, after complete keratinization, Zn was firmly bound to sulfhydryl groups in such a way that a turn-over did not occur. Considerable differences between the Zn concentrations of hair from individual subjects suggested that the formation of hair was dependent upon physiological mechanisms which in turn were effected by the state of nutrition, in particular the availability of nutrients such as Zn. This theory has been supported in part by the effects of Zn-deficient diets on the content of Zn in hair (87,88).

Evidence has been obtained which indicates that Zn is firmly bound in hair and that the Zn content ordinarily undergoes little change over long periods of time. Other, more limited studies have indicated that changes did occur over relatively long periods of time; these changes were attributed to the natural growth processes and/or environmental contamination. Bate and Dyer (89) reported that fractions of many elements, one of them being Zn, are tightly bound in hair. From this, the authors concluded that it was these elements in hair which would be least affected by normal hair washing. Hildebrand and White (90) stated that once minerals were incorporated into the hair, they were no longer in any dynamic equilibrium with the rest of the body. These authors further pointed out that concentrations of

minerals found in hair were not reflective of the status of minerals in the body at the time the sample was collected, but at some prior time.

To date, relatively little information has been available concerning the factors that regulate the trace element content of hair. Considering the results of many studies, it was apparent that, although a large number of factors may influence the hair concentration of trace elements, each element was most probably effected by different factors. It has been postulated that as the list of influential factors increases, the reliability and use of hair analysis for trace metal content will increase. Age (82,84,88,90,93) and sex (81,84,90,92-94) were among the factors most frequently reported to influence the concentration of trace elements in hair. Additional factors reported included: geographic variations (97), ethnic origin (81), seasonal variations (87), time (98), and between-persons variability (106).

Reinhold et al. (88) in a study of the zinc content of hair from Iranian subjects attempted to match their urban and rural groups for age but admitted some lack of success. Anke and Schneider (94) reported that age influenced the zinc content of hair of the German population they studied. This was in agreement with the data reported by Klevay (82), who indicated that significant differences existed between age groups; changes in age were associated with changes in hair zinc. These changes in hair zinc with age were apparent in scatter diagrams which indicated a gradual decrease in the first decade, followed by a rise in the second decade. Such changes were independent of the age interval selected; consequently, the values were reported for half

decades. As no trend with age was noted over age 20, all the data on the older subjects were combined. By contrast, while investigating possible geographic variations of zinc concentrations in hair, Klevay (97) reported that no significant differences due to age were demonstrable for males in the Panamanian population studied.

The hair copper values for males 8 or more years old, reported by Klevay (82), were similar in magnitude to those reported by Gibbs and Walshe (92), Rice and Goldstein (99), and Martin (100) for industrialized populations, and Eminians et al. (101) for non-industrialized populations. The data presented by Klevay (82) for children less than 8 years old indicated hair copper levels higher than those control subjects in the kwashiorkor studies of MacDonald and Warren (102), Gopalan et al. (103), and Lea and Luttrell (104). These authors reported control values of between 10 and 20 ug/g. As Klevay (82) pointed out, it was improbable that these data and the data he presented were samples of the same statistical universe, and thus were not comparable.

Petering et al. (84) reported that the zinc content of hair in male subjects increased from age 2 to age 12 and thereafter declined slowly to age 80. This author also noted that the average content of zinc in the hair of females was very similar to that in the hair of males of comparable. However, the content of copper in the hair of females apparently followed a different pattern from that in the hair of males with respect to age. A seemingly slight increase, with age, of copper content in the hair of females was not statistically

significant; the decrease with age of copper content in males was significant (P=0.01).

Klevay's data (82,83) showed with considerable consistency that for males, at least, there were two age ranges with differing influences on the concentrations of zinc and copper in the hair, namely, the group younger than 12 years and the group over 12 years. Within either of these two groups, the concentrations of the respective metals were closely related to age. The decrease of zinc and copper levels in the hair of subjects from 12 to 80 years of age was of interest. Klevay suggested that such a decrease may have been a reflection of changes in nutrition with increasing age or it may have been indicative of environmental effects on trace metal metabolism in the adult population. Quite possibly, both nutritional and environmental factors may act in either an additive or synergistic manner.

Creason et al. (91) reported that copper rapidly decreased with age in women's hair. This was in agreement with data reported by Schroeder and Nason (93) and Petering et al. (84). Schroeder and Nason (93) reported that the copper concentration in male hair gradually declined from the second decade thereafter. Even at this, the data presented by these two investigators (93) on the trace element content of hair was difficult to analyze with respect to age and thus seemed inadequate to provide a definite indication of possible age associated changes.

Several investigators have reported sex differences in the trace element content of hair, with females most often higher than males.

The greater copper content of female hair compared with male hair was confirmed by the work of several authors, although not all differences were statistically significant (81,84,91). Briggs and Briggs (81) reported an increased copper content in the hair of female subjects studied. Gibbs and Walshe (92), in examining the copper content of hair for possible differences associated with Wilson's disease, reported that in all groups studied, females had a slightly higher hair copper concentration than the males. Schroeder and Nason (93) reported that female hair showed higher concentrations of copper; similar hair concentrations of zinc were found for males and females. This was in contrast to the study of Anke and Schneider (94) who reported that sex influenced the zinc content of hair of their German population. It must be pointed out, however, that Schroeder and Nason (93) have presented inconclusive evidence on sex variation in the group over 30 years of age, as females in their population represented only about 16% of the total sampling. Creason et al. (91) found that sex was the most important covariate influencing the trace element content of hair.

It has been postulated that this tendency for hair from females to have a higher concentration of several metals than the hair of males may be related to the higher average inorganic ash content for hair from females (95,96). Klevay (97) pointed out that samples of hair from females were generally longer than were those from males; consequently, information obtained from females was representative of a longer period of time than that obtained from males.

Reinhold et al. (88) found that the hair copper content of Iranian males and females varied depending on whether they lived in rural or urban areas. In investigating possible geographic variations of zinc concentrations in hair, Klevay (97) reported that no significant differences due to place of residence were demonstrable for males in the Panamanian population studied. The differences in the amount of zinc in hair among the several regions of Panama were considerably greater however, than those noted by Hammer et al. (105) in a study of the hair of boys from cities in the United States with or without a zinc smelting industry. Creason et al. (91) emphasized that future hair reports on various geographic areas may aid in clarifying the utility of hair as a possible community exposure monitor.

Briggs and Briggs (81) reported that ethnic origin significantly ($P < 0.05$) influenced the zinc concentrations in hair samples from 222 subjects, with Asiatics reportedly having the highest values and Negroes the lowest. Strain et al. (87) collected hair samples periodically throughout an entire year from 6 normal male subjects in an attempt to determine the range of any seasonal variation. These authors reported a definite seasonal variation with the highest hair zinc level being attained in the summer months. Based on results from samples of hair taken from an individual over a 14 year span, Perkons and Jervis (98) found that very large differences occurred for samples taken one or more years apart.

The majority of hair trace element analyses have been made to measure between-person variability. Based on hair analyses from 50

individuals, Perkons and Jervis (98) concluded that differences between individuals were greater than the within-person variability. Jervis (98) pointed out that the within-person variability appeared to be much larger when measurements were based on single hairs rather than milligram quantities. Briggs and Briggs (81) reported that individual variations within a group were large for the elements determined and further stated that because of this wide range of variability, it was doubtful if measurements of trace elements in hair could be of any value as a diagnostic aid, though such measurements could provide information on surveys of large groups.

Since the concentrations of essential metals may vary, a firm basis for comparison of data obtained in different laboratories has become essential. Recent investigations (89,91,106) have recognized the necessity for standardization of collection and analytical procedures. Analytical methods for the determination of metals in samples of biological origin should be evaluated with respect to the recovery of the metal added to a standard sample. This procedure could then serve to improve the validity of comparison of data obtained in different laboratories with regard to biological significance. Sorenson et al. (106) developed a technique whereby meaningful inter-laboratory comparison of analytical data could be made. These researchers presented a technique for the preparation of a standard reference hair sample. This standard reference hair was then used to demonstrate that concentrations of copper and zinc could be measured with precision and accuracy.

It has long been realized that, after collection and preceding analysis, it was essential to wash hair samples to remove oils, dust, and other possible surface contaminants that may contain the element(s) to be examined. However, laboratory washing of hair prior to metal analyses has been a point of contention. Harrison et al. (107) reviewed the methods of hair washing and have suggested the use of a non-ionic detergent. However, use of ionic detergents gave similar results. Methods using incipient boiling in ethylenediaminetetraacetic acid (EDTA) solutions were considered and discarded in the belief that hair metal, in addition to surface metal, would be removed. Washing with organic solvents alone was also discarded in the belief that inorganic surface contamination would not be removed.

McKenzie (108) described a washing and analytical procedure for the analysis of zinc and copper concentrations in hair samples. This author reported that, in all cases, unwashed hair had higher zinc and copper concentrations than did washed hair. Nonionic detergent removed more adsorbed zinc and copper than did ionic detergent, while EDTA was reportedly more effective than either of them in removing adsorbed zinc and copper. Nonionic detergent followed by EDTA washing removed the most adsorbed zinc and copper. No mention was made, however, of previous investigators' findings regarding EDTA.

In McKenzie's study (108) an apparent loss of zinc occurred in the reference stock hair soaked in deionized water, while tap water caused no alteration. Copper concentrations in the reference stock hair almost doubled when soaked in tap water. The decreased zinc

concentration in the hair soaked in deionized water might have indicated a leaching of zinc. Perkons and Jervis, quoted by Harrison et al. (107) suggested that some elements would be partially removed from hair by soaking in some solutions, and in distilled water. Tap water was considered to be a significant source of copper in hair in the study by McKenzie (108).

Creason et al. (91) reported that available evidence did not support the hypothesis that zinc was lost from their hair specimens. (90,107). Second, hair specimens stored for several decades have exhibited typical values with (109) or without (110) laboratory washing. Third, treatments that removed zinc from hair samples also removed copper (111).

Bate and Dyer (89) found that water removed large amounts of trace elements from hair that had been washed with organic solvents, but organic solvents alone were inefficient for removing inorganic contamination. These researchers tried a number of detergents and commercial shampoos and reported that each gave comparable results. Probably the most cogent reason given for using detergents for washing the hair samples was because of normal use by individuals when washing their hair; detergents should therefore cause no more (or less) changes in trace element composition than is caused by in situ washing. The authors concluded that hair should be thoroughly cleaned of surface contamination before trace elements are measured; they felt this could best be accomplished by washing the hair with either a nonionic detergent or an organic solvent followed by distilled water.

Hildebrand and White (90), in reporting on the effects of hair sample washing, noted that zinc concentrations were unchanged after most wash procedures, but were significantly ($P < .05$) decreased (10-65% decrease) by a chelating-agent wash procedure, which indicated that the chelating agent removed more than just surface contamination. EDTA containing washes appeared to remove a considerable portion of the true hair minerals and were not recommended. The use of strong detergents was also questionable because of their leaching effects. No statistically significant trend was reported for copper. The authors recommended a mild ion-free detergent for washing untreated hair before it was analyzed for metals. These investigators concluded, nonetheless, that it appeared doubtful that accurate values for the minerals investigated could be obtained, regardless of the wash treatment used.

Bate and Dyer (89), using a washing procedure involving detergent and demineralized water, and using neutron activation analysis to determine zinc and copper levels in hair found no evidence that their washing procedure caused any great loss of zinc or copper. These authors, however, asserted in this paper, as did Bate (111) in a later publication, that washed hair could adsorb (or absorb) zinc and copper from a simulated sweat solution. Their results were less than convincing, since they subjected washed hair to a 16 to 24 hour period of soaking in a solution which contained an excess of the elements in question. It was believed that this unphysiologic procedure bore questionable relevance to the problem of determining the significance

of metal content of hair.

The findings of a study by Petering et al. (84) showed that their washing procedure efficiently removed particulate zinc adhering to the surface of the hair of laboratory rats maintained in galvinized cages, leaving only the metals contained within the hair structure for the subsequent analyses. Such confinement in the galvinized cages was considered to reflect the possibility of environmental contamination from this metal. The method used by Petering et al. (84) washed hair samples with ionic detergent, followed by successive rinses with water. The detergent used in this procedure was demonstrated to be similar in effects to commercial shampoos, which agreed with the findings of Bate (111) for nonionic detergents. In an earlier Environmental Protection Agency hair study (112), a metal chelating agent (EDTA) was included in the hair washing procedure, but use of this reagent was not recommended in a subsequent report (113), and other investigators agreed (90,107). Dilute acid has been reported to remove metals from hair (112).

Klevay (82) believed it to be necessary to treat hair samples with an organic solvent to remove fatty materials, lacquers, and any adhering particulate matter on samples. The use of an ionic detergent was desired over a nonionic one, as this investigator was apparently most interested in removing metal ions which could be adsorbed onto the hair shaft. Sodium lauryl sulfate was preferred for this purpose over nonionic detergents since it was important that the divalent heavy metals, as well as alkaline earth metal ions, be solubilized.

The extensive washing with demineralized water was of additional importance in removing all detergents and soluble metal salts.

Hilderbrand and White (90), in an evaluation of the effect of prior cosmetic and various commonly cited sample washing treatments on the zinc and copper concentrations of hair samples, reported that the values observed were greatly affected by some of the typical treatments given to human hair, and that such changes were not corrected by the commonly used sample wash procedures. These authors concluded that for these reasons, hair samples could not be expected to accurately indicate the concentrations of these metals that would otherwise be present.

The analysis of human hair samples in attempts to assess the degree of exposure of individuals or populations to certain metallic elements is becoming more common. One important criterion for the use of hair levels as an index of nutritional status for a specific element is that the hair content of the element measured after standard washing procedures should be endogenous in origin and not derived from external environmental contamination. Environmental adsorption may be a significant source of trace elements in hair.

Klevay (97) in an attempt to determine a geographic variation of zinc content in hair, pointed out that industrial exposures were assumed to be low in Panama, and thus it seemed likely that the nutritional and demographic influences upon zinc in hair exceeded those of industry. This author noted a slight differential effect of residence and postulated that some of the males studied may have had occupational exposures to zinc that may have obscured the effect of

geography.

It has been suggested that exogenous copper may make a major contribution to the hair copper content, at least after several months of exposure to the external environment, and that this exogenous copper was not effectively removed by routine sample washing procedures. Several investigators have concluded that exogenous copper may contribute to the hair copper content, and that this can result in a significant and progressive increase in copper levels in the hair shaft with increasing duration of exposure to the external environment. Bate (111) ascertained that washed hair could adsorb (or absorb) zinc and copper from a simulated sweat solution. This same researcher performed a study to determine how extensively the trace element content of hair might be modified by the adsorption of environmental trace elements and to see if solvents could be found that would remove adsorbed elements. The procedure used was to place a sample of hair in a solution containing measured quantities of an element and a radiotracer. After 16 hours, the sample was removed from the solution, rinsed, and then washed. The radioactivity that remained after the wash was taken as a measure of the quantity of the adsorbed element. All solutions were prepared to approximate the composition and pH of sweat. Both copper and zinc were found to adsorb, with the extent of the adsorption being reported as pH dependent. The results obtained by Bate showed that hair could adsorb many elements on its surface, and normal methods of cleaning hair did not remove them.

In studying the effect of environmental exposure on trace element

content in hair, Creason et al. (91) concluded that the absence of a demonstrable relationship between values for hair and for the media in the case of some elements in their study was not definitive, but may have simply indicated that: a) the exposure differences were not sufficient for a correlation with values for trace elements to be observed in hair; or b) that the media indexes of environmental exposure used were not representative of the overall trace element exposure for the population sampled.

Previous investigators (90,111) have argued that trace element content of hair was largely dependent on external hair treatments. But it has been acknowledged that, under contrived laboratory conditions (90,111,114), hair did adsorb and release trace elements. Such adsorption could explain the increasing concentration of elements in long strands of hair.

Obtaining a representative sample for analysis presents another problem not ordinarily encountered. Mineral concentrations in hair may not be the same because of differences in length of hair or other factors that have not been evaluated. In long hair strands an increase, with distance from the head, in the concentration of most elements has been observed. It has been suggested that time and increased external contamination may have caused a build-up. In most cases, past studies have not involved particular parts of the hair, although Pearson and Pounds (115), Renshaw et al. (116), and Valkovic et al. (117) have studied the variation of elements along the length of hair with resolution in the millimeter range.

Hambidge (118), in a comparison of the concentrations of hair copper in proximal sections of the hair shaft, adjacent to the scalp, with that of more distal sections from the samples, reported that the mean for the more distal section was in each case significantly ($P<0.005$) greater. The higher mean copper concentration of that part of the hair shaft that has been exposed to the external environment for the longest duration suggested that exogenous copper may have made a major contribution to the hair content of this element. The results of this study, though not negating entirely the potential value of hair copper as an index of copper nutriture, indicated that caution was required in the selection of sample material and in the interpretation of analytical data. No increases in the mean concentrations of zinc were observed by Hambidge (118) with increasing distance from the scalp in the same hair samples. In contrast to these results, MacKenzie (108) reported that increasing distance of the hair from the scalp was associated not only with a regular increase in copper concentration but also with increases in zinc concentration for most subjects.

Hambidge (118) pointed out that although an abnormally low concentration of endogenous copper in human hair may result from a copper deficiency, other factors may increase the total hair copper content of the same samples to within the normal range. In order to minimize this error, Hambidge (118) concluded that hair sampling should be restricted to recently grown hair within 2 cm from the scalp. The magnitude of the variations in hair copper levels observed by this researcher in the same individuals, depending on the distance from the

scalp, was comparable to the differences between mean values reported by different investigators (82,84,87-89,99,100,102-104), and also to reported differences related to age (84,88) and sex (83,93). It was possible therefore that these differences may have been related to variations in sampling procedures.

Perkons and Jervis (98), who analyzed hair from five positions on the head of four subjects, concluded that the concentration of most elements varied only slightly between positions. Kerr (119) measured the trace elements in 80 single hairs from one individual to see if their concentrations differed in growing and non-growing hair. This investigator reported that the variability was small. A limited number of measurements have also been made on sectioned portions of long strands of women's hair (98). Some elements varied by a factor of 10 or more from one end of the strand to the other.

Hambidge (118) referred to the work of Schroeder and Nason (93) who reported significantly ($P<0.001$) higher copper concentrations in female hair than in male hair. In that particular study, samples from male subjects were taken with clippers from the side and back of the head, whereas female samples were collected from the ends of the hair of unspecified length. Harrison et al. (107) have suggested collecting the hair from the nape of the neck. However, Strain et al. (87) have recently presented a detailed study of the seasonal variation encountered in the use of this collection method. It has been recommended that, in future reports of human hair analyses, precise information on sampling techniques should be included.

Some evidence has indicated that trace element content of hair can reflect whole body content (120-122) or content in specific tissues (82,83,87,122-124). When values for hair did not reflect values for tissues (93,120,125-127), hair may have been reflecting the metabolic or health status (120,128-130), while the values for blood and other tissues may not have. During the investigation of parakeratosis in swine, Lewis et al. (131) reported that the tissue zinc status seemed to be measured better by the hair zinc than by the plasma zinc content. Addink and Frank (132), in examining the zinc content of hair from carcinoma patients, reported that any deviations found, though not significant, did not run parallel with deviations found in blood investigations. The authors explained their findings by ascribing the decreased zinc level of whole blood to a reduction of the zinc content of the erythrocytes, in particular their carbonic anhydrase content. Hilderbrand and White (90) found a wider range for trace metals in hair samples from individuals than would be expected from the range found in other tissues from the same individuals. Goldblum et al. (133) have presented evidence that copper and zinc occurred in hair, nails, and skin of human beings, each at levels which were similar in the three tissues.

The most recent published research dealing with Klevay's zinc/copper ratio theory (34) was a study done by Helwig et al. (134). These investigators examined the effects of varied zinc/copper ratios on egg and plasma cholesterol levels in White Leghorn hens. The manipulation of energy content or of the zinc and copper content of the

practical diets studied did not result in any alteration in cholesterol metabolism, i.e., there were no observed correlations between the levels of cholesterol observed in the egg and plasma cholesterol or dietary composition. This inability to support Klevay's theory (34) in a species other than the rat indicated that further studies, possibly with human subjects, should be undertaken before such a theory is accepted for humans.

Klevay (32) has associated an altered ratio of zinc to copper, as characterized by high zinc to copper, with the risk of CHD. To the author's knowledge, this research study examining the relationship between zinc and copper nutritional status and risk factors associated with cardiovascular disease, will be the first to examine Klevay's hypothesis (32) in human subjects. That this research will be supportive of Klevay's zinc/copper ratio theory is doubtful. It is anticipated, however, that some relationship will be shown to exist between subjects' age and cholesterol and triglyceride values, and the hair zinc concentrations. Such results would be verification of those previously reported in the literature.

MATERIALS AND METHODS

A. Description and Procurement of Subjects

As males appear to exhibit an increased incidence of CVD, and in an attempt to eliminate the interferring variable of sex, the subjects of this study were males on a continuum ranging from 19 to 59 years of age. Not every subject had all the aforementioned risk factors present. Their presence, however, was considered as an indication, in terms of conditional probability, of the development of CVD. The risk factors considered in this study were compared to the values of the cardiovascular disease risk profile (33) seen in Table 1 to determine the level of risk associated with each factor. The relative importance of specific risk factors has been shown to differ among individuals in a population. Any given individual may have more than one risk factor and the combined effect of two or more risk factors may increase an individual's susceptibility for CVD either by an additive or a synergistic means.

Subjects were obtained, on a volunteer basis, from the university community. Two forms of recruitment were employed. A short article was submitted to the Collegiate Times (V.P.I. & S.U.'s biweekly student newspaper) and to the Daily Bulletin, published by the V.P.I. & S.U. Communications Department. The content of the printed versions can be found in Appendix A. The first article was intended to reach the student population, both undergraduate and graduate, whereas the second notice was an attempt at reaching faculty and staff

Table 1

CARDIOVASCULAR DISEASE RISK PROFILE

	Increasing Risk					
Age	25	35	45	55	65	75
Family History	None	1 Blood Relative		2 Blood Relatives		
Cigarette Smoking	None	10/Day	10-20/Day	20-30/Day		30/Day
Blood Pressure						
Systolic	120	140	160		180	
Diastolic	85	90	95	100	105	110
Obesity (% Fat)	20%	25%	30%	35%	40%	
Blood Chemistry						
Cholesterol	220	240	260	300	350	
Triglycerides	100	200	300	400	500	
Glucose	100	140	160		180	
Physical Activity Habits	Active	Moderately Active				Inactive
C-V Fitness Category	High	Good	Average	Fair		Poor
Resting Heart Rate	65	75	85	95		
Electrocardiogram						
Resting	Normal		Borderline			Abnormal
Exercise	Normal		Borderline			Abnormal
Stress and Tension	Under Control		Fair Control			Poorly Controlled

Decreasing Risk

members. In addition, subjects, who had already volunteered, were encouraged to solicit additional volunteers from among their friends, colleagues, and associates.

It was not the intention to limit the sample population solely to the above mentioned groups. Originally, it had been anticipated that subjects would be recruited from among the participants in the V.P.I. & S.U. Cardiac Rehabilitation and/or Cardiac Intervention program(s). These particular individuals would have been at somewhat of a physician-defined risk, some of them having already suffered a heart attack.

B. Data Collection

All samples were collected in the Human Nutrition Metabolic Laboratory (Solitude House) located on the V.P.I. & S.U. campus. Data were collected during the winter quarter on four separate occasions from the hours of 7:00-9:30 a.m. Subjects were scheduled at their convenience in 15 minute intervals, with no more than four subjects scheduled at any one time period, so as to allow for a steady flow and to limit the amount of time required of each subject. A follow-up call was made one to two days prior to the scheduled appointment to serve as a reminder.

The following samples were collected from each subject: a fasting (12-14 hr.) blood sample, a hair sample ($\sim$1g), height, weight, and blood pressure measurements, and information concerning family history of CVD, cigarette smoking, and exercise-activity level. The order of sample collection was as it appears on the Sample Collection

Checklist in Appendix B. Upon sample collection completion, subjects were served breakfast, as a compensation for their time and fasting.

Assisting trained personnel and their responsibilities included: a medical technologist (blood sample collection), a registered nurse (blood pressure measurements), three cosmotology students (hair sample collection), and three graduate students (height-weight measurements, questionnaire explanation and assistance, and breakfast). Hair and blood samples were collected, and height, weight, and blood pressure measurements were made by the same individuals throughout the entire collection period to eliminate the possibility of between-person variability.

C. Description of Questionnaire and Exercise-Activity Level Assessment

A sample questionnaire can be found in Appendix C. A professor of scaling and measurement techniques was consulted on questionnaire design and evaluation. This self-administered questionnaire was designed to elicit as accurate a response as possible regarding the subjects' family history of CVD, cigarette smoking habits, medications, occupation, and exercise-activity level. A section was also provided for recording the blood pressure and height and weight measurements. It would have been necessary to conduct a personal follow-up interview to obtain more accurate data by further probing for specific differences. As all subjects were volunteers, available time was a limiting factor.

In investigating the physical activity level, it was not feasible to employ an external criterion such as direct assessment of energy

cost, recording the heart rate, or directly observing subjects' performances of various activities. As an alternative, an objective system was developed for the evaluation of this risk factor.

Subjects were requested to check those activities listed on the questionnaire in which they had participated over the past twelve months. Activities listed included a combination of occupational, sports, and leisure activities. Each activity was assigned a numerical value. These values were obtained primarily from tables (135) of metabolic costs for various occupational and leisure tasks, expressed as a ratio of work metabolism to basal metabolism (WMR/BMR). Data on energy costs of some activities, included on the questionnaire used in this study, were lacking and estimates were made by comparison and extrapolation of previous data published (136) on the energy cost of physical activities. Values for the exercise-activity level were obtained by calculation of the WMR/BMR units in conjunction with a consideration of frequency (days/wk.), duration (hrs.), and intensity (degree of sweating associated with the particular activity). Four categories were included for this latter variable to eliminate the likelihood of a tendency for subjects to check an intermediate (moderate) level of three possibilities.

As with the study by Reiff et al. (135), this index was used to eliminate the necessity of considering the subjects' body weight, converting the work to calories, etc. The method assumed that a task performed by a heavy person would raise his metabolism to the same extent as the same task performed by a person weighing less, even

though the caloric expenditure might be different. Since much of the activity recorded involved the movement of one's own body weight, errors in making this assumption were probably not serious. Reiff et al. (135) further pointed out that the energy cost for the same task varies from individual to individual depending upon skill, walking surface, equipment, clothing, environmental conditions, terrain, and perhaps many other factors. Nonetheless, this investigator reported rank order correlations ranging from 0.96 to 0.99, which illustrated consistency in ranking among judges using this method of physical activity assessment.

The values for this exercise-activity score were expressed in somewhat arbitrary quantitative terms. Such a score was not meant to be an exacting one, but rather was intended to classify subjects into low, moderate, and high categories. These categories were developed merely as a possible indication of susceptibility to CVD from this risk factor. The mean score formed the criterion for the development of an objective ranking system for the three levels of activity. Values were considered to be low or high if they exceeded one-third or three times this mean, respectively.

As the questionnaire was merely an attempt to estimate the physical activity level during the preceding year, if the pattern of activity had been followed for a shorter or longer time span, the respondent was requested to indicate this for the specific activity. In addition, the questionnaire accounted for the subjects' occupation, length of employment, and the extent of an occupational effect (if any)

on the exercise-activity level in terms of frequency, duration, or intensity.

All subjects requesting results of the study were supplied with such. The format for these reports can be seen in Appendix F.

D. Analytical Methodology

Several methods have been reported for the determination of copper (83) and zinc (82) by atomic absorption spectroscopy. Previous workers have shown the determination of these elements to be almost totally free of inter-element interferences when a-c instruments were used with conventional burners and air-acetylene flames. Siggia (137), using atomic absorption, reported relative detection limits (ug/ml) of 0.005 and 0.002 for copper and zinc, respectively. Atomic absorption spectroscopy has facilitated studies of zinc and copper metabolism in man. Employing this method, Klevay (82,83) designed experiments to test the hypothesis that hair could be used as a biopsy material for the assessment of zinc (82) and copper (83) nutriture in humans; in these investigations, hair was compared with other means of nutriture evaluation, i.e., the zinc and copper content of red blood cells and plasma. In order that results could be compared, the utilization of the same technique, atomic absorption spectroscopy, for trace element (zinc and copper) analysis was employed.

Hair samples were collected and analyzed for their zinc and copper content by a modification of the method described by Petering et al. (84). All glassware used for the analysis was thoroughly

washed in a 50% nitric acid solution and carefully rinsed with deionized water to ensure removal of possible metal contaminants. Duplicate hair samples (93 to 350 mg in size) were washed successively with sufficient acetone, ether, and acetone to cover each sample several millimeters after the samples had been placed in 150 ml pyrex beakers. The hair was in contact with the aforementioned organic solvents for at least ten minutes each at room temperature; samples were stirred periodically with a clean glass rod. After each wash, the solvent was decanted and discarded. Following the second acetone wash, which was repeated only if turbidity or color was still present in the liquid, the hair was considered to be free of oils, particulate matter, and lacquers.

Each sample was then stirred with a 1.0% solution of sodium lauryl sulfate (ionic detergent) at temperatures of 25 to 40^{o} C for 30 minutes; again, this step was repeated only if the wash water was colored or highly turbid. Following the detergent washing, the hair samples were placed in deionized water, stirred, filtered (acid-washed filter paper) and rinsed with deionized water until it was apparent that all the detergent solution was removed. The samples were then rinsed once more with acetone to remove any final traces of the detergent solution; they were placed between clean (acid-washed) filter papers and allowed to air dry for a period of 18 to 24 hours. Following the drying phase, each sample was placed in individual labeled, clean plastic (polyethylene) containers, capped and stored until further analysis.

Duplicate aliquots (50 mg in size) of the washed and dried hair were placed in 50 ml watch-glass covered pyrex beakers and pre-digested in 5 ml of nitric acid; samples were evaporated to near dryness on a hot plate at 250-350° C. The samples were then wet ashed with 10 ml of nitric acid, 5 drops of sulfuric acid, and 2 ml of 70% perchloric acid solution. Perchloric acid additions and wet ashing were performed under the perchloric acid ashing hood. This hood was washed down 20-30 minutes prior to each use and again 20-30 minutes following each use. Appropriate precautionary safety regulations were adhered to at all times for this portion of the procedure. Samples were wet ashed on a hot plate initially set at 400° C. At the first sight of white fumes (45 minutes to a hour), the temperature was reduced to 350° C. After samples cleared in color and the perchloric-nitric acids reaction was complete (1-2 ml solution remaining), watch glasses were removed and samples were evaporated to near dryness. When white fumes were barely visible, the hot plate temperature was further reduced to 250° C to prevent possible charring of the samples likely to occur at higher temperatures. Samples were then evaporated to dryness. If the residue was colored (brown to black), nitric acid and 30% hydrogen peroxide were added in varying quantities, beakers were again covered with watch glasses and heated until the sample cleared. Blanks, corresponding to sample treatments, were wet ashed as outlined above.

The ashed residues were taken up in a 10% nitric acid solution to a 5 ml volume. Copper values were read on the atomic absorption spectrophotometer (Perkin-Elmer 305) at the 324.7 nm resonance line

and the absorption was recorded on a strip chart recorder. These values were then converted to copper concentrations from a calibration graph (standard curve) prepared with copper sulfate standard stock solutions (.1,.3,.5,1.0 ppm) and run with each group of copper samples analyzed.

As the zinc content of the samples was present in higher concentrations, a further dilution (1:10) was made on the copper solutions with 10% nitric acid. The zinc values were read on the atomic absorption spectrophotometer (Perkin-Elmer 305) at the 213.8 nm resonance line and this absorption was also recorded on the strip chart recorder. These values were subsequently converted to zinc concentrations from a standard curve prepared with zinc sulfate standard stock solutions (.1,.3,.5,1.0 ppm) and run with each group of zinc samples analyzed.

The zinc values obtained with the 1:10 dilution of 10% nitric acid were believed to be contaminated by the additional nitric acid, as duplication of samples was, in many cases, well above $\pm$ 25%. A reading of the 10% nitric acid solution produced a resulting concentration of nearly .3 ppm. To obtain a more representative value of the zinc concentrations present, and to eliminate the possible interferences due to the nitric acid solution, a new dilution (1:10) was made on the copper solutions with deionized water.

In future experimentation employing this method for the analysis of zinc and copper content of hair, it is recommended that the ashed residues be taken up in a 10% hydrochloric acid solution for copper readings, and a 1:10 dilution with deionized water be made from these

solution for zinc readings.

Serum samples were analyzed in triplicate for serum cholesterol and triglyceride values by means of the Technicon Autoanalyzer II.

E. Calculation of Results

Zinc, copper, cholesterol, and triglyceride values were determined through the use of standard calibration curves, prepared from solutions of stock standards. For zinc and copper values, sample weights and dilution factors were accounted for in the calculations. Calculation of % error was determined by the following formula.

$$\frac{\text{high conc.} - \text{low conc.}}{\text{mean conc.}} \times 100\% = \% \text{ error}$$

The exercise-activity score was determined by a multiplication of the WMR/BMR value for each activity (see Appendix D) checked times the frequency, duration, and intensity values for that particular activity. The duration variable was expressed in terms of blocks of time of unequal intervals. For calculation purposes, the mean of each of these values was used; these means were then converted to hours or portions thereof. Specific activities (see Appendix D) were considered seasonal and were calculated accordingly. A total figure was obtained by addition of the values calculated for all activities checked by an individual. Values calculated were expressed in terms of WMR/BMR/hr./wk./intensity.

The value for the risk from obesity was calculated by a simple ratio, with table values (146) for 'ideal' height for weight being compared to the measured values recorded on the questionnaires.

Criteria for overweight and obesity were considered as greater than 10% and greater than 20% over 'ideal' weight, respectively.

F. Statistical Analysis

Statistical analysis of data consisted of a correlation run between the zinc/copper ratio and each risk factor (age, cholesterol, triglycerides, blood pressure, cigarette smoking, obesity, and the exercise-activity level) individually. A further analysis to determine the relative importance of each of the risk factors, with respect to the zinc/copper ratio, was achieved by the use of stepwise regression. Additional correlations were run between the zinc and copper values and each risk factor, as well as between age and cholesterol and triglycerides values, and between occupation and exercise-activity level. Analysis of variance was applied to hair zinc concentrations, the zinc/copper ratio, cholesterol, and triglyceride values with age (by decades) to test for significant differences.

RESULTS AND DISCUSSION

A breakdown of the subjects by age and occupation is supplied in Table 2. As can be seen from the total figures, the subjects were primarily students (46.9%) or faculty members (34.4%). Other occupations reported on the questionnaires included ROTC personnel and two retired individuals. Thirty subjects (46.9%) were under the age of 30, with another thirteen subjects (20.3%) being under the age of 40. Such an age distribution no doubt accounted for the low mean age of 34.7 years.

A compilation of data from the questionnaires concerning family history of CVD is shown in Tables 3 and 4; Table 3 also contains information on the number of medications taken, as reported by the subjects. Eighteen subjects (28.1%) reported no family history of CVD; twenty-seven (42.2%) reported one male member in their immediate family as having a medical history of CVD; ten subjects (15.6%) reported 2 male members; and, the remaining nine subjects (14.1%) reported 3 or more male members with a medical history of CVD. Fifty-five subjects (85.9%) reported that they took no medications. Only seven subjects (10.9%) reported that they took one medication on a regular basis. The remaining two subjects reported taking 2 or 3 medications daily. Two of the reported medications, Aldomet and Catapres, were antihypertensive agents and one reported medication, Atromid, was an antihypercholesterolemic agent. The remaining drugs reported were not specifically related to CVD.

The family history of CVD as reported by condition and male

Table 2

POPULATION DESCRIPTION (NUMBER OF SUBJECTS) BY AGE AND OCCUPATION

Age (Decades)	Occupation				n[1]
	Student	Faculty	Staff	Other	
0-19	1				1
20-29	26	1	1	1	29
30-39	2	8	3		13
40-49	1	8		3	12
50-59		5	2	2	9
TOTAL	30	22	6	6	64

[1]Number of Students

Table 3

SUBJECT FAMILY HISTORY OF CARDIOVASCULAR DISEASE AND MEDICATIONS[1]

	Frequency of Occurrence							
Variables	0	1	2	3	4	5-8	9	10
Family History	18	27	10	5	2	0	1	1
Medications	55	7	1	1	0	0	0	0

[1]Number of Subjects

Table 4

FAMILY HISTORY OF CARDIOVASCULAR DISEASE
BY CONDITION AND RELATIONSHIP[1]

	Relationship				
Condition	Paternal Grandfather	Maternal Grandfather	Father	Brother(s)	Uncle(s)
Angina Pectoris		1	2	1	2
Arrhythmia			4		
Arteriosclerosis		2	3		2
Atherosclerosis	1				
By-Pass Surgery				1	
Congestive Heart Failure		2			
Coronary Artery Disease			1		3
Coronary Thrombosis	1		4		
Enlarged Cardiac Artery				1	
Hardening of the Arteries			2		
Heart Attack	1	3	4		2
Hypertension	2	1	9	5	5
Myocardial Infarction		1	1		1
Stroke	3	1	2		1
Non-Specific	2		1		1

[1]Reported Frequency of Occurrence

relation can be seen in Table 4. Fourteen separate conditions were reported, with hypertension being the condition most frequently reported by subjects. This condition was followed in frequency of reporting by heart attack, arteriosclerosis, stroke, and angina pectoris. Fathers were the relation most frequently recorded when the subjects considered their family history of CVD, with thirty-three of the forty-six subjects who reported a family history, reporting such a condition in their father.

The mean concentrations (±S.D.) of zinc and copper obtained from the analyzed hair samples are listed in Table 5. As can be noted from this table, zinc values ranged from 42.7 ug/g to 407.6 ug/g, with a mean concentration of 171.2 ± 65.2 ug/g; copper values ranged from 15.1 ug/g to 38.4 ug/g, with a mean concentration of 27.4 ± 5.3 ug/g. A wide range of concentrations have been reported as 'normal' for zinc and copper in human hair. Tables 6 and 7 present a compilation of some of these reported values. The mean values of zinc and copper obtained in this study were in general agreement with results reported in the literature.

Briggs et al. (81), in attempting to determine the effects, if any, of some of the factors believed to exert an influence on trace metal content of hair, reported the values seen in Tables 6 and 7 for 97 subjects. The zinc and copper concentrations (mean ±S.D.) were reported as 184 ± 66 ug/g and 16 ± 9 ug/g, respectively. The mean copper values determined in the present study (27 ± 5.3 ug/g) approached the upper range of the copper values obtained by these

Table 5

ZINC AND COPPER CONTENT OF HAIR AND ZINC/COPPER RATIO

	Mean ± S.D.	Range
Zinc (ug/g)	171.2 ± 65.2	42.7 - 407.6
Copper (ug/g)	27.4 ± 5.3	15.1 - 38.4
Zinc/Copper Ratio	6.2 ± 1.8	2.4 - 12.0

Table 6

REPORTED VALUES FOR NORMAL CONCENTRATIONS OF ZINC IN HAIR[1]

Sample	Age (Years)	Number of Observations	Zinc Mean ± S.D.	Reference
Males	19-21	2	189 ± 4	(108)
Females	21-24	3		
Males	---[2]	97	184 ± 66	(81)
Males	0-10	81	115 ± 1.6	(97)
Males	11-20	43	137 ± 1.2	(97)
Males	> 20	64	133 ± 1.3	(97)
Males	> 20	64	142 ± 43	(82)
Males	0-5	52	147 ± 108	(82)
Males	6-10	31	127 ± 49	(82)
Males	11-15	29	126 ± 27	(82)
Males	16-20	14	163 ± 27	(82)
Males (Iranian Villagers)	---	19	139 ± 16.4	(88)
Males (Controls)	---	20	181 ± 36.3	(88)
Males	2	--	105	(84)
Males	12	--	180	(84)
Males	80	--	125	(84)
Males	1-102	117	165 ± 9	(93)
Males and Females (Tennessee)	Adults and Children	33	177	(89)
Males (Napier, New Zealand)	6-12	33	126	(89)
Males (Hastings, New Zealand)	6-12	33	132	(89)

Table 6 (continued)

Sample	Age (Years)	Number of Observations	Zinc Mean ± S.D.	Reference
Females (95%)	---	1,500	189 ± 1.0	(106)
Males (Normal Egyptians)	27-40	12	103.3 ± 4.4	(87)
Males (Normal, New York)	23-37	6	119.6 ± 4.6	(87)
Males (Egyptian Dwarfs, Treated)	16-20	10	121 ± 4.8	(87)
Males (Egyptian Dwarfs, Untreated)	16-20	8	54.1 ± 5.5	(87)
Males	0-15	224	90.5	(91)
Males	≥ 16	167	108.5	(91)
Males	Children	---	83.9	(91)
Males	Adults	---	106.7	(91)

[1]All values are in ppm (ug/g) in dry hair.

[2]Data not supplied.

Table 7

REPORTED VALUES FOR NORMAL CONCENTRATIONS OF COPPER IN HAIR[1]

Sample	Age (Years)	Number of Observations	Copper Mean ± S.D.	Reference
Males	---[2]	97	16 ± 9	(81)
Kwashiorkor	Children	10	16.4 ± 2	(104)
Kwashiorkor	Children	---	18.0 ± 0	(142)
Kwashiorkor	Children	---	19.3 ± 1.23	(143)
Healthy Controls	Children	3	8.9 ± 0.1	(104)
Males (Black)	---	2	15.0 ± 0	(142)
Males (Black)	---	2	25 ± 6	(143)
Males (Normal)	---	8	16.0 ± 4	(100)
Males (Wilson's Disease)	---	3	12.3 ± 0	(100)
Males	> 8	121	22.4 ± 16	(83)
Males	---	18	15.0 ± 9.3	(99)
Males (Iranian Villagers)	---	19	11.8 ± 5.5	(88)
Males (Controls)	---	20	11.7 ± 2.6	(88)
Males	---	20	14.3	(92)
Proximal Hair Sections Males	4-32	8	11.8	(118)
Proximal Hair Sections Females	4-32	19	11.8	(118)
Distal Hair Sections Males	4-32	8	20.7	(118)
Distal Hair Sections Females	4-32	19	20.7	(118)
Males	1-102	117	18 ± 2	(93)
Males and Females (Tennessee)	Adults and Children	33	34.1	(89)
Males (Napier, New Zealand)	6-12	33	30	(89)
Males (Hastings, New Zealand)	6-12	33	15.5	(89)

Table 7 (continued)

Sample	Age (Years)	Number of Observations	Copper Mean ± S.D.	Reference
Males	0-15	279	12.1	(91)
Males	≥16	204	18.2	(91)
Males	Adult		13.9	(91)
Males	19-21	2	26.3 ± .9	(108)
Females	21-24	3		

[1]All values are in ppm (ug/g) in dry hair.

[2]Data not supplied.

researchers, whereas the corresponding mean zinc value (171 ± 65 ug/g) determined was somewhat lower.

In an investigation of the copper content of hair in kwashiorkor, Lea and Luttrell (104) reported the average copper content (mean ±S.D.) of the kwashiorkor group to be 16.4 ± 2.0 ug/g. This was similar to normal values of 18.0 ug/g found by Goss and Green (138) and by Gopalan (103) of 19.3 ± 1.23 ug/g. The average of the small (3 subjects) healthy control group, reported as 8.9 ± 0.1 ug/g, was relatively low. By comparison, the copper values determined in this study (27 ± 5.3 ug/g) were higher than those reported by the latter three groups of investigators. As the subjects of these studies were, for the most part, children, the possible influence of age should be considered in such a comparison of values.

In another study, Goss and Green (138), in attempting to relate the color of hair to its copper content, found a wide range of values, 15-47 ppm, for this trace element in hair. The highest value, 47 ppm, was obtained from the hair (red) of a 3 year old child. The lowest value, 15 ppm, was obtained from the hair of a Negroid male, consistent with previous values reported by Briggs et al. (81). However, Kikkawa et al. (139) found a much higher value, 31 ppm, in the hair of a Negroid male. The range of values reported by Goss and Green (138) was much larger than the range of copper concentrations reported in the present investigation (15.1-38.4 ug/g).

Martin (100), in an attempt to determine the copper content of hair and nails of normal individuals and of patients with hepato-

lenticular degeneration (Wilson's disease), reported a mean ±S.D. value of 16.0 ± 4.0 ug/g, with a range of 7.1-19.4 ug/g for 8 normal male subjects. As before, these copper concentrations were much lower than those reported here. In examining the zinc content of hair from carcinoma patients, Addink and Frank (132) concluded that hair from the head of these individuals did not show any decrease in zinc level when compared with healthy hair samples. Comparison of these investigators' results with those reported in this study was difficult as the zinc content of hair was expressed in terms of percentage by weight.

In investigating possible geographic variations of zinc concentrations in hair of a Panamanian population, Klevay (97) obtained values consistently lower than those determined in this study, when considering age as an influential factor. Zinc concentrations (geometric mean ±S.D.) reported were 115 ± 1.61 ug/g for 81 male subjects ranging in age from 0-10 years, 137 ± 1.21 ug/g for 43 male subjects ranging in age from 11-20 years, and 133 ± 1.30 ug/g for 64 male subjects over 20 years of age. The amounts of zinc in the hair of Panamanian boys was also generally less than those found by Hammer et al. (112). No groups had mean values less than the 70 ug/g suggested by Hambidge et al. (147) as being indicative of zinc deficiency.

Klevay (82), in examining hair as a potential biopsy material for the assessment of zinc nutriture, reported concentrations of zinc (mean ±S.D.) as 142 ± 43 ug/g for 64 male subjects greater than

20 years of age. As before, this mean value was somewhat lower than the corresponding mean determined for the population in this study. However, these values reported for the zinc content of hair of this Panamanian population were of similar magnitude to those reported by Addink and Frank (132) and from Iranian subjects by Reinhold et al. (88). Again, no group values fell below the 100 ug/g reported by Strain et al. (87) from a population of Egyptians judged to be zinc deficient.

Rice and Goldstein (99), in an attempt to determine if an increase in copper concentration occurred in the hair of individuals with Wilson's disease, reported values for 18 normal male subjects to be 15.3 ± 9.3 ppm. These values were again lower than those found in the present study. Goldblum et al. (133) have recorded ranges of 13-44 ppm for male subjects; these ranges are quite comparable to the copper range of 15.1-38.4 ug/g reported in this study. Reinhold et al. (88), in an examination of zinc and copper concentrations in the hair of Iranian villagers, reported zinc concentrations (mean ±S.D.) of 139 ± 16.4 ug/g for 19 male villagers and 181 ± 36.3 ug/g for 20 male control subjects, and copper concentrations (mean ±S.D.) of 11.8 ± 5.5 ug/g for the 19 male villagers and 11.7 ± 2.6 ug/g for the 20 male control subjects. The hair zinc concentrations obtained in the present study (171 ± 65 ug/g) were not too disimilar from those of the 20 male controls; the hair copper concentrations in this study (27 ± 5.3 ug/g) were once again higher than those reported for both groups, villagers and controls.

Gibbs and Walshe (92), in an effort to determine if a difference existed between the hair copper concentration of normal individuals and patients with Wilson's disease, reported a mean copper concentration of 14.3 ug/g, with a range of 5.4-55.7 ug/g for 20 normal male subjects. Although the range reported by these investigators was much more extensive than the range of 15.1-38.4 ug/g reported in the present study, the mean value of 14.3 ug/g obtained by Gibbs and Walshe (92) was close to half that of the mean value determined for copper in this study.

In a comparison of the concentrations of copper in hair samples varying in distance from the scalp, Hambidge (118) reported that for each of the 27 samples studied, the concentration was greater in the more distal sections. The mean copper concentration for the 27 proximal sections, ranging in length from 1 to 5 cm, was 11.8 ppm, and for the more distal sections 20.7 ppm ($P<0.005$). The mean hair copper concentrations reported in the present study, although still somewhat higher, were most comparable to the value reported for the more distal section. These values were most probably a reflection of the hair sampling collection techniques employed in the present investigation.

Petering et al. (84), in a consideration of age and the zinc content of hair, noted that the content of zinc in the hair of males increased from 105 ppm at age 2 to 180 ppm at age 12, and thereafter declined slowly to 125 ppm at age 80. As the subjects in the present study were all 19 years of age and older, no such comparable age

difference or association would have existed. Data presented by Petering et al. (84) for the zinc concentration of hair were most readily compared with those of Schroeder and Nason (93), who determined the levels of trace elements in the hair of 117 males and 47 females from a small New England community. Their population was heavily weighted with males below 30 years of age, the age group with some of the highest values reported by Klevay (82). Such was the case in the present study, with 30 of the 64 subjects (46.9%) under the age of 30. This may partially account for some of the higher values obtained in the present study, although no statistically significant relationship was established between age and zinc concentrations of the hair samples. On the other hand, Petering et al. (84) stated that, in carrying out their washing procedure (same method employed in the present study), values reported were somewhat low; but in a number of studies using the procedure, their results were consistent.

Bate and Dyer (89), measuring trace element concentrations in hair by activation analysis, showed a variation among the individuals sampled. These investigators reported hair zinc and copper concentrations (mean,range), respectively as follows: 177 ppm, 51-602 ppm and 34.1 ppm, 7.8-234 ppm for 33 subjects (adults and children of both sexes) in Tennessee; 126 ppm, 101-186 ppm and 30.0, 7-93 ppm for 33 boys (5-12 years of age) in Hastings, New Zealand; and 132 ppm, 85-166 ppm and 15.5 ppm, 8-150 ppm for 33 boys (5-12 years of age) in Napier, New Zealand. Although the ranges for both zinc and copper values obtained from the first group (Tennessee) were much more

extensive than those determined in the present research, the mean hair zinc concentration was comparable; the mean hair copper concentration, however, was, in contrast, much higher than not only the mean concentration found in the present study, but also higher than most of the previously reported values. Caution must be applied in comparing values from the second and third groups due to the age differences.

Sorenson et al. (106), in an examination of possible interferences in the determination of zinc and copper in human hair, using atomic absorption spectrophotometry, reported values for 1,500 samples, 95% of which were female subjects. The values reported for zinc and copper concentrations were 189.4 ug/g for zinc and 70.7 ug/g for copper. As sex has been directly related to the copper content of hair, high values for copper are most likely attributable to the preponderance of female subjects in the study. Comparisons of values with results of the present study were not conducted for this reason.

Strain et al. (87), in an investigation of the zinc content of hair as related to zinc deficiency, reported the average zinc concentration (±S.D.) in the hair of 12 normal male Egyptians, age 27 to 40 years, to be 103.3 ±4.4 ppm. The zinc content of hair for 6 normal males residing in New York, age 23 to 37 years, was reported as (mean ±S.D.) 119.6 ± 4.6 ppm. Both of these mean values were well below the mean hair zinc concentration of 171 ± 65 ug/g reported in this study. In the same study, the mean hair zinc concentration in 10 untreated dwarfs, ranging in age from 16 to 20 years, was 54.1 ± 5.5 ppm; oral zinc sulfate therapy produced an average hair zinc level in

8 treated dwarfs of 121.1 ± 4.8 ppm.

Cookson and Pilling (140), using a proton induced x-ray method of determination, examined zinc and copper concentration distributions across the diameter of human hair. The mean concentration of copper at the surface of the hair was 290 ppm, while the value obtained in the center of the hair was only 11 ppm, a 26-fold variation. Corresponding values for zinc concentrations were 340 ppm at the surface and 160 ppm in the center, a 2.1-fold variation. This particular distribution of copper in the subject's hair was illustrated by a sharp copper peak near each surface of the hair and very little intensity within the hair. The presence of a strong copper x-ray peak in an analysis of some shampoo used on the subject's hair encouraged the theory that in this case the copper was in the form of a deposit very near the surface. Cookson and Pilling's study may indicate a possible explanation as to the copper values (higher, in most cases, than those reported in the literature) obtained in the present study. It is recommended that, in future studies of this nature (trace element concentration determinations of human hair samples), a prior history (cosmetic treatments, shampoo, creme rinse, etc.) of the hair samples to be analyzed, be obtained.

Creason et al. (91) studied trace element concentrations in hair, as related to exposure in metropolitan New York. For adults of both sexes, 16 years of age or older, the mean hair copper concentration was reported as 18.25 ug/g for 204 subjects and the mean hair zinc concentration was reported as 108.54 ug/g for 167 subjects. In a further

breakdown of subjects by age and sex, these investigators reported a mean hair copper level of 13.87 ug/g for adult males and a mean hair zinc level of 106.7 ug/g for the same group. The average values for zinc in the hair examined by Creason et al. (91) were lower than the range of 151-220 ug/g reported in some publications (93,107). However, other investigators have reported mean values between 75-197 ug/g (97), 82-190 ug/g (141), and 88-180 ug/g (147). The zinc values reported in the present study were somewhat higher than those found by Creason and co-workers (91).

McKenzie (108), in describing a washing and analytical procedure for the analysis of zinc and copper concentrations (mean ±S.D.) in hair samples, reported a value of 189 ± 4 ug Zn/g dried hair and a value of 26.3 ± 0.9 ug Cu/g dried hair obtained from five subjects (two male and three female). These values were most comparable with the zinc and copper results obtained in the present study. In their study of the trace metal content of human hair, Schroeder and Nason (93) reported zinc and copper concentrations, respectively, (mean ±S.D.) of 167.0 ± 5.09 ug/g for 82 male subjects and 16.1 ± 1.19 ug/g for 79 male subjects. The mean zinc level varied only slightly from the mean zinc concentration reported in this study, whereas the mean copper concentration obtained by these investigators was again lower than the copper values determined.

The zinc/copper ratio (mean ±S.D.) determined in this study was 6.2 ± 1.8, with a range of 2.4-12.0. As no literature source was located which specifically referred to, or listed values for, the

zinc/copper ratio, comparison of results obtained in this study was achieved by calculating the zinc/copper ratio from various studies which reported both 'normal' mean hair zinc and copper levels for subjects of the same age group and sex.

The zinc/copper ratio values most closely comparable to the mean value of this study, were 6.9 and 7.2, obtained from 5 subjects (2 males,3 females) and a reference stock hair sample, respectively (108). As the sampling population was relatively small in this particular study, it would be difficult to draw definite conclusions.

Petering et al. (84) reported that the ratio of zinc to copper obtained from 211 hair samples from male and female subjects, ranging in age from 1 to 80 years, was a variable factor which was closely related to age and which probably differed between the sexes. In males, the ratio decreased during the ages of 2 through 12, and thereafter increased gradually with age. As no specific mean zinc or copper values were reported by age group or sex, comparison of results with this study was difficult.

The lowest zinc/copper ratio, calculated from the work of Hildebrand and White (90), was 4.2; the subjects in this study were 33 elementary school boys. Such a value was in agreement with Petering's postulated decrease in the ratio for this age group. However, a zinc to copper ratio twice that value, 8.5, obtained from the same age group, sex, and number of subjects but from a different city, was reported in the same study (90).

A zinc/copper ratio of 11.5 was calculated for 97 male subjects

in the study by Briggs et al. (81). By comparison, this ratio, although still within the upper range, was nearly twice the mean value reported in this study. A similar ratio of 11.8 was obtained from 19 male subjects (Iranian villagers) studied by Reinhold et al. (88). The highest zinc to copper ratio calculated, 15.5, was obtained for 20 male control subjects in the same study.

A value of 10.4 was calculated from the work of Schroeder and Nason (93) on hair zinc concentrations determined in 82 males and hair copper concentrations determined in 79 males. This value is in general agreement with the work of Briggs et al. (81) and Reinhold et al. (88). Zinc/copper ratios of 7.7 and 5.2 were obtained from male subjects studied by Creason et al. (91) and Hilderbrand and White (90), respectively. Both of these values were well within the range of the mean value reported in this study.

Serum cholesterol and triglyceride values (mean ±S.D.) can be found in Table 8. A wide range of variation in this sample population was evident for these two parameters of risk. Although serum cholesterol values were well within normal limits of reported values (142-144), by comparison, the mean triglyceride value obtained in this study was somewhat higher than reported literature values (142,144,145).

Scatter diagrams were initially constructed for each individual subject's values (rounded of the the nearest integer) of zinc, copper, zinc/copper ratio, cholesterol, and triglycerides with age to determine if an emerging pattern was obvious. The zinc, copper, and zinc/copper ratio values by age group can be found in Table 9. Figure 1 illustrates

Table 8

REPORTED VALUES (MEAN ± S.D.) AND RANGES OF CARDIOVASCULAR RISK VARIABLES IN SUBJECTS OF THIS STUDY

Variable	Units	Mean ± S.D.	Range
Age	Years	34.7 ± 11.6	19.0 - 59.0
Cholesterol	Mg. %	225.4 ± 44.8	131.3 - 320.6
Triglycerides	Mg. %	115.2 ± 73.0	23.5 - 431.0
Smoking	(Cigarettes/Day)	1.6 ± 5.9	0 - 25.0
Obesity	(% 'Ideal' Wt.)	107.3 ± 12.1	84.8 - 148.2
Exercise	WMR/BMR/hr./wk./intensity	117.4 ± 121.1	0 - 624.0

Table 9

ZINC, COPPER, AND ZINC/COPPER RATIO
VALUES BY AGE GROUPS

Age (Decades)	n[1]	Zinc(ug/g) Mean	Zinc(ug/g) Range	(Copper(ug/g) Mean	(Copper(ug/g) Range	Zinc/Copper Ratio Mean	Zinc/Copper Ratio Range
0-19	1	117.7	---	24.0	---	4.9	---
20-29	29	175.4	42.7-344.3	26.9	15.1-37.3	6.5	2.8-12.0
30-39	13	175.9	96.7-407.6	27.2	16.9-36.7	6.3	4.6-11.1
40-49	12	156.5	66.7-210.1	28.0	18.0-33.0	5.6	2.4- 7.7
50-59	9	176.2	114.0-393.4	28.9	19.6-38.4	6.0	3.6-10.2

[1]Number of subjects

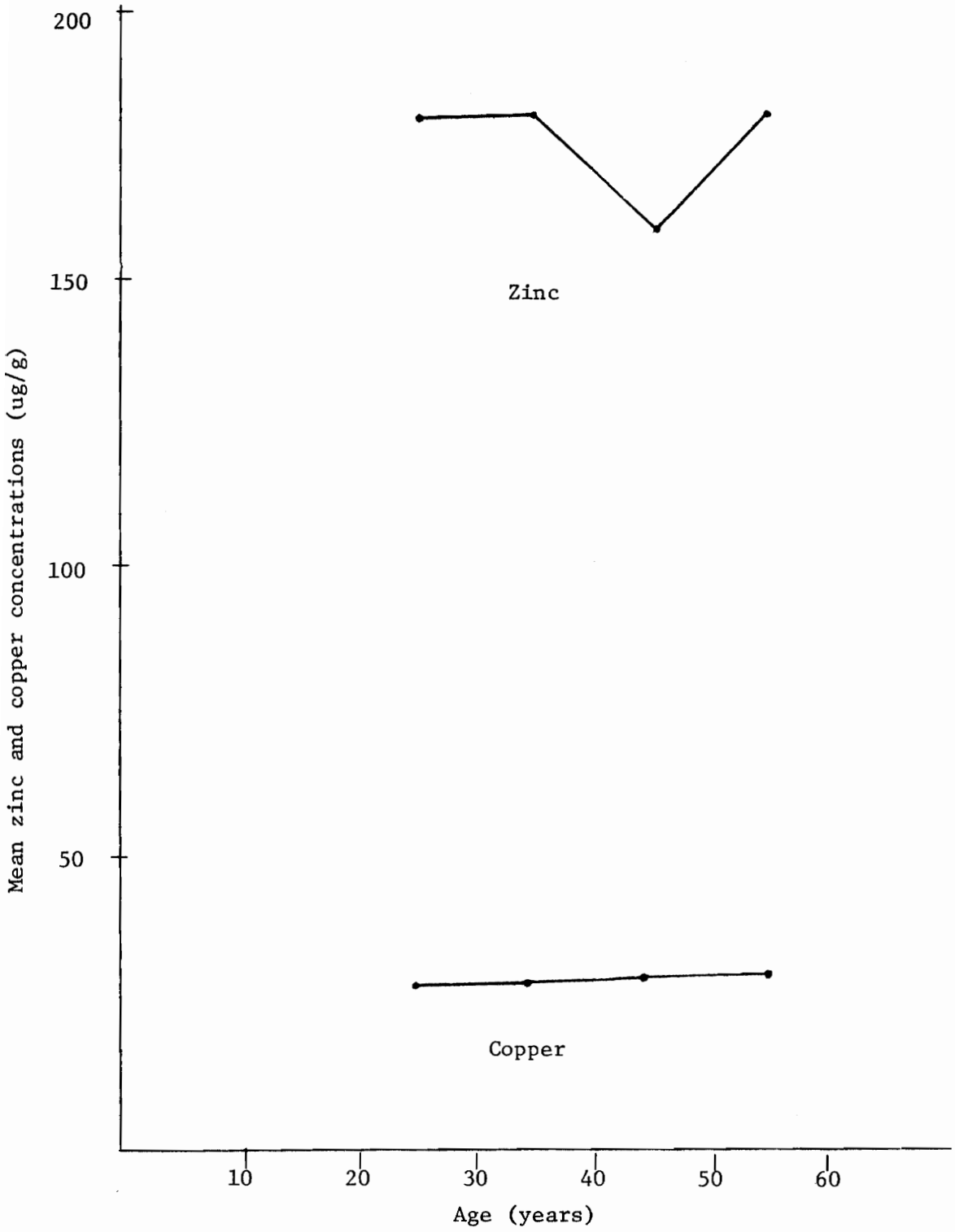

Figure 1. Relationship of hair zinc and copper concentrations to age.

the effect of age on mean zinc and copper concentrations in hair. No relation was shown to exist between copper concentrations in hair and age for these male subjects (see Figure 1). Such results were in keeping with reported literature values (93,97). The zinc values, however, showed changes associated with age, as indicated by the sharp decline from the third to fourth decade followed by an equally as sharp rise from the fourth to fifth decade. Highest mean zinc concentrations were obtained for this latter group. Such a variable fluctuation was in conflict with several investigators (82,84,93) who have reported an age related decrease in the zinc content of hair.

The mean zinc/copper ratio for each age group appeared to parallel the changes in mean zinc concentrations with increasing age, as illustrated by the same sharp decrease from the third to fourth decade, followed by the rise from the fourth to fifth decade (see Figure 2). At that, the highest zinc/copper ratio was obtained for the second decade. Whether the basis for such a similarity was merely due to the higher zinc values with age, or is of some possible physiologic significance was not established.

Serum cholesterol and triglyceride values by age group are reported in Table 10. By comparison, normal ranges for cholesterol and triglyceride values in male subjects based on age can be seen in Table 11. Figure 3 depicts the changes in mean cholesterol and triglyceride values associated with increasing age. As can be seen from this graph, both cholesterol and triglyceride changes paralleled each other. Both of these values followed a strikingly similar pattern,

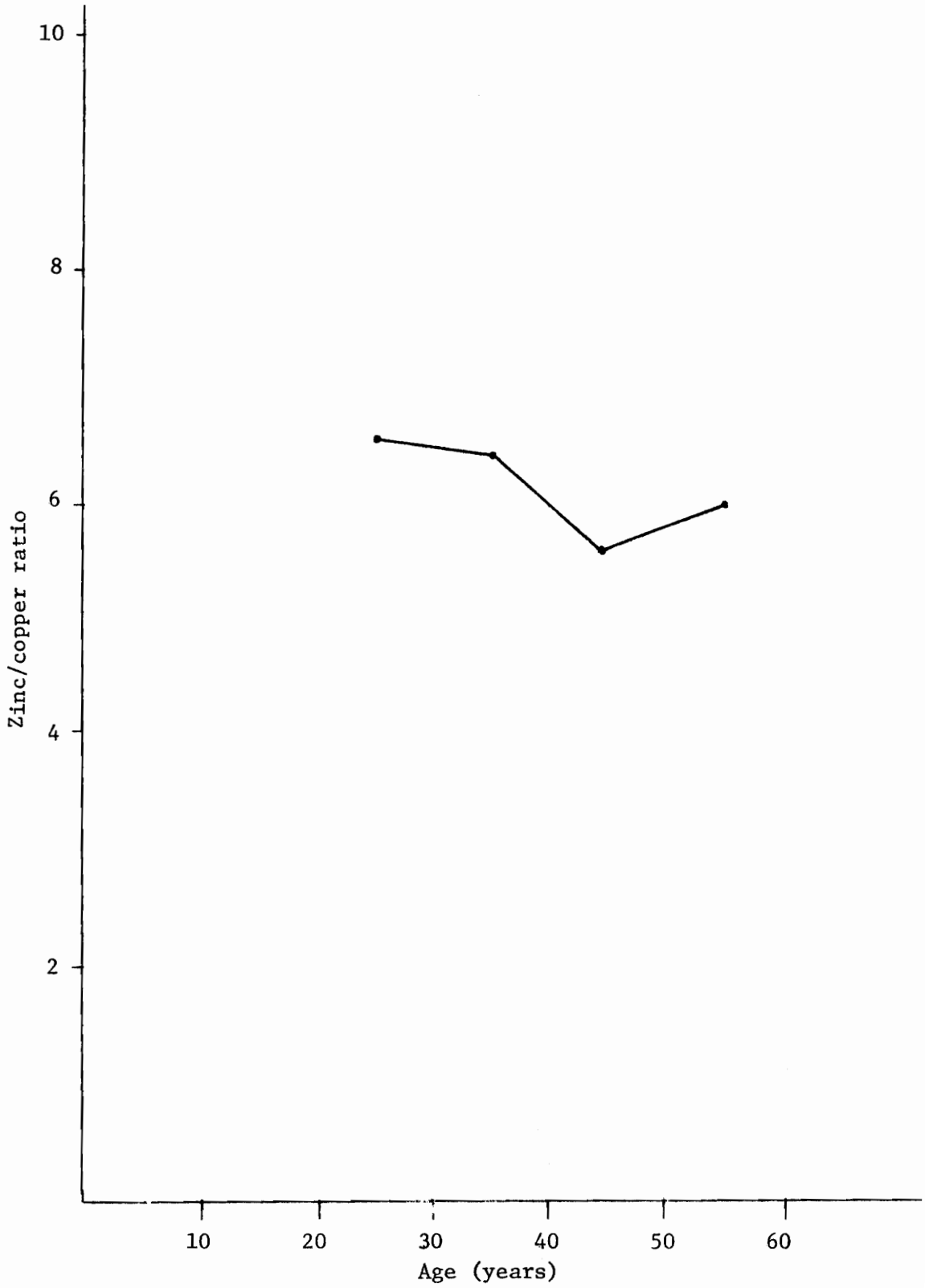

Figure 2. Relationship of zinc/copper ratio to age.

Table 10

SERUM CHOLESTEROL AND TRIGLYCERIDE VALUES
BY AGE GROUPS

Age (Decades)	n[1]	Serum Cholesterol (mg%) Mean	Serum Cholesterol (mg%) Range	Triglycerides (mg%) Mean	Triglycerides (mg%) Range
0-19	1	195.5	---	111.6	---
20-29	29	198.4	131.3-264.5	85.5	23.5-194.1
30-39	13	251.8	144.4-320.6	137.5	31.4-311.6
40-49	12	236.5	154.9-281.6	130.6	66.7-272.5
50-59	9	262.5	210.7-311.6	158.8	63.7-430.9

[1]Number of subjects

Table 11

NORMAL RANGES FOR CHOLESTEROL AND TRIGLYCERIDE VALUES IN MALE SUBJECTS BASED ON AGE[1]

Age	Cholesterol (mg/100 ml)	Triglycerides (mg/100 ml)
0-19	172 ± 34	61 ± 34
20-29	183 ± 37	73 ± 32
30-39	210 ± 33	78 ± 39
40-49	230 ± 55	60 ± 41
50-59	240 ± 48	104 ± 45

[1]Fredrickson *et al.* (142)

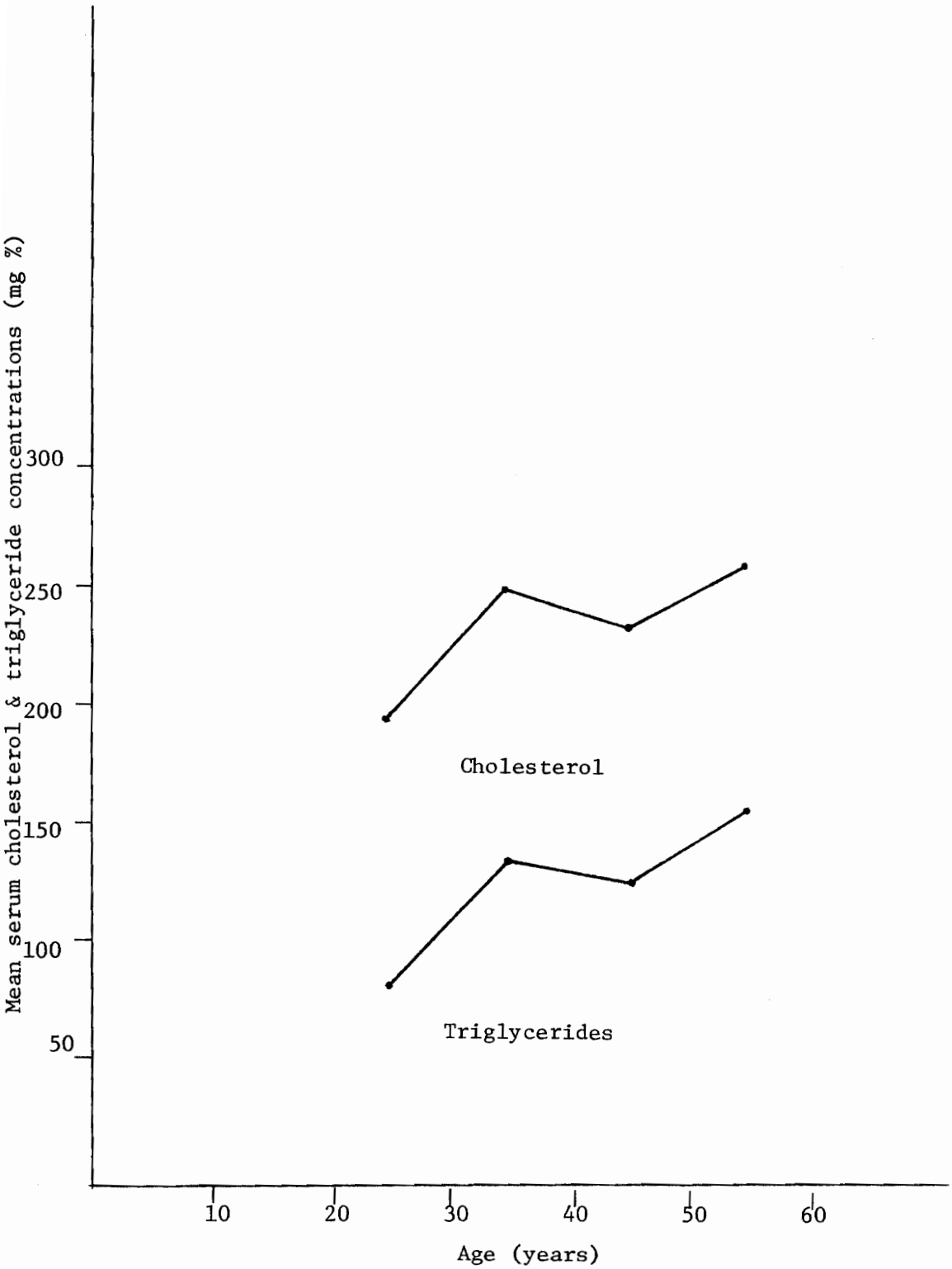

Figure 3. Relationship of serum cholesterol and triglycerides to age.

as illustrated by a sharp rise in mean concentrations from the second to third decade, followed by a slight decrease in values to the fourth decade, and rising again slowly to the highest concentrations in the fifth decade. Such a pattern was not entirely consistent with values reported in the literature (142-145) for these two parameters. The majority of investigators in this area have reported a gradual but steady rise in both cholesterol and triglyceride values with increasing age.

Although the plotting of mean values by age groups, for zinc, zinc/copper ratio, serum cholesterol, and triglyceride values appeared to indicate changes with age, a statistical analysis did not detect significant differences among these various means. Such results were most likely attributable to the wide range of variation within the groups. From these data it appeared that the concentration of zinc and copper in human hair, as well as serum cholesterol and triglyceride values, may vary considerably from individual to individual.

The mean exercise-activity score (mean ±S.D.) was 117.4 ± 121.1 WMR/BMR/hr./wk./intensity, with a range of 0-624 WMR/BMR/hr./wk./intensity. The exercise-activity score was evaluated as a risk factor in terms of low, moderate, and high levels of activity. The exercise-activity level by age is reported in Table 12. Forty-six subjects (71.9%) fell under the moderate level category, thirteen subjects (20.3%) were placed in the low level category, and only five subjects (7.9%) were placed under the high level category.

Table 13 shows the breakdown of exercise-activity score and

Table 12

EXERCISE-ACTIVITY LEVEL BY AGE[1]

Age (Decades)	n[2]	Exercise/Activity Level[3]		
		Low (38.8)	Moderate (117.4)	High (352.1)
0-19	1		1	
20-29	29	1	23	5
30-39	13	4	9	
40-49	12	4	8	
50-59	9	4	5	
TOTAL		13	46	5

[1]Number of Subjects

[2]Number of Subjects/Group

[3]WMR/BMR/hr./wk./intensity

Table 13

OBESITY AND EXERCISE-ACTIVITY
SCORE BY AGE

Age (Decades)	n[1]	Obesity[2] Mean	Obesity[2] Range	Exercise Activity Score[3] Mean	Exercise Activity Score[3] Range
0-19	1	101.6	---	135.8	---
20-29	29	104.7	84.8-148.2	176.7	24.2-624.0
30-39	13	109.1	98.2-118.7	68.5	4.4-178.5
40-49	12	112.4	93.6-123.6	64.3	1.1-151.6
50-59	9	107.2	95.4-118.6	65.4	0 -135.1

[1]Number of Subjects

[2]% 'Ideal' Weight

[3]WMR/BMR/hr./wk./intensity

obesity by age. The highest mean exercise-activity score, 176.7 WMR/BMR/hr./wk./intensity, was attained by the 20-29 age group. The remaining three decades, 30-39, 40-49, and 50-59, all had similar means. When the obesity risk factor was expressed in terms of age, notable differences between groups were not apparent. A total of twenty-four of the sixty-four subjects (37.5%) were > 10% over the 'ideal' weight for height; fifteen of these twenty-four subjects (23.4%) were determined as being overweight, with the remaining nine subjects (14.1%) determined as being obese.

Hypertension and cigarette smoking were insignificant as risk factor variables in this study. Only five of the sixty-four subjects (7.8%) were cigarette smokers, and only eight of the sixty-four subjects (12.5%) were hypertensive, with five of these eight subjects exhibiting blood pressure values only 5% above normal for their age.

Correlation coefficients revealed no discernable relationship between the risk factors associated with susceptibility for CVD and either the zinc and copper content of hair as a reflection of their nutritional status, or the zinc/copper ratio obtained from these values (see Table 14).

A comparison of two methods, atomic absorption spectrophotometry and neutron activation analysis, on five selected hair samples can be found in Table 15. The neutron activation analysis was performed by the Nuclear Activation Analysis Laboratory of the College of Engineering at V.P.I. & S.U. Two of the hair samples were from the Neutron Activation Analysis Laboratory Director and a neutron

Table 14

CORRELATION COEFFICIENTS FOR SEVEN RISK FACTORS WITH ZINC, COPPER, AND ZINC/COPPER RATIO

Risk Factors	Zinc	Copper	Zinc/Copper Ratio
Age	-0.029	0.125	-0.124
Cholesterol	-0.120	-0.034	-0.124
Triglycerides	-0.139	-0.044	-0.133
Blood Pressure	-0.093	-0.009	-0.108
Cigarette Smoking	0.035	0.081	-0.001
Obesity	-0.245	-0.089	-0.247
Exercise-Activity	-0.162	-0.051	-0.169

Table 15

COMPARISON OF ATOMIC ABSORPTION SPECTROPHOTOMETRY AND NEUTRON ACTIVITATION ANALYSIS FOR DETERMINATION OF ZINC AND COPPER CONCENTRATIONS IN HAIR

Sample No.	Zinc Concentrations AAS[1] (ug/g)	Zinc Concentrations NAA[2] (ppm)	Copper Concentrations AAS (ug/g)	Copper Concentrations NAA (ppm)
32	184.8	167	22.6	21
34	169.1	155	22.7	15
36	150.7	150	21.3	20
46	279.5	319	23.4	20
59	144.5	180	27.3	27
Mean	185.72	194.2	23.5	20.6

[1]Atomic Absorption Spectrophotometry

[2]Neutron Activation Analysis

activation analysis laboratory technician; the remaining three samples were randomly selected. All five hair samples underwent the washing procedure, as outlined in the analytical methodology section, prior to the neutron activation analysis. Zinc and copper values determined by the two different methods were variable and not consistently in agreement. In the case of copper, atomic absorption spectrophotometry resulted in concentrations which were 1.1 to 51.3% higher than those reported for the neutron activation analysis. In the case of zinc, atomic absorption spectrophotometry analysis yielded two concentrations which were higher and two concentrations which were lower than those found by the other method; the fifth sample was in agreement. Differences in exposure to trace metals was not a consideration in this study as all subjects were from the university community in the same small town.

If this particular study were repeated and/or expanded, several changes would be recommended. First of all, a place should be included on the questionnaire for self under the family history of CVD section. A section should also be included for prior hair sample treatment(s), e.g., shampoo used. As fluctuations are known to occur in blood pressure, blood pressure measurements should be taken in triplicate, with an average of the best two considered as the value for this variable. Thirdly, the CVD risk profile evaluated obesity as a risk factor in terms of % fat; as it was known that some of the subjects were greater than 20% of their 'ideal' weight and thereby classified as obese, they were nonetheless of a muscular build and as such would

most likely have had a lower % fat content. It is therefore recommended that any future analyses of obesity as a risk factor contain anthropometric measurements, such as skinfold thickness. Finally, serum cholesterol as a risk factor may not have been definitive in itself. Indeed, new findings are requiring a re-evaluation of blood lipid risk factors for CHD. While it appears that the total serum cholesterol level remains a good indicator for individuals under 50 years of age, it no longer appears to be a valid measure for older subjects. Research data has revealed that a high level of HDL (high density lipoproteins) may be associated with a decreased risk of CVD. If this study were repeated or expanded, fractionation of the blood into HDL, LDL, and cholesterol would be performed, at least in the older individuals, to give a better indication of risk attributable to the presence or lack of the HDL fraction. Future research in this area should also take into consideration standardization of sampling and analytical techniques for determinations of trace mineral content of human hair. There is no doubt that hair will become much more useful diagnostically as more careful and controlled research studies are done.

It is doubtful that Klevay's zinc/copper ratio theory would have been verified, even with an expanded sampling population by this experimental design. As an extension of the examination of this theory in humans, controlled intakes of zinc and copper, of varying levels and ratios, with a consideration of other nutrient interrelationships, e.g., phytate and protein content of the diets, might

be fed to individuals. Serum cholesterol and lipoprotein fractions could be monitored to test for any significant changes over time with varying intakes of zinc and copper, i.e., will the subjects become hypercholesterolemic with a high zinc to copper ratio ingested?

SUMMARY AND CONCLUSIONS

Zinc and copper content of hair samples obtained from sixty-four male subjects, ranging in age from 19 to 59 years, as well as a zinc/copper ratio calculated from these values, were determined and tested for a significant relationship with risk factors (obesity, hypertension, elevated serum cholesterol and triglycerides, cigarette smoking, and exercise-activity level) associated with susceptibility to cardiovascular disease. Correlation coefficients revealed no discernable relationship between either the zinc and copper content of hair, or the zinc/copper ratio obtained from these values.

Although a graphical plotting of mean values by age groups (decades), for zinc, the zinc/copper ratio, serum cholesterol, and triglyceride values appeared to indicate changes associated with age, a statistical analysis of variance detected no significant differences among these various means. Such results were attributed to the wide range of variation within groups for these parameters as determined in this sample population. Even at that, the concentrations, as analyzed for these factors, were within the realm of previously reported literature 'normal' values.

Whether or not Klevay's zinc/copper ratio theory is of some physiological significance in the etiology of cardiovascular disease was not established in this experimental research. The results of this study appeared to indicate that such an altered ratio was not even associated with any of the established risk factors presently related to the condition. It is doubtful that, even with an expansion

of sampling population, Klevay's hypothesis would have been verified.

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Appendix A
Subject Recruitment Notices

Collegiate Times

Males Needed For Heart Study

by Bob Lazo
Staff Writer

The department of human nutrition and foods is seeking subjects for a study to discover the relationship between trace minerals and risk factors usually associated with heart disease.

Subjects needed are males between the ages of 20 and 65. A blood and hair sample will be taken, as well as measurements of height, weight, and blood pressure.

Subjects will also be asked to complete a brief questionnaire concerning family history of heart disease, cigarette smoking, and the subject's exercise-activity level.

The entire experiment should last 15 to 20 minutes.

Results of each analysis will be available upon request for those interested in feedback about their health status.

Interested people are now being scheduled. For more information, contact Jane Geders, 951-5375 during the day, or 951-4994 after 5 p.m.

Daily Bulletin

MEN WANTED

Department of human nutrition and foods needs volunteer males between 20 and 65 to participate in study to determine relationship of trace minerals to presence of risk factors associated with susceptibility to cardiovascular disease. Will need from each subject blood and hair sample, height measurements, weight and blood pressure as well as information concerning family history of cardiovascular disease, cigarette smoking and exercise level. Should take no more than 20 minutes. For further information, contact Jane Geders at 5375 during day or at 951-4994 after 5 p.m.

Appendix B

SAMPLE COLLECTION CHECKLIST

1. Questionnaire _____
2. Blood Pressure _____
3. Height/Weight _____
4. Hair Sample _____
5. Blood Sample _____
6. Breakfast _____

(Code No. _____)

Appendix C

I. QUESTIONNAIRE

Name _____ Age _____

1. Which of the following male members of your immediate family have had any medical history of cardiovascular disease? Please indicate the nature of the condition.

Relationship	Nature of the Condition*
Paternal grandfather	_____
Maternal grandfather	_____
Father	_____
Brother(s)	_____
	_____
	_____
Uncle(s)	_____
	_____
No known family history	_____

* Coronary Artery Disease, Angina Pectoris, Coronary Thrombosis, Rheumatic Fever, Cardiac Enlargement, Valvular Heart Disease, Arteriosclerosis, Myocardial Infarction, Hypertension, Arrhythmia, Other (please specify)

2. How many cigarettes do you smoke per day?

   Number of cigarettes smoked/day _____

   Non-smoker _____

3. How long have you been smoking cigarettes? _____

4. Are you taking any medications (i.e., antihypertensive drugs, etc.) on a regular basis?

   _____ Yes _____ No

5. If so, please specify the name and daily dosage of the medication(s).

(Code No. _____)

6. Exercise-Activity Level

Please check the following exercises/activities in which you have participated over the past 12 months.

Activity	Frequency (days/wk.)	Duration (mins.)	Intensity
Baseball ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Basketball ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Bicycling ____ (on level roads)	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Bicycling ____ (on graded roads)	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)

(Code No. _____)

6. Exercise-Activity Level

Please check the following exercises/activities in which you have participated over the past 12 months.

Activity	Frequency (days/wk.)	Duration (mins.)	Intensity
Bowling ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Calisthenics ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Canoeing ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Carpentry ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)

(Code No. _____)

6. Exercise-Activity Level

Please check the following exercises/activities in which you have participated over the past 12 months.

Activity	Frequency (days/wk.)	Duration (mins.)	Intensity
Chopping Wood ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Farming ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Gardening ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Golfing ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)

(Code No. _____)

6. Exercise-Activity Level

Please check the following exercises/activities in which you have participated over the past 12 months.

Activity	Frequency (days/wk.)	Duration (mins.)	Intensity
Dancing:	____ 1 ____ 4	____ 1	____ Light (no sweating)
		____ 5-10	
Square ____	____ 2 ____ 5	____ 15-30	____ Mild (limited sweating)
		____ 30-45	
	____ 3 ____ 6	____ 45-60	
		____ 90(1½ hr.)	____ Moderate (sweating ≤ 5 mins.)
	Daily ____	____ 120(2 hr.)	
		____ 180-300(3-5 hr.)	
		____ >5 hr.	____ Vigorous (profuse sweating)
	____ 1 ____ 4	____ 1	____ Light (no sweating)
		____ 5-10	
Clogging ____	____ 2 ____ 5	____ 15-30	____ Mild (limited sweating)
		____ 30-45	
	____ 3 ____ 6	____ 45-60	
		____ 90(1½ hr.)	____ Moderate (sweating ≤ 5 mins.)
	Daily ____	____ 120(2 hr.)	
		____ 180-300(3-5 hr.)	
		____ >5 hr.	____ Vigorous (profuse sweating)
	____ 1 ____ 4	____ 1	____ Light (no sweating)
		____ 5-10	
Moderately ____	____ 2 ____ 5	____ 15-30	____ Mild (limited sweating)
		____ 30-45	
	____ 3 ____ 6	____ 45-60	
		____ 90(1½ hr.)	____ Moderate (sweating ≤ 5 mins.)
	Daily ____	____ 120(2 hr.)	
		____ 180-300(3-5 hr.)	
		____ >5 hr.	____ Vigorous (profuse sweating)
	____ 1 ____ 4	____ 1	____ Light (no sweating)
		____ 5-10	
Vigorously ____	____ 2 ____ 5	____ 15-30	____ Mild (limited sweating)
		____ 30-45	
	____ 3 ____ 6	____ 45-60	
		____ 90(1½ hr.)	____ Moderate (sweating ≤ 5 mins.)
	Daily ____	____ 120(2 hr.)	
		____ 180-300(3-5 hr.)	
		____ >5 hr.	____ Vigorous (profuse sweating)

(Code No. _____)

6. Exercise-Activity Level

Please check the following exercises/activities in which you have participated over the past 12 months.

Activity	Frequency (days/wk.)	Duration (mins.)	Intensity
Ice Skating ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Mountain Climbing ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Ping pong ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Pitching horseshoes ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)

(Code No. _____)

6. Exercise-Activity Level

Please check the following exercises/activities in which you have participated over the past 12 months.

Activity	Frequency (days/wk.)	Duration (mins.)	Intensity
Raquetball ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Running:			
Cross-country ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
On level surface ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
On graded surface ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)

(Code No. _____)

6. Exercise-Activity Level

Please check the following exercises/activities in which you have participated over the past 12 months.

Activity	Frequency (days/wk.)	Duration (mins.)	Intensity
Shooting pool ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Skiing ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Soccer ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Sprinting ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)

(Code No. _____)

6. Exercise-Activity Level

Please check the following exercises/activities in which you have participated over the past 12 months.

Activity	Frequency (days/wk.)	Duration (mins.)	Intensity
Swimming:	____ 1 ____ 4	____ 1	____ Light (no sweating)
		____ 5-10	
Normal ____	____ 2 ____ 5	____ 15-30	____ Mild (limited sweating)
		____ 30-45	
	____ 3 ____ 6	____ 45-60	
		____ 90(1½ hr.)	____ Moderate (sweating ≤ 5 mins.)
	Daily ____	____ 120(2 hr.)	
		____ 180-300(3-5 hr.)	
		____ >5 hr.	____ Vigorous (profuse sweating)
	____ 1 ____ 4	____ 1	____ Light (no sweating)
		____ 5-10	
Back-stroke ____	____ 2 ____ 5	____ 15-30	____ Mild (limited sweating)
		____ 30-45	
	____ 3 ____ 6	____ 45-60	
		____ 90(1½ hr.)	____ Moderate (sweating ≤ 5 mins.)
	Daily ____	____ 120(2 hr.)	
		____ 180-300(3-5 hr.)	
		____ >5 hr.	____ Vigorous (profuse sweating)
	____ 1 ____ 4	____ 1	____ Light (no sweating)
		____ 5-10	
Breast-stroke ____	____ 2 ____ 5	____ 15-30	____ Mild (limited sweating)
		____ 30-45	
	____ 3 ____ 6	____ 45-60	
		____ 90(1½ hr.)	____ Moderate (sweating ≤ 5 mins.)
	Daily ____	____ 120(2 hr.)	
		____ 180-300(3-5 hr.)	
		____ >5 hr.	____ Vigorous (profuse sweating)
	____ 1 ____ 4	____ 1	____ Light (no sweating)
		____ 5-10	
Side-stroke ____	____ 2 ____ 5	____ 15-30	____ Mild (limited sweating)
		____ 30-45	
	____ 3 ____ 6	____ 45-60	
		____ 90(1½ hr.)	____ Moderate (sweating ≤ 5 mins.)
	Daily ____	____ 120(2 hr.)	
		____ 180-300(3-5 hr.)	
		____ >5 hr.	____ Vigorous (profuse sweating)

(Code No. _____)

6. Exercise-Activity Level

Please check the following exercises/activities in which you have participated over the past 12 months.

Activity	Frequency (days/wk.)	Duration (mins.)	Intensity
Walking:	____ 1 ____ 4	____ 1	____ Light (no sweating)
		____ 5-10	
Slowly ____	____ 2 ____ 5	____ 15-30	____ Mild (limited sweating)
		____ 30-45	
	____ 3 ____ 6	____ 45-60	
		____ 90(1½ hr.)	____ Moderate (sweating ≤ 5 mins.)
	Daily ____	____ 120(2 hr.)	
		____ 180-300(3-5 hr.)	
		____ >5 hr.	____ Vigorous (profuse sweating)
	____ 1 ____ 4	____ 1	____ Light (no sweating)
		____ 5-10	
Moderately ____	____ 2 ____ 5	____ 15-30	____ Mild (limited sweating)
		____ 30-45	
	____ 3 ____ 6	____ 45-60	
		____ 90(1½ hr.)	____ Moderate (sweating ≤ 5 mins.)
	Daily ____	____ 120(2 hr.)	
		____ 180-300(3-5 hr.)	
		____ >5 hr.	____ Vigorous (profuse sweating)
	____ 1 ____ 4	____ 1	____ Light (no sweating)
		____ 5-10	
Rapidly ____	____ 2 ____ 5	____ 15-30	____ Mild (limited sweating)
		____ 30-45	
	____ 3 ____ 6	____ 45-60	
		____ 90(1½ hr.)	____ Moderate (sweating ≤ 5 mins.)
	Daily ____	____ 120(2 hr.)	
		____ 180-300(3-5 hr.)	
		____ >5 hr.	____ Vigorous (profuse sweating)
	____ 1 ____ 4	____ 1	____ Light (no sweating)
		____ 5-10	
Upstairs ____	____ 2 ____ 5	____ 15-30	____ Mild (limited sweating)
		____ 30-45	
	____ 3 ____ 6	____ 45-60	
		____ 90(1½ hr.)	____ Moderate (sweating ≤ 5 mins.)
	Daily ____	____ 120(2 hr.)	
		____ 180-300(3-5 hr.)	
		____ >5 hr.	____ Vigorous (profuse sweating)

(Code No. _____)

6. Exercise-Activity Level

Please check the following exercises/activities in which you have participated over the past 12 months.

Activity	Frequency (days/wk.)	Duration (mins.)	Intensity
Tennis ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Volleyball ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Weight lifting ____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)
Other (please specify) _____	____ 1 ____ 4 ____ 2 ____ 5 ____ 3 ____ 6 Daily ____	____ 1 ____ 5-10 ____ 15-30 ____ 30-45 ____ 45-60 ____ 90(1½ hr.) ____ 120(2 hr.) ____ 180-300(3-5 hr.) ____ >5 hr.	____ Light (no sweating) ____ Mild (limited sweating) ____ Moderate (sweating ≤ 5 mins.) ____ Vigorous (profuse sweating)

(Code No. _____)

With the above answers, if longer than 12 months, how long have you followed this pattern of activity? (Indicate for which specific activity)

7. What is your occupation? _____

8. How long have you been employed at this occupation? _____

9. To what extent (if any) does your occupation effect your activity level? Consider frequency, duration, and intensity.

Would you like the results of your analysis?

_____ Yes _____ No

II. Blood Pressure _____/_____ mm Hg

III.

Height _____ in. _____ cm.

Weight _____ lb. _____ kg.

Appendix D
WMR/BMR Values for Exercise-Activity Scoring

Activity	WMR/BMR
Baseball (S-6)[1]	3.0
Basketball	8.0
Bicycling (level)	4.0
Bicycling (graded)	6.0
Bowling	3.0
Calisthenics	4.5
Canoeing (S-3)	3.5
Carpentry	4.0
Dancing:	
Square	6.0
Clogging	6.395
Moderately	5.0
Vigorously	6.79
Chopping Wood (S-6)	5.5
Farming	3.74
Gardening (S-6)	5.0
Golfing (S-6):	
Walking	5.0
Power cart	3.5
Ice Skating (S-3)	7.0
Mountain climbing	8.0/4 hr.
Ping pong	4.0
Pitching horseshoes	3.0
Raquetball	12.0
Running:	
Cross-country	11.85
Level	14.05
Graded	16.86
Shooting pool	2.5
Skiing (S-3):	
Water	6.0
Snow	8.0
Soccer (S-6)	12.0
Sprinting	19.84
Swimming:	
Normal	4.0
Back-stroke	2.14
Breast-stroke	2.91
Side-stroke	3.36
Walking:	
Slowly	3.17
Moderately	4.0
Rapidly	5.99
Upstairs	15.70

Activity	WMR/BMR
Tennis	7.0
Volleyball	4.0
Weight lifting	6.0
Other:	
Karate	10.0
Push-ups	4.5
Sailing (S-6)	3.5
Pit digging	8.0
Yoga	3.0
Handball	12.0

[1]S-considered a seasonal activity, corresponding number of months

Appendix E
DATA

Subject #	Zinc (ug/g)	Copper (ug/g)	Zn/Cu Ratio
01	164.24	27.34	06.01
02	113.14	17.96	06.30
03	210.07	35.75	05.88
04	184.53	25.40	07.26
06	134.29	19.62	06.84
07	170.12	33.22	05.12
11	256.03	36.10	07.09
12	066.71	27.36	02.44
13	156.85	23.05	06.80
14	196.12	34.39	05.70
15	216.58	37.32	05.80
16	086.20	20.44	04.22
18	168.10	29.98	05.61
19	114.00	26.90	04.24
20	142.02	24.86	05.71
23	153.19	28.35	05.40
24	204.59	25.16	08.13
25	164.13	24.27	06.76
26	208.75	23.48	08.89
27	120.67	38.43	03.14
28	152.13	26.52	05.74
29	153.19	32.97	04.64
30	136.05	29.26	04.65
31	136.05	23.99	05.67
32	184.82	22.64	08.16
33	206.08	28.56	07.22
34	169.07	22.75	07.43
35	189.36	27.15	06.97
36	150.71	21.33	07.06
37	185.50	28.47	06.52
38	119.16	16.86	07.07
39	042.74	15.10	02.83
40	177.76	28.21	06.30
41	096.69	25.43	03.80
43	193.43	27.22	07.10
44	188.65	29.47	06.40
45	117.67	24.01	04.90
46	279.52	23.37	11.96
47	096.69	18.47	05.23
49	171.62	23.98	07.16
50	393.42	38.42	10.24
51	197.09	25.73	07.66
52	407.56	36.67	11.12
53	117.51	23.43	05.02
54	170.04	27.66	06.15

DATA cont'd.

Subject #	Zinc (ug/g)	Copper (ug/g)	Zn/Cu Ratio
55	203.09	27.93	07.27
56	143.69	30.71	04.68
57	213.40	30.58	06.98
59	144.49	27.33	05.29
60	132.66	33.43	03.97
61	221.07	30.09	07.35
62	188.39	30.01	06.28
63	215.08	30.69	07.01
64	098.16	19.68	04.99
65	102.43	18.18	05.63
66	116.17	27.77	04.18
67	344.27	31.34	10.99
68	203.09	29.71	06.84
69	144.92	25.51	05.68
70	168.62	26.81	06.29
72	117.51	29.50	03.98
73	119.01	32.67	03.64
74	211.58	34.11	06.20
75	204.59	30.47	06.71

DATA

Subject #	Age (yrs.)	Cholesterol (mg/100 ml)	Triglycerides (mg/100 ml)
01	29	247.3	088.8
02	48	229.7	161.9
03	49	237.3	148.9
04	21	206.0	046.9
06	57	290.4	097.1
07	35	231.3	048.1
11	28	164.3	067.0
12	42	247.6	089.3
13	24	156.3	083.2
14	31	262.8	115.6
15	20	174.0	194.1
16	26	206.2	070.7
18	45	154.9	080.5
19	54	311.6	431.0
20	52	233.2	085.5
23	44	264.7	171.6
24	25	206.0	073.0
25	26	189.2	099.4
26	23	192.0	086.6
27	25	264.5	135.6
28	27	200.9	153.3
29	45	224.1	116.6
30	37	209.8	089.5
31	22	259.8	045.7
32	29	223.4	082.0
33	27	190.4	100.8
34	53	273.0	211.0
35	33	264.3	101.7
36	27	174.5	061.0
37	25	202.7	045.7
38	33	286.2	200.9
39	27	204.4	074.1
40	37	222.8	230.8
41	26	160.1	064.6
43	30	144.4	067.1
44	35	229.6	060.4
45	19	195.5	111.6
46	24	197.0	100.2
47	37	241.9	235.9
49	24	185.5	079.4
50	54	239.1	153.8
51	48	261.0	066.7
52	36	270.5	055.3
53	23	131.3	063.2

DATA cont'd.

Subject #	Age (yrs.)	Cholesterol (mg/100 ml)	Triglycerides (mg/100 ml)
54	53	293.7	170.6
55	24	219.5	099.4
56	20	184.4	052.1
57	22	206.0	154.8
59	22	157.4	080.4
60	51	276.7	118.9
61	25	195.4	137.0
62	48	237.3	082.8
63	25	256.6	067.6
64	31	285.2	031.4
65	47	260.0	178.3
66	26	248.2	023.5
67	25	151.0	049.3
68	46	281.6	112.4
69	30	303.7	311.6
70	37	320.6	239.4
72	49	257.2	272.5
73	59	210.7	063.7
74	55	234.0	097.8
75	45	182.1	085.1

DATA

Subject #	Blood Pressure (mm Hg)	Obesity (% 'ideal' wt.)	Exercise-Activity Score WMR (BMR/hr./wk./intensity)
01	138/090	094.9	145.2
02	132/070	116.0	049.8
03	130/090	123.0	004.3
04	120/080	091.7	055.6
06	130/080	095.8	118.0
07	112/060	101.2	098.5
11	112/070	107.8	024.2
12	142/090	119.7	099.7
13	130/070	091.1	146.3
14	126/062	111.2	146.4
15	124/078	108.6	365.8
16	128/060	103.2	220.7
18	138/074	109.2	005.5
19	140/090	105.5	064.2
20	120/080	136.5	118.7
23	110/068	114.9	046.9
24	112/064	093.6	259.9
25	120/070	099.3	063.1
26	118/060	112.7	047.0
27	122/070	100.6	170.8
28	090/038	148.2	123.5
29	126/080	093.6	120.0
30	124/070	116.7	021.5
31	128/078	121.7	093.5
32	130/038	104.7	154.6
33	112/078	110.5	104.9
34	132/082	108.6	013.4
35	146/100	110.4	069.8
36	118/072	107.2	179.6
37	120/064	104.6	583.4
38	122/078	110.2	051.5
39	124/090	108.9	360.2
40	136/070	108.5	058.2
41	118/062	089.0	071.2
43	138/088	105.3	020.6
44	106/070	109.0	114.9
45	118/070	101.6	135.8
46	120/070	098.1	164.3
47	102/068	112.2	004.4
49	110/078	126.1	187.4
50	140/094	097.4	000.0
51	130/086	098.0	035.5
52	120/084	104.7	049.7
53	112/060	097.0	086.5

DATA cont'd.

Subject #	Blood Pressure (mm Hg)	Obesity (% 'ideal' wt.)	Exercise-Activity Score WMR (BMR/hr./wk./intensity)
54	160/100	101.9	098.4
55	110/072	092.6	065.7
56	150/084	133.5	624.0
57	110/078	106.1	078.3
59	152/070	100.0	111.2
60	132/088	118.6	011.9
61	128/078	084.8	045.6
62	138/088	114.3	108.1
63	114/078	085.7	052.6
64	128/080	111.8	050.0
65	130/090	123.6	151.6
66	110/060	112.3	381.9
67	120/060	100.8	158.2
68	118/070	121.5	001.1
69	128/078	118.7	178.5
70	128/074	098.2	026.3
72	130/070	120.3	064.0
73	108/060	104.7	135.1
74	140/088	095.4	029.3
75	124/070	094.8	084.8

DATA

Subject #	Cigarette Smoking (#/day)	Occupation[1]	Family History[2]	Medications[3]
01	00	2	01	0
02	00	5	01	0
03	00	4	02	0
04	00	1	00	0
06	00	5	00	1
07	00	4	01	0
11	00	4	02	0
12	00	4	02	0
13	00	2	02	0
14	00	3	02	0
15	00	1	01	0
16	00	2	04	0
18	00	4	03	0
19	00	3	09	0
20	00	3	01	3
23	00	4	01	0
24	00	2	01	0
25	00	2	00	0
26	00	2	02	0
27	00	2	01	0
28	25	2	00	1
29	00	4	01	0
30	00	4	00	0
31	00	2	01	0
32	00	2	00	0
33	00	2	00	0
34	00	4	02	0
35	00	3	00	0
36	00	3	00	0
37	00	2	01	0
38	00	4	01	1
39	00	2	00	0
40	00	4	03	0
41	00	2	01	0
43	00	4	03	0
44	00	4	02	0
45	00	1	01	0
46	00	2	01	0
47	00	3	00	0
49	00	2	01	0
50	00	4	01	1
51	00	2	01	1
52	00	4	01	1
53	00	2	00	0

DATA cont'd.

Subject#	Cigarette Smoking (#/day)	Occupation[1]	Family History[2]	Medications[3]
54	00	4	10	2
55	00	1	01	0
56	00	1	00	0
57	00	1	01	0
59	00	1	01	0
60	00	4	03	0
61	25	3	01	0
62	00	4	03	0
63	00	5	02	0
64	00	2	01	0
65	00	4	00	0
66	00	2	00	0
67	00	2	01	0
68	25	4	00	0
69	00	4	02	0
70	18	4	01	0
72	00	5	01	0
73	13	5	00	0
74	00	4	04	1
75	00	5	00	0

[1]1-undergraduate student
2-graduate student
3-staff
4-faculty
5-other

[2]number of male members of immediate family who have had any medical history of cardiovascular disease

[3]number of medications taken on a regular basis

Appendix F
Subjects' Results-Report Format

The results of your blood analysis for cholesterol and triglyceride concentrations were:

	Cholesterol (mg/100 ml)	Triglycerides (mg/100 ml)
Your value	_____	_____
Normal range (based on your age group)	_____	_____
Range reported in this study (based on your age group)	_____	_____

Thank you again for your participation in this research study. Additional results will be forwarded at a future date.

Jane M. Geders, Department of Human Nutrition & Foods

VITA

Jane M. Geders was born May 24, 1950 in St. Louis, Missouri.

She received her Associate of Arts degree from St. Petersburg Junior College, where she was a member of Phi Theta Kappa, a scholastic honorary fraternity. She was the recipient of a Bachelor of Science degree in Home Economics Education from Florida State University in August, 1972 and a Master of Science degree in Foods and Nutrition from the same institution in March, 1974. She is presently a candidate for the Doctor of Philosophy degree in Human Nutrition and Foods at Virginia Polytechnic Institute and State University.

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Jane M. Geders

RELATIONSHIP BETWEEN ZINC AND COPPER NUTRITIONAL STATUS AND RISK FACTORS ASSOCIATED WITH CARDIOVASCULAR DISEASE

by

Jane M. Geders

(ABSTRACT)

A study was conducted to assess the relationship between the zinc/copper ratio and risk factors (hypertension, obesity, elevated serum cholesterol and triglycerides, cigarette smoking, and exercise-activity level) associated with susceptibility to cardiovascular disease. A questionnaire technique for assessment of physical activity was presented. The zinc/copper ratio was determined from the zinc and copper concentrations of hair samples obtained from sixty-four male subjects, ranging in age from 19 to 59 years. Correlation coefficients revealed no discernable relationship between either the zinc and copper content of hair, or the zinc/copper ratio obtained from these values.

Serum cholesterol and triglyceride values, zinc concentrations in hair and the zinc/copper ratio were tested for significant differences associated with age. Statistical analysis indicated that these parameters were not significantly different. Such results were attributed to the wide range of variation within groups for this sample population.

Data was not supportive of Klevay's altered zinc/copper ratio hypothesis as the major factor in the etiology of cardiovascular disease.