DIETARY ANALYSIS OF ARCHAEOLOGICAL HAIR SAMPLES FROM PERU

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by
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This thesis is lovingly dedicated to the memory of my mother, Marian Murray Brewer. I miss you everyday.

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Abstract

The purpose of this research project was to determine if the effects of diet are distinguishable from diagenesis through trace element analysis on hair samples from ancient inhabitants of Peru. Factors were identified that defined patterns derived primarily from meat sources, vegetable and grain sources, salt sources, or diagenesis. Factor analysis identified two dietary components which varied as predicted from archaeological analyses of diet by site. The factor score means varied significantly by location, location by sex for the meat sources intersection, and by vegetable and grain sources. Factor score means also varied significantly by sex and location by sex for the sources of salt. Age at the time of death was significantly correlated with meat sources. Because sex and age varied across pooled samples from locations and two of the five factors covariated with age at the time of death, the concentrations of trace elements found in these samples cannot primarily result from diagenesis. Log (Ba/Sr) means varied significantly for location and for sex by location where marine resources were available either through trade or directly where there was strong marine component in their diets. Further confirmation of a nondiagenic source for the elements was shown through their different patterns across the longitudinal length of the hair.

Introduction

The purpose of this thesis was to determine if the effects of the diet are distinguishable from diagenesis in hair samples from ancient inhabitants of Peru through trace element analysis. Previously collected hair samples were digested and analyzed on an Inductively Coupled Plasma – Mass Spectrometer (ICP-MS). The data was analyzed using the SPSS statistical package (v. 10.0 for Windows).

Early Physical Analysis of Hair

The earliest studies were mentioned by Brothwell and Spearman. They presented a history of pre-1900 research done on ancient hair in a chapter of the 1963 edition of *Science and Archaeology*. The first known study was reported in 1860. Browne analyzed human hair samples from South American, from Lima, Pachacamac, Arica, Pisco, Mexico, and Brazil (1960). In 1877 Pruner-Bey studied Egyptian and Peruvian specimens for pigmentary and structural variation. The fair hair of Guanche mummies was used as evidence to support Shurbsall's hypothesis that the Canary Islands had a population of fair-haired individuals in the past (Brothwell, et al., 1963).

In the 1920's, Hrdlicka studied "Old Americans" to compare skin, hair, and eye color, with respect to each other and to gender, anomalies of pigmentation, and regional distribution (1922). He also looked at the graying of hair and hair loss, finding that men turn grey faster and lose their hair faster than women, although there is wide individual variation. Trotter studied the life cycles of human hair in order to better understand irregularities of hair growth, and noted that the cycles of

that the cycles of individual hair follicles are of relatively constant duration (1924). Wynkoop (1929) collected information on cuticle scales, medullas, and shaft diameters to determine the correlation with the age of the individual donating the hair. He found that there is no correlation between age and the morphological type of hair, the size of cuticle scales, or the hair shaft diameter. In the 1930's Kneberg sought to improve cross sectional techniques for determining hair color, form, and texture and reported that a hair index is not a reliable racial criterion (Kneberg, 1935).

In the 1940's Steggerda looked closely at hair morphology in relation to "racial" groups. While several groups were studied, intraracial variation in dimensions was found to exceed the interracial differences observed (Steggerda and Seibert, 1941). It appears that Steggerda did not follow up this method of study because he later describes his work with hair and statistics as merely showing the possibilities for future research (Steggerda, 1943). By the end of the 1940's studies of hair became more common. Trace element studies of hair minerals began to be presented in the 1960s.

Structure of Hair

Human hair growth starts during the third month of fetal life. Hair fibers are then created through a cyclic pattern of cell proliferation and differentiation that continues through life (Sen, 1996). Hair consists of a strong exterior layer or cuticle, a cortex, and an inner layer called the medulla. Most trace elements detected in hair reside in the cuticle, which is made up of polymerized protein in a homogeneous high-sulfur protein matrix. This protein matrix consists primarily of the amino acid

the amino acid cysteine (Al-Hashimi, et al, 1992). While hair is growing, the matrix cells at the papilla of the follicle have a high rate of metabolic activity, which causes them to absorb elements introduced in the diet.

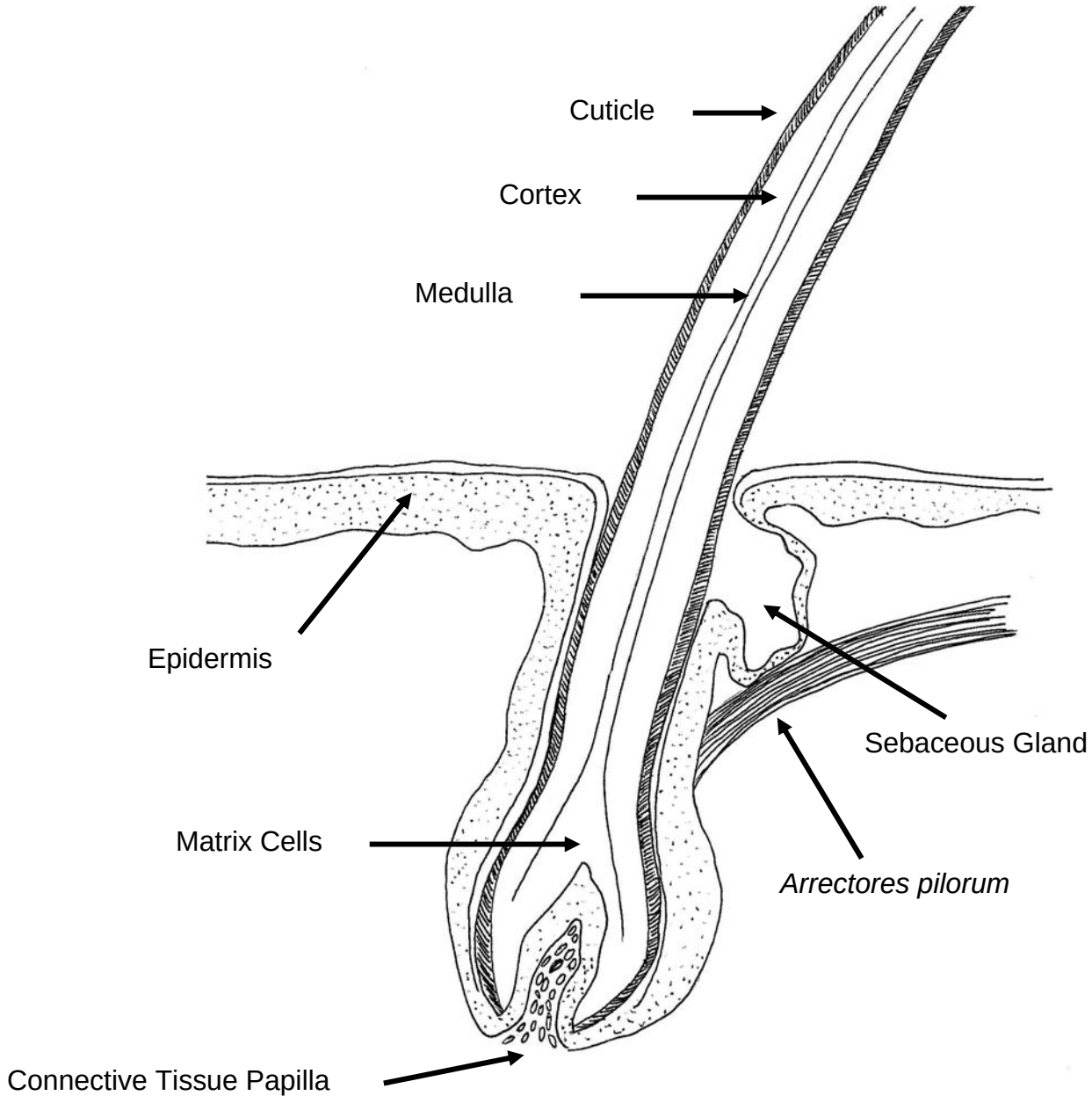


Figure 1: Human Hair Follicle and surrounding tissues.

The growing hair is exposed to this activity for a short time as it approaches the skin's surface and hardens through keratinization. The trace elements accumulated during growth are then sealed into the hair (Katz and Katz, 1992). Once it grows past the skin's surface, hair is isolated from the body's ongoing metabolic activity (Graf, 1978). Hair differs from bone in that the organic part of hair is made up of keratin, a cross linked protein, while bone is primarily comprised of collagen (Wilson et al., 2001). Studies on bone collagen and hair keratin show that isotopic values in both are related to dietary protein isotopic values (O'Connell and Hedges, 1999).

Osseous (bone) and hair tissue also differ in that as a whole, the chemical analysis of bone represents an average of the isotopic composition of the diet over a long period of time (O'Connell and Hedges, 1999). In contrast, hair grows at a constant rate. Once it is formed, biogenic turnover stops, thereby setting the biological makeup of any segment of hair as a snapshot in time of that individual's life (Wilson et al., 2001). Each hair follicle is associated with a minimum of one sebaceous gland. Minute muscle bundles, the *Arrectores pilorum*, also associated with hairs are involuntary and cause "goose-bumps". When the muscles contract to form these bumps sebum is compressed into the hair follicle (Sanford, 1993). Including sebum, there are five primary sources of elements to growing hair which include matrix cells, the connective tissue papilla, eccrine secretions, apocrine secretions, and the epidermis (Sanford, 1993).The elements obtained through ingestion or environmental exposure are applied to hair through endogenous deposition from the connective tissue papilla and matrix cells. Eccrine sweat

cells. Eccrine sweat exogenously deposits salts high in sodium and potassium on the hair. Other trace elements deposited through sweat include nitrogen, calcium, phosphorus, magnesium, copper, manganese, and iron (Sanford, 1993). According to Katz and associates, the link between hair growth and heavy metal concentrations in the body suggests hair functions as an excretory organ for arsenic and other potentially toxic metals (Katz and Katz, 1992). This suggestion has been confirmed (Foo, et al., 1993).

Even though sebum is not believed to contribute directly to the application of elements in hair, the wax and lipids excreted on hair aid in the physical binding of trace elements from other sources. The chemical composition of keratin also keeps trace elements tightly bound in the hair, making it difficult for these elements to be lost from the hair's structure (Sanford, 1993). No consensus exists on the average number of strands a human sheds. A range from 100 to 200 strands per day can be assumed based on different sources (Wilson, et al., 2001, Cahill, 1996, Bonnichsen and Schneider, 1995). Bonnichsen suggests that an average person, with a 60 year lifespan could produce over 4,000,000 hairs in a lifetime (Bonnichsen, et al, 2001). This explains the relative abundance of human hair in archaeological sites.

The endogenous deposition sources previously mentioned are not the only sources of elements on hair. Exogenous depositions of trace elements also occur and are influenced by exposure to soil, water, air, and bacteria (Doi, et al, 1988; Mikasa, et al., 1988). All of these factors have to be considered when studying hair, especially when studying samples that have been exposed to the elements post-

post-mortem, such as those recovered from archaeological sites.

Archaeological hair samples have been found at several sites across the globe. Unfortunately, many researchers have failed to realize the possible dietary significance of studying elements and their isotopes in human hair, preferring human bone tissue instead. Because it is flexible and durable, hair can be folded into sample containers and studied by non-destructive techniques, such as Neutron Activation Analysis (NAA). Other destructive analyses, such as Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) analysis can be useful for studying hair, bone, teeth, and other human tissues.

Scientists from fields such as molecular biology, genetics, and geochemistry can make contributions to the field of archaeology by studying hair samples. Hairs from an archaeological site can be analyzed for abundance patterns, the types left by different organisms, DNA, and archaeological information (Barnaby, 1999).

DNA analysis of hair can provide important information. Current research is being done to compare modern hair samples from Mongolia to 10,000 year old hair samples from a site in Montana. Through DNA analysis, it is hoped to determine the origin of these early inhabitants of North America (Cahill, 2003).

Bonnichsen, an archaeologist and the current director of the Center for the Study of the First Americans in Texas, is interested in early Holocene and late Pleistocene peoples. Bonnichsen and Bolen (1985) point out that while shed hair does not survive some preservation conditions, it does preserve in Artic permafrost, dry and arid caves, and damp, cold environments such as those found in high

found in high altitude limestone caves. While sieving sediments at a 14,500 year old site, Bonnichsen noticed several hairs clinging to the screen. A single, long, black hair was also found at a 10,000 year old site. Working with soil scientists, Bonnichsen developed a method to remove hair from sediment. Besides hair fragments, this method has also collected bird feathers, fish scales, plant matter, beetles, and other organic material (Bonnichsen and Schneider, 1995). This research had to be discontinued when several Indian tribes claimed the hair samples as human remains subject to repatriation under the Native American Graves Protection and Repatriation Act of 1990. Because of this the Bureau of Land management has stopped the excavations at the Mammoth Meadow site in Southwest Montana and any further testing on previously recovered human hair.

Methods of organic matter recovery have led to the finding of human hairs in cave sediments from four caves in the Dordogne Valley and near the French Pyrenees, in France. These sites have been studied because they also contain cave art but little other information about the individuals who created that art (Holden, 1998). DNA analysis from these samples may help scientists discover if these individuals were Paleo-Frenchmen or ancestors of the Laplanders or another group (Holden, 1998).

Modern Research

Besides trace element studies, a single strand of hair can be used to determine drug use, diet, toxicological studies, and mitochondrial DNA sequence (Wilson, et al., 2001). According to Wilson, the pigment of hair captures an individual's trace metal and drug history. Stable isotope and trace element analysis

analysis can provide information on diet, health, environment, and a geographical record.

Because hair is made up of carbon, and radiocarbon accelerator mass spectrometry has been improved to the point that analysis can be performed on samples as small as 20 micrograms, radiocarbon dating can be performed on as little as a 2 - 4 cm segment of hair (Hodges, et al, 2001). The range of techniques used to analyze hair, both ancient and contemporary, have also included microscopic analysis, fluorescence microscopy, pigmentation, anthropometric variability, and parasitism (Brothwell, et al., 1963).

Hair Chemistry: Isotope Studies

All plants, with the exception of succulents, are either C3 or C4 plants. C4 plants include maize, sorghum, and millet. These retain more of the ^{13}C isotope from the atmosphere. Because of this, during photosynthesis, C4 plants retain a higher $^{13}C/^{12}C$ ratio than C3 plants. C3 plants include wheat, barley, rice, fruits, vegetables, and nuts. The isotopic composition of the plant is passed on to consumers and therefore can be followed along the food chain (White, et al., 1999). Other elements that have been utilized for stable isotope analysis are nitrogen, oxygen, strontium, (Larsen, 1997) and sulfur (Macko, et al., 1999).

Macko et al. (1999), have performed stable isotope analysis on a wide variety of archaeological hair samples. Their studies of a hair sample from the Tyrolean Iceman found in the Italian/Austrian Alps suggested the individual ate a primarily vegetarian diet. However, this was later contradicted by at least two different studies focusing on the Iceman's intestinal contents and will be discussed under

discussed under Methodological Concerns.

Other findings by Macko include a huge range of food eaten by Egyptian Copts from 700 AD and a significantly narrower range of food for Middle Kingdom Egyptians living several thousand years earlier. He has also reported that hair from 7000 BC Chinchorro mummies found on the West coast of Chile indicated these individuals ate less seafood the further they lived from the coast (Barnaby, 1999). Macko's team has also studied amino acid composition of both modern and ancient hairs and found the relative percentages of individual amino acid to be similar in both samples.

Among many investigations, the use of carbon isotope analysis to determine trade routes in the Nile valley is illustrative (White, et al., 1999). White and associates studied hairs from six Roman-Byzantine Period (410 - 700 AD) individuals and found that they ate significantly greater quantities of C3 foods than C4 foods. C4 foods were not produced locally and were apparently not imported through trade with northern Sudanese populations. This has been confirmed through historic documentation and archaeological data on diet. A diet consisting of more C4 foods was consumed by individuals of the Wadi Halfa area of Nubia. Changes along the strands of hair from the latter indicate shifting patterns between C3 and C4 plants. This indicates seasonal fluctuations in the level of the Nile (White, et al., 1999). Although isotope analysis was not performed on the Peruvian hair samples of this study, the work cited suggests it may be a beneficial line of research to pursue.

Hair Chemistry: Trace Elements

According to Aras and Ataman, trace elements serve two general functions in the human body (2006). The first is as a structural material and the second is in regulating the numerous biological activities of the body. A summary of the significance of several trace elements in hair and the functions in the body of living people are given below. The elements presented include those discussed by Chatt and Katz (1988), Aras and Ataman (2006), and those analyzed in this research. Research on trace elements of different tissues, such as bone, hair, finger nails, and others provide a connection between how the body utilizes trace elements and give clues in the diagnosis of disease. Different tissues also provide different times spans of analysis. For example, tissues such as bone midshaft specimens produce a lifetime average, whereas teeth sample childhood, and hair, the last year or so of life. Trace element analysis of other tissues also may direct future hair analysis.

Aluminum

Aluminum concentrations in hair may reflect environmental exposure (Chatt and Katz, 1988). Higher levels of aluminum were found in hairs from dyslexic children in comparison to control children (Capel, et al., 1981). By sampling hair from rabbits injected with aluminum lactate, Yokel found a variable increase in the aluminum concentration in the hairs. It was suggested that hair may be a useful indicator of aluminum body burden in such aluminum induced conditions as dialysis encephalopathy (Yokel, 1982).

Antimony

Elevated levels may reflect occupational exposure and/or systemic intoxication (Chatt and Katz, 1988). Exposure to antimony may be monitored through hair.

Arsenic

The chief function in the body for arsenic is increased arginine urea and ornithine, as well as metabolism of methyl compounds (Aras and Ataman, 2006). Elevated levels may reflect systemic intoxication (Chatt and Katz, 1988). Hair samples have been studied to monitor arsenic exposure in locations all over the world, such as West Bengal, India (Das, et al., 1985), Antofagasta, Chile (Borgono, et al., 1977), and Zimapan, Mexico (Armienta, 1997), to name a few. Increased manganese and arsenic concentrations were found in hair samples of school children who scored poorly in tests to assess general intelligence, visual motor skills, receptive language, verbal memory, non-verbal problem solving and behavioral problems (Wright, et al., 2005). In the study, a significant manganese by arsenic interaction was found.

Barium

Rosborg and associated (2003) compared hair samples from women living in two areas of Sweden, one with acid in water because of acid rain and the other with more alkaline water. In both cases the women got their drinking water from unfiltered private wells. The researchers found that the hair concentration of barium and boron were significantly higher for women living in the acid region.

Boron

In the body boron aids in the control of membrane function, nucleic acid biosynthesis, and lignin biosynthesis (Aras and Ataman, 2006). Boron has been found to be higher in women living in an acid area of Sweden (Rosborg, et al., 2003) than an alkaline area.

Cadmium

Cadmium stimulates elongation in ribosomes (Aras and Ataman, 2006). Elevated levels in hair may reflect systemic intoxication (Chatt and Katz, 1988). Exposure to cadmium has been shown to decrease fertility in men (Gennart, et al., 1982).

Calcium

Calcium plays a major role in human physiology, as the principal constituent of bones and teeth. It is also involved in muscle contraction and relaxation, nerve function, blood clotting, and blood pressure (Aras and Ataman, 2006). Concentration levels in hair may be related to cystic fibrosis, myocardial infarction, and/or nutritional status (Chatt and Katz, 1988).

Chlorine

Chlorine is part of stomach acid, necessary for proper digestion (Aras and Ataman, 2006).

Chromium

In the body, chromium is associated with insulin and required for the release of energy from glucose (Aras and Ataman, 2006). Concentration levels may be

may be related to diabetes mellitus (Chatt and Katz, 1988). Davies and associates (1997) sampled 51,665 hair, sweat, and serum samples obtained from 40,872 patients and found a significant decrease in chromium levels in diabetes. It was suggested that reduced chromium levels may increase diabetes mellitus as well as coronary artery disease.

Cobalt

Cobalt is part of vitamin B_{12}, which is involved in nerve function and blood formation (Aras and Ataman, 2006).

Copper

Copper has an effect of adsorption of iron in the body and is part of several enzymes (Aras and Ataman, 2006). Concentration levels in hair may reflect nutritional status (Chatt and Katz, 1988). Deficient copper concentrations in hair and serum have been found to be related to chronic diarrhea in children, especially when accompanied by malabsorption (Rodriquez, et al., 1985). Vir and associates noted an increase in hair and serum copper concentrations of pregnant women (1981). These levels persisted throughout pregnancy and early postpartum.

Fluorine

Fluorine is important for the formation of bones and teeth, and helps make teeth resistant to decay and bones resistant to mineral loss (Aras and Ataman, 2006). Elevated levels in hair may reflect environmental exposure and/or systemic intoxication (high in ocean fish) (Chatt and Katz, 1988).

Iodine

In the body iodine is part of thyroxin, which regulates metabolism (Aras and Ataman, 2006). Iodine is rapidly incorporated into the external layers of hair roots and can be easily removed during washing. Because of this, hair is not a good source for biological monitoring of iodine exposure (Zareba, et al., 1995).

Iron

Iron is an important part of haemoglobin formation in the body. It is also part of myoglobin and used in energy utilization (Aras and Ataman, 2006). Comparing the iron concentration of hair to the body mass index of 180 young women in Taipei, Taiwan, Wang and associates (2005) found that the lower the iron concentration, the higher the body mass index. The authors further suggest that individuals with lower iron concentration also had lower cognitive performance. In another study, inhalation exposure of iron in workers in the iron and steel industry led to increased high blood pressure and hypoglycemia (Chai, 2004).

Lead

In the body lead plays a role in many enzyme effects (Aras and Ataman, 2006). Elevated levels in human hair may reflect systemic intoxication (Chatt and Katz, 1988). Research has found that hair lead levels can be considered an indicator of exposure to lead pollution (Sen, 1996; Sanna, et al., 2003). In pre-industrial archaeological hair, lead is an indicator of contamination.

Lithium

Lithium plays a role in the body's control of sodium (Aras and Ataman, 2006). Low levels of lithium have been found in mothers of children with autism and in children with autism (Adams, et al., 2006).

Magnesium

Magnesium plays a major role in human physiology, involved in bone mineralization, protein synthesis, enzyme action, normal muscular contraction, and nerve transmission (Aras and Ataman, 2006). In human hair, depressed bound magnesium may be related to cystic fibrosis (Chatt and Katz, 1988). An inverse relationship has been found between magnesium concentration in hair and body mass index (Wang, et al., 2005). Looking at children with selected neurological disorders (hyperexcibility, loss of consciousness, and epileptiform convulsions of an unknown origin), Lech (2002) found significantly reduced magnesium levels in their hair and suggested the reduced magnesium may be the cause of these disorders.

Manganese

In the body, manganese facilitates enzyme functions and many cell processes (Aras and Ataman, 2006). Elevated levels may reflect occupational exposure (Chatt and Katz, 1988). Increased manganese and arsenic concentrations were found in hair samples of school children who scored poorly in tests to assess general intelligence, visual motor skills, receptive language, verbal memory, non-verbal problem solving and behavioral problems (Wright, et al., 2005). In archaeological bone, manganese is often correlated with aluminum (Pate and Hutton, 1988).

Mercury

Elevated levels of mercury may reflect environmental exposures and/or systemic intoxication (Chatt and Katz, 1988). Batista (1996) and colleagues sampled 233 children in Tarragona Province, Spain for mercury. They found a significant correlation between an increased frequency of eating fish and seafood and an increase in the concentration of mercury in the hair. They also found that girls had more mercury in their hair than boys.

Similar results were reported for women living in the Amazon Basin (Dolbec, et al., 2001). The authors also suggested that mercury concentration in hair also varied due to the seasonality of the fish being consumed.

Molybdenum

In the body, molybdenum facilitates enzyme functions and many cell processes (Aras and Ataman, 2006). Cao and associates (1998) compared hair and serum molybdenum concentrations to patients with gastric cancer and found that concentrations from both samples were lower for patients with gastric cancer than control specimens. They suggested that molybdenum deficiency may be a risk factor for gastric cancer. Positive correlations between molybdenum in water and in hair samples from an acid and an alkaline region of Sweden led researchers to stress the importance of intake from minerals in water (Rosborg, et al., 2003).

Nickel

Environmental exposure of nickel was suggested by Spruit and Bongaarts (1977) when they noticed a decrease in nickel concentration blood and serum of

individuals on vacation compared to concentrations during work times. Hair concentrations were also included in this study. Nickel is a constituent of urease and is a factor in reduced haemopoiesis in the body (Aras and Ataman, 2006). Elevated concentration levels in hair may reflect environmental exposure (Chatt and Katz, 1988).

Phosphorus

In the body, phosphorus is part of every cell and is involved in pH buffering (Aras and Ataman, 2006). As reported earlier, Mierkeley and associates (2001) noted increased calcium and phosphorus levels in patients with endocrinologic pathologies.

Potassium

Potassium facilitates many reactions in the body, including protein synthesis, nerve transmission, and muscle contraction (Aras and Ataman, 2006). Elevated levels in human hair may be related to cystic fibrosis or celiac disease (Chatt and Katz, 1988). Wang and associates (2005) noted that women with the highest body mass index had the highest levels of potassium. Higher concentrations of potassium and sodium in the body may inhibit the passage of potassium and sodium ions through cellular membranes and lead to edema and an individual becoming overweight. The authors further suggest that potassium and sodium content in the body should be monitored to prevent a person from becoming overweight.

Rubidium

Goulle and associates (2005) determined reference values though Inductively

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) techniques on whole blood, plasma, urine, and hair. This work suggested that ICP-MS is a fast and effective toxicological tool.

Selenium

Selenium helps protect body compounds from oxidation (Aras and Ataman, 2006). Elevated levels in hair may reflect occupational or environmental exposures (Chatt and Katz, 1988). Spallholz and associates (2005) studied the effect of selenium on arsenic concentration. To show that selenium has the effect of reducing arsenic toxicity, they studied hair samples from five countries. Countries with high arsenic concentrations showed low concentrations of selenium, with the exception of Nepal, where the source of high selenium was suggested to come from the use of henna on hair.

Sodium

Sodium helps maintain ionic strength of body fluids (Aras and Ataman, 2006). Elevated levels in hair may be related to cystic fibrosis or celiac disease (Chatt and Katz, 1988).

Sulfur

In the body, sulfur is a component of certain amino acids, part of biotin, thiamin, and insulin (Aras and Ataman, 2006).

Thallium

In an effort to find a successful tool to determine thallium poisoning, Frode Maurice and associates (2002) analyzed hair using isotope dilution electrothermal vaporization inductively coupled plasma mass spectrometry. The authors found that

authors found that thallium concentrations in exposed individuals decrease along the hair shaft with concentration being highest at the hair root. Individuals who had not been exposed to thallium had concentrations below the detection limit for the method.

Tin

In the body, tin interacts with riboflavin (Chatt and Katz, 2006). Environmental studies have been done to compare tin concentrations in two diverse populations. Shah and associates (2006) studied hair samples from Pakistani and Libyan populations and found that tin concentrations were higher in the Libyan population.

Uranium

Karpas (2001) suggested that hair and nail analysis may overcome some of the drawbacks of analysis of urine for determination of uranium. In a study of 205 individuals living in Finland and drinking from privately drilled wells, Karpas and associates (2005) suggested that analysis of hair, nails, and urine could be used to monitor uranium exposure through the ingestion of drinking water.

Vanadium

Vanadium is important in the body in its role of controlling of the sodium pump and inhibition of ATPase (Aras and Ataman, 2006). A study was performed to compare body mass index to vanadium concentration in hair from students at the Medical University of Bialystok, Poland (Stefan'ska, et al., 2005). Female students

Female students had a higher concentration of vanadium compared to male students. Students with higher body mass index had lower amounts of vanadium levels.

Zinc

In the body, zinc is part of many enzymes, present in insulin, involved in making genetic material and proteins, immunity, Vitamin A transport, taste, wound healing, making sperm, and normal fetal development (Aras and Ataman, 2006). Depressed levels in hair may be related to nutritional deficiency (Chatt and Katz, 1988). Individuals who consume distilled beverages have been found to have increased hair zinc concentrations in a study of chronic alcoholics by Gonzales-Reimers and associates (2002). In adults in New Zealand, hair concentrations of zinc showed no relation to seasonality, reported dietary variety, ethnicity, gender, and hair color (Rush, et al., 2003).

Longitudinal Studies of Hair by Sampling Location on Shaft

Human hair grows at different rates, depending on location and kind (Sanford, 1993). Fine hair grows at roughly 1.5mm/week while coarse hair grows at 2.2mm/week. The average growth rate is about 1 cm per month. Passwater and Cranton (1983) confirmed that hair grows at the rate of approximately 1 millimeter every three days, also about one cm per month.

Location on the hair shaft indicates the precise time during growth of the hair when deposited. The distribution of essential trace elements, sulfur, iron, copper, zinc, and selenium appear relatively consistent along the length of the hair, but vary widely when analyzed as absolute values (Sky-Peck, 1990). Yujawa and associates

Yujawa and associates (1984) profiled trace elements along the length of hair and found that iodine, magnesium, calcium, and copper increased from the scalp to the tip, as concentrations of chlorine and bromine decreased. After embedding hairs in resin, Cookson and Pilling (1975) used a proton induced X-ray method to determine element concentrations across the diameter of the hairs. They noted that concentrations changed depending on the time of year when the hair was collected and that concentrations of various elements are not constant in various parts of the hair strand. These findings were attributed to a change in the person's health or "mode of life". Samples studied were chosen because the individuals were known to have high amounts of either lead from an unknown origin or arsenic from therapeutic use. The arsenic concentration was found to be uniform but the lead concentration was found to vary greatly.

Hambidge and Droegemueller (1974) compared concentrations of zinc, copper, chromium, and manganese in the hair and plasma of 20 women during their pregnancies. The concentrations decreased between 16 and 38 weeks gestation for some elements in the hair samples. For the plasma samples there was an increase in the copper, chromium, and manganese concentrations, while zinc and iron concentrations decreased.

Benfer compared hair samples from ancient Peruvian specimens, finding irregular increases over time in concentrations of iron, magnesium, calcium, sodium, potassium, strontium, chromium, manganese, cadmium, and lead, but not in copper or zinc, as well as high concentrations of chromium that indicates a lack of diabetes among the sampled individuals (Benfer, et al., 1978). A later study showed that five

study showed that five prehistoric hairs from Paloma and Chilca I (dating from 7,800 to 4,700 BP), each of which could be divided into scalp, proximal, middle and distal segments, had an increase proximally in chromium and copper. Cadmium showed increases in three of the samples (Benfer, 1986).

However, criticisms of trace element analysis have been made for hair and other tissues (Sillen, et al., 1989, Steindel, et al., 2001). Therefore, I proceed here with caution. Studies of the behavior of elemental concentrations of prehistoric peoples, whose diets differed drastically from today should help us find the advantages and usefulness of the method. Trace elements have been studied for specific diseases, as discussed above. Some studies deserve further review since they are more pertinent to prehistoric studies.

Health Studies

Tavakkoli and associates (2000) analyzed hair samples using INAA from an Iranian population. They found significant correlations between aluminum and manganese, aluminum and vanadium, magnesium and calcium, and magnesium and vanadium. Furthermore, there was no significant age correlation with any element concentration. Comparisons were made to studies done in England and New Zealand where it was discovered that the Iranian concentrations of aluminum, calcium, chlorine, sulfur, potassium, and vanadium were higher than those in the other countries. To aid in the diagnosis of toxic elements in patients, hair samples from a group of patients in a clinic in Brazil were studied (Saiki, et al., 1998). Saiki and associates suggested that reference ranges should be used with caution as a normally healthy population living in a distinct region may have a variation in

a variation in nutritional and environmental conditions, and that factors such as sex and age may play a role in the accumulation mechanism of trace elements in hair.

Hair chromium studies continue to be a topic of interest in health studies. In several studies Hambidge and his associates have studied the concentrations of chromium in the hair of children with juvenile diabetes mellitus and normal children, as well as comparing the levels in women who have and have not had children (Hambidge, et al., 1968, 1969). Gestational diabetes appears to be associated with much higher levels of chromium than for not diabetic pregnant women (Aharoni, et al., 1992).

Findings showed that chromium levels in adults appeared to be influenced by both sex and parity, and that mean levels of chromium in adults were lower than the levels in normal children. More recent investigations of a very large sample showed that chromium decreased with age in the hair of individuals suffering from type II diabetes mellitus (Davies, et al., 1997).

High levels of chromium in hair may suggest the presence of diabetes, however, longitudinal variation may be a better predictor of the disease. Stupar and associates (2007) studied longitudinal hair chromium (H-Cr) profiles in a group of patients with type II diabetes mellitus and healthy elderly subjects. The subjects were matched by age and sex and were measured by solid sampling electrothermal atomic absorption spectrometry, providing data on the magnitude of variation of Cr content along the hair length. Their findings suggested that the longitudinal H-Cr profile resembles the variation in Cr metabolic rate over the time span of growing

time span of growing hair, which was not appreciably affected by external contamination. This suggests that glucose intolerance (type II diabetes mellitus) is an important factor that disturbs Cr metabolism.

Burger studied the hair of patients of Kwashiorkor, a disease of young children (1974). Frequently, victims of Kwashiorkor experience hair changes such as a thinning of their hair, a lack of shine and luster, and a change in color to either red or grey. Because of the known changes in hair during this disease, trace element and mineral analysis were performed to determine the effect of treatment of the disease on the hair of the victims. A comparison of the trace elements in hairs of Kwashiorkor patients upon admission to a hospital and at discharge showed an increase in concentrations of bromine, copper, molybdenum, chromium, cesium, and cobalt. The same comparison saw a decrease in sodium, chlorine, vanadium, manganese, iodine, barium, selenium, cadmium, and zinc.

Graf (1978) compared 29 matched hair samples from mother-infant pairs, from Turku, Finland, for 8 elements; zinc, copper, iron, manganese, chromium, lead, cadmium, and manganese. This study took into account the mother's age group and presence of diabetes, as well as number of previous offspring, prematurity, and sex of the infant. Graf found the number of births affected the concentration of iron in the infant's hair and the sex of the infant affected the concentration of magnesium in the infant's hair. The same method has been used to determine trace element deficiencies in potential mothers prior to conception (Barlow, et al. 1985). This analysis was proposed to improve women's health before conception and prevent

women's health before conception and prevent birth defects. It was found that the concentration of magnesium was lower in mothers who did not have diabetes than those who did.

Health analysis can also be considered for an individual as well as a population. Trace element analysis of the hair of Duke Mirko Petrovich-Njegos showed a reduced concentration of zinc, a known symptom of a person suffering from cholera. The presence of elevated levels of mercury (Hg) indicate the Duke was being treated with Calomel (Hg_2Cl_2), one of the two known medications of the time used to treat cholera. Both results verified previous speculation that the duke was suffering from cholera at the time of death (Stupar, et al., 2005).

Another case of a post-mortem health study is the analysis of hair from Napoleon Bonaparte. It has long been suspected that Napoleon died from arsenic poisoning. Lin and colleagues analyzed a hair sample taken at the time of Napoleon's death and a hair sample taken seven years prior to his death for nineteen different elements (Lin, et al., 2004). Their findings showed elevated levels of arsenic and mercury in both samples. The elevated mercury is possibly due to Napoleon taking certain medicines. There was no explanation for the elevated levels of arsenic. Because the hair samples were taken several years apart and both contained elevated levels of arsenic, this was thought to reduce the possibility of arsenic poisoning as the cause of Napoleon's death. However, more recent investigation shows that Napoleon was exposed to arsenic over a period of time (Kintz, et al., 2002). Studies of elemental concentrations in hair always are problematic due to contamination. Historic and prehistoric specimens may be

may be expected to show more contamination.

Possible Sources of Contamination

According to Limic and Valkovic, body stores, genetic effects, body fluids, and environment are the factors that influence the concentration of trace elements in human hair (Limic and Valkovic, 1986). Exposure studies have focused on the effects of outside contaminants on hair. Limic and Valkovic (1986) attempted to create a model where concentration profiles of trace elements were compared to environmental contributions. It was determined that cross sectional and longitudinal distribution of trace elements can be predicted as long as diffusion parameters are known.

Mercury contamination was studied in Brazilian Indians living in Xingu Park, a reservation. This study employed INAA and cold vapor atomic absorption spectroscopy to determine if the mercury content was the result of environmental contamination from gold mining activities in the Amazonic region. High levels of mercury were found, which were speculated to have entered the systems of the individuals tested through fish consumption (Vasconcellos, et al., 2000).

Environmental studies such as the changes in methyl mercury exposure from preindustrial to modern times, are also of interest. Inorganic Mercury (Hg) is a naturally occurring element in the Earth's crust. The distribution of mercury across the globe has changed due to human activities such as coal burning and garbage incineration. Mercury is methylated by microorganisms and enters the food chain through marine food stuffs. Egeland and associates (1999) found that ancient

ancient human and animal hair show lower levels of methylated and total mercury in comparison to samples of hair from current residents living near the archaeological sites. However, a study of samples taken from the Karluk One Archaeological site near the current Karluk village on the Kodiak Archipelago of Alaska showed higher than expected levels of Hg with low MeHg/Hg ratios. The authors suspected contamination from the soil contributed to the high levels of mercury (Egeland, et al., 1999).

Hsiung (1974) compared copper and zinc concentrations in hair from a semi-rural and urban Missouri population. It was found that while zinc concentrations were higher in a southeastern population, copper concentrations were higher in the St. Louis population. Hsiung's results showed that females in both regions had higher copper and zinc concentrations than males. The most striking difference between the two populations was a greater concentration of copper in children age 0-12 years in the urban population, which decreased with age. In the semi-rural population the copper concentration did not change with age indicating a dietary copper deficiency in individuals in the Bootheel. Air pollution or another factor was suggested as the cause of increased copper content in the St. Louis area.

The possibility of using human hair as a pollutant dosimeter has been studied by Al-Hashimi, Krishnan, and Jervis (1992). Their purpose was to determine to what capacity hair absorbs pollutants from the environment. Samples of heavy metals were applied to human hair and the concentrations were determined by neutron activation analysis (NAA). Low levels of heavy metals were absorbed into the hair

metals were absorbed into the hair samples. Rauf and Jervis (1002) considered environmental factors when studying Indonesian, Canadian, and Canadian-of-Indonesian-origin individuals for eleven elements using instrumental neutron activation analysis. Their results indicated that differences in trace element concentrations in hair can result from moving from one location to another. The Canadian-of-Indonesian-origin group mainly reflected the same environment as the Canadian group.

Because the potential for environmental exposure of lead, zinc, and cadmium is large in mining regions of Poland, Chlopicka and associates studied the hair and blood of 158 children for these trace elements. It was found that boys accumulated more lead and cadmium in their hair and blood than girls (Chlopicka, et al., 1998). These studies are pertinent for archaeological specimens from post-industrial age societies. However, even simpler societies may have had technologies such as metal or ceramic production, which could introduce contamination into living tissue.

In order to understand whether there has been contamination or instead concentrations are related to health, diet, disease, or hair treatment, we have to know what is the range of values expected in normal subjects.

Reference Studies

Senofonte and associates found significant differences for specific trace element concentrations with respect to age and sex on normal Roman boys from 3 – 15 years old (Senofonte, et al., 2000). They also reported overall reference values for 19 minor trace elements. Sakai and associates studied the concentration of zinc,

concentration of zinc, copper, manganese, and iron from 418 subjects ranging in ages from 6 months to 20 years (2000). Reference values were reported as comparable to previous studies, and were analyzed versus age and sex differences. They concluded that a diet consisting of higher concentrations of copper, manganese, and zinc is required for growing children, especially adolescents.

A study of 336 healthy subjects, age 16 or less, from urban areas in Southern Italy were compared for elemental concentrations by age and sex (Perrone, et al., 1996). An overall difference in concentrations of bromine, iron, and zinc were found between male and female children, and concentrations of bromine, copper, and chromium between male and female adolescents. The authors stressed the need for a matched control group when analyzing trace elements in hair for information on nutrition and health (Perrone, et al., 1996). The effects of age and sex on trace element concentrations on an urban population in Pakistan were studied. The goal of this study was to collect baseline information and to evaluate trace element levels with respect to the nutritional status of hair donors in terms of industrial, agricultural, and occupational exposure (Asharf, et al., 1994). It was reported that higher levels of arsenic, mercury, and potassium were found in the rural population compared to the urban population. The increased arsenic and mercury were associated with pesticide and herbicide use in the rural areas (Asharf, et al., 1994). Batzevich studied hair samples from 17 ethnic groups in the former USSR. Because of variation in local factors, Batzevich was unable to identify racial and ethnic groups.

groups. However, abnormal nutrient intake was identified through hair trace element analysis (Batzevich, 1995).

Herber and associates (1983) compared trace element information from 183 eight-year-old children in Amsterdam. The trace element concentrations were compared among ethnic groups within the study. Nutritional status, anthropometric variables, and hematological variables were also studied for trace element concentrations. In general, their findings corresponded to findings previously reported in literature. They suggested that the concentration of iron in hair is related to the individual's daily dietary intake of iron.

The studies above differ in methodology, a subject that requires careful discussion.

Methodological Concerns

There is a danger of contamination affecting the study of prehistoric hairs. While studying the degradation of hair, scientists from the department of Archaeological Science and the Department of Biomedical Sciences, at the University of Bradford, UK, suggested that hair suffers from selective break down (http://www.sciencenet.org/uk/slup/CuttingEdge/Dec99/hair.html, 1999). Even though archaeological hair appears to be completely intact, the internal structure of the hair may degrade, leaving only the outer material. With the loss of its internal structure, the outer layer is more susceptible to contamination from the burial environment.

Other research has challenged the isotopic findings of Stephen Macko. In contrast to Macko's findings, studies of the Tyrolean Iceman found meat remains in his colon and other evidence the individual was omnivorous (Dickson, et al., 2000).

Using DNA extraction and reconstruction of samples from the intestines of the Iceman, Rollo and associates (2002) also found evidence that the Iceman ate red deer meat and cereals for his last meal. The Iceman also had previously eaten ibex meat, different species of dicots, and cereals. Both studies reduce the credibility of isotope analysis of human hairs.

Sanford and Kissling (1994), while encouraged by the possibility of trace element analysis on hair and bone samples, are also cautious about expecting too much from this method of study. They point out that many of the basic assumptions used in trace element research concerning archaeological samples are untested, and recommend that more experimentation be performed. The present study used a design that permits identification of effects independent of contamination. These can be recognized when samples pooled across archaeological sites and different burial environment show expected properties by sex and age. A further test of the contamination hypothesis is offered by comparison of expected change in concentrations along individual hair shafts.

Chapter 2: Materials and Methods

Hair Samples were provided from the archaeological sites of Chongos, Buena Vista, and Huaca Malena.

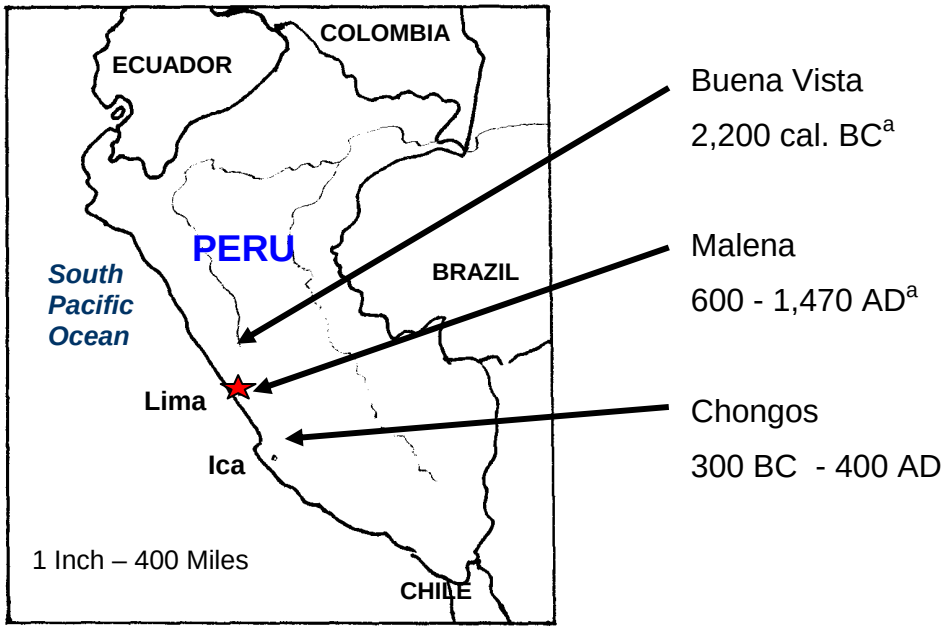


Figure 2. Map of the Sample Sites, Peru, South America. [a]Dates listed indicate the approximate age of the samples, not the occupation of the sites.

The Chongos site is located in the Pisco River Valley, approximately 220 Km South of Lima. This site was first discovered by Dwight Wallace and was excavated in 1985, when the National Institute of Culture in Peru sponsored a salvage excavation (Peters 1987, 1988). Approximately 70 sets of remains of humans were recovered and are housed at the Ica Museum of Anthropology in Ica, Peru. Preservation is good to excellent with natural mummification in many instances (Dietz and Bergfield, 2001). Hair samples were collected by Michael Dietz in August 1999, June 2000, and May 2001.

Huaca La Malena is located in the Valley of Asia, between the valleys of Mala

Mala and Cañete. The coastal side of the Valley of Asia has occasionally become swampy but overall has very little vegetation. The coast would be arid without canal irrigation, which was part of the subsistence pattern during the occupation of the site (Mejia Xesspe, 2000). The site is know to possess components that span the Middle Horizon period, 600-1000 AD, and the Late Intermediate period, 1000-1470 AD (Angeles and Pozzi-Escot, 2000a, 2000b). Sample collection was supervised by Robert A. Benfer. Hair samples were collected during July 2002.

Buena Vista is located north of Lima, on the north bank of the Chillon river. The site was originally identified as having a preceramic component by Engel (1987). The site's transitional Preceramic/Initial Period component, from which samples were drawn, has been confirmed through field work directed by Benfer (http://web.missouri.edu/~nad2b1/BuenaVista/Index.html, 2004, Duncan, et. al, 2004). Sample collection occurred in 2004, under the supervision of Benfer.

Methods of Analysis

The hair samples were handled as little as possible in the field. They were taken from a sterile dusty matrix. Plastic tweezers and rulers were predominately used to manipulate the samples. Stainless steel scissors and spatulas were used when necessary.

Figure 3. Photo of Hair Sample RAB003 (RB3001 – RB3005 for serial sections) from B.P. 1800, Ent. 2. Female, Commingled Remains, Chongos, Peru.

To prepare hair samples for digestion, a visual inspection was performed and any visible particulate matter or connected tissue was scraped away before cleaning. The hair samples were cleaned by placing each sample in a vial of 10mL acetone. The samples were then sonicated for two minutes and the rinsate was poured off. At least two successive sonications in 10mL of 10X distilled water were performed. Samples were determined to be clean if no discoloration or particulate matter was observed in the rinsate. Cleaning was continued until these criteria were met. Once the samples were cleaned they were allowed to air dry. Samples that continued to show a colored rinsate or particulate were repeatedly rinsed and sonicated until they were determined to be clean. Excessively dirty samples received a second sonication in acetone on top of successive sonications in distilled water. Some samples were also soaked overnight in distilled water.

Three separate digestions were performed. The initial digestion took place on eleven samples from Chongos and one modern control. The samples were digested using an Ethos Plus Microwave Labstation. The digestion tubes were cleaned by adding 10mL of ultra pure concentrated nitric acid (HNO_3) which were allowed to reflux in the microwave for at least 20 minutes at 180ºC. This process was performed twice.

Each hair sample was weighed and placed in a clean digestion tube with 3ml of ultra pure concentrated HNO_3. One blank sample was prepared using 3ml of concentrated HNO_3. The samples were then digested for 20 minutes at 190ºC. Once cooled, the resulting liquid was transferred to a centrifuge tube that had been pre-rinsed with 2% HNO_3. The sample solutions were weighed and diluted to approximately 10mL with 2% HNO_3.

The second digestion included 23 samples, also from Chongos, which were digested in the microwave as described above. Some of the samples were longer and contained more hairs than those of the original digestion. If it could be determined which end of the sample was proximal to the scalp, the sample was divided into serial sections.

Samples were divided into 2.54cm (1 inch) segments to maximize sample size. Each segment was treated as a separate sample for digestion and analysis. For the purpose of this study, each segment was considered to represent approximately two months worth of growth.

The third digestion set included samples from Malena, Buena Vista, and a modern comparison. It was determined that microwave digestion was not needed to completely digest these samples. Fifty-four samples and one blank were digested by placing them in pre-rinsed centrifuge tubes and adding 3mL HNO_3. The samples were allowed to sit for eight days. At the end of this time there were no visible hair fragments. However, some tubes still contained what appeared to be proteinaceous material floating in the acid. One milliliter of 30% H_2O_2 was added and weighed to remove this material. After sitting for another day no proteinaceous material could be seen. The samples were diluted to 10mL with 2% HNO_3.

Each digestion procedure included a blank sample consisting of 10X distilled water. After each digestion the samples were analyzed using a VG Axiom high resolution Inductively Coupled Plasma - Mass Spectrometer. This analysis was performed by Robert J. Speakman at the MU Research Reactor, at the University of Missouri – Columbia campus. Blank values, if detected, were subtracted from the data during analysis.

Statistical analysis was performed using SPSS software. The original elemental concentrations were transformed to the natural log (Ln) of the data to reduce skewness. Missing values were next estimated using SPSS 14, using the series mean method. The percent missing ranged from 0% to 42% for elemental concentrations. The data were then analyzed for outliers by converting the values to z-scores. Values that were three standard deviations away from the means for each element were removed before further analysis. Factor Analysis was performed using

was performed using the design found in Table 1. Tables 2 and 3 show the sample numbers by location, sex group, and age group.

Table 1: Research Design		
Factor Analysis		
Parameter		Setting
Data Reduction		Factor Analysis
Descriptives		Initial Solution
Extraction Method		Principle Component
		Correlation Matrix
		Rotated Solution
Number of Factors Equal to		5
Maximum Iterations for Rotations		25
Rotation Method		Varimax
Factor Scores		Save as Variables
Options		Coefficient Display Format
		Sorted by Size
Suppress Absolute Values Less Than		0.300
General Linear Model		
General Linear Model		Univariate
Dependent Variables		Factor Scores
Fixed Factors		Location, Sex, Location*Sex
Model		Full Factorial
Significance Level		0.05
Confidence Intervals		95%

Table 2: Research Design - Location and Sex Group		
Location Group	**Sex**	**N**
Buena Vista (B)	f (Female)	1
	m (Male)	4
	u (Undetermined)	7
Chongos (C)	f	14
	m	9
	u	14
Huaca Malena (M)	f	15
	m	3
	u	2

Table 3: Research Design - Age Group		
Group	**Age Range in Years**	**N**
0	Unknown	13
1	1 - 8	13
2	9 - 18	4
3	19 – 30 (Young Adult)	28
4	$\geq$ 31 (Old Adult)	11

Chapter 3: Results

The first step in interpreting this data was to determine if analysis of the modern samples falls within acceptable ranges. A comparison of the 17 modern samples with the reported trace and minor element concentrations of hair can be seen in Table 4.

Table 4: Reported Ranges for Modern Trace Elements in Hair

Element	Iyengar and Iyengar (mg/Kg)*	Doctor's Data**[a]	MineraLab**[a]	Thesis Cont (mg/kg)[c,d,e]
Arsenic (As)	0.10-0.30	0.4	2-3	0.07-0.15
Barium (Ba)	0.40-2.00	-	-	65.34-175.8
Cadmium (Cd)	0.25-1.00	1.6	1-2	0.15-9.24
Cesium (Cs)	0.10-1.00	-	-	0.0005-0.00
Chromium(Cr)	0.30-1.20	1.03-3.23	0.5-1.5	1.69-22.51
Cobalt (Co)	0.05-0.30	-	0.2-1	0.15-1.47
Copper (Cu)	15-25	17-67	12-35	119.91-386.4
Iron (Fe)	30-60	21-50	20-50	49.57-470.2
Lead (Pb)	2-20	15	20-30	7.42-40.63
Magnesium (Mg)	-	29-137	25-75	4,301.34-11,992.92
Manganese (Mn)	0.50-1.50	0.62-1.97	1-10	1.01-6.40
Nickel (Ni)	0.02-0.20	1.8	1-2	0.54-22.69
Potassium (K)	-	42-430	75-180	5.79-46.63
Rubidium (Rb)	1.00-2.00	-	-	0.02-0.08
Selenium (Se)	0.50-1.00	0.08-0.64	3-6	1.29-4.37
Sodium (Na)	-	346-1080	150-350	1.91-261.62
Thallium (Tl)	10-50[b]	-	-	0.0013
Uranium (U)	0.003-0.03	-	-	0.75-1.60
Vanadium (V)	0.05-0.15	-	0.5-1	0.42-1.55
Zinc (Zn)	150-250	104-288	160-240	886.99-1,207.13

*(Iyengar and Iyengar, 1994)
**(Katz and Chatt, 1988)

[a]Assumed mg/kg
[b]Uncertain
[c]Of the 24 elements tested, 10 elements from the modern control had one segment, sample RB7001, a minimum of 100% higher in concentration than the highest concentrations of the remaining segments. Because of this, Sample RB7001 was removed from all ranges listed, except for thallium where that segment was the only segment to show a recorded concentration.
[d]Hair was dyed sometime prior to analysis.
[e]Only analyzed values listed. No imputed values were included.

Elements whose concentrations fell either partially or fully within the range of one of the reported concentrated ranges included: arsenic, cadmium, cesium, chromium, cobalt, iron, lead, manganese, nickel, potassium, selenium, sodium, and vanadium. Elements whose concentrations were higher than the reported ranges were barium, copper, magnesium, and zinc. Elements whose concentrations were lower than the reported ranges were rubidium and thallium.

Longitudinal Analysis

Next, a comparison of trends was performed to see if serial sections of ancient hair followed the same pattern as modern hair. From the end proximal to the scalp to the distal end the concentration of copper, lead, nickel and arsenic is reported to increase (Katz and Chatt, 1988) along with iodine, magnesium, and calcium (Yujawa et. al, 1984). Yujawa also reported that concentrations of chlorine and bromine decreased from scalp to tip. Figures 3 - 6 show a comparison of serial sections from each of the sites listed to serial sections of modern hair for copper, lead, magnesium, and nickel. Concentration of arsenic over time was not plotted because only a few hair segments from the modern sample showed any recordable concentration for comparison. These figures indicate that each sample of hair from which serial sections were taken shows the expected trend as reported in literature

the expected trend as reported in literature and seen by comparison with a modern sample.

Figure 7 shows a slight decrease in selenium concentration as it grows away from the scalp for all samples. This indicates that the archaeological samples, like the modern sample, do not have increasing concentrations for all elements over time. Changes in element concentrations across the hair shaft may be due to increasing exposure to the environment. It is also possible that the loss of major elements may increase the presence of minor elements.

Figure 4

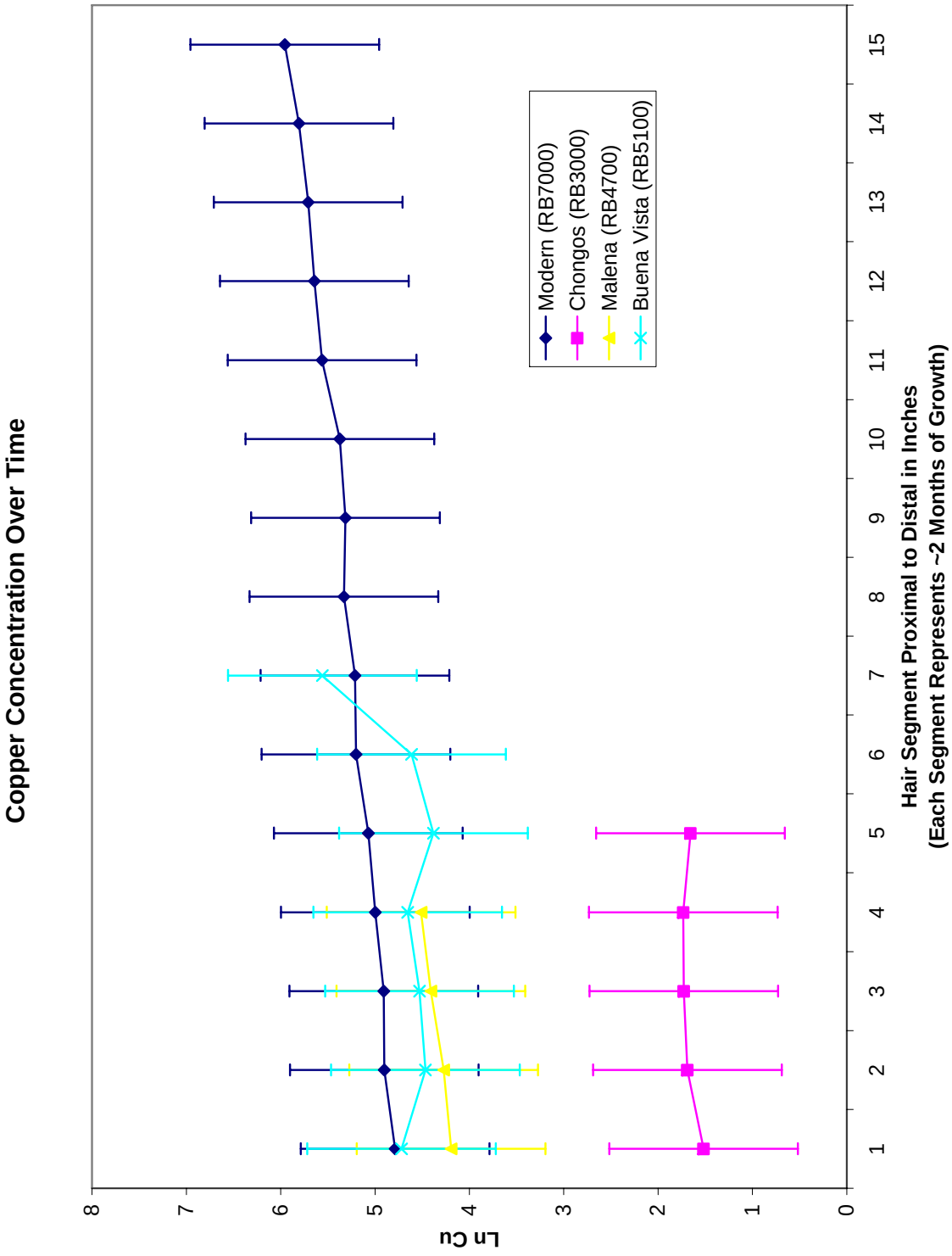

Copper Concentration Over Time
Ln Cu
0
1
2
3
4
5
6
7
8
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Hair Segment Proximal to Distal in Inches
(Each Segment Represents ~2 Months of Growth)
Modern (RB7000)
Chongos (RB3000)
Malena (RB4700)
Buena Vista (RB5100)

Figure 5

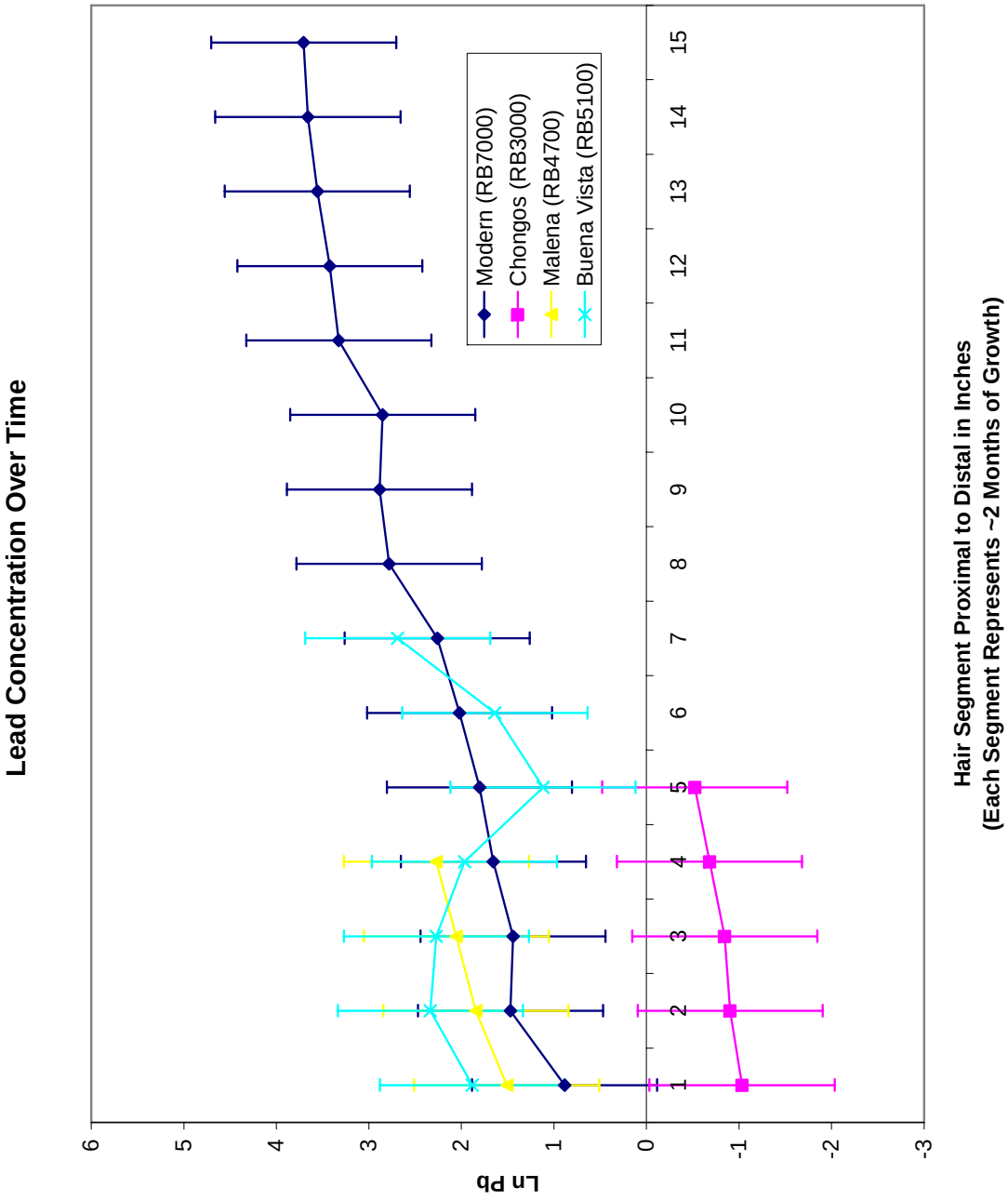

Lead Concentration Over Time
Ln Pb
6
5
4
3
2
1
0
-1
-2
-3
Modern (RB7000)
Chongos (RB3000)
Malena (RB4700)
Buena Vista (RB5100)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Hair Segment Proximal to Distal in Inches
(Each Segment Represents ~2 Months of Growth)

Figure 6

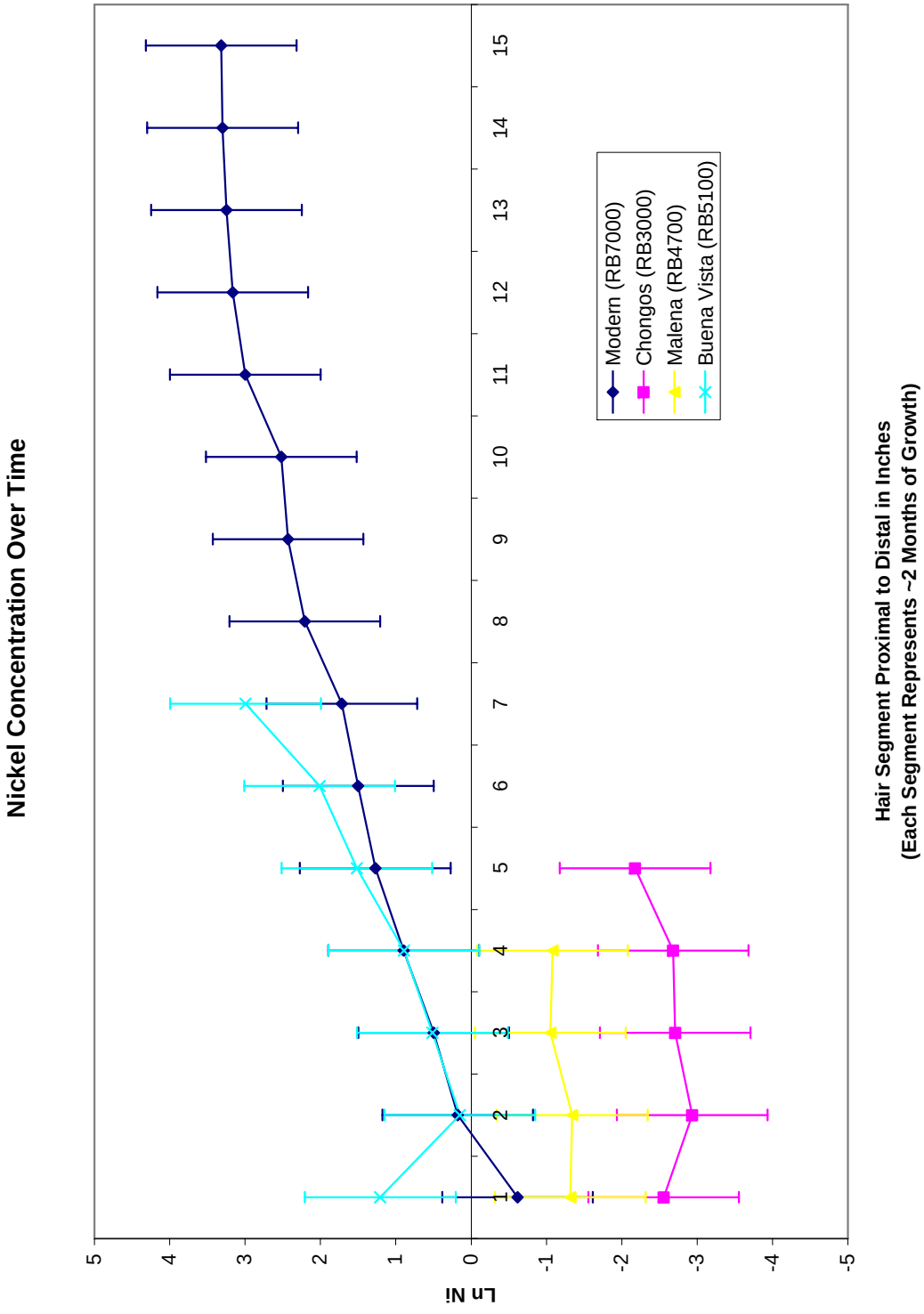

Nickel Concentration Over Time
Ln Ni
5
4
3
2
1
0
-1
-2
-3
-4
-5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Hair Segment Proximal to Distal in Inches
(Each Segment Represents ~2 Months of Growth)
Modern (RB7000)
Chongos (RB3000)
Malena (RB4700)
Buena Vista (RB5100)

Figure 7

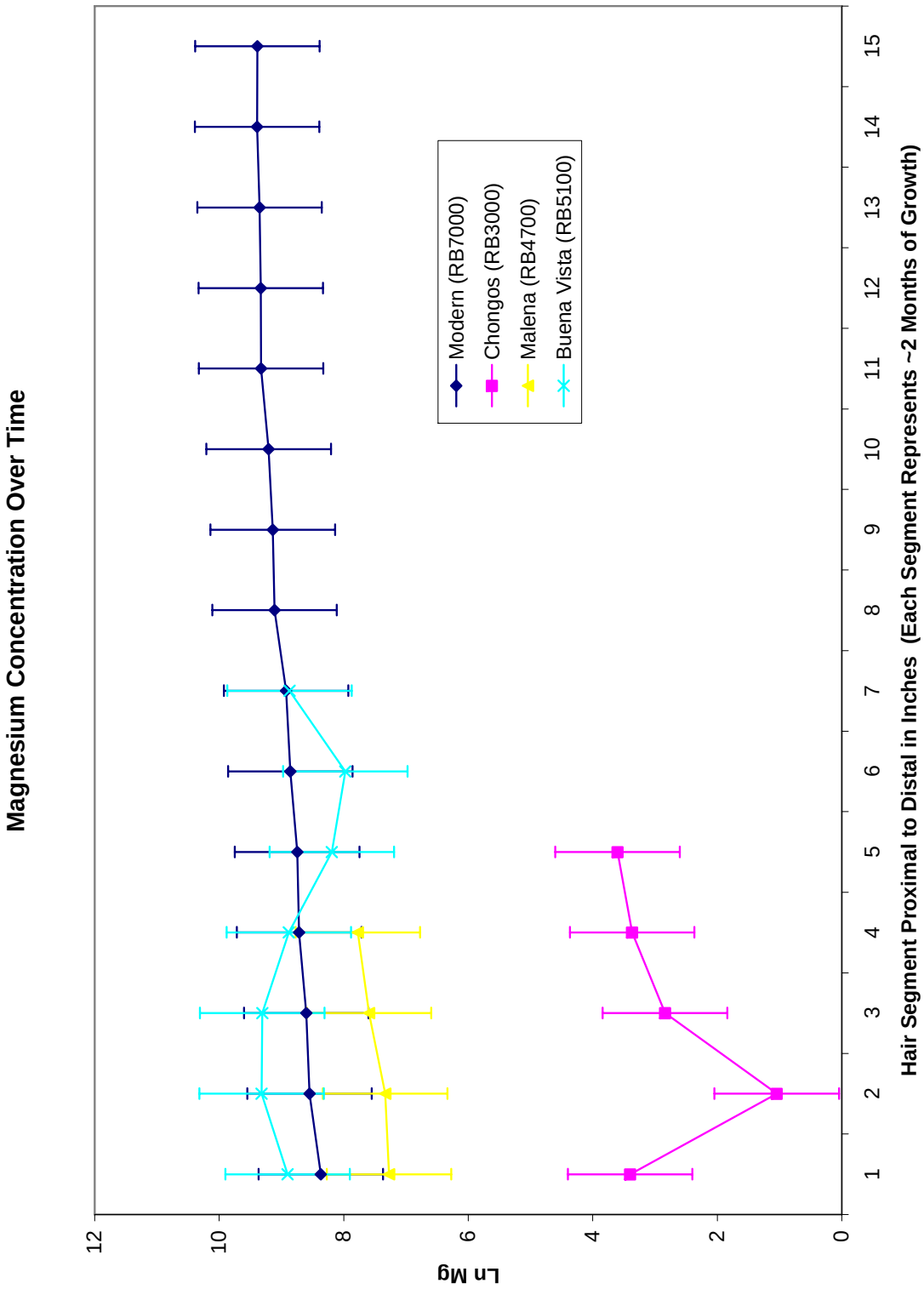
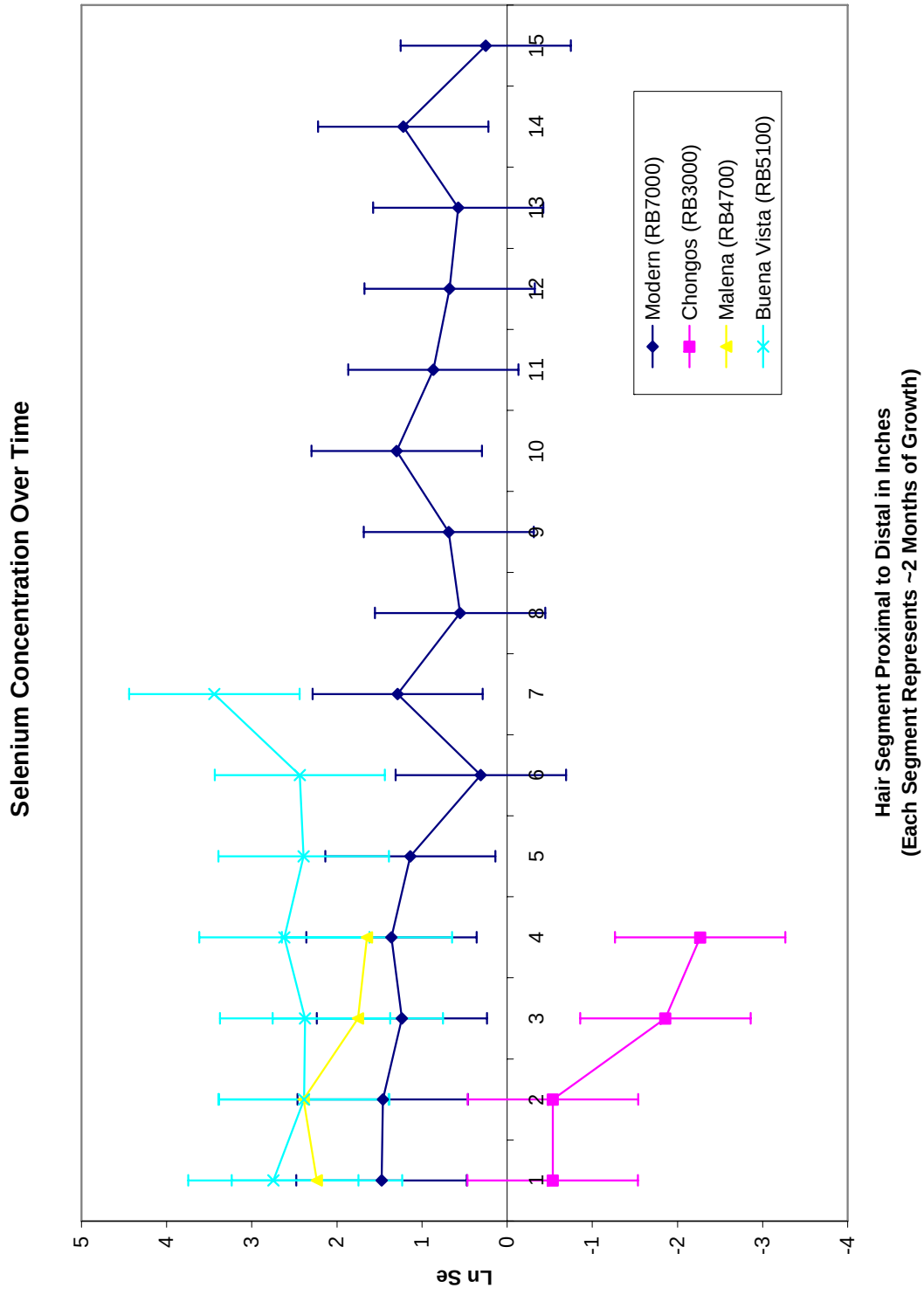

Magnesium Concentration Over Time
Ln Mg
0
2
4
6
8
10
12
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Hair Segment Proximal to Distal in Inches (Each Segment Represents ~2 Months of Growth)
Modern (RB7000)
Chongos (RB3000)
Malena (RB4700)
Buena Vista (RB5100)

Figure 8


Selenium Concentration Over Time
Ln Se
5
4
3
2
1
0
-1
-2
-3
-4
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Hair Segment Proximal to Distal in Inches
(Each Segment Represents ~2 Months of Growth)
Modern (RB7000)
Chongos (RB3000)
Malena (RB4700)
Buena Vista (RB5100)

Factor Analysis

The next step was to search for broad patterns of interrelations, correlations, among the elements pooled across the samples. Factor analysis was performed of the correlation matrix to produce 5 factors.

Table 5: Factor Analysis Statistics			
	Initial Eigenvalues		
Component	Total	% of Variance	Cumulative %
	12.	5	5
	2.		6
	2.		7
	1.		7
	1.		8

Extraction Method: Principal Component Analysis

As seen above, five factors with Eigenvalues greater than 1.03 accounted for 83.1% of the variance within the data.

Table 6: Rotated Component Loadings					
Component Matrix: Rotated[a]					
	Component				
Element	1	2	3	4	5
	0.		0.3		
	0.	0.			
	0.				
	0.				0.
	0.	0.			0.
	0.	0.			
	0.	0.			
	0.			0.	
	0.	0.	0.		
	0.	0.			
	0.	0.			
		0.			
		0.			
		0.			
	0.	**0.**			
	0.	**0.**	0.		
		0.		0.	
	0.	**0.**			
	0.	**0.**			0.
			0.		
			0.		
				0.	
			0.	**0.**	
					0.

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization. (Loadings <0.30 not presente

[a] Rotation converged in 8 iterations.

The important elements for each factor, those >|0.60|, are discussed below. Using an univariate general linear model, each factor score was analyzed for variation in mean values by location, sex, and location by sex.

Table 7: Analysis of Factor Score 1 Loadings by Location, Sex, and Locatio by Sex					
Dependent Variable: Regression Factor Score 1					
Source	**Type III Su of Squares**	**df**	**Mean Squa**	**F**	**Sig.**
Corrected Model	43.96		5.4	17.8	0.0
Intercept	3.1		3.1	10.1	0.0
Location	26.3		13.1	42.7	0.0
Sex	2.0		1.0	3.2	0.0
Location * S	11.8		2.9	9.6	0.0
Error	16.0		0.3		
Total	60.0				
Corrected Total	60.0				
[a] R Squared = 0.733 (Adjusted R Squared = 0.692)					

As seen in Table 7, for factor score 1, location and location by sex was the most significant. The effect of sex independent of location was slightly significant. As Figure 8 shows, Chongos differs dramatically and significantly from the other two sites in that males show a lower median score than that of females and unknowns. The opposite is true for Buena Vista and Malena.

The significant elements correlated with component 1 are zinc, copper, silver, cadmium, lead, bismuth, arsenic, selenium, iron, manganese, and gallium. Of these, arsenic, cadmium, copper, iron, manganese, selenium, and zinc are recognized as having a role in human physiology (Aras and Ataman, 2006). Copper, iron, selenium, and zinc reflect the amount of meat in the diet. High concentrations of manganese are indicative of a diet including vegetables, fruits, and cereals, but might reflect contamination (Meador, 1992). Cadmium is typically delivered to the body through diets of cereals and other vegetables. However, shellfish and kidneys have

However, shellfish and kidneys have a higher cadmium concentration than other meats (Aras and Ataman, 2006). In summary, component 1 measures a dietary component, but it is complicated with contamination in the form of silver, bismuth, gallium, and possibly manganese.

Figure 9

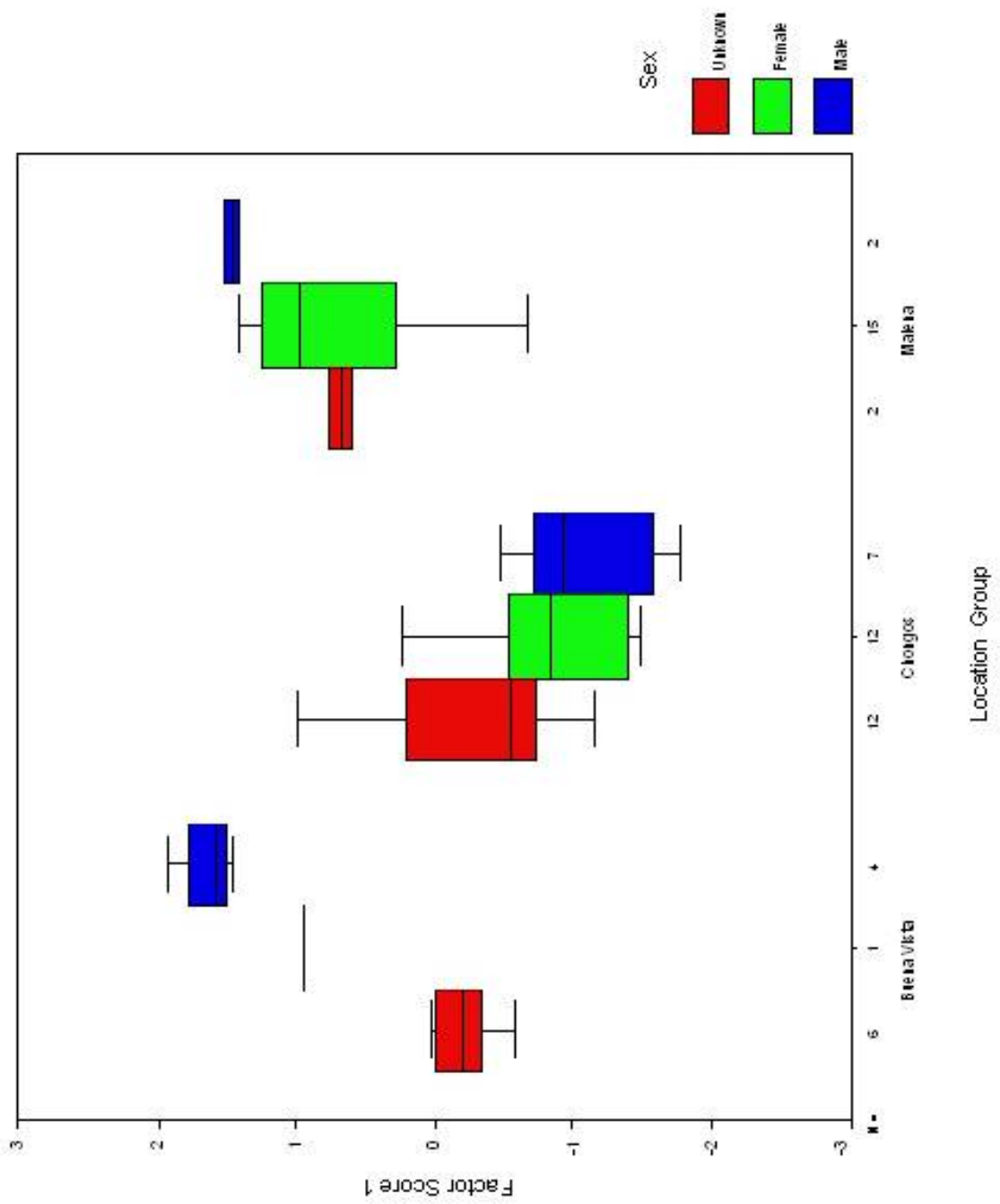

Sex
Unknown
Female
Male
Factor Score 1
3
2
1
0
-1
-2
-3
Buena Vista
Chogos
Malela
Location Group

Table 8: Analysis of Factor Score 2 Loadings by Location, Sex, and Locatio by Sex					
Dependent Variable: Regression Factor Score 2					
Source	**Type III Su of Square**	**df**	**Mean Squa**	**F**	**Sig.**
Corrected Model	29.62		3.7	6.3	0.0
Intercept	1.6		1.6	2.8	0.0
Location	10.3		5.1	8.8	0.0
Sex	1.2		0.6	1.0	0.3
Location * S	8.7		2.1	3.7	0.0
Error	30.3		0.5		
Total	60.0				
Corrected Total	60.0				
[a] R Squared = 0.494 (Adjusted R Squared = 0.416)					

Table 8 shows the general linear model results for factor score 2. Again, the effect of location and location by sex is significant for factor 2. Figure 9 shows that the males in Buena Vista have a lower median for factor 2, compared to women and samples from individuals of unknown sex. The opposite is true for Chongos and Malena. The effect of sex, independent of location, is not significant, showing the importance of site in influencing average concentrations by sex.

The significant elements for component 2 are strontium, cobalt, vanadium, uranium, chromium, magnesium, nickel, and barium. Of these magnesium is considered a major element in human physiology (Krebs and Hambidge, 1997). Chromium, cobalt, nickel, and vanadium also play a role (Krebs and Hambidge, 1997; Aras and Ataman, 2006). Of the elements found to be important for factor 2, high values for barium, cobalt, magnesium, nickel, and vanadium are all associated

associated with a vegetarian diet. However, strontium is especially associated with a marine life diet in coastal Peru (Meador, 1992), so this suggests diets high in fish were also high in plants, which seems unlikely, but possible. Chromium is also known to be found in vegetable tissue (Aras and Ataman, 2006). This data indicates that factor 2 has a dietary component that also includes some contamination in the form of uranium; the role of strontium remains unclear.

Figure 10

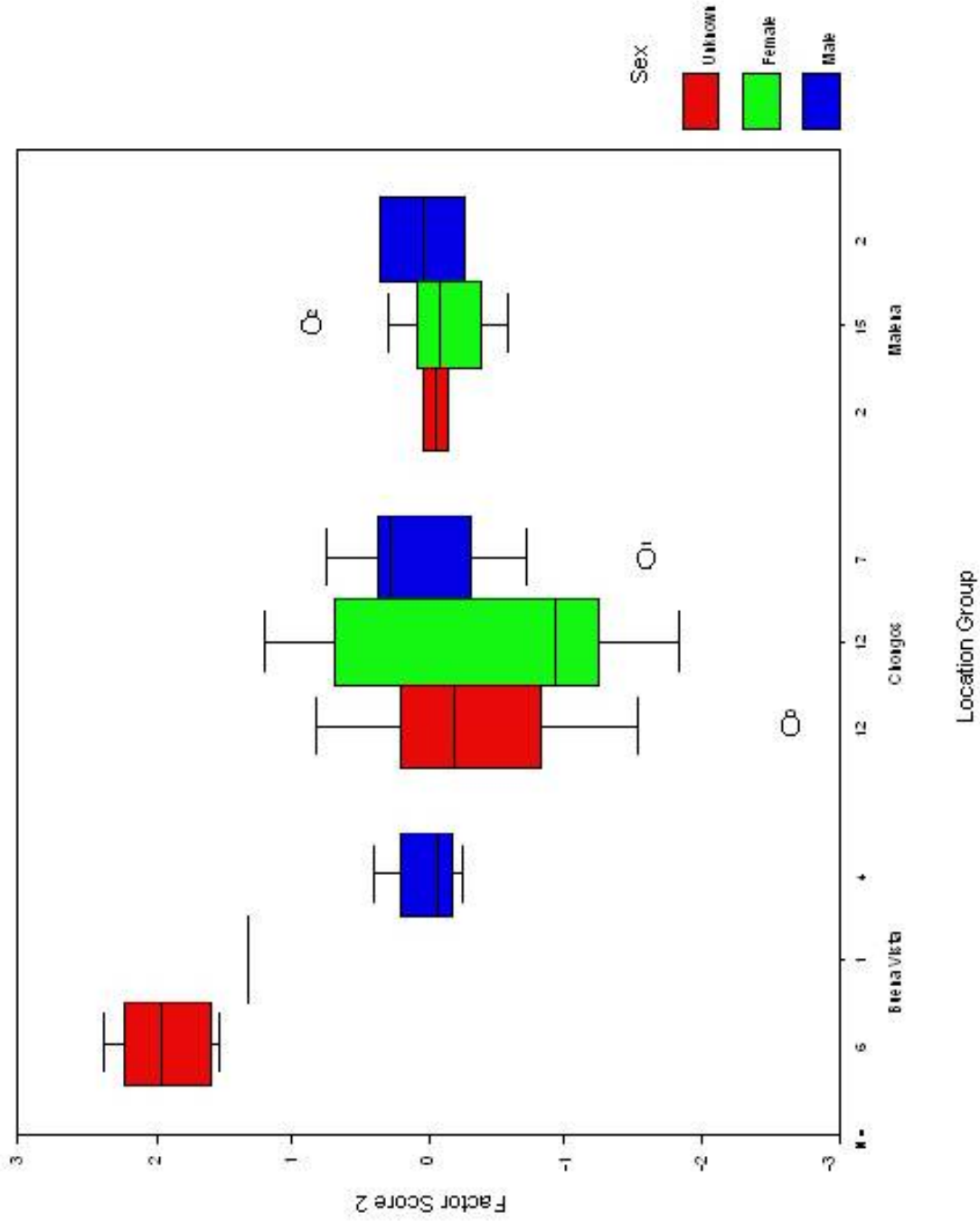

Sex
Unknown
Female
Male
Factor Score 2
3
2
1
0
-1
-2
-3
N =
6
1
4
12
12
7
2
15
2
Bella Vista
Chongos
Makita
Location Group

Table 9: Analysis of Factor Score 3 Loadings by Location, Sex, and Locatio by Sex					
Dependent Variable: Regression Factor Score 3					
Source	Type III Sum Squares	df	Mean Squa	F	Sig.
Corrected Model	26.4		3.3	5.1	0.0
Intercept	0.1		0.1	0.3	0.5
Location	3.3		1.6	2.5	0.0
Sex	11.6		5.8	9.0	0.0
Location * Se	6.9		1.7	2.6	0.0
Error	33.5		0.6		
Total	60.0				
Corrected To	60.0				
[a] R Squared = 0.441 (Adjusted R Squared = 0.355)					

The effect of sex on factor score 3 varies by sex, sex by location, but not location independent of sex (Table 9). Male-female differences are distinctive by site (Figure 10). In Chongos, no differences are observed, possibly reflecting contamination. Females have much greater average score for factor 3 than males at Malena. With only one female at Buena Vista, nonetheless, one sees similar values and pattern to Malena.

This factor score is based on the elements sodium and potassium. While these elements are strongly associated with salt and contamination (Edwards and Benfer, 1992), the fact that there is a strong effect of sex across location removes contamination as the explanation for the effect of these elements. Both sodium and potassium are major elements in human physiology (Krebs and Hambidge, 1997, Aras and Ataman, 2006). Sex differences could be activity differences in production of sweat, the effects of males as divers, or hair styles that differ between men and

that differ between men and women. Also, sodium and potassium may be indicators of purposeful contamination from funeral practices as salt may have been used for preservation (Benfer, 1990).

Figure 11

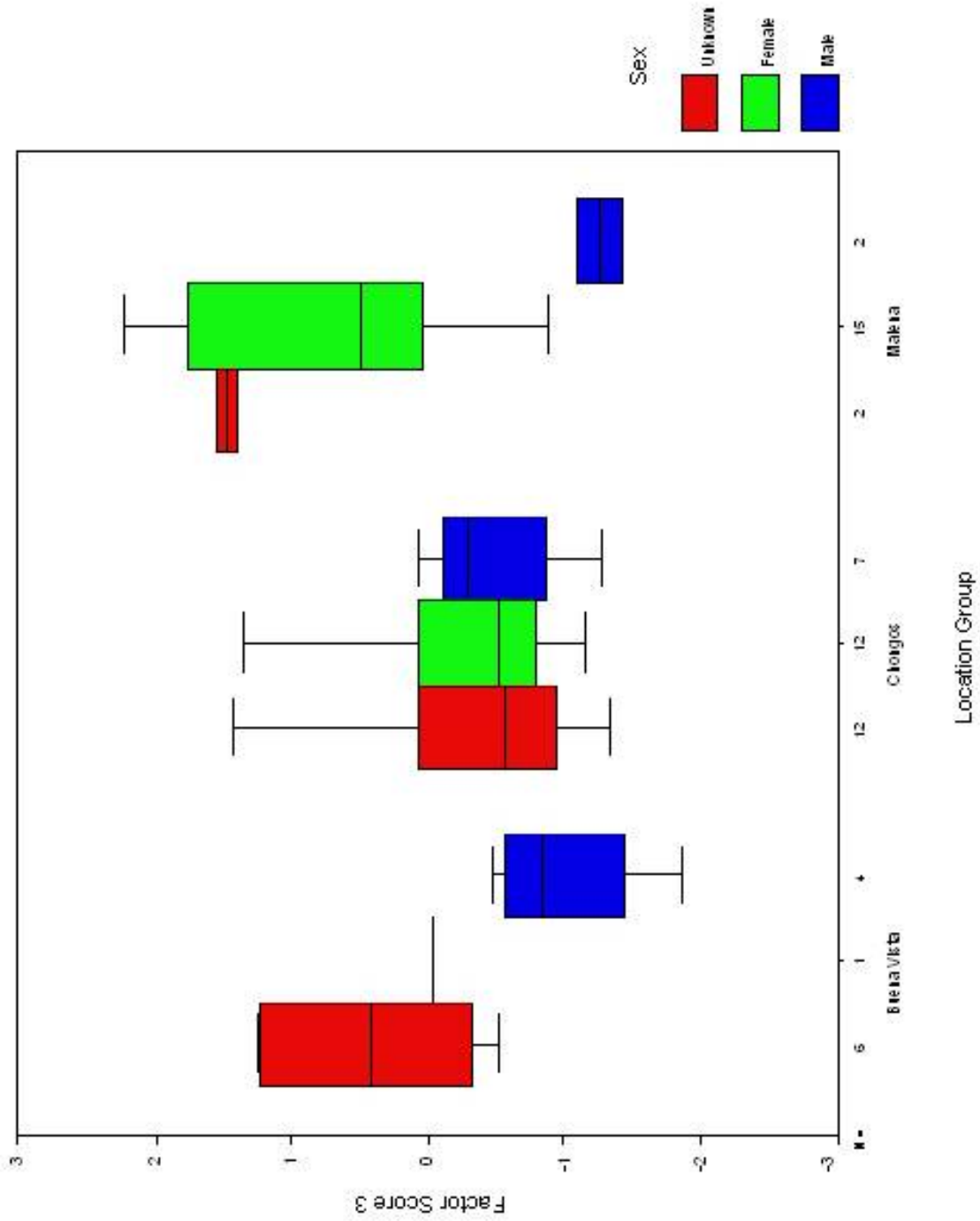

Sex
Unknown
Female
Male
Factor Score 3
3
2
1
0
-1
-2
-3
M=
6
1
4
12
12
7
2
2
15
Buena Vista
Chogos
Makia
Location Group

Table 10: Analysis of Factor Score 4 Loadings by Location, Sex, and Locati by Sex					
Dependent Variable: Regression Factor Score 4					
Source	Type III Sum Squares	df	Mean Squa	F	Sig.
Corrected Model	16.1		2.0	2.3	0.0
Intercept	1.3		1.3	1.5	0.2
Location	7.4		3.7	4.4	0.0
Sex	8.877E-		1.438E-	0.0	0.9
Location * Se	4.4		1.1	1.3	0.2
Error	43.8		0.8		
Total	60.0				
Corrected To	60.0				
[a] R Squared = 0.269 (Adjusted R Squared = 0.157)					

The elements associated with factor 4, cesium and rubidium show significance across location only (Table 10, Figure 11). The presence of rubidium is associated with contamination in bone (Edwards and Benfer, 1992). Cesium and rubidium also fall in the Alkali Group of the periodic table of the elements. Elements within the same vertical group of the periodic table have similar physical and chemical properties (Kotz and Purcell, 1987). This implies that both are associated with diagenesis, suggesting highest soil values at Buena Vista and lowest at Malena.

Figure 12

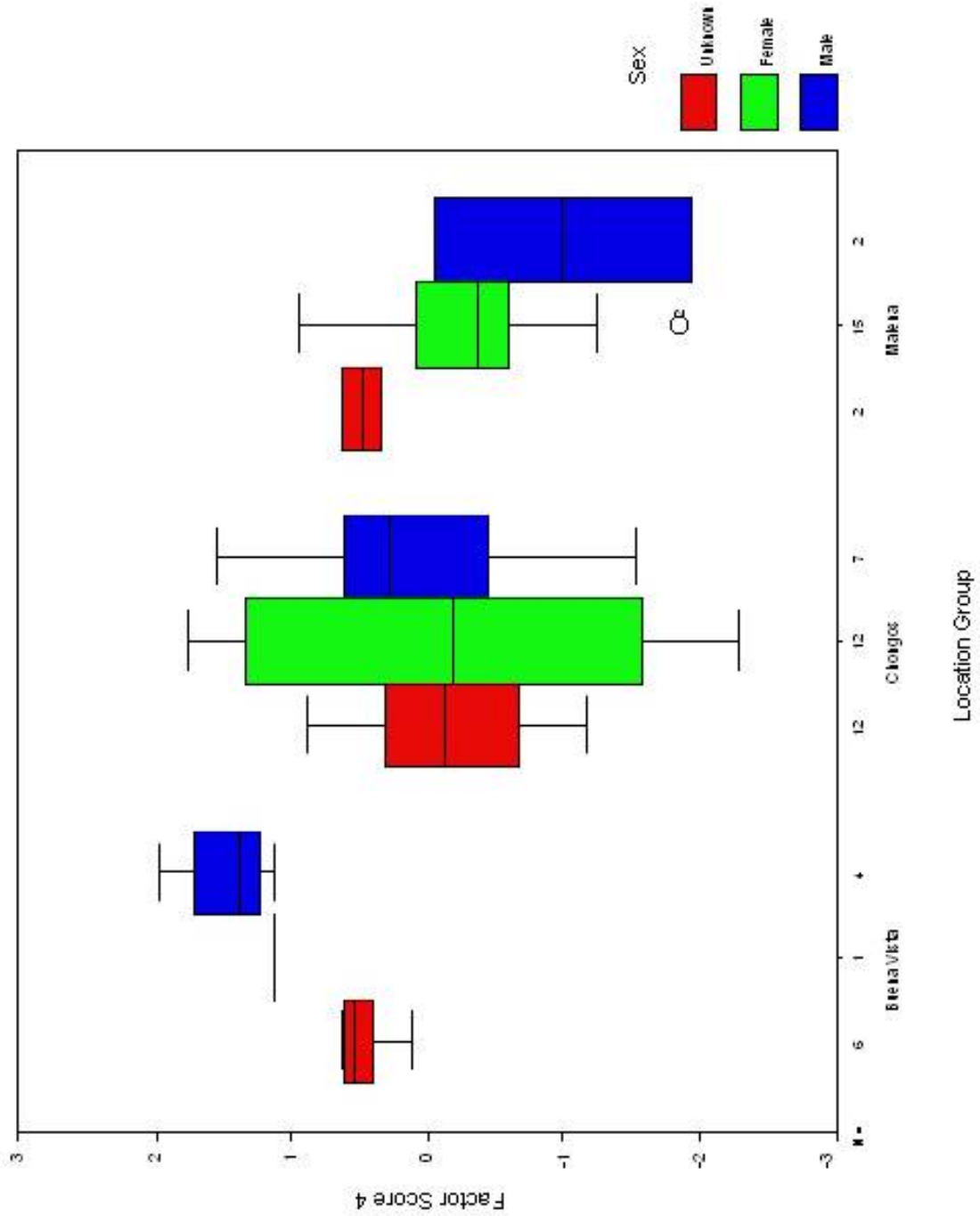
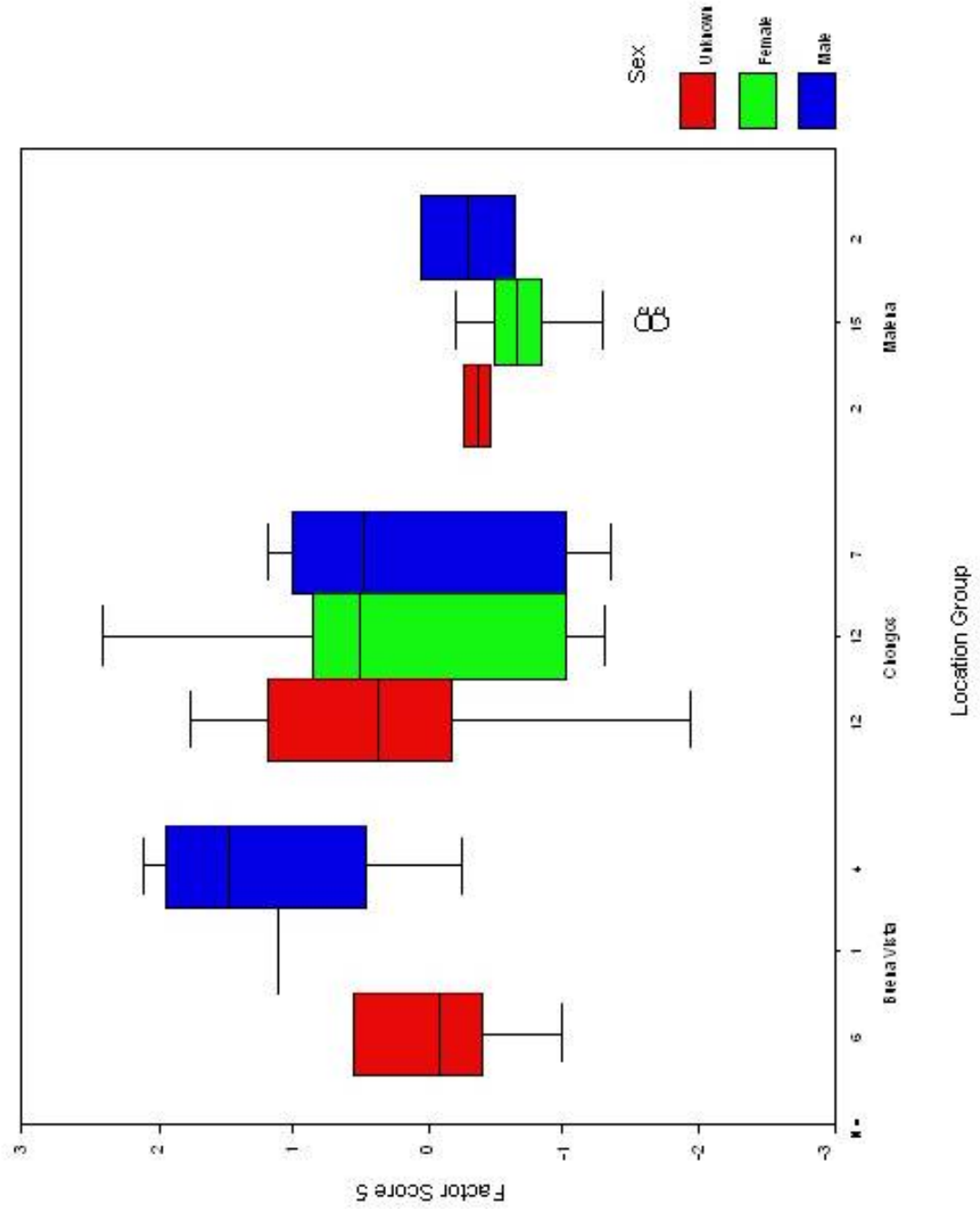

Sex
Unknown
Female
Male
Factor Score 4
3
2
1
0
-1
-2
-3
N =
6
1
4
12
12
7
2
15
2
Buena Vista
Chiloguin
Malin
Location Group

Table 11: Analysis of Factor Score 5 Loadings by Location, Sex, and Locati by Sex					
Dependent Variable: Regression Factor Score 5					
Source	**Type III Su of Square**	**df**	**Mean Squa**	**F**	**Sig.**
Corrected Model	18.2		2.2	2.8	0.0
Intercept	0.7		0.7	0.9	0.3
Location	5.5		2.7	3.4	0.0
Sex	0.6		0.3	0.3	0.6
Location * S	5.0		1.2	1.5	0.1
Error	41.7		0.8		
Total	60.0				
Corrected Total	60.0				
a. R Squared = 0.305 (Adjusted R Squared = 0.198)					

Table 11 shows the elements associated with factor 5. This element, thallium, shows no significant differences across sex, but, like Factor 4, a small by significant variation in average value by location (Figure 12). A review of related literature indicates that it is not considered an essential element (Frieden, 1984). It likely represents contamination. The varimax rotation used in the factor analysis produces factors with a zero intercorrelation among them. Thus, these two sources of contamination have been removed permitting the dietarily interesting pattern of the first three factors to be studied by sex and site (location).

Figure 13


Sex
Unknown
Female
Male
Factor Score 5
3
2
1
0
-1
-2
-3
N =
6
1
4
12
12
7
2
15
2
Bella Vista
Chongos
Makela
Location Group

Age Effects

A bivariate correlation was performed between age group and the factor scores (Table 12). General Linear Model analysis was rerun with age as a covariate for factor score 1 and 4, where age was determined to correlate significantly with the factor score. The results may be seen in Table 13.

Table 12: Correlation Analysis of Age Group by Factor Scores

		Age Group	Reg. Factor Score 1	Reg. Factor Score 2	Reg. Factor Score 3	Reg. Factor Score 4	Reg. Factor Score 5
Age yea	**Pearson Correlation**	1.0	0.46	-0.1	-0.0	-0.27	0.1
	Sig. (2-tailed)		0.0	0.2	0.2	0.0	0.3
	N						

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

Correlation analysis shows that factor score 1 is quite significant for age. In comparison to the general linear model results found in Table 7 to those adjusted for age (Table 13), location remained significant, as did location by sex. However, sex became less significant when the mean differences were adjusted for age. As discussed earlier, regression factor score 1 represents a diet of meat as well as some contamination. This indicates that there is a difference across locations with respect to age group. This would not be possible if the source of the elements listed were due to diagenesis.

In comparing the general linear model results for factor score 4, as found in Table 10 with those found in Table 13, location became slightly more significant while sex and location by sex became considerably significant. The significance of factor

significance of factor score 4 is that the effect of rubidium and cesium is not completely associated with diagenesis. There is some effect on the individuals based on age before death, possibly related to age specific mortuary customs. The significant variation among means for factor score 4 (Table 10) may be in part due to diet, not just diagenesis.

Because two additional parameters are estimated with the covariate analysis (the slope and intercept of the regression equation), the resulting loss of degrees of freedom reduces the statistical power of the analysis unless the correlation between the covariate and variate is high. Given that the correlation with age and elements varied from -0.278 to 0.462, it would seem that the control by covariate analysis has caused the main analysis to be more conservative.

Table 13: Analysis of Factor Scores 1 and 4 Loadings by Age Group Location, Sex, and Location by Sex						
Dependent Variables: Regression Factor Scores 1 and 4						
Source	**Dependent Variable**	**Type III Su of Square**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	Factor Score 1	48.8		5.4	16.7	0.0
	Factor Score 4	37.1		4.1	7.9	0.0
Intercept	Factor Score 1	0.3		0.3	1.0	0.3
	Factor Score 4	16.6		16.6	31.9	0.0
Age Group	Factor Score 1	1.9		1.9	6.1	0.0
	Factor Score 4	15.0		15.0	28.7	0.0
Location	Factor Score 1	20.2		10.1	31.2	0.0
	Factor Score 4	11.4		5.7	10.9	0.0
Sex	Factor Score 1	0.7		0.3	1.1	0.3
	Factor Score 4	7.9		3.9	7.6	0.0
Location* Sex	Factor Score 1	5.2		1.3	4.0	0.0
	Factor Score 4	14.7		3.6	7.0	0.0
Error	Factor Score 1	19.1		0.3		
	Factor Score 4	30.8		0.5		
Total	Factor Score 1	68.0				
	Factor Score 4	68.0				
Corrected Total	Factor Score 1	68.0				
	Factor Score 4	68.0				

a. R Squared = 0.719 (Adjusted R Squared = 0.676)
b. R Squared = 0.546 (Adjusted R Squared = 0.477)

Barium – Strontium Analysis

A comparison of Barium (Ba) and Strontium (Sr) has long been recognized as an indicator of a marine diet (Burton and Price, 1990). Decreased Log_{10} (Ba/Sr) indicates a marine diet while the opposite indicates a terrestrial diet.

Table 14: Analysis of Log_{10} (Ba/Sr) by Age Group, Location, Sex, and Location by Sex

Dependent Variable: Log_{10} (Ba/Sr)

Source	Type III Sum Squares	df	Mean Squar	F	Sig.
Corrected Model	1.76		0.1	5.6	0.0
Intercept	0.4		0.4	14.0	0.0
Age Group	6.735 x 1		6.735 x 1	0.0	0.9
Location	0.7		0.3	10.3	0.0
Sex	1.978 x 1		9.889 x 1	0.2	0.7
Location * S	0.3		8.574 x 1	2.4	0.0
Error	1.9		3.446 x 1		
Total	11.1				
Corrected Total	3.6				

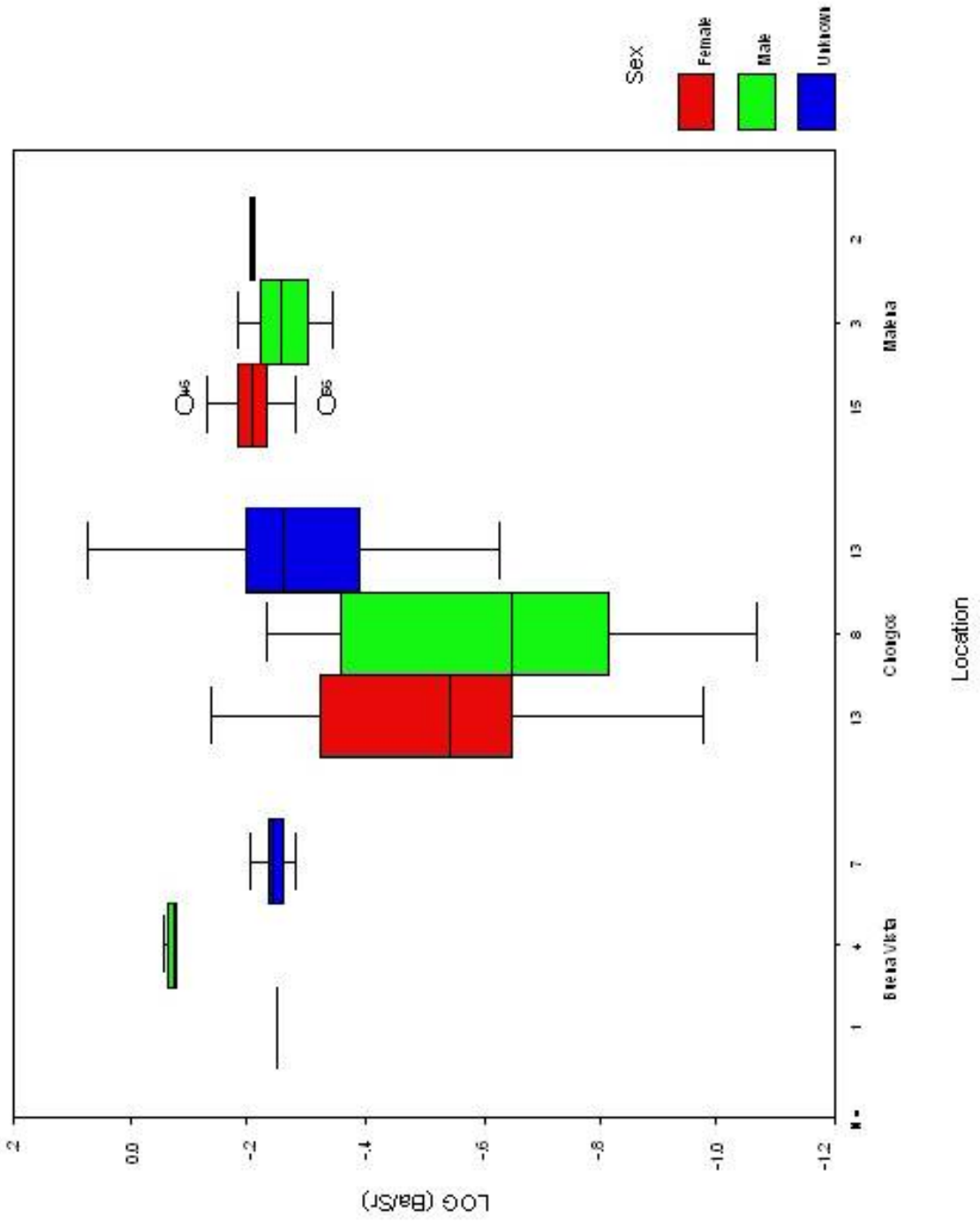
a. R Squared = 0.478 (Adjusted R Squared = 0.394)

The Log_{10} (Ba/Sr) was significant for Location and less significant for location by sex (Table 14). It was not significant for sex alone or age. Figure 14 shows a comparison of the Log_{10} (Ba/Sr) ratios over location and sex. As can be seen, Buena Vista differs from the other sites in that the median score for males is higher than that for females and unknowns, however, there is only one female sample for Buena Vista. An opposite trend can be seen for both Chongos and Malena. This would indicate that males at Chongos and Malena have a higher marine diet than

marine diet than females at those sites who presumably gardened to collect more plant foods.

Chongos and Malena have a significant marine component to their diets, both being approximately 5 Km from the coast of Peru. Chongos had intensive agriculture and fishing (Peters, 1987). Malena had intensive maize production and other cultigens with a significant marine component (Angeles R. Pozzi-Escot D., 2000a, 2000b). In contrast, Buena Vista had an early flood-plain agriculture with, unlike the other two sites, no maize (Duncan, et. al., 2003). A marine component was also present from trade with other groups. According to Figure 14, men were eating more fish in Chongos and Malena than the females in the same locations. However, men were eating less fish than females in Buena Vista, 45Km from the coast. These values conform to the model for such ratios in human bone, as reported by Burton and Price (1990).

Figure 14


Sex
Female
Male
Unknown
LOG (Ba/Sr)
2
0.0
-2
-4
-6
-8
-10
-12
N=
1
4
7
13
8
13
15
3
2
Bella Vista
Chorgos
Maketa
Location

Chapter 4: Discussion and Conclusions

This study determined the effects of diet are at least partly distinguishable from diagenesis through trace element analysis on hair samples from ancient inhabitants of Peru. Statistical analysis produced the following results:

1) Using factor analysis, factor scores separated out elements based on primarily meat sources, vegetable and grain sources, salt sources, and elements associated with diagenesis as sources of trace elements.

2) The factor scores varied significantly by factor scores for location and location by sex for meat and vegetable and grain sources of trace elements.

3) Factor scores varied significantly by sex and location by sex for the sources of salt trace elements.

4) Age was significant for the factor scores associated with the meat sources of trace elements.

Log_{10} (Ba/Sr) showed a significant difference between location and location by sex. While all three had access to marine resources, Buena Vista's access was limited to trade. For the other areas, where there was intensive fishing or a marine component, men ate more fish than women.

Observed values for elemental concentrations were mostly in the range of modern values, themselves based on a much more narrow variation in diet than prehistoric specimens. Increasing and decreasing trace element concentrations distally along the hair shaft of the analyzed prehistoric specimens conform to trends observed in modern hair. Contamination may have been present due to soil contamination or contamination during the preparation of samples for analysis.

analysis. However, the difference in trace element concentrations across the hair shaft would be difficult to explain by soil or other contamination.

Sex and age varied significantly across locations, so the concentrations of trace elements found in these samples cannot be the result of diagenesis alone. Hair is thus a promising tissue for dietary studies. Analyzed in segments it conserves a record of the previous month's diet. Both increasing and decreasing concentrations were observed along the shaft in the same order as shown in modern specimens. This effect would be impossible to explain through diagenesis.

Future work on prehistoric hair should focus on more specimens from individuals whose sex, age, health status, and disease can be compared. Segments should be preserved for analysis to permit studies of aging and seasonality. Future work should also take into consideration associated soil analysis, isotope analysis, and a parallel analysis or coprolites whenever possible.

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Appendix A

Sample Information

Sample #	Burial	Location	Date Collected	Notes	S	Approx. Ag
RAB001	BO 1800, ENT.	Chongos	8/2/19		M	17 - 19
RAB002	BO 1800 #8	Chongos	8/1/19		U	4-5 years
RAB004	BO 1820 #3	Chongos	8/1/19	#4	U	0.75-1
RAB003	BP 1800, ENT.	Chongos	8/12/19	Left Temp.	fem	unk. Remain comingled
RAB006	BP 1820, ENT.	Chongos	8/12/19			0.5 - 1
RAB008	BP 1820, ENT.	Chongos	8/12/19		u	6 - 7 yr
RAB007	BU 1460 #2	Chongos	8/1/19	Left Frontal, 8	u	3 - 4 yr
RAB011	BW 1500	Chongos	8/1/19	Bolsa 14, Upp Right Occipita 17	fem	18-20
RAB016	BW 1500	Chongos		Estrado 1, bol 20, Central Occipital and Loose hair	u	1-1.5
RAB013	BW 1500 #10	Chongos	8/2/19	Occipital or Parietal, 13	u	2 years
RAB015	BW 1500 #20	Chongos	8/6/19	Sagittal Line, Top of Head,	u	Birth
RAB017	BW 1500 #26	Chongos	8/6/19	Bolsa 58, Parietal Near Saggital, 15	u	0-0.5
RAB005	BW 1500 #4	Chongos		Right Upper Temp. or lowe Parietal, 10	fem	45-55
RAB009	BY 1780, ENT.	Chongos	8/12/19	Rt. Temporal,	m	19-21
RAB012	BY 1780, ENT.	Chongos	8/8/19	Bolsa 23, Rt. Parietal, 19	Fem	18-19
RAB014	BY 1780 #2	Chongos	8/9/19	Bolsa 24, 20	M	13-15
RAB010	BY 1780 #3	Chongos	8/11/19	Bolsa 25 + 26 22	m	50-55
RAB019	BW 1500, ENT.	Chongos		Hair, Left Fron with Scalp		3 - 4 years
RAB020	BP 1800, ENT.	Chongos	5/30/20	Cabello	m	25-35
RAB031	BW 1500, ENT.	Chongos	6/15/20	Cabello, Mues 8	u	1 - 2 years

Sample #	Burial	Location	Date Collected	Notes	S	Approx. Ag
RAB022	DE 100 - 120, Sector 4	Chongos	12/6/2(	Muestra 5, DE 100-120, Sect 4	fem	12 - 13 year
RAB023	BW 1500, ENT.	Chongos	6/20/2(	Muestra 23, Cabello, Tight Temoral and Pariatal	(	4 - 5 years
RAB024	BP 1820, ENT.	Chongos	5/22/2(	Cabello, Parie Der.	(	5 - 6 years
RAB025	BP 1820, ENT.	Chongos	5/24/2(		u	3 - 4 years
RAB026	BO 1820, ENT.	Chongos	5/23/2(	Cabello, Fron	fem	14 - 16
RAB027	BW 1500, ENT.	Chongos		Cuaericula, Material Cabe De Cuy, Mues 10	u	1 - 2 years
RAB040	HMFF-38	Huaca Maler		08.20999.5g	fem	20-24
RAB041	HM-H2-015	Huaca Maler		08.10996 4g h and scalp	fem	17-20
RAB042	HM-H2-0018	Huaca Maler		Sct I, Subset 2 08.10998, 4.5 Adult #1	fem	17-20
RAB043	HM U1 T4	Huaca Maler	11/7/2(	Hair/Scalp Sample 08.11061 5g	fem	30-40
RAB044	HM-H2-0015	Huaca Maler		Adult male Ha sample? 08.10994 4g (Fine fluffy yellowish hair with brown threads mixed in.)	m	30-40
RAB045	HM-U2-T4	Huaca Maler	10/7/2(	Hair/Scalp sample, 08.11000 4g	m	40-44
RAB046	HM-U2-T5 N34	Huaca Maler	13/09/02	Scalp/hair Nir 08.10995 6g	u	5

Sample #	Burial	Location	Date Collected	Notes	S	Approx. Ag
				6g		
RAB047	HM-U2-T5	Huaca Maler	13/09/02	scalp/hair de adult (HM-U2- on paper tag inside) 08.109 5g	fem	18-22
RAB048	HM-UT-T6	Huaca Maler	10/7/2(	Hair sample, scalp, 08.110(4g	m	30-40
RAB049	HM-U2-T10	Huaca Maler	12/7/2(	08.11003, 6.5 (HMU2T0 on paper tag insi	fem	20-25
RAB050	BV Dis B7 (T10	Buena Vista		8.10	fem	20-24
RAB051	11b-1X-390	Buena Vista	(6/18/02	5ec.D, uIla #200, 18jnuio '02 (Just excavated hai	unkno	unknown
RAB052	BV Dis B13 Tl 0	Buena Vista	18/07/02	08.10746, Buena Vista 1 11b-1X-390 T	m	37-47
RAB700	modern	U.S.A.	3/1/2(	modern	fem	35