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Before the Inca: Prehistoric Dietary Transitions in the Argentine Cuyo

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by

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For Roy E. Shelnut

My father, my inspiration, my friend

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ABSTRACT

A dietary reconstruction was performed in order to understand changing prehistoric subsistence patterns in the Central Andean geographical area of the Argentine Cuyo that includes the provinces of San Juan and Mendoza. Archaeologically, the Cuyo is also known as a boundary between Andean agriculturalists and the foragers of Patagonia. One hypothesis being tested is whether this area was one of the last South American cultural groups to convert to maize cultivation, probably around 2000 BP. The process of stable isotope analysis is used to reconstruct the diets of individuals, as it reveals the relative proportions of C_3 and C_4 plants and the contribution of aquatic resources to otherwise terrestrial diets, as well as variations in trophic level of the foods consumed.

In this study the bones, teeth, hair, and flesh from 45 individuals were tested to address specifically total and protein diets, as well as seasonal variation and changes between childhood and adulthood. This process, when used in combination with previous analyses, such as midden or faunal analysis, allows researchers to evaluate the results of those previous studies, and thus compose a more thorough reconstruction of the lifestyles of a prehistoric culture.

Information garnered from this study indicates that the times of dietary transition were variable, with seasonal patterns becoming more stable over long periods. Furthermore, some members of the study population demonstrate the existence of

nutritional stress indicators, such as dental caries, that can be viewed in relation to the dietary shifts that may have been a cultural adaptation to the environment of the Cuyo. Overall, this study shows the early adoption of maize agriculture in central western Argentina and recommends future studies that analyze the relationships between agriculture, diet, and nutrition in the New World.

Chapter One: Introduction

This thesis focuses on the interplay between human biological systems and their corresponding environments, both natural and cultural. Stable isotope analysis has been undertaken to examine prehistoric dietary transitions in an area of central west Argentina called the Cuyo. In a broad sense, this thesis examines the ways that the choices humans make, as members of their distinct societies, affect the way they live and, in turn, their future descendents.

By considering a few remarkable changes in cultural systems as defining moments in the human past, one can begin to explain and possibly predict the wide range of effects that might occur. Transitions such as those from nomadism to sedentism, foraging to farming, and even the relatively recent occurrences of the Industrial Revolution and globalization can be viewed as a series of events that have assisted in shaping the human career. For instance, the development of transcontinental trade has been associated with a wealth of epidemics, including the black plague (Magerison and Knüsel 2002). Also, the shift from nomadic to sedentary existence has been linked to various alterations in artistic expression, craft specialization, social organization, and the spread of chronic disease (Larsen 2002; Schoeninger and Schurr 1994). Along similar lines, the move from hunter-gatherer to agricultural lifestyles in many populations can be seen as a way in which humans have attempted, and in some ways achieved, a control over the natural environments in which they exist. However, this transition has not been a one-way process. Along with the adoption of agriculture, some populations have experienced increases in nutritional pathologies, such as anemia and osteoporosis (Gilbert

and Mielke 1985; Larsen 2002). Pearsall (1994) emphasizes that the introduction of maize agriculture into a society has important biological and cultural implications.

Diet as a Key to Understanding Culture

Although anthropologists have frequently used dietary studies to understand social and economic patterns, researchers are now beginning to address the potential impacts upon the human species that result from the transition of hunter-gatherer to agricultural lifestyles. Poor health conditions are increasingly being associated with changes in human diet and associated sedentism (Milton 2002; Schoeninger, DeNiro and Tauber 1983). The recently published volume, *Human Diet: Its Origin and Evolution*, begins with similar thoughts:

> In essence "diet" is a key to understanding our past, present, and future. Much of the evolutionary success of our species can be attributed to our ability to procure, process, and consume a wide range of foods. However, recent changes in our diet (e.g., increased intake of such things as saturated fat, refined carbohydrates, and sodium, and decreased intake of nonnutrient fiber) may lie at the root of many of the health problems swamping our health care systems [Ungar and Teaford 2002: 1].

In regard to chronic disease and diet, Eaton et al. (2002: 12-16) outline five ways in which contemporary diets have affected modern human health disadvantageously. These consist of high blood pressure due to marked increases in sodium intake; severely reduced consumption of cancer preventing fruits and vegetables, in exchange for cereal grains, which have demonstrated no evidence of such effects; lack of energy output in exchange for caloric intake; increased dietary fat associated with coronary heart disease; and a relationship between docosahexonic acid (DHA) deficiencies and reduced brain sizes.

Similarly, the transition to farming often involved an overwhelming dependence on key domesticates such as rice, barley, and maize. While these staple crops have allegedly played historic roles in the broad cultural systems of societies, reliance on these cultigens can often supersede intake of basic nutritional resources such as fresh meats, fruits, and vegetables, and result in a dietary lack of iron and essential amino acids. Maize-based diets are of particular interest, as iron, protein content, and niacin absorption are all characteristically low. Lack of these essential nutrients has been linked to increases in anemia and osteoporosis, declining oral health, and reduced growth rates throughout various societies (Larsen 1995, 2002; Steele and Bramblett 1988; Ungar and Teaford 2002; White 2000).

The Thesis

This work provides much needed information that will assist those trying to understand the effects of cultural transitions on human biological systems. It focuses on a range of prehistoric populations on the verge of major cultural, biological, and subsistence strategy shifts. Dietary reconstruction is used to understand changing subsistence patterns in the Central Andean geographical area of the Argentine Cuyo (Figure 1.1). While the idea of a transition from hunter-gatherer to agricultural lifeways has been suggested by previous archaeological research, stable isotope analysis provides clearer data on the area of study.

In recent times, researchers interested in studies of prehistoric diets have been limited to mostly indirect methods of investigation; for example, the analysis of floral and

Figure 1.1. Map of South America. The geographical region of the Argentine Cuyo is highlighted in red

faunal remains left in middens, cultural iconography, and ceramic use (Keene 1985; Schoeninger, DeNiro and Tauber 1983; Schoeninger and Schurr 1994). The few methods from which more lucid evidence can be obtained include residue or lipid studies of pottery (Charters 1993), coprolite analysis (Reinhard and Bryant 1992), or the very rare incidences in which mummies are recovered and can be autopsied to discover the contents of their stomachs (Holden and Núñez 1993). While many of the indirect methods have proven useful for group reconstructions (e.g., study of Mendoza by Lagiglia 1977), their results are in some ways incomplete, as they are only capable of identifying the food sources in a given environment, which individuals may or may not have eaten extensively. Here lies the distinction between menu and diet. Among others, Armelagos (1994: 235) clarifies the difference between these two terms; menu refers to the variety of foods available to a population, whereas diet refers to what is actually eaten. The relative importance of these dietary components can be hypothesized by studies of ratios of floral and faunal materials found in archaeological context, but one can only posit that, in practice, the remains being recovered were actually consumed.

In contrast, the diet of individuals can be reconstructed through the use of stable isotope analysis, which reveals the relative proportions of C_3 and C_4 plants and the contribution of aquatic resources to otherwise terrestrial diets, as well as variations in trophic level of the foods consumed. During the last 30 years, stable isotope analyses have enabled researchers to document physically the transition from hunter-gatherer to agricultural subsistence patterns by comparing the proportions of diet represented by resources with differing photosynthetic processes (Krueger and Sullivan 1984; Schoeninger, DeNiro and Tauber 1983; Schoeninger and Moore 1992; Schwarcz and

Schoeninger 1991; Tykot 2004; van der Merwe and Vogel 1978; Vogel and van der Merwe 1977). This process, when used in combination with other analyses, allows researchers to evaluate the results of previous studies, and thus compose a more thorough reconstruction of the lifestyles of a prehistoric culture.

In areas such as South America, where a written language did not exist prior to the contact period, stable isotope analysis of human skeletal material becomes an indispensable method of tracking diachronic trends in subsistence patterns (Isbell 1997; Pearsall 1992; Schwarcz and Schoeninger 1991). Isotopic analysis can be used to investigate transitions from a subsistence pattern based primarily on hunter-gatherer diets, to intermediary, and eventually maize-based diets. Isotopic analyses of teeth and bone have also been used to understand patterns of food distribution, and compare juvenile and adult paleodiets (e.g., Aufderheide et al. 1994; Cohen 1977; Dupras 2001).

One critical limitation of stable isotope analysis is that the results of such studies represent ratios of certain food types, rather than actual diet; thus, one might postulate about the relative proportions of maize in an individual's diet as compared to other dietary resources, but they will never be able to give precise lists of what that individual actually ate during their lifetime. Also, researchers are typically confined to the examination of hard tissues, such as bone and teeth, which have far slower rates of decomposition than soft tissue due to the nature of the materials (Mays 1998). The analysis of the bone samples portrays the average diet over the last several years of an individual's life, while that of tooth enamel reflects diet during the age of crown formation. This combination of analyses is useful for contrasting the juvenile and adult diets of individuals, but conclusions are limited to the representation of average diets

from considerable periods of time (Larsen 1997). However, soft tissues have a much more rapid turnover rate. They are particularly advantageous in that the relatively new procedures of isotopic analyses of hair and flesh samples can reveal seasonal, or possibly even monthly dietary variations (O'Connell and Hedges 1999a). The excellent preservation conditions of the materials in this study (Figure 1.2) allow a unique opportunity to study soft tissues, as many are the result of natural mummification, caused by the arid conditions of the environment in which they were interred at death (White and Schwarcz 1994). This analysis was selected because it had the capability to reflect varying dietary patterns of individuals, and sheds light on previously abstract ideas about prehistoric South American lifestyles. Here, a dietary reconstruction is used to test explicitly models of seasonal variation, identify any evidence of ecological stress, and add to scientific knowledge. Further, this work contributes to the establishment of a dietary baseline in the archaeological record of Argentina.

Human samples were provided, along with funding for analysis, by the Museo de Historia Natural, Argentina. The biological samples from 45 individuals range in age from approximately 4100 BP (before present) to 200 BP (Table 1.1). These samples are thought to be representative of the greater human population from multiple prehistoric sites located in the present day provinces of San Juan and Mendoza. These cultural, geographical, and temporal differences allow for the examination of long-term patterns in what is thought to be an area that encountered substantial dietary changes around 2000 BP. Gil (2003), among others, has hypothesized that around this time, many cultures in the surrounding area shifted from hunter-gatherer to agricultural lifestyles, with an increasing dependence upon maize as the primary crop. This thesis evaluates the

Figure 1.2. The naturally mummified remains of an infant, recovered from the province of Mendoza, Argentina

hypothesis of dietary change around the time of 2000 BP with questions including: (1) Did a transition from forager to agricultural subsistence occur in the Argentine Cuyo? (2) If so, when? (3) Is there a difference between the two provinces of San Juan and Mendoza which are thought to have retained separate dietary practices in prehistoric times? And (4) what were the effects of this transition if it did indeed take place?

As previously mentioned, the samples available for analysis are particularly unique in that many of the individuals were naturally mummified. Bone and/or teeth were analyzed for all individuals, while scalp hair, skin or muscle tissue, and/or fingernails were also analyzed for the mummified persons (Appendix A). These materials were processed at

Table 1.1. Chronology and site location of human samples

Museum Sample #	Province	Site	Chronology*
AF-1083	Mendoza	Arbolito 7	100
ENT-2	Mendoza	Capiz Alto	400
12	Mendoza	Caverna de las Brujas	3850
2038	Mendoza	El Desecho	
AF-2036	Mendoza	India embarzada	
AF-505	Mendoza	La Matancilla	
AF-2000	Mendoza	C Negro del Escorial	580
AF-2018	Mendoza	Canada Seca	1700-1400
AF-2019	Mendoza	Canada Seca	1700-1400
AF-2020	Mendoza	Canada Seca	1700-1400
CS-10001	Mendoza	Canada Seca	
AF-508	Mendoza	Cerro Mesa	
AF-510	Mendoza	Cerro Mesa	300-200
ENT-3	Mendoza	El Chacay	
AF-673	Mendoza	El Manzano	
AF-13894	Mendoza	Gruta del Indio	2300
AF-2021	Mendoza	Gruta del Indio	510
AF-828	Mendoza	Gruta del Indio	580
AF-830	Mendoza	Gruta del Indio	3860
GIRA-27	Mendoza	Gruta del Indio	
GIRA-70	Mendoza	Gruta del Indio	
GIRA-71	Mendoza	Gruta del Indio	
GIRA-831	Mendoza	Gruta del Indio	
JP/J4	Mendoza	Jaime Prats	2100-1700
JP-1155	Mendoza	Jaime Prats	2100-1700
JP-1352	Mendoza	Jaime Prats	2100-1700
AF-8	Mendoza	La Olla	
AF-2072	Mendoza	Las Ramadas	970
AF-681	Mendoza	Medano Puesto Diaz	2000
AF-500	Mendoza	Rincon del Atuel	1760
AF-2025	Mendoza	Tierras Blancas	200
AF-2022	Mendoza	Ojo de Agua	1200
AF-503	Mendoza	RA-1	1760
MGA-1	Mendoza	RQ-1	
SJ10-ENT1	San Juan	Angualasto	600
SJ4-ENT2	San Juan	Angualasto	640
SJ5-ENT2	San Juan	C Calvario	880
SJ2	San Juan	Calingasta	800
SJ6-ENT8	San Juan	Gruta 1 Morrillos- Ansilta	2000
SJ8-ENT5	San Juan	Gruta 1 Morrillos- Ansilta	2000
SJ7-ENT2	San Juan	Gruta 1 Morrillos- Ansilta	4070
SJ1-ENT7	San Juan	Gruta Morrillos	7900-4200
SJ9-ENT1	San Juan	Hilario	1400-1200
SJ3-ENT3	San Juan	Punta del Barro	590

*Given in years BP

**Samples submitted for radiocarbon dating, for which the results have not yet been received

the University of South Florida (USF) Laboratory for Archaeological Science under the direction of Dr. Robert H. Tykot.

Drs. Adolfo Gil and Gustavo Neme, specialists in the field of South American prehistory, were also consulted during the various stages of the study. Gil and Neme, archaeologists from the Museo de Historia Natural, were present for many of the previous excavations in Mendoza and are involved in ongoing work on the gathered materials. The following chapters add to scientific knowledge in the fields of archaeology, stable isotope analysis, Argentine prehistory, and South American archaeology, while building upon the prior work of Gil, Neme, and their associates. This research can also assist those interested in present-day populations that are struggling with nutrition related diseases, such as anemia and osteoporosis. Knowledge of ancient diet patterns may lead to a better understanding of the beginnings of human disease. Stable isotope analysis provides data regarding the transition to agriculture that can be viewed in relation to diet, nutrition, and disease in both past and present populations. In addition, this research has a public component, in that it provides a greater understanding of New World prehistory for both academics and Argentineans. The interpretation of the results of this study are incorporated in the exhibits of the Museo de Historia Natural, Argentina.

The subsequent chapter, *Background of the Cuyo*, begins with a description of the study area and explains the geographical boundaries of the Cuyo. The various cultures acknowledged as previous inhabitants of the environmental zones that make up the region are portrayed. This culture history also discusses the plant and animal resources that have played important roles in past diets, and briefly assesses the development of agriculture in the region. Models are conferred for the origin of domesticated maize,

aspects influencing South American archaeology, and theoretical orientations. Further explanation is given of the research goals with a detailed account of the samples that have been selected for analysis.

Next, *Principles of Stable Isotope Analysis* reviews the fundamentals of stable isotope analysis and its initial studies. This process is not complete without a clarification of the biological processes affecting the development of bones, teeth, hair, muscle and skin tissue. Carbon, nitrogen, and strontium isotope analysis and fractionation are explained. Sources of error that may affect isotope ratios if not taken into account are also considered. This explanatory section enables a greater understanding of the subsequent chapters.

The fourth chapter, *Stable Isotope Analysis Methods*, details the various preparation procedures that were used to process the numerous sample materials at the USF Laboratory for Archaeological Science. Laboratory procedures discussed include those that were employed to prepare bone apatite, tooth enamel, bone collagen, scalp hair, skin/muscle, and resource samples. This description also consists of information regarding the instruments of analysis used at the USF St. Petersburg facilities.

The *Results and Discussion* chapter reports the numerical figures obtained from stable isotope analysis and the results are examined for accuracy. SPSS 13.0 and Microsoft Office Excel 13.0 were used to perform quantitative analysis and create graphical elements. Intra-population relationships are considered and seasonal changes in diet are examined. Quantitative analysis was used to study inter-populations relationships, including temporal and spatial trends.

Finally, the *Conclusion* summarizes the work that has been done. Future methodological considerations for isotopic studies are reported, and a section has been included on the scholarly and educational importance this study. Admittedly, this study is but one small segment of ongoing research in the area of interest. The information presented in this thesis is by no means intended as an ultimate portrayal of dietary changes and effects in the Cuyo. It is, however, hoped that the work that has been done will significantly contribute to understanding of this region and its associated peoples. Chapter 6 specifies the directions of future studies in greater detail.

Chapter Two: Background of the Cuyo

The Andes are composed of high peaks, deep basins, low valleys, and rolling grasslands. A diversity of vegetation and climatic zones results, because the Andes are made up of three parallel mountain chains that were formed by volcanic activity in the Late Cenozoic era (Lamb 2004). These mountain chains are referred to as the Cordillera Principal, Frontal Cordillera, and the Precordillera, and are divided by tectonic valleys and deep, mountain fed rivers (Compagnucci and Vargas 1998). Interestingly, the seismically active fields of the Andes may have affected both the ways that previous people used their landscapes, and what present day archaeologists know about the sites that remain (Bruhns 1994).

In the central Andes, high-altitude lakes, such as Lake Titicaca and Lake Junin, are common. To the east of the mountains, many rivers and valleys flow toward the Amazon, separated by vast grasslands (*punas)* and high summits. Moving southward, altitude increases in a variety of punas and valleys, and then decreases into the coastal deserts of present day Chile along the coast of the Pacific Ocean (Clapperton 1993; Isbell 1997; Bruhns 1994; Willey 1971).

The Southern Andes include the majority of present day Chile, southern Bolivia, and western Argentina. The Argentine Andes are generally dry, and primarily composed of barren slopes, with semi-deciduous scrub forests filling the region (Figure 2.1). Moving south of the La Plata Basin, a terrain of basins and plains, the vegetation changes to *pampas* of mesquite brush land (Figure 2.2). This area of western Argentina

Figure 2.1. Dry mountain slopes of Argentina

Figure 2.2. Mesquite brushlands of the Argentine pampas

encompasses a greater number of mountain streams (Compagnucci and Vargas 1998). As a consequence of the increased access to water, this was the chief area of human occupation throughout prehistoric times, and is often referred to as the "southern cone" (Bruhns 1994; Pearsall 1992).

Geography of the Cuyo

The western Andean geographical area of the Argentine Cuyo lines the Central Andes and extends southward beginning with the aforesaid southern cone. The term Cuyo might be based on the indigenous Huarpe word "cuyum," meaning dry or sandy earth (Muñoz and Lillo 2001). The area consists of three provinces: San Juan to the north; Mendoza to the south; and to a lesser extent, San Luis to the east (Figure 2.3). Although San Luis is politically defined as part of the Cuyo, it is far less dry and mountainous than either the San Juan or Mendoza provinces, ecologically dissimilar, and is not thought to have been occupied as thoroughly throughout prehistoric time. The relatively few known sites from San Luis will not be considered in this study.

The provinces of San Juan and Mendoza are known for their complex ecosystems, diverse floral and faunal resources, and occasional earthquakes. The Cuyo borders the central Andes and sits southeast of the Atacama Desert. It is shadowed by Mount Aconcagua, the second highest mountain peak in the world (6,960 m asl) and experiences somewhat frequent hailstorms. Climate and the availability of resources change remarkably over short distances in this region of the world (Gil et al. 2005). The nearby Atacama Desert of Chile serves as an example of one of the most extreme climates of South America, and is the occupation area of the Chinchorro, a well-known population

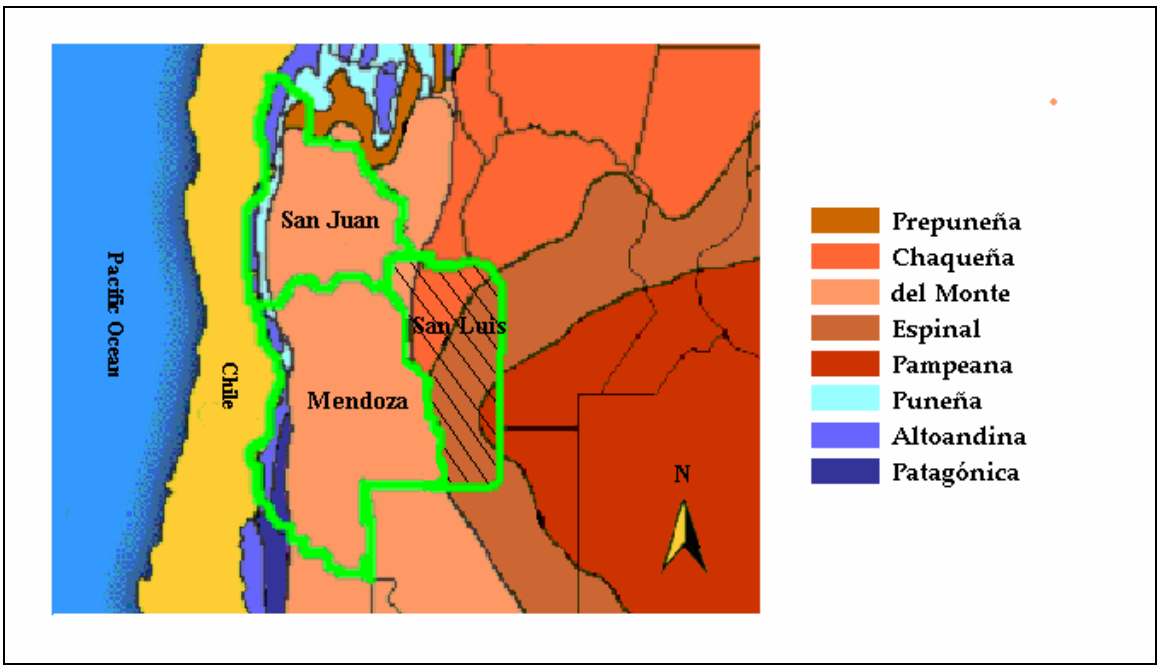

Figure 2.3. Phytogeography of the Cuyo. Political boundaries of modern day provinces are highlighted in green.

documented by Uhle (1922). This coastal group is known for their unique mortuary practices (Figure 2.4), and might have been the first culture to mummify their infants and children (Arriaza et al. 1998; Aufderheide 1993; Vreeland 1998). Some (Gil, personal communication, 2004) have even suggested that the Chinchorro introduced the practice of maize agriculture to the area of the Argentine Cuyo; however no substantial studies have been undertaken thus far to address this issue and no archaeological remains have been found which would support this argument.

The Mendoza-Neuquen region is defined as an environmental and cultural subdivision of the Southern Andes, with an elevation between 1200 and 3000 m and extending from 32 to 37 degrees south and 70 to 67 degrees west. This diverse ecological region includes mountains, plains, volcanic fields, and deserts. The highlands of the Andes Cordillera are mountainous at 32 degrees south, with eastern piedmonts

Figure 2.4. Chinchorro mummy recovered from the Atacama Desert of Chile

extending toward large plains from 32 to 34 degrees. Melting glaciers and mountain snowfall feed the fluvial systems of the Río Diamante, Río Atuel, and Río Grande throughout the volcanic fields of La Payunia in southern Mendoza at 34 to 37 degrees (Compagnucci and Vargas 1998; Gil et al. 2005; Willey 1971). In the past, the area that is now known as southern Mendoza represented a unique transition zone between the hunter-gatherers of Patagonia and the semi-sedentary agricultural populations of the Andes. Mendoza is thought to have been one of the last cultural areas to cultivate maize and other prehispanic crops, probably around 2000 BP. Further north, in the province of San Juan, inhabitants were thought to have adopted agriculture somewhat earlier, although still relatively late when compared to the continent of South America as a whole (Gil 2003). The present study employs the method of stable isotope analysis to test those hypotheses; to examine what differences existed among contemporary populations of the Cuyo, and to document what dietary transitions took place over the broad time span of approximately 6000 BP to 200 BP.

It is important to note that sections of the provinces of Mendoza and San Juan are desert environments, with some areas receiving less than 100 mm of rain annually

(Compagnucci and Vargas 1998). This is an ideal environment for natural mummification. Vreeland (1998) defines three types of mummification that occur in the New World: natural mummification caused by environmental factors such as aridity, extreme hot or cold temperatures, and/or lack of air in burial situation; intentional natural mummification, resulting from human exploitation of said natural processes; and artificial mummification, which is produced exclusively through human manipulation. While the second process, intentional natural mummification, typifies the cultural practices of residents of the Atacama Desert of Chile, the arid climate of the Argentine Cuyo provides for frequent occurrences of natural mummification regardless of human intervention, thereby preserving soft tissues and hair (Gil et al. 2005). This preservation of soft tissues allows for greater understanding of dietary complexity in prehistoric systems, including analysis of possible seasonal variation.

South American Archaeology

Previous archaeological research in South America has a history of strong Marxist orientation (Funari 1997; Politis 1999, 2002). This theoretical perspective has resulted in many cultural neo-evolutionary models (Bruhns 1994) that either implicitly or explicitly suggest all cultures have passed through a similar progression of advancement. South American archaeologists working with previous archaeologists' records are commonly confronted with neo-evolutionary models and many are actively working to develop more flexible and more plausible temporal and chronological schemes. The research this thesis undertakes is an attempt to develop a regionally specific model, while still interpreting

humans as a species that interacts with its environment in a multitude of ways; both influencing and being influenced by its surrounding ecosystems.

Research Frame

This research fits into a human ecology framework by viewing human subsistence activities as interactions with corresponding ecosystems. Just as environmental variations affect the availability of dietary resources and lead species to adapt to these changes, dietary transitions, such as the adoption of agriculture in a region, can be seen as behavioral adaptations to a surrounding environment. Butzer (1982) argues that human ecology theory applies well to many archaeological studies, as the analysis of human remains and associated artifacts demonstrate activities that may be perceived as interactions with corresponding ecosystems.

Similarly, stable isotope analysis of human remains serves to reflect subsistence activities that may be perceived as reactions to, or interactions with, surrounding environments. Stable isotope analysis not only tracks long-term diachronic changes, but the naturally mummified samples that have been analyzed in this study allow for the examination of seasonal dietary variation. The following study documents an intrinsic relationship with the lands where prehistoric inhabitants of the Cuyo subsided, foraged, and later cultivated crops.

Gilbert (1985: 340) defines three categories of stress that may influence a population or an individual to make changes in their survival techniques: directly environmental, indirectly environmental, and psychosocial. While direct environmental changes can include climatic changes, such as those that may have influenced the

inhabitants of the Cuyo in the mid-Holocene (see below), indirect stresses include disease or nutritional stress - also likely products of environmental change. The third category of stress, psychosocial, includes pressure from other members of an individual's culture.

Here, it is important to recognize the concept of human agency in a decision making process (Dobres and Robb 2000). Again, one does not want to fall prey to the sort of determinism that plagued stage theorists such as Morgan; in reality, the possibility exists that no action at all may be taken in response to any of the stress sources indicated above. Conversely, adaptations can be made either consciously or subconsciously. Certainly, adaptations to stress can be biological, but they can also be behavioral. The concept of human agency is epitomized in the Cuyo. Not only might some cultures have chosen to take on maize agriculture (e.g., the inhabitants of San Juan), others (e.g., the inhabitants of Mendoza) may have remained foragers well up until the arrival of Spanish conquistadors.

Additionally, not all intentional adaptive decisions necessarily result in biological or cultural success (Gilbert 1985). Intentional alterations in diet may be unintentionally maladaptive. Many anthropologists (Larsen 1995, 2002; Steckel et al. 2002b) have pointed to detrimental changes in health that correspond to the adoption of maize agriculture, such as anemia and osteoporosis. Moreover, some (e.g., Steckel et al. 2002a) note agricultural societies were the first to fall victim to the health epidemics brought on by the arrival of Spanish conquistadors. This suggests that the success of the European invasion might have been due to weakened immune systems of natives brought on by inadequate diets, thereby making indigenous cultures such as the Inca easy prey for European disease vectors. Furthermore, some members of the study population

examined in this thesis demonstrate the existence of nutritional stress indicators, such as dental caries, that can be viewed in relation to the dietary shifts that may have been a cultural adaptation to the environment of the Cuyo. For these reasons, it is important to question not only when dietary transitions occurred, but also what the impetus might have been for these changes, and how they may have affected the populations that undertook them.

Models for the Adoption of Agriculture

Multiple competing models accounting for the adoption of agriculture have been proposed (see Steckel et al. 2002a for a thorough review). Many such models interpret the transition to food production in various regions of the world and can be grouped under the broad heading of evolutionary ecology. One such perspective (Price and Gebauer 1995) argues that populations made very conscious decisions to adopt agriculture as a sort of reserve method in otherwise satisfactory ecological systems. Thus, they adopted primary food production because they could *afford* the risk. Assuming this gamble was successful, increased crop quantities supported larger population sizes, thereby allowing for the development of increasingly complex political systems such as the pre-Hispanic Inca Empire.

Another interpretive stance incorporates the view that humans were forced to begin practicing agriculture, because of environmental scarcity or nutritional deficiencies (Gilbert 1985). On a regional scale, climate changes during the Holocene epoch are thought to have lessened the availability of protein resources in the Cuyo when large

game became extinct as grazing lands diminished. This may be one reason some cultures eventually decided to adopt maize agriculture, while others chose to hunt smaller animals and continue to gather wild plants. It has also been suggested (Cohen 1977; Steckel et al. 2002a) that population increases in the Neolithic took place *prior* to the adoption of agriculture, thereby providing a type of social stress, in the form of population pressure that was combated with the behavioral adaptation of landscape modification in the form of agricultural systems. Thus, food production might be viewed as an attempt to compensate for some sort of perturbation in a culture's nutritional system. Keene (1985) points to dietary shifts as an indicator of nutritional stress, noting that many hunter-gatherer groups were completely capable of sustaining and even exceeding their energy needs in appropriate environments. Keene views the transition to agriculture as a risk that would only be undertaken in otherwise unbalanced or insufficient ecosystems, that were, for some reason, suddenly incapable of supporting human populations.

Price and Gebauer (1995) further illuminate these alternating viewpoints by grouping the potential motivations for adoption of agriculture into exogenous and endogenous factors. Endogenous factors include social change and allude to a conscious alternation of subsistence strategies. Exogenous influences include climate changes and population pressure, thereby viewing the adoption of food production in response to environmental perturbations. They, too, suggest that agriculture was first adopted in areas of abundant resources; however, they reason that significant population is a condition for, rather than a cause of, food production. Their global analysis indicates no presence of remarkable population growth immediately prior to a dietary transition.

Some of the cultural changes that are thought to have taken place in the provinces of San Juan and Mendoza are examined below. A causal relationship between environmental/social stressors and cultural changes is *not* assumed; however, it is important to acknowledge these transitions so that any existing correlations may be taken into account and examined in further detail.

Cultural Chronology

In general terms, little is known about South American prehistory when compared to the rest of the New World, or most certainly to the Old World. In part, this may be due to the lack of an indigenous writing system. Native Americans of the Andes did not have writing systems as are known today, and their knotted string records, called *quipus*, may never be fully understood (Isbell 1997; Urton 2003). Quipus are thought to have been a Huari invention that served as mnemonic devices for the Inca's class of scribes, called the quipucamayoc (Bruhns 1994). While archaeologists (Quilter and Urton 2002) now recognize the numbering system that these knotted strings might represent, the meaning of any individual quipu is not known (Urton 2003: 161-164), and their translators are now extinct without having passed down their specialized knowledge, due in part to the invasion of Spanish conquistadors.

Furthermore, the research that has been undertaken in South America has predominantly focused on information from Peru, or Inca populations (Pearsall 1994; Politis 1999, 2001). To this effect, relatively little is known about Argentina. While it might be helpful to examine changes in the Cuyo compared to broad continental movements, few horizon lines - cultural chronologies defined by distinct artifacts

indicative of the rapid spread of a culture over a wide period of time - exist for South America in general. Experts commonly define the four central Andean horizons as Inca, Tiahuanaco-Huari, Cuzco, and Chavin (Bruhns 1994; Vreeland 1998). While this information is spatially consistent with the area of the Cuyo, the full prehistory of Argentina has not been considered in detail, nor is the temporal span particularly relevant. There exists a predominance of information regarding Inca sites, with few previous reports centering on the time period being consideration in this study. For this reason, continued research in Argentina is especially important and should include, but not be limited to, the scope of this thesis.

Paleoindian and Archaic sites

It is now recognized that areas of South America, such as the pre-Clovis site Monte Verde in present-day Chile, were occupied at least 12,500 years ago, with later sites occurring much more frequently (Dillehay 1999). Thus far, little is known about South American inhabitants east of the Andes mountain range. However, finds throughout northeast Argentina, Paraguay, and Uruguay suggest the region was occupied throughout the early Holocene (Gil et al. 2005; Bruhns 1994). The information that does exist includes the analysis and interpretation of Archaic sites including Ayampitín and Inithuasi, with the Ayampitín tool industry recognized as a distinct style of early points and flakes. Named after the site of Ayampitín, an open air camp in Cordoba (to the north of the Cuyo), this grouping includes distinctive willow leaf points, flakes, and grinding tools. While tools of this type have been recovered from a number of sites, the most

well-known assemblage is from Intihuasi Cave (González 1959), because the clearly stratified deposits make relative dating possible.

Another early tool industry is the Ampajango, first identified in the northwest Argentine province of Catamarca (Bruhns 1994). The Ampajango tool industry is primarily defined by a group of percussion flakes; however, disagreement exists regarding the temporal scheme of these tools. While some have argued for an earlier date because tools of this industry are made with minimal modification and lack projectile points, Bruhns (1994) states that this general lack of sophistication is due to the poor quality of rock available in the area, and not some sort of incompetence by tool makers of the early Holocene.

The Holocene Epoch

The progression from the Late Pleistocene to the early Holocene is known for worldwide perturbations in climatic and environmental conditions. These global changes might have placed environmental stress on human populations, and thereby contributed to dietary transitions. While climates varied widely, many areas experienced increased temperatures resulting in a diminution of grasses, decreased grazing lands (Sandweiss et al. 1999; Zárate 2002), and the eventual extinction of some large mammals, notably the ground sloth (Long et al. 1998). These climate changes were particularly pronounced throughout the Andes, resulting in a number of environmental changes that probably affected the early inhabitants of Argentina. Higher snowfall in the upper Andes led to neoglacial advances, resulting in decreased water flow in the major rivers of the Cuyo that are ordinarily fed by melting snow and ice. Meanwhile, fewer summer rains at the

piedmont level probably led to a concentration of human populations along large fluvial valleys. Further, these changes directly affected the availability of floral and faunal resources throughout the Cuyo. Intensity and location of human occupations changed, with lowland sites most likely abandoned. Gil et al. (2005) hypothesize that these environmentally stressful conditions brought on by decreasing water resources in the already dry environment of the Cuyo, led to human responses of a movement upland, where the increased snowfall provided for comparatively greater water availability.

Silencio Arqueológico

The aforementioned occupational hiatus of lowland Argentina is suggested by a lack of sites from this period, and corresponding low densities of materials (Gil et al. 2005). Alternate explanations, such as disturbance by modern populations and site formation processes, have been examined with negative results (Gambier 2000). It is believed that as environmental conditions became increasingly dry from the Late Glacial to the mid-Holocene, many South America populations declined in number and/or adapted culturally. The period of 9000-4500 BP is sometimes referred to as "silencio arqueológico", or archaeological silence, in the most affected areas (Gil et al. 2005; Nuñez et al. 2001). This occupational hiatus likely took place in the most affected environments of southern Mendoza. Essentially, environmental perturbations influenced human populations to abandon previous occupation sites and relocate during this period. Alternatively, Lagiglia (2001, 2002) has proposed models of continuous occupation in southern Mendoza throughout this time.

Gil et al. (2005) tested this hypothesis by analyzing 97 radiocarbon dates from the time interval of 14,000 to 200 years BP. After examining these carbon-14 dates they found that the archaeological record does not support Lagilia's (2001, 2002) earlier assumption of a continuous occupation in southern Mendoza. Rather, they agree with a significant hiatus during the period of 7000-6000 BP. Further, they cite the aforementioned geomorphological processes of the Holocene as the impetus for this cultural trend.

While less is known about the early cultural chronology of San Juan (Table 2.1) than Mendoza, the archaeological record is currently being examined, and this thesis will contributes to the construction of a more thorough cultural chronology based, in part, on cultural transitions such as dietary shifts.

Moving toward the late Holocene, rainfall increased again and major fluvial systems were once again hydrated. Humans began to move back into the Cuyo, while simultaneously modifying their subsistence strategies to adapt to the "new" environments they were living in. It is emphasized that the extent of the silencio arqueológico varies with the scale of spatial analysis. Again, the most climatically affected areas of San Juan and Mendoza are thought to have been abandoned for the longest amount of time, with less fragile environments being reoccupied earlier. The oldest recorded cultigens in Mendoza occur in a funerary context as early as 2200 years B.P., at sites such as Gruta del Indio. Subsequent indications of agriculture from various archaeological contexts do not come until 1,000 or more years later. This information has been used to argue that maize may have had a special cultural meaning before becoming a dietary staple (Gil 2003). Disparities in the adoption of maize agriculture also occurred in different

Table 2.1. Chronology and associated cultures of San Juan, Argentina

Chronology (BP)	Cultural period	Cultural group
8500-8200	Early hunter-gatherer	La Fortune Industry
7900-4200	Late hunter-gatherer	Los Morrillos
3800-1950	Early farming	Ansilta
1950-1400	Early agropastoralists	Punta del Barro cultural phase
1400-1200	Early agropastoralists	Calingasta
1200-900	Middle agropastoralists	La Aguada influence
750-460	Late agropastoralists	Angualasto/late Calingasta
460-420	Inca	Inca, local group with Inca influence
420-388	Local indigenous	Huarpes/capayanes and yacampis

environmental regions, such as the Argentine highlands and areas located to the direct east of the mountains. This thesis tests these hypotheses, questioning when, if at all, maize was adopted and in what areas. While Mendoza and San Juan are sometimes considered separately - with San Juan thought to have converted to agriculture earlier than Mendoza - this thesis tests this hypothesis with quantitative data obtained from stable isotope analysis.

Dietary Resources

Although the Cuyo is a dry, desert-like environment, it is also known for its diversity of floral and faunal resources (Gil et al. 2005). Certainly, the abundance of available dietary resources was utilized by previous inhabitants of the Cuyo, and may have led to the sustained tradition of hunter-gatherer subsistence patterns in the province of Mendoza. Dietary resources that are considered to have been particularly important prior to, and in addition to, maize include guanaco (Figure 2.5), rhea, squash, and various fruits.

Figure 2.5. Modern-day guanacos, a staple of the prehistoric Argentine diet

While this thesis primarily focuses on the analysis of human samples, it is important to acknowledge the available resources humans may have also been eating. A growing number of South American archaeology projects are incorporating botanical recovery methods (e.g. Barberena 2002; Hastorf 1999; Hastorf and Johannessen 1994; Pearsall 1992) into their research and this has resulted in an exponential increase in knowledge of past dietary options (Bruhns 1994). In the Cuyo, a registry of carbon and nitrogen stable isotope values for alleged dietary staples is being built. Table 2.2 lists floral and faunal species that were analyzed to this effect. The analyses of resource samples will define dietary values in an attempt to rule out the possibility of any abnormal carbon or nitrogen results that may affect human values irregularly.

South American archaeologists are also looking towards advanced laboratory methods to document the actual inception of maize agriculture. The crop that may have had the greatest effect in Argentine prehistory is maize (*Zea mays*). As discussed in the previous chapter, the adoption of food production has been associated with a multitude of

Table 2.2. Analyzed samples of floral and faunal resources

Latin Name	Common Name
Cassia arnottiana	Cassia
Chaetophractus villosus	Armadillo
Chenopodium sp.	Chenopodium
Cholephaga melanoptera	Andean goose
Cucurbita maxima	Winter squash
Geoffroea decorticans	Chanal
Lagenaria sp.	Gourd
Lagidium viscacia	Chinchilla
Lama guanicoe	Guanaco
Phaseolus vulgaris	Common bean
Prosopis sp.	Prosopis tree
Pterocnemia pennata	Lesser rhea
Rhea americana	American rhea
Schinus polygamus	Pepper tree
Zea mays	Maize

cultural, political, and biological changes (Gilbert 1985; Steckel et al. 2002). Therefore, this thesis focuses predominantly on the history of this particular crop in the study area.

Prior to the use of stable isotope analysis, archaeologists struggled to evaluate the transition to maize agriculture throughout South America; without any type of chemical analysis, researchers often had to settle for the few artistic depictions of maize that exist on limited numbers of ceramics and sculptures (Bruhns 1994; Pearsall 1994). This proved problematic for a number of reasons, including that few cultures produced such stylistic renderings, and those that did (e.g., the Inca) were relatively late. Another indirect method of documentation that was used in the central and southern Andes was the mere presence (or absence) of manos and metates or other grinding materials. Bruhns (1994) has argued that this method of interpretation is highly inaccurate, as many South American people either did not grind corn, or did not use manos and metates to do so.

Beyond this, the subject of how and when maize species reached South America has previously served as a subject of much disagreement.

Origins of Domesticated Maize

In general, there exist three competing schemes regarding the origins of maize domestication. Beadle (1980) and Galinat (1983) have argued that a wild grass, called teosinte, is the ancestor of all maize variations. This suggests a single origin of maize, that must have traveled to spread across all of the Americas. Alternatively, Mangelsdorf (1947) presented a model that contends for multiple and separate origins of maize, including teosinte, from wild tripsicum grass in multiple environments. This position also allowed for an independent center of origin in the central Andes. Finally, in 1983, Iltis presented a radical theory that asserts an almost instantaneous evolution of maize with the sexual reversal of the male tassle of teosinte grass. This alteration could occur in a number of manners, including random mutation, environmental disturbance, or human domestication.

The final two models are made more plausible by the observation that South American corns possess rather dissimilar characteristics from Mexican and Central American corns. Bruhns (1994) draws attention to this divergence in shape and size, arguing that all flour corns originated in South America. However, Pearsall (1994), an expert in the topic of maize domestication, favors a single origin. A common explanatory theme in the multiple origin models is the spread of maize seeds by migratory animals such as birds. Understandably, Pearsall sees these as archaeologically unverifiable and notes it is plausible that several varieties of maize may have originated and subsequently

vanished in the past. Because the temporal scheme of this thesis is significantly later than the time at which this would be of major concern, the more common interpretation of the origin of maize will be assumed. In addition, one could refer to Pearsall (1994) for further exploration of this topic.

It is now generally agreed that maize was domesticated from teosinte in Mesoamerica between 10,000 and 5,000 B.C. Long and colleagues (1989) used accelerator mass spectrometry (AMS) to show that the earliest maize remains are at least 4700 years old. Benz and Long (2000) later used morphological analysis and genetic tests on Mesoamerican maize to argue that the domestication of maize might have occurred even sooner, perhaps earlier than 5400 BP (Benz 2001). The species then moved through Central America into South America, eventually adapting to elevations of 3,000 m in the Andes (Bray 2000; Pearsall 1994). The first scientific discoveries of pre-ceramic maize in the Andean region was made by Willey and Corbett in 1941 and 1942 (Willey 1953), however, it took researchers many years to grasp the importance of these discoveries. It was not until the 1950s when Lanning broke new ground with his research in the central and north-central coastal areas, that knowledge of pre-ceramic maize in the Central Andes truly became a focus of New World archaeology (Lanning and Patterson 1967). Today, there is a mass of information pertaining to maize from prehistoric Peru, identified from the southern Andes, including information regarding Argentinean agriculture (Bonavia and Grobman 1989; Isbell 1997; Keene 1985). Furthermore, maize may have been utilized in different ways throughout the Andes, and not every culture that

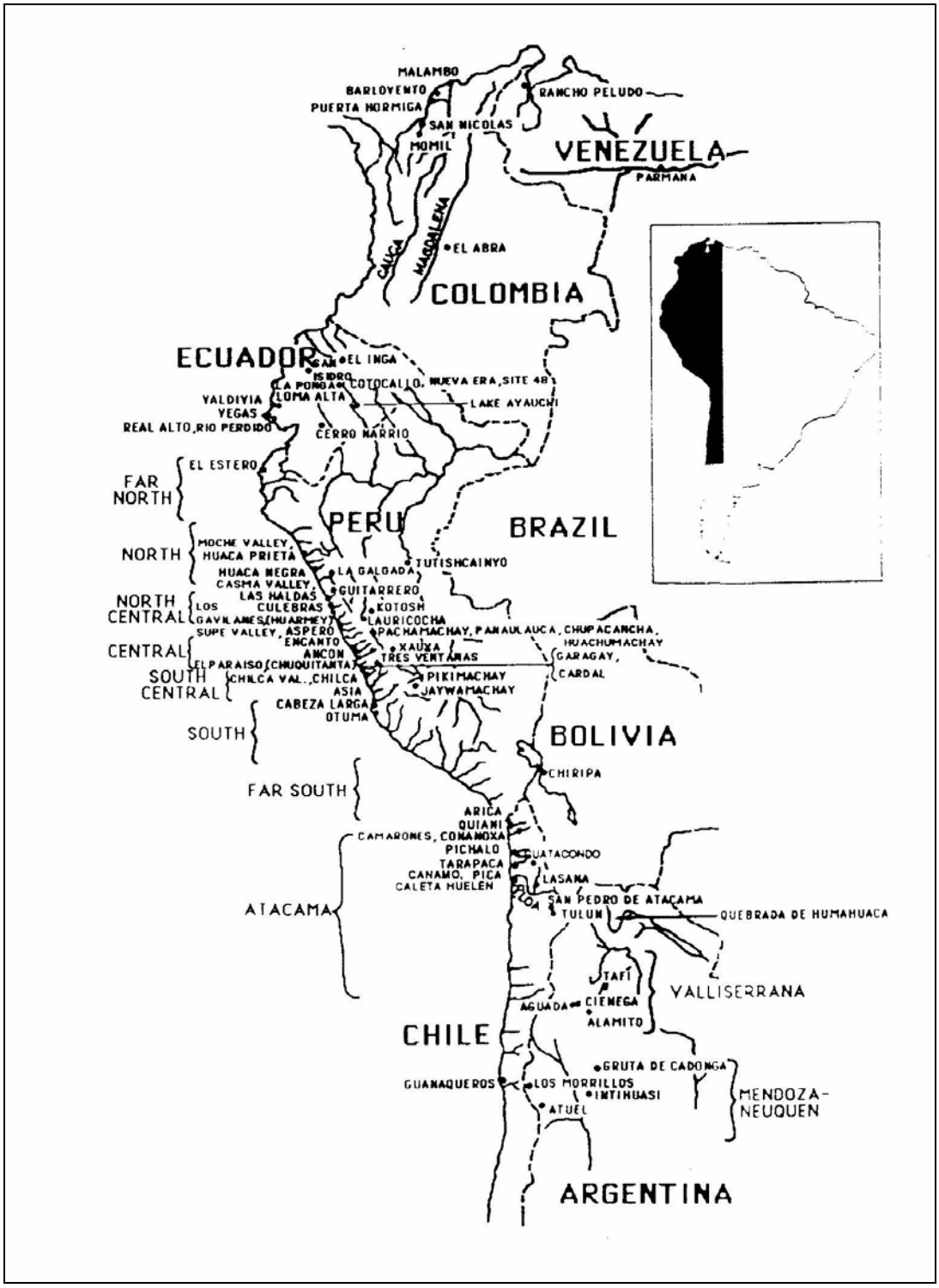

Figure 2.6. South American sites with early plant remains. From Pearsall (1992:176)

knew of maize necessarily adopted it (Pearsall 1994). For these reasons, it is important to look at the adoption of maize agriculture on a regional scale, such as the study area of the Argentine Cuyo.

Sample Description

Adolfo Gil, Gustavo Neme, and Humberto Lagiglia, of the Museo de Historia Natural, San Rafael were primarily responsible for the collection of archaeological materials throughout Mendoza. Samples were selected in an attempt to define site locations and to construct a broad temporal and spatial inventory of the Argentine Cuyo, with an emphasis placed on sites with high levels of preservation and little post-depositional disturbance. This thesis continues recent exploratory work from the province of San Juan, with many samples being selected from current museum collections. The excellent preservation of the museum samples aids in the experimental analysis of soft tissues materials. The majority of human samples that have already been processed are now in the Museo de Historia curation, with a minimal amount of processed material remaining at the University of South Florida Laboratory for Archaeological Science.

Tissue selection

Bones and teeth are the most frequently preserved biological materials, other than shells and charcoal, in any given archaeological context, as they have much slower rates of decomposition than soft tissue. Bone is the most common of all tissues, and is composed of an organic matrix formed by collagen, which is sometimes referred to as

gelatin; and an inorganic portion termed hydroxyapatite, which is commonly called apatite (Armelagos 1994; Boutton 1991; Schwarcz and Schoeninger 1991). Collagen represents a large fraction of bone (approximately 20 to 25 percent in fresh bone), is relatively insoluble, and contains both nitrogen and carbon. Apatite represents 75 to 80 percent of fresh bone, but contains no nitrogen (DeNiro and Schoeninger 1983).

The analyses of the bone matrices, collagen and apatite, portray the average diet over the last several years of an individual's life, while those of tooth enamel reflect diet during the age of crown formation. This combination of analyses is useful for contrasting the juvenile and adult diets of individuals, but conclusions are limited to the representation of average diets. The materials in this study provide a unique opportunity to study soft tissues, as the arid conditions of the environment where they were interred often result in natural mummification. Soft tissues have a particular advantage, in that the relatively new procedures of isotopic analysis of hair and flesh samples may reveal seasonal, or even monthly, dietary variations (O'Connell and Hedges 1999a, 1999b).

While the arid conditions of the Cuyo do provide for some natural occurrences of mummification, there was unsurprisingly a predominance of hard tissue available for this study. Whenever possible, all available materials were analyzed for each individual, including both soft and hard tissues. Soft tissues that existed for analysis included scalp hair, fingernails, skin and muscle, all of which originated from individuals of the San Juan province (Table 2.3).

Table 2.3. San Juan samples available for soft tissue analysis (Gil, pers. comm.)

Sample	Site	Sex	Age	Chronology (years BP)	Hair	Skin/ Muscle	Nail
SJ1-ENT7	Gruta Morrillos	F	-	7900-4200		X	
SJ2	Calingasta	-	-	800	X	X	
SJ3-ENT3	Punta Del Barro	-	-	590	X	X	X
SJ4-ENT2	Angualasto	-	-	640		X	
SJ5-ENT2	Calvario	-	-	880	X	X	
SJ6-ENT8	Gruta Morrillos- Ansilta	F	Adult	2000	X	X	X
SJ7-ENT2	Gruta Morrillos- Morrillos	M	Adult	4070	X		
SJ8-ENT5	Gruta Morrillos- Ansilta	M	-	2000	X	X	X
SJ9-ENT1	Hilario	F	Adult	1400-1200	X	X	X
SJ10-ENT1	Angualasto	F	-	600	X	X	X

Temporal distribution

Radiocarbon dates have been obtained for sites throughout both provinces of study whenever possible (Gil et al. 2005). This method of dating helps establish chronology that is otherwise determined through indirect analysis of archaeological materials such as pottery and tool typologies. Human remains that have been analyzed range in antiquity from approximately 6000 to 200 BP. This wide temporal span encompasses a number of cultural transitions that may also represent dietary shifts (see Table 2.1). This thesis seeks to evaluate the changes from hunter-gatherer to agricultural and pastoral societies that have been assumed for the province of San Juan, and to determine if these transitions are reflected in the dietary record of the Mendoza province. The stable isotope analyses reported here compare variation for these periods from both provinces.

Table 2.4. Sex distribution by province

	Province	
	San Juan	Mendoza
Female	4	7
Male	2	7
Unknown	4	21
Total	10	35
Sex Ratio	2:1:2	1:1:3

Spatial distribution

Human and resource samples were selected from sites throughout both provinces, with the majority of samples originating from the Mendoza province. A total of 27 sites were sampled, 21 of which were located in Mendoza and six from San Juan. Mountainous, piedmont, and lowland sites were sampled to gather information from a wide variety of environments (Gil et al. 2005; Neme 2002). Many of the sites are rockshelters or situated along banks of the major rivers that run throughout the dry environment of the Cuyo. It is emphasized that work has only just begun in San Juan, and the analysis that is presented here just begins the effort that will be undertaken to document the prehistory of the San Juan province.

Sex distribution

Sex determinations were made on the basis of morphometric analysis performed by Barrientos, Perez, and Novellino (unpublished data). The selected samples appear in Table 2.4. Whenever possible, human samples were assigned to male or female categories; unfortunately, a large number of skeletons were incomplete, juvenile or

otherwise found indeterminable, and were simply labeled as "unknown". Whereas only individuals of known sex were used to test sex differences, individuals of unknown sex were also analyzed to evaluate whether those individuals had similar dietary patterns to those of known sex.

Age distribution

Barrientos, Perez, and Novellino (unpublished data) were also responsible for determining the ages of individuals based on morphometric and dental analyses. However, many individuals could not be assigned ages, and those that were assigned age groups presented a discontinuous sequence. Furthermore, while some individuals from Mendoza were determined to be children, very few juveniles were available from either province. Therefore, dietary differences regarding age distribution were analyzed only on the basis of individuals' apatite to enamel comparison, which yields information on adult and juvenile diets, respectively. In order to further understand these processes, the following chapter discusses the principles of stable isotope analysis, including isotope fractionation, fractionation between trophic levels, and sources of error that may affect isotope ratios.

Chapter Three: Principles of Stable Isotope Analysis

The science of isotopic analysis represents a fusion of two diverse fields, biomedical research and archaeology (Sullivan and Krueger 1984). Procedures originally developed for radiocarbon dating have given researchers the ability to examine the chemical makeup of biological material and examine the human past in a direct, quantitative manner. This practice serves as an excellent example of the holism of the field of anthropology and is founded on the principle that nearly all biochemically significant elements exist as a mixture of two or more isotopes with differing numbers of neutrons, and identical numbers of electrons and protons (Schwarcz and Schoeninger 1991). Methods of stable isotope analysis have been defined for elements including carbon (C), nitrogen (N), and strontium (Sr). Researchers use mass spectrometers to measure the isotopic ratios of a sample. These results are then compared with the isotopic ratios of a standard. The results yielded from this type of analysis may speak volumes about diet, nutrition, and migration patterns of otherwise prehistoric cultures (Eaton et al. 1988; Ericson 1985; Fogel and Tuross 2003; Larsen 2002). Carbon and nitrogen isotope analyses are often used to reconstruct past foodways and the information garnered can then be extrapolated to supplement dietary studies, whereas strontium analysis is often used to examine the "home range" of an individual and extract data regarding relocation or migration affairs.

Biological Materials

The adult human skeleton is composed of approximately 206 bones and accounts for approximately 14 percent of a living person's body weight (Steele and Bramblett

1988). As briefly discussed in the introductory chapter, the inherent properties of hard tissues (bones and teeth) allow for greater preservation in the archaeological record than their counterparts, the soft tissues. As a result, the majority of stable isotope studies performed thus far have used bone and/or tooth materials for analysis.

The exception to this generalization is an extreme environment. Climates with anaerobic conditions, hyper-aridity, or freezing temperatures often allow for higher levels of preservation (O'Connell and Hedges 1999a). The present study is unique in that many of the human samples originated from a desert environment that runs parallel to the Argentine Andes. Thus, soft tissue materials (e.g., hair, skin, muscle tissue, and fingernails) were available for a proportion of the study population. This range of materials allows for greater understanding of prehistoric diet in the Argentine Cuyo, as well as advances in isotopic studies as a whole. Prior to explaining the chemical processes involved in such analysis, a brief discussion of the composition of human biological materials aids comprehension of the study as a whole.

Bones

The two complexes of bone, collagen and apatite, compromise for bone stress from different activities, such as tension, compression, and bending. Collagen, the more flexible portion formed primarily from protein, allows bone the ability to bend and compress; a collagen deficiency, such as that caused by nutritional maladies, may cause brittleness in bones that make them more apt to fracturing. Alternatively, apatite is composed of all dietary components and protects bone from compression. Dietary or

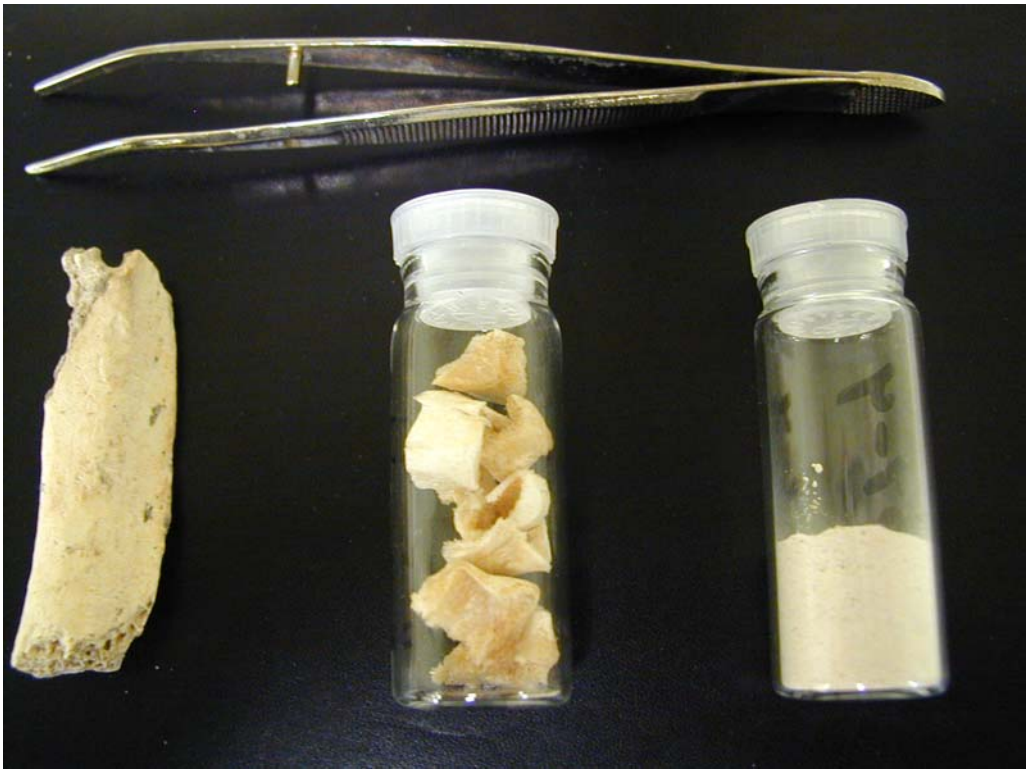

Figure 3.1. Three forms of archaeologically preserved bone. From left to right: complete bone, bone collagen, bone apatite.

nutritional deficiencies, particularly a lack of calcium, may cause a demineralization of bone, decreasing the ability to compensate for body weight. Bones may bend and flex in excessive ways without the support of appropriate amounts of apatite. An example of this condition is rickets, a condition commonly experienced by children who lack sufficient amounts of vitamin D (Larsen 1997; Steele and Bramblett 1988).

Early stable isotope studies, such as those by van der Merwe and Vogel in 1977 and 1978, focused on collagen, the organic portion of bone. However the inorganic portion, apatite, is less susceptible to deterioration than collagen. Methods were soon developed to extract apatite for analysis (Sullivan and Krueger 1981). Apatite analysis is

somewhat disadvantageous in that it does not allow for nitrogen studies (Table 3.1), but it may be resorted to when preservation is less than ideal, and is certainly useful for a more reliable depiction of the prehistoric diet as a whole (Schoeninger et al. 1983). Both complexes are susceptible to the constant decomposition and regeneration of bone that is the work of osteoblast, osteocyte, and osteoclast cells (Ortner and Putschar 1981); this results in an artificial "homogenization" of stable isotope values throughout the period it takes for each complex to recycle itself. Thus, stable isotope values obtained from bone samples represent the average diet of the last few years, approximately seven to 10, of an individual's life (White 1993).

Teeth

Samples available for this study included teeth from individuals of varying ages and it is important to note the differences between juvenile and adult dentition. The juvenile dental set is comprised of 20 deciduous teeth. These "milk teeth" are subsequently replaced by the permanent teeth throughout childhood (White 2000).

The adult dental set is typically comprised of 32 teeth, subsequent to variation based on factors such as number of third molars. Each tooth is bound to the jaw via a periodontal ligament surrounded by cementum (Figure 3.2). Cementum is a somewhat softer tissue than the other materials composing teeth and is often lost in the archaeological record. Extending from the roots of the teeth upwards thru the bulk of the crown is a hard tissue called dentin that is approximately 30 percent organic. A layer of

Table 3.1. Types of stable isotope analysis for biological samples

Element	Form of results	Material	Complex	Turnover rate
Carbon	$\delta^{13}C$	Bone	Collagen	7-10 years
			Apatite	7-10 years
		Tooth	Enamel	Variable depending on time of formation
		Skin/muscle	Collagen	Variable depending on depth
		Hair	Collagen	$\geq$ 12 days
Nitrogen	$\delta^{15}N$	Bone	Collagen	7-10 years
		Skin/muscle	Collagen	Variable depending on depth
		Hair	Collagen	$\geq$ 12 days
Strontium*	Sr^{87}/Sr^{86}	Tooth	Enamel	Variable depending on depth

*Not analyzed in this study

enamel then superimposes the entire structure (Steele and Bramblett 1988). It is this portion of the tooth that is most commonly sampled for isotopic analysis.

The composition of tooth enamel is similar to that of bone apatite, but teeth do not recycle themselves once they are formed. Thus, stable isotope values obtained from tooth samples represent diet at the time of formation. Teeth form at different times throughout an individual's life, with third molars typically developing sometime between 10 and 12 years of age, and other tooth types forming between zero and two years (Mays 1985; White 2000). Juvenile values might even reflect the diet of their mother, from either the period in the womb or from the nursing period (Dupras 2001). Alternatively, adult dentition reflects the values of corresponding juvenile diets that will vary in age depending on the tooth type.

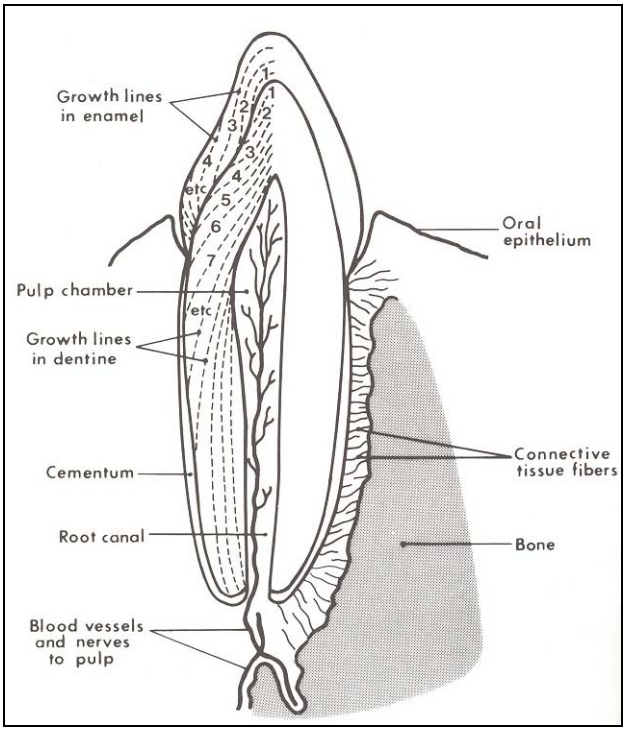

Figure 3.2. Lateral view of an adult tooth. From Steele and Bramblett (1988:72).

Skin/Muscle tissue

White and Schwarcz (1994) demonstrated the utility of soft tissues to document shifting seasonal dietary patterns in Nubian mummies. Subsequent studies, such as those by O'Connell and Hedges (1999a, 1999b) and by Fenner (2002), have built upon their work and supported the value of such studies, but soft tissue analysis is still fairly uncommon. Whereas hard tissue studies can be useful for determining average diets over the last few years of an individual's life, soft tissues experience continuous regeneration (Uzuka and Sakamoto 1967). This is particularly advantageous for the study of short-term, or even seasonal, diet. Variation in growth is linked to nutritional stress and physiological dynamics, but is not easily influenced by aspects such as environmental

season or equatorial zone (White 1993). Soft tissues include hair, skin and muscle tissues, and finger and toe nails.

Skin is composed primarily of collagen, but also contains lipids and the protein keratin. Factors including irregular, and therefore unpredictable, depletion of lipids and amino acids may bias isotopic values when soft tissue begins to decay (O'Connell and Hedges 1996; White and Schwarcz 1994). Thus, a lipid extraction was performed on soft tissue samples prior to mass spectrometric analysis in an effort to ensure greater reliability of results. Alternatively, muscle is composed almost completely of protein and is often left unprocessed prior to analysis, if any analysis does indeed take place. Not only were muscle samples analyzed in this study, they were also processed to remove any possible contaminants prior to analysis (White and Schwarcz 1994). This lipid extraction will be discussed in greater detail in the subsequent chapter.

The depth of skin is sometimes difficult to determine in mummified individuals and it is quite possible to confuse desiccated muscle and skin when obtaining samples. It should be noted that while there are major differences between the composition of muscle and skin tissue, the researchers who took the samples from the Argentine population are not entirely confident in their differentiation between the two types. While this is unfortunate, it would be very precarious to forge ahead assuming all samples were properly assigned to their categories when researchers have expressed some doubt. Thus, skin and muscle samples are discussed together throughout the remainder of this thesis. While results of analyses are discussed, the main benefit of this particular portion of the study is intended to be the contribution of additional knowledge of laboratory methods for skin and muscle processing where the scientific record is lacking.

Hair

Hair, much like muscle, is composed of almost pure keratin protein. It is exceptional for tracking short-term dietary changes due to its constant generative state. Samples taken along the shaft of the hair's length correspond to diet prior to an individual's mortem, with the area nearest to the scalp yielding stable isotope values representative of the period closest to death.

Scalp hair typically grows from .8 to 1.4 cm per month, with an accepted rate of 1.1 cm per month, or .35 mm per day, considered standard. Lacking a naturally monotonous diet, the isotopic values of hair samples reflect variation within 12 days, with isotopic equilibrium attained in an extra two to five months. Jones et al. (1981) caution researchers that the use of whole strands for analysis can confuse results, as older values will mingle with newer ones. For these reasons, a 20 mm section is used regularly to analyze carbon and isotope values for two-month periods (Fenner 2002; White 1993).

Carbon Isotope Analysis

Little is known about the initial acceptance of maize into the human diet although it has long been recognized as a staple crop in South America. The importance of maize in relevance to other foods can be measured by analyzing a ratio of the content of two stable isotopes of carbon, ^{12}C and ^{13}C, in human bones (Fogel and Tuross 2003; Pearsall 1994; Schoeninger and DeNiro 1982; Schoeninger and Schurr 1994; Smith 1995; Sullivan and Krueger 1981); this ratio reflects the isotopic ratios of the foods eaten by an animal, and statements can be made concerning the implementation of increasing levels of maize into prehistoric diets, and subsequently, the history of agriculture.

Carbon occurs in three isotopic forms: ^{14}C, ^{13}C and ^{12}C. Carbon 14 is the carbon isotope used for radiocarbon dating; it decays (at a known rate) and is therefore known as radioactive. ^{13}C to ^{12}C are carbon's stable isotopes (van der Merwe 1982). The ratios of ^{12}C to ^{13}C vary among plant communities, atmospheres, and trophic levels. Mass spectrometric techniques can measure these ratios of carbon isotopes in a given plant, e.g., maize (Boutton 1991; Schwarcz and Schoeninger, 1991). It is this variation among carbon isotope contents in plants that allows researchers to assess the importance of specific food products in an individual's diet.

Although there are three photosynthetic types, most plants consumed by humans can be grouped into one of two terrestrial types: C_3 plants use the process known as Calvin-Benson, and convert carbon dioxide into a phosphoglycerate with three atoms during photosynthesis; and C_4 plants enable a photosynthetic pathway called Hatch-Slack, and convert carbon dioxide into dicarboxylic acid (Larsen 2000; Schoeninger and DeNiro 1982; Schoeninger and Schurr, 1994; van der Merwe 1982). An additional type, Crassulacean Acid Metabolism (CAM), occurs predominantly in non-terrestrial plants and is rarely consumed by humans, the exception of such being seaweed and cactus (Boutton 1991b). Carbon isotopes are strongly fractionated when plants metabolize carbon dioxide during these types of photosynthesis and plants consequently demonstrate either high proportions of ^{13}C or ^{12}C (DeNiro and Schoeninger 1983; Dupras 2001; Hoefs 1980). C_3 plants are typically found in moderate climates and include Old World domesticates such as barley, wheat, and rye, as well as fruits, tubers, and various shrubs and trees (Figure 3.3). C_4 and CAM plants such as maize, sorghum, millet, and other cereal grasses are often found in tropical areas. C_3 plants use the enzyme RuBP

carboxylase, which discriminates against $^{13}CO_2$, during photosynthesis and are thus known to have lower carbon stable isotope ratios than C_4 and CAM plants, that use the enzyme PEP carboxylase (Boutton 1991b). C_4 and CAM plants are more efficient at conserving water as their photosynthesis is performed at night, and thus thrive in increased temperatures and/or desertic environments (Smith and Epstein 1971; O'Leary 1988). The warm, arid climates encountered throughout South America have yielded exceptionally suitable environments for maize agriculture.

Stable carbon isotope ratios are reported as $\delta^{13}C$ values in ‰ (read, parts "per mil"), relative to an accepted reference standard, and are derived from the following formula:

$$\delta^{13}C = [\{(^{13}C/^{12}C)_{sample} / (^{13}C/^{12}C)_{PDB}\} - 1] \times 1000‰$$

The Pee Dee Belemnite (PDB) carbonate is the commonly agreed reference standard for $\delta^{13}C$ measurements; it is derived from a piece of Cretaceous marine fossil from the Pee Dee formation in South Carolina (Larsen 2000; Schoeninger 1985; van der Merwe 1982). The National Bureau of Standards now relates current standards as the original limestone has been completely expended. C_3 plants have lower $\delta^{13}C$ values (approximately –26‰) than C_4 plants (approximately –14‰). CAM plants range from –28 to –10‰, but most commonly yield values of –20 to –10‰. Schoeninger and Schurr (1994) postulate that a hunter-gatherer ("pre-maize") diet would be represented by a human bone collagen value of approximately –21‰, a 50 percent maize diet would be represented by a $\delta^{13}C$ value of -14‰ , and a $\delta^{13}C$ value of -7‰ would indicate a diet consisting only of maize (see Figure 3.3), although nutritionally unfeasible. It is also important to note that atmospheric CO_2 has decreased from deforestation and combustion of fossil fuels by

about 1.3 to 1.5‰ since the Industrial Revolution of the 1800s (Boutton 1999b; Friedl et al. 1986). Thus, individuals that lived prior to the industrial era will demonstrate slightly "enriched" values if evaluated by comparable modern day samples.

Nitrogen Isotope Analysis

The analysis of stable isotopes of nitrogen (^{14}N and ^{15}N) by mass spectrometry has proven particularly useful for comparing terrestrial versus marine-based diets, as nitrogen is another essential element for plants. While most terrestrial plants absorb nitrogen that is deposited in the soil by decaying vegetation, marine plants absorb their nitrogen directly from the air; this produces different ratios of stable nitrogen isotopes in each type of plant (Larsen 2000; Mulvaney 1993).

Nitrogen isotope ratios are reported as $\delta^{15}N$ values relative to the standard of atmospheric nitrogen (N_2) known as ambient inhalable reservoir (AIR), and are derived from the following formula:

$$\delta^{15}N = \{[(^{15}N/^{14}N)_{sample} - (^{15}N/^{14}N)_{AIR}] / (^{15}N/^{14}N)_{AIR}\} \times 1000‰$$

Marine plants are known to have higher stable nitrogen isotope ratios than terrestrial plants, by approximately 4‰ (Schwarcz and Schoeninger 1991). These values are passed through the trophic levels, with marine animals also expected to have higher $\delta^{15}N$ values than terrestrial animals due to the type of plants they consume (Schoeninger et al. 1983). Schoeninger et al. (1983) note that humans on marine-based diets display bone collagen values of approximately 17 to 20‰, while $\delta^{15}N$ values of 6 to 12‰ indicate terrestrial diets.

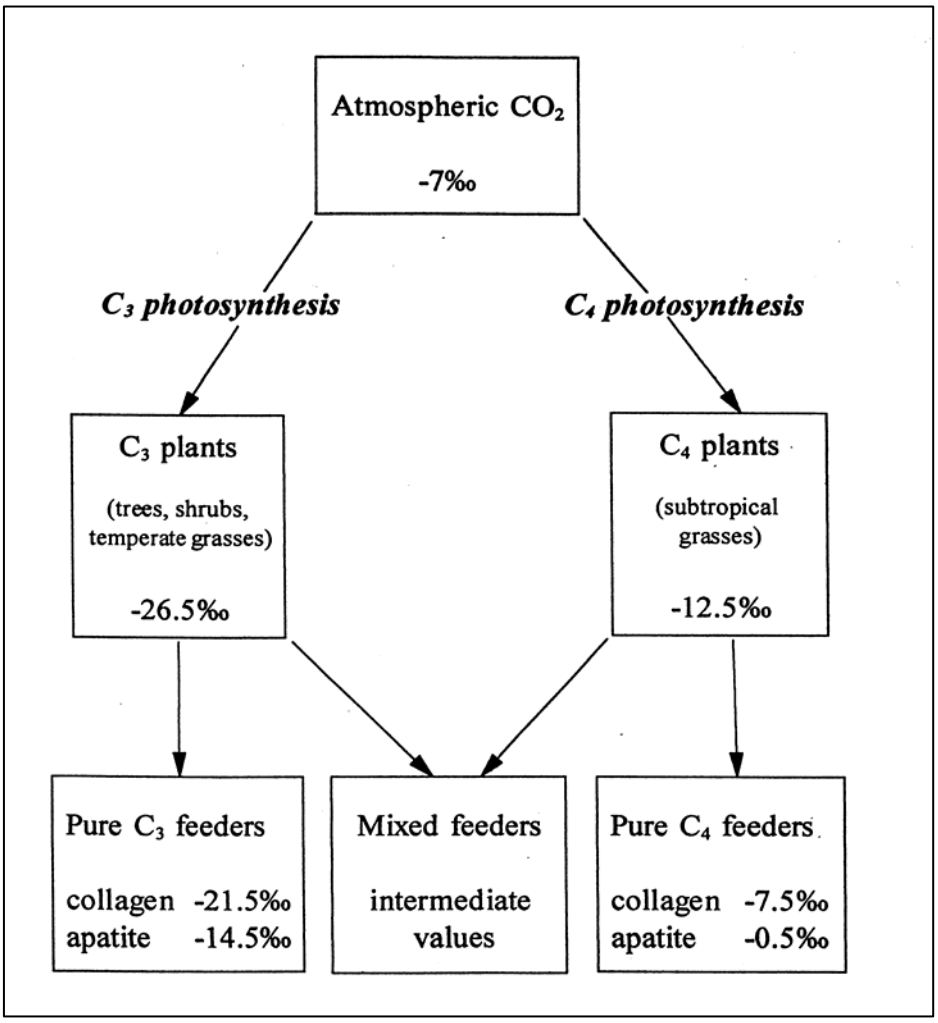

Figure 3.3. Carbon isotope fractionation in terrestrial plants. From Tykot (2004: 435.)

In prehistoric coastal settings, humans are expected to have high stable nitrogen isotope ratios due to a diet based on marine-resources (Mulvaney 1993; Schoeninger 1985). Low nitrogen ratios were expected in this study due to location of the sites in question. Although Argentina has historically been linked to Chile and Peru through exchange, the time period is too early to expect a high degree of marine resources in human diet.

Strontium Isotope Analysis

The three types of isotopic studies most commonly undertaken in archaeological analysis employ carbon, nitrogen, and strontium elements. Strontium isotope ratios ($^{87}Sr/^{86}Sr$) portray the local geology of an individual, by representing the strontium isotope levels of the soils of one's home range or catchment, that have been absorbed by the plants in the local ecology (Schwarcz and Schoeninger 1991).

Proportions of ^{87}Sr and ^{86}Sr can be valuable when studying migration patterns, as different strontium isotope levels in human tooth enamel may indicate a resettlement during the individual's lifetime, particularly if the individual were to move to and from heterogeneous geological areas (Ericson 1989; Schoeninger 1985). Analyzing tooth enamel, which is formed during various periods as a juvenile, from multiple permanent teeth of the same individual has the potential to identify resettlement to a detailed annual age (Ericson 1985).

Problems with strontium isotope analysis are outlined by Ericson (1989). They include: geological variability, home range definition, and home range characterization. The documentation of migration patterns requires that an individual move to and from noticeably heterogeneous areas and that they are not extremely mobile, so as not to render chronologies extremely perplexing. Due to the difficulties associated with this type of destructive analysis, samples were not used to analyze strontium isotope ratio variation; thus strontium studies that are typically used to characterize migration patterns will not be further discussed in this dietary analysis. Further studies may explore this topic if a high degree of relocation becomes suspected of the subject population.

Sources of Error

Isotopic ratios may be mistakenly interpreted if sources of error are not taken into account. It is thus important to note factors that should be taken into consideration, as well as research that demonstrates isotope ratios do not naturally vary by either sex or age. DeNiro and Schoeninger (1983) outline three key factors that are important to consider when reconstructing the diet of a population: (1) the isotopic composition of an individual does not significantly differ from the mean value of the remainder of the population; (2) isotopic ratios from different bones of the same individual are virtually analogous with variation of only about 1‰ between multiple bones; and (3) isotopic ratios of males and females fed the same diet are identical.

Additionally, Tieszen and Fagre (1993) and Schoeninger and Schurr (1994) advise of potential inconsistencies of $\delta^{13}C$ values when using multiple samples from a broad temporal range; these discrepancies can be caused by anthropogenic addition of CO_2 into the modern day atmosphere, environmental fluctuations, and the absorption of water. They recommend that floral remains found in association at a site are analyzed as well as the human samples themselves, whenever possible. All practical matters have been taken into consideration when comparing the $\delta^{13}C$ values of associated floral remains to those of biological materials. This study also analyzes floral remains, found in association with the above-mentioned individuals, to establish the extent of variation in isotopic levels.

Issues of interpretation can be further complicated if a lack of osteological materials limits sample size. Thus, when choosing individuals to be statistically representative of their respective populations, samples have been chosen that are

expected to be homogenous to their total group. Isotopic ratios have been tested from as many materials as possible to ensure equal value, and both males and females have been tested, when available, to evaluate whether they are feeding on the same diet with equal isotopic values, from the largest number of individuals possible. Seasonal variation in the southern hemisphere has been taken into account, with $\delta^{13}C$ values expected to be approximately six months out of phase with the northern hemisphere (Boutton 1991b).

Perhaps the most probable contaminants of stable isotope results are biological inclusions, such as another bone matrix, fatty oils commonly called lipids, and humics - residual components of decaying plant matter (White and Schwarcz 1994). Proper procedures for the removal of such matter are discussed in the following chapter, as well as the various methods that were used to process the samples.

Chapter Four: Stable Isotope Analysis Methods

The materials supplied by the Museo de Historia Natural, Argentina provide an excellent opportunity for stable isotope analysis as they were obtained from a desert environment and therefore exhibit high levels of preservation. The mummified soft tissue remains of the individuals in this study allow analysis of seasonal variation, migration patterns, and differing metabolic processes, with minimally destructive techniques (Fenner 2002; O'Connell and Hedges 1999a, b; O'Connell et al. 2001). Samples available include human bone, teeth, muscle and skin tissue, and hair. There has been little documented analysis of soft tissue materials and, while this research is not entirely experimental, it will add to scientific knowledge by allowing examination of areas that are currently underrepresented in the literature. Objectives for this study included defining a catalogue of prehistoric stable isotope values for the Argentine provinces of San Juan and Mendoza, documenting any dietary transitions including those from foraging to agriculture, and examining the possible existence of separate dietary practices in the two provinces.

Procedures were developed (Tykot 2002) to prepare samples for mass spectrometry and separate the complexes of materials described in Chapter Three. Proper execution of these techniques is necessary to ensure accurate results. Without such methods, isotopic samples could be affected by contaminants like residues of decomposed plants, called humates or humic acids, bacterial residues, or other similar biological complexes. For example, an intended sample of bone apatite would demonstrate inaccurate values if poorly preserved or if another biological material that portrays different dietary ratios is included in the final product for analysis. The

procedures outlined in this chapter are believed to be the best possible methods to remove such impurities and obtain accurate results.

Sample Preparation

The following pages detail the sample preparation that was performed at the USF Laboratory for Archaeological Science in the Fall 2003 and Spring 2004 semesters, where there exist appropriate facilities for stable isotope analysis and an experienced faculty member, Dr. Robert H. Tykot, knowledgeable of dietary studies in the study region. Separate methods were required for each of the biological complexes discussed in the previous chapter before samples were transported to the USF Marine Science Laboratory in St. Petersburg for mass spectrometry.

Bone apatite

Bone apatite is typically the first complex obtained, because the collagen preparation process is minimally destructive and removes the entire mineralized portion of bone. To begin, bones that had been previously treated for preservation required an extra step of processing. Those samples were soaked in acetone and rinsed with distilled water to eliminate any conservation chemicals. Once those materials were presumably removed, segments of bone weighing approximately 1 g were cut from all samples using a circular saw. These portions were then cleaned manually and ultrasonically to remove any loose particles of soil. Distilled water was always used in the cleaning process to prevent possible contamination of any additional organics. Whenever necessary,

ultrasonic cleaning was repeated until water ran clear. The clean bone was then dried a minimum of 6 hours (h) or overnight in a drying oven set at 60°C. Dried samples were digitally photographed and any abnormalities were recorded.

Next, approximately 10 mg of powder were drilled from the clean, dry bone samples. Bone powder was weighed on a Mettler Toledo AT261 DeltaRange scale and the initial weight was recorded on the USF Bone Apatite Sample Processing form (Appendix C) and in the Laboratory for Archaeological Science database that also assigns laboratory numbers to samples.

Bone powder was transferred into a 1.5 ml centrifuge tube labeled with the USF number and any additional material was stored in case further analysis becomes necessary. Extra portions of whole bone that were not drilled were stored in containers that were labeled with corresponding collagen analysis numbers, with the intent of minimal destruction. Then, 1 ml of 2 percent bleach solution was added to all apatite samples to remove collagen, bacterial proteins, and humates.

The bleach solution was removed with a pipette and replaced by distilled water after a 72 h soaking period. This rinsing procedure was repeated four times before removing the water and placing the bone powder in the drying oven for another six hour to overnight period. Once dry, samples were measured again and weights were recorded on apatite processing sheets.

Apatite samples were next treated with 1 ml of buffered acetic acid solution for 24 h. This process removes non-biogenic carbonates. Again, samples were centrifuged to pour off acetic acid and replaced with distilled water four times before placing in the drying oven. Finally, dry samples were removed from the oven, weighed to 1 mg

samples and transferred into containers for transport to the mass spectrometer lab. Any excess bone powder was saved, placed in a sterile container, labeled as processed apatite, and is stored in the USF lab.

Tooth enamel

The outer portion of a human tooth, called enamel, is composed of the same essential minerals as bone apatite and thus requires similar processing methods. Both complexes can be reduced to the general formula (Ambrose 1990): $Ca_5[PO_4]_3OH$. Enamel is much harder than apatite because it contains less organic material; in fact, it is the hardest substance in the human body. The only difference in laboratory procedures of tooth enamel (Appendix D) and bone apatite is that enamel powder does not need to remain in the bleach solution for as long a period due to the reduced portion of organics to be removed and any contaminants. After cautiously drilling (Figure 4.1) for enamel and avoiding any dentin, the powder is soaked for only 24 h to remove bacterial proteins and humates, rather than the 72 h required for apatite powder.

Bone collagen

Although collagen typically represents an approximate 20 - 25 percent of fresh bone weight and is relatively insoluble, preservation conditions can greatly affect the amount of collagen that remains in preserved bone. Thus, laboratory procedures most often emphasize both maximum potential yields of samples and the removal of all possible contaminants (White 1991). The most common impurities that might affect

Figure 4.1. Drilling for tooth enamel sample

collagen samples are other bone complexes (e.g., apatite), lipids, and humic inclusions. Collagen samples were prepared after first removing apatite samples through the procedure described above. Once apatite sampling was complete, the remaining 1 g of bone was processed to remove as many of these other materials as possible while still maintaining a relatively large portion of the collagen itself.

Laboratory procedures (Appendix E) began by soaking each sample in 50 ml of a solution of 0.1 M sodium hydroxide (NaOH) for a period of 24 h. This process removes humic acids before all samples are rinsing thoroughly in distilled water (Figure 4.2). Then, 50 ml of 2 percent 2 M hydrochloric acid (HCl) was added to remove the mineral portion of bone. This solution must be replaced every 24 h for at least three days to remove all remaining hydroxyapatite. When the bone was completely dimineralized, the HCl was removed and the bone was thoroughly rinsed. Another cycle of NaOH for 24 h was used to ensure the elimination of any residual humic inclusions.

Figure 4.2. Collagen samples and the chemical solutions used for processing

The fat content or lipids of the bone were removed with a defatting solution consisting of a mixture of 2 parts methanol, 1 part chloroform, and .8 parts of distilled water. This solution was allowed to soak for 24 h before being disposed of in special waste containers. Finally, the remaining collagen was rinsed extra thoroughly, cut into small portions, and transferred into two-dram vials labeled with indelible marker. Samples were dried overnight before collagen yields were calculated and 1 mg portions were weighed and transferred into aluminum containers for transport to the mass spectrometer lab. As with the apatite samples, any excess collagen was saved, placed in sterile containers, labeled as processed collagen, and is stored in the USF Laboratory for Archaeological Science.

Resource samples

Thirty-eight prehistoric samples of both floral and faunal resource species were analyzed as well as control samples (Figure 4.3), in an attempt to rule out the possibility of any abnormal carbon or nitrogen results that might affect human values irregularly. Table 2.2 of Chapter Two lists the types of samples that were analyzed to this effect. All of the species named have been recovered through archaeological investigation and are thought to have played strong roles in the diets of native Argentineans.

The bones of animals such as prehistoric armadillos, Andean geese, and guanacos were prepared in the same manner as human samples; both collagen and apatite samples were taken from these bones whenever possible. Thus, the procedures for non-human animal bones are the same as those listed in Appendices D and E that were originally developed for human bones. Botanical samples consisting of plants such as maize, winter squash, and the common bean were crushed into a powdered form using a mortar and pestle. All samples were then weighed into appropriate sample sizes and sent to the St. Petersburg laboratory.

Hair

Analysis of scalp hair and skin samples is relatively uncommon due to intrinsic preservation issues, with a few notable studies having been performed by researchers such as Schwarcz and White (2004) and Macko et al. (1999a). The preliminary study included in this thesis is meant to address issues of preparation and interpretation. Samples consist of eight individuals from a wide temporal span of 4070 to 590 BP, representing a number of cultural periods of the Argentine Cuyo (see Table 2.3).

Figure 4.3. Archaeological samples for stable isotope analysis. Clockwise from upper left: muscle tissue; winter squash; and human mandible with teeth *in situ* for tooth enamel, bone apatite and collagen analysis.

Admittedly, this short study requires much more work and many more samples from different time periods before any conclusions can be made. Although this portion of the study concentrates primarily on methods, motivating insights were provided by the work done thus far and will be analyzed in the following chapter.

Procedures to prepare hair samples (Appendix F) for mass spectrometry followed those of Fenner (2002), after O'Connell and Hedges (1999a). It is particularly important to wear either a hairnet or a ball cap when processing hair samples to prevent possible contamination by the modern-day researcher's own hair segments. Accordingly, proper laboratory gear was worn during all procedures. To begin, a hair sample of approximately 15 strands was selected from one individual. Locks of hair were carefully lined up and cut along the shaft into 2 cm samples representing two month growth

periods, or approximately 57 days, based on a growth rate of 0-35 mm per day (White 1993). It is noted that the time periods represented by hair samples are not "months" in a strict calendar sense (e.g., January through February); rather the term month is used to denote an approximate growth period of 28 days. While some individuals (Table 4.1) had as few as three samples available (representing six months), others had as many as eight (or 16 months).

Appropriate cleaning procedures were performed on the Argentine samples to minimize the presence of any possible contamination factors, including body fats, soil residues, or even scalp lice. Hair is almost entirely composed of the protein keratin, and thus requires very little processing to prepare. Some researchers have even chosen to simply clean samples ultrasonically and then move on to mass spectrometry. However, amino acids residing in endogenous lipids have the potential to affect isotopic values unpredictably (O'Connell and Hedges 1999a). Thus, it was decided prudent to employ a brief lipid extraction. This short procedure takes less than one day, plus drying time, and it may allow for greater reliability in the results although others, notably White (1991), have shown no differences in isotopic values. Nevertheless, hair is very valuable archaeologically and, because the process was largely unlikely to cause any unintended harm to the samples, the lipid extraction was carried out to remove any variables that could not be accounted for otherwise.

Each 2 cm was placed in a test tube with a sample identifier prior to execution of the lipid extraction. The test tubes were then filled with distilled water and samples were cleaned ultrasonically. Next, the water was exchanged for a 2:1 solution of methanol and chloroform, similar to the defatting solution used for collagen samples. Hair samples

Table 4.1. Number of hair samples per individual

Museo Sample #	USF Sample Series #s	Total # of Samples	Total # of "months" represented
SJ2	7105.1 - 7105.4	4	8
SJ3 – ENT3	7109.1 - 7109.5	5	10
SJ5 – ENT2	7145.1 - 7145.5	5	10
SJ6 – ENT8	7146.1 - 7146.5	5	10
SJ7 – ENT2	7148.1 - 7148.3	3	6
SJ8 – ENT5	7153.1 - 7153.5	5	8*
SJ9 – ENT1	7156.1 - 7156.8	8	16
SJ10 - ENT1	7157.1 - 7157.8	8	16

*Results not available for one sample due to a mechanical error in mass spec analysis

were sonicated in this solution for 15 min, before the solution was refreshed and allowed to sonicate for another 15 min. The samples were then rinsed with distilled water before being placed in a drying oven set to 60°C to dry for 48 h.

Afterward, hairs were examined microscopically to confirm cleanliness. Samples were ultimately weighed (Figure 4.4), folded into tin foil squares (Figure 4.5), and sent for mass spectrometric analysis. Unlike the bone collagen tests, where two samples are taken from one individual and both run to determine reliability, hair is unlikely to have as much trophic level variation within one area as any skeletal element, with only a 1-2‰ difference expected between $\delta^{13}C$ values of hair samples and dietary proteins (O'Connell and Hedges 1999a; White 1993). Also, there is often a limited amount of material, so it was deemed necessary to run only one sample for each period of two months. Results discussed in the subsequent chapter confirm that corresponding values were dependable.

Figure 4.4. A hair sample representing a two-month growth period

Figure 4.5. Hair samples are placed in tin foil squares

Skin and muscle

Stable isotope analysis of skin and muscle tissue analysis is relatively uncommon, not only due to preservation issues, but also because of difficulties including lack of established processing procedure; ambiguity in separating complexes as was discussed in Chapter Three; and complexity in interpreting results, which can be escalated again by uncertainty in skin or muscle sample type. This study used techniques based on the work of Schwarcz and Schoeninger (1991) to process soft tissue samples before mass spectrometric analysis.

Skin and muscle samples were first cleaned ultrasonically with distilled water, and examined macro and microscopically to determine their context. Please note that while the researchers who originally sampled the mummifed tissues believe they have appropriately differentiated between skin and muscle tissue samples, the depth of skin can be quite challenging to determine in mummified individuals and it is possible to confuse the two types when obtaining samples. Thus, all samples were photographed and assigned to categories of skin or muscle (Table 4.2) but, because it was not entirely clear what lipids and amino acids would be included in the tissues based on type, a short lipid extraction was performed on all samples. Tissues were soaked in a 2:1 methanol/chloroform defatting solution for 1 h, before being thoroughly rinsed, and placed in the drying oven overnight.

A homogenized sample was considered most desirable given the conditions of the samples. Different levels of skin and muscle depth represent various dietary periods because soft tissues constantly regenerate. Thus, it is very problematic to analyze diet if the exact depth of the tissues is not known. Additionally, there are many factors that may

Table 4.2. Skin and muscle sample types

Museum Sample #	USF Skin #	USF Muscle #
SJ1-ENT7	7103	-
SJ2	-	7107
SJ3-ENT3	-	7383
SJ4-ENT2	7141	-
SJ5-ENT2	7144	-
SJ6-ENT8	-	7147
SJ7-ENT2	-	7149
SJ8-ENT5	-	7154
SJ9-ENT1	7155	-
SJ10-ENT1	7158	-

further affect width, including differences in tissue growth, loss of external skin layers in burial, and natural decomposition. Therefore, tissue samples were ground into a fine powder before being analyzed.

The dried soft tissues proved to be very tough and fibrous, particularly after the lipid extraction was performed. Archaeologically valuable sample could have easily been lost if ground into the necessary powdered form with the typical mortar and pestle. To combat this, samples were frozen with liquid nitrogen. This converted the samples into very brittle, yet durable frozen states, allowing them to be ground quickly before being weighed and placed into tin cups for analysis. Finally, all samples were transported to USF Tampa Marine Sciences in St. Petersburg for mass spectrometric analysis.

Instrumentation

Collagen and other organic samples were processed using a CHN analyzer in combination with a Finnigan MAT stable isotope ratio mass spectrometer. The CHN analyzer switches between gases of carbon dioxide and nitrogen while samples are on the way to the mass spectrometer. This allows for samples to be mechanically transferred into the machine in ordered rows and efficiently processed. The mass spectrometer then combusts the samples and measures them in comparison to the gas standards discussed in Chapter Three. Proper operation of the machinery is necessary to ensure precision, and values of carbon to nitrogen are analyzed after processing to confirm integrity of results.

Apatite samples were processed using an automated Kiel III individual acid bath device connected to another Finnigan MAT mass spectrometer. The Kiel system sequentially drops 90°C phosphoric acid into each sample vial. The calcium carbonate in bone apatite reacts and becomes carbon dioxide, which is then measured in the mass spectrometer against the PDB standard. Results around 0.1‰ precision are typically given by both mass spectrometers (Tykot 2004).

Chapter Five: Results

This chapter explores the results of the stable isotope analyses. Very well preserved human and non-human animal remains ranging in age from approximately 4100 to 200 BP were provided by the Museo de Historia Natural. A total of 198 samples from 45 human individuals recovered from the provinces of San Juan and Mendoza were analyzed to examine dietary patterns in the prehistoric Argentine Cuyo. Materials consisted of bone collagen and apatite, tooth enamel, hair, and skin/muscle tissue. Human samples were analyzed, as well as 15 different species of potential food resources, such as llamas, rheas, maize, and squash, with numerous materials tested for all.

Archaeologically, the Cuyo is thought to represent a boundary between the hunter-gatherers of Patagonia and the sedentary populations of the Andes. While San Juan is known as an area of moderate agricultural production, Mendoza is thought to have been one of the last South American culture areas to adopt maize agriculture, probably around 2000 BP (Gil 2003). This chapter addresses the questions previously stated in Chapter Two by attempting to document whether an agricultural transition occurred, when it may have happened, and whether there were dietary differences between the two provinces in prehistoric times.

Quantitative analysis was performed using SPSS 13.0 software for Windows at the USF Laboratory for Archaeological Science. Graphical elements, such as tables and charts, were composed using both SPSS 13.0 and Microsoft Office Excel 2003. All mathematical operations were run at least twice to protect against any computational error. Complete results of the stable isotope analyses are provided in Appendix B.

Resource Sample Results

It is important to consider the results obtained from the dietary resource samples that were analyzed before examining the values provided by the analysis of human samples. While the main focus of this thesis is human values, a database of resource values is also being built. Stable isotope analysis operates on the basic principal that varying isotopic values of human samples reflect different dietary patterns. However, this is complicated by the effects of trophic level: for example, a human who eats animals that have high $\delta^{13}C$ and/or $\delta^{15}N$ values, will have higher isotope values than a human who only consumes animals that have low isotope values. Thus, isotopic values could be misinterpreted if high $\delta^{13}C$ and $\delta^{15}N$ values of dietary resources are not taken into account before further analysis.

Prehistoric samples of both floral and faunal resource species were analyzed in an attempt to rule out the possibility of any abnormal carbon or nitrogen ratios that might affect human values. The results of these analyses are reported in Table 5.1 and illustrated in Figure 5.1. When this information is compared to the expected values given in Figure 3.3, it is shown that the very low $\delta^{13}C$ values of floral samples tested is consistent with standard values for C_3 plants. These results suggest that all wild herbivorous animal diets were based only on C_3 plants. Thus, it is assumed that the dietary resources obtained from the Argentine Cuyo compose a reasonably valid dataset, without abnormal resource values affecting human values irregularly. Moreover, where two or more samples from the same species have been analyzed, they have resulted in similar values, particularly for $\delta^{13}C$.

The majority of species variation is seen in $\delta^{15}N$ values. This variation could

Table 5.1. Results of analysis of floral and faunal resource samples

Museum Sample #	USF Lab #	$\delta^{13}C$	$\delta^{15}N$	Species	Material
R-A-1	5905	-10.7		*Lama guanicoe*	Apatite
R-A-1	6170	-19.0	4.3	*Lama guanicoe*	Collagen
R-A-1	7368	-14.2	9.0	*Lama guanicoe*	Collagen
R-A-2	5906	-6.8		*Lama guanicoe*	Apatite
R-A-2	6171	-14.7	5.0	*Lama guanicoe*	Collagen
R-A-2	7369	-18.3	6.7	*Rhea americana*	Collagen
R-A-3	5907	-11.1		*Lama guanicoe*	Apatite
R-A-3	6172	-19.4	4.6	*Lama guanicoe*	Collagen
R-A-3	7370	-18.1	5.6	*Lama guanicoe*	Collagen
R-A-4	6173	-9.1		*Lama guanicoe*	Enamel
R-A-5	5908	-11.5		*Cholephaga melanoptera*	Apatite
R-A-5	6174	-22.0	4.1	*Cholephaga melanoptera*	Collagen
R-A-6	5909	-11.8		*Rhea americana*	Apatite
R-A-6	6175	-20.0	5.7	*Rhea americana*	Collagen
R-A-7	5910	-12.1		*Pterocnemia pennata*	Apatite
R-A-7	6176	-20.6	4.6	*Pterocnemia pennata*	Collagen
R-A-8	5911	-9.1		*Lagidium viscacia*	Apatite
R-A-8	6177	-19.3	3.7	*Lagidium viscacia*	Collagen
R-A-9	5912	-11.1		*Chaetophractus villosus*	Apatite
R-A-9	6178	-17.7	5.6	*Chaetophractus villosus*	Collagen
R-A-10	5913	-8.9		*Lama guanicoe*	Apatite
R-A-10	6179	-18.8	4.3	*Lama guanicoe*	Collagen
R-A-11	5914	-11.5		*Pterocnemia pennata*	Apatite
R-A-11	6180	-21.0	4.9	*Pterocnemia pennata*	Collagen
R-V-1	6181	-9.7	3.4	*Zea mays*	Plant
R-V-2	6182	-9.6	3.9	*Zea mays*	Plant
R-V-3	6183	-23.2	13.1	*Cucurbita maxima*	Plant
R-V-4	6184	-25.4		*Lagenaria sp.*	Plant
R-V-5	6185	-27.6	6.9	*Chenopodium sp.*	Plant
R-V-6	7376	-24.3	7.0	*Cucurbita maxima*	Plant
R-V-6	6186	-23.9		*Prosopis sp.*	Plant
R-V-7	6187	-25.4	1.6	*Cassia arnottiana*	Plant
R-V-8	6188	-24.0	5.5	*Phaseolus vulgaris*	Plant
R-V-9	7379	-24.2	9.8	*Cucurbita maxima*	Plant
R-V-9	6189	-20.2	14.0	*Geoffroea decorticans*	Plant
R-V-10	6190	-20.8		*Geoffroea decorticans*	Plant
R-V-11	6191	-24.9	11.6	*Prosopis sp.*	Plant
R-V-13	6193	-24.4	1.6	*Schinus polygamus*	Plant

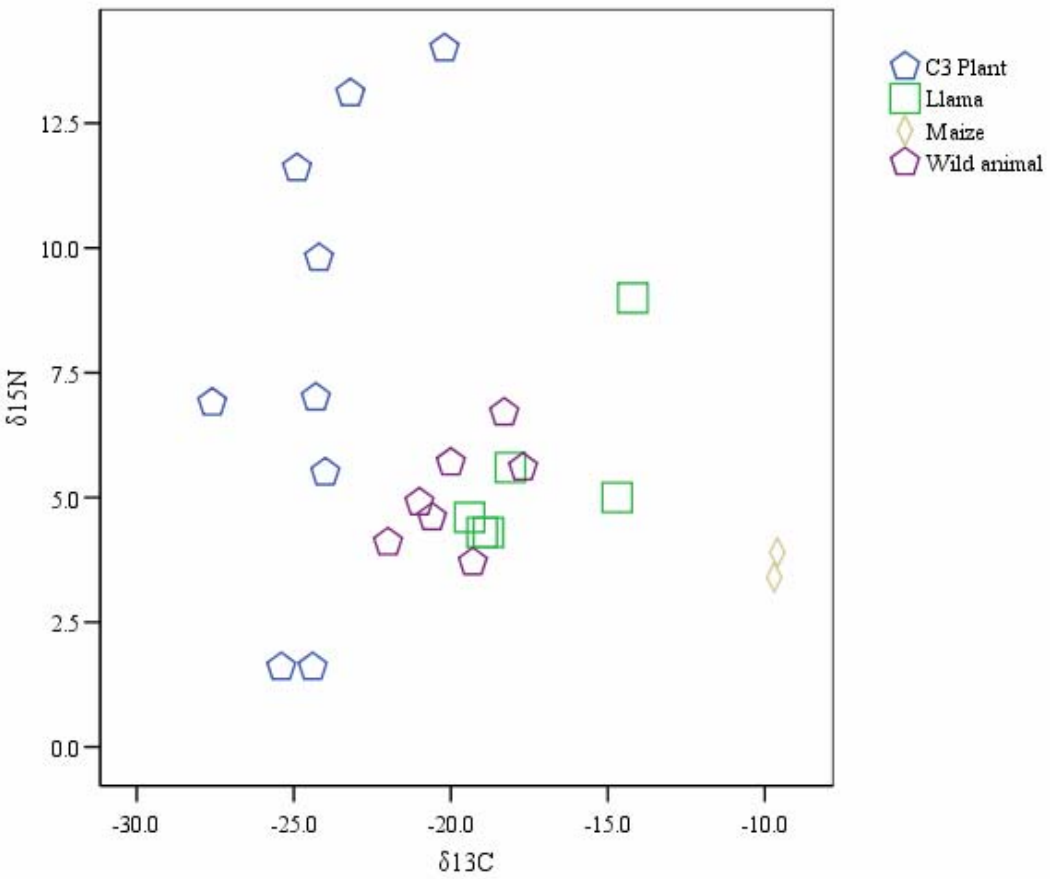

Figure 5.1. δ13C vs δ15N values for resource samples

reflect the high elevation of the area, as nitrogen varies naturally by altitude, rather than just diet (DeNiro and Schoeninger 1983). Although this is not an issue for many stable isotope studies, the mountainous terrain of the Andes may have affected these values.

The domesticated llama, however, must have consumed a significant amount of C_4 plants, presumably cultivated maize, given their relatively high $\delta^{13}C$ values. This is in keeping with hypotheses by Gil (2003) and Pearsall (1992) that humans used maize to feed their livestock, potentially before consuming the maize themselves, and should be kept in mind when investigating the isotopic values that are provided by the human

samples of the Argentine Cuyo. Thus, high $\delta^{13}C$ values in human samples may be an indicator of either a diet based directly on maize, secondary consumption of maize by eating animals who consumed maize, or a combination thereof.

Human Hard Tissue Sample Results

Next, the collagen, apatite, and tooth enamel results of all human samples from both provinces are examined. Stable isotope results from bone represent the last seven to 10 years of an individual's life, whereas tooth samples represent diet at the time of tooth formation, which varies depending upon the dentition type (Mays 1995; White 1993, 2000). Most (n=36) bone samples yielded both collagen and apatite results, with two producing only apatite values (Table 5.2). This is most likely due to the more resilient nature of bone apatite, as discussed in Chapter Three. Tooth enamel testing was highly successful, with all processed samples (n=23) yielding isotope carbon results.

Bone collagen

Bone collagen is formed mostly from the protein that is consumed as part of an individual's diet (Ambrose and Norr 1993). Therefore, collagen values largely represent changes in protein diet, of animals and/or plants. Collagen samples do, however, produce nitrogen values, whereas apatite and tooth enamel samples do not. These values can be useful in contrasting terrestrial versus marine and freshwater fish based diets, as well as trophic level.

First, all samples were plotted by $\delta^{13}C$ versus $\delta^{15}N$ values (Figure 5.2) with $\delta^{13}C$ values for the entire dataset ranging from –18.8 to –12.3‰. It is evident that San Juan

Table 5.2. $\delta^{15}N$ and $\delta^{13}C$ results from human samples

		δ15N	δ13C		
District	Individual	Collagen	Collagen	Apatite	Enamel
Mendoza	12	11.4	-15.9	-11.3	
Mendoza	2038	6.4	-18.8	-11.2	
Mendoza	AF-1082	12.9	-16.5	-11.3	
Mendoza	AF-1083	9.5	-16.1	-10.6	-9.8
Mendoza	AF-13894	9.8	-15.0	-10.1	
Mendoza	AF-2000	8.9	-14.8	-7.4	-5.5
Mendoza	AF-2018	11.5	-14.3	-9.8	-9.0
Mendoza	AF-2019	10.4	-14.5	-10.1	-8.8
Mendoza	AF-2020	11.3	-14.3	-9.5	-8.7
Mendoza	AF-2021				-6.1
Mendoza	AF-2022	10.5	-18.5		
Mendoza	AF-2025	9.5	-15.5	-8.2	-10.8
Mendoza	AF-2036	9.7	-17.5	-10.1	-10.8
Mendoza	AF-2072		-13.7	-7.6	-6.2
Mendoza	AF-500	10.3	-13.5	-8.0	
Mendoza	AF-503	9.4	-13.8	-7.8	-9.9
Mendoza	AF-505	11.9	-16.0	-10.1	
Mendoza	AF-508	10.8	-17.9	-12.1	
Mendoza	AF-510	10.9	-17.9	-13.0	-12.7
Mendoza	AF-673	10.2	-17.2	-12.5	-12.8
Mendoza	AF-681	8.7	-15.6	-10.2	-10.7
Mendoza	AF-8	11.7	-17.6		
Mendoza	AF-828	9.8		-7.6	
Mendoza	AF-830			-12.0	
Mendoza	CS-10001	11.6	-15.7	-9.0	
Mendoza	ENT-2	11.7	-14.9	-10.6	-9.6
Mendoza	ENT-3	7.9	-16.2	-9.2	-10.2
Mendoza	GIRA-27			-11.9	
Mendoza	GIRA-70			-9.8	
Mendoza	GIRA-71	10.8	-14.0		
Mendoza	GIRA-831			-10.5	
Mendoza	JP/J4	9.8	-17.4	-13.5	-13.5
Mendoza	JP-1155	10.6	-16.8	-10.2	-8.6
Mendoza	JP-1352	9.9	-16.3	-10.6	-11.2
Mendoza	MGA-1	10.9	-14.2	-8.9	-8.7
San Juan	SJ10-ENT1	9.9	-12.3	-8.2	
San Juan	SJ1-ENT7	9.7	-17.3	-13.1	
San Juan	SJ2	9.5	-13.8	-10.1	-6.3
San Juan	SJ3-ENT3	9.5	-13.3	-9.8	-9.0
San Juan	SJ4-ENT2	10.1	-13.8	-10.3	
San Juan	SJ5-ENT2				-8.3
San Juan	SJ6-ENT8	8.1	-17.3	-14.0	
San Juan	SJ7-ENT2	10.8	-15.3	-12.2	-9.2

values tend to cluster towards the more positive side of the figure than those of the entire Mendoza group which are more widely dispersed. Recall that values of –21.5‰ would indicate a diet that is entirely based on C_3 plants. This suggests that San Juan diets more commonly included some maize, or animals that consumed maize, than those of Mendoza, which show a greater range of values.

$\delta^{15}N$ values for the entire dataset range from 6.4 to 12.9‰. These values are within the terrestrial diet range suggested by Schoeninger et al. (1983), and the resource samples previously discussed. They also correspond with the low $\delta^{15}N$ values that were expected in this study, since the study area is blocked from access to the nearest ocean by the South American Andes. However, a weakness of the present study is the lack of analysis of any freshwater fish samples. Given the proximity of many sites to the mountain fed rivers that run throughout western Argentina (Compagnucci and Vargas 1998), it is not unreasonable to think that some freshwater fish may have been consumed throughout the year. Future analyses of aquatic resource samples may lead to greater understanding of the $\delta^{15}N$ values obtained from this project.

Although human $\delta^{15}N$ values are not high, they do tend to vary; previously, aquatic resources were not expected to have been of great importance throughout the desert environment of the Cuyo. These findings suggest it may be useful to reevaluate the relative importance of aquatic resources in sites throughout the study area. Perhaps aquatic resources were of greater importance to some prehistoric residents of Mendoza and San Juan than previously thought. This study did not test any samples of freshwater fish, although it seems that this type of analysis would be useful in the future.

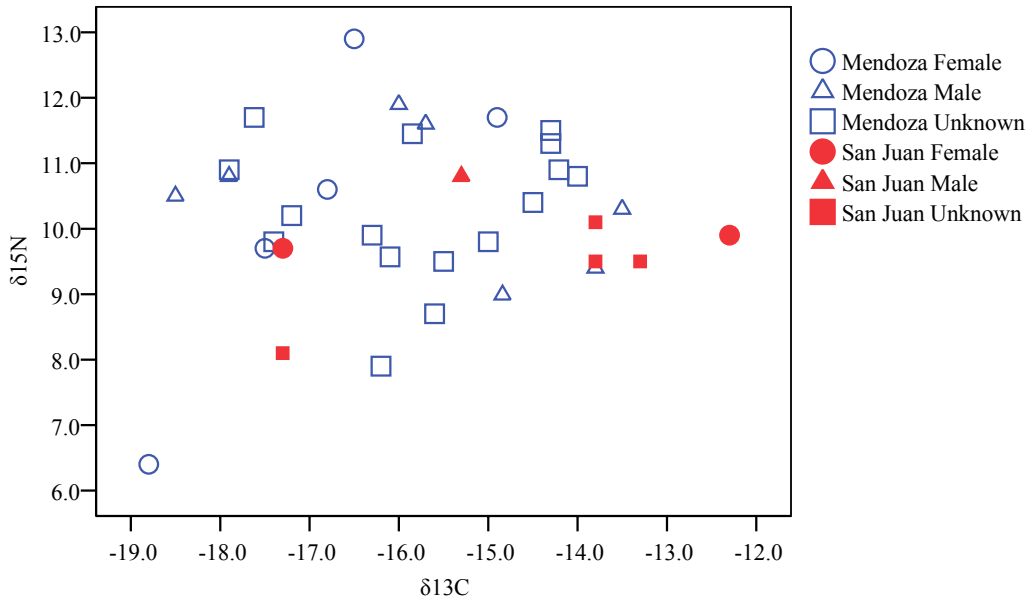

Figure 5.2. δ13C vs. δ15N values for human bone collagen samples

Bone apatite

Bone apatite is much less susceptible to deterioration than bone collagen, and is also useful for evaluating human diets as a whole, rather than just the protein components that are addressed by collagen samples. A disadvantage involved when sampling this type of material is that it does not allow for $\delta^{15}N$ studies. Thus, $\delta^{15}N$ values from collagen samples have been plotted against $\delta^{13}C$ values obtained from apatite in Figure 5.3 to allow for graphical representation and compare protein versus whole diet.

$\delta^{13}C$ results from apatite samples ranged from –14.0 to –7.4‰. On first glance, the more positive apatite $\delta^{13}C$ values may indicate subsistence patterns increasingly based on maize. Because apatite samples reflect the total diet, it should be noted that the values given should equally represent the consumption of both animals that ate maize, as well as

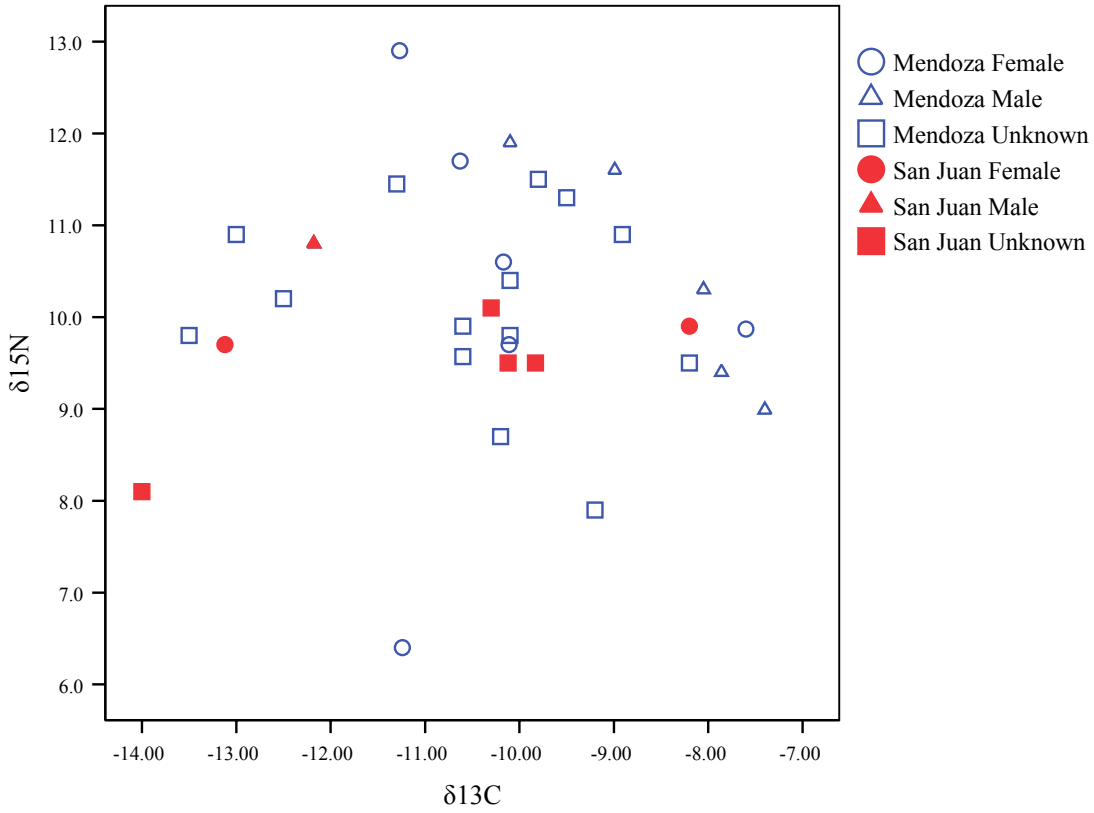

Figure 5.3. δ13C of bone apatite vs. δ15N values of bone collagen samples

direct intake of maize by humans themselves. However, ranges of $\delta^{13}C$ values for both apatite and collagen samples were similar (6.6‰ and 6.5‰, respectively). The similarity of the ranges for both apatite and collagen suggests plant foods may have dominated dietary intake.

Tooth enamel

Tooth enamel is very similar to apatite in that it is often well preserved in the archaeological record and composed of similar material. Stable isotope analysis of both tooth enamel and bone apatite yields only $\delta^{13}C$ values, but teeth reflect the diet of an

individual at the time of tooth formation, rather than the last seven to 10 years of an individual's life.

Because the time period represented varies by tooth type, researchers often choose to sample only one type of tooth from their entire population, typically the third molar (M_3). However, issues with availability of materials, such as the case of juveniles whose M_3s have not yet erupted, sometimes make this unachievable. Such was the case for this study, as the sample population was selected from a museum collection that possessed mixed materials from various individuals; nevertheless, whenever possible, an M_3 was used.

A benefit of using tooth enamel to evaluate diet is the ability to contrast juvenile, or even pre-natal, diets with those of an adult (Dupras 2001). This could not be precisely executed to the extent of an individual's lifetime with the existing materials, but comparing the two material types allows for a generalized picture of childhood versus adult dietary patterns. The boxplot in Figure 5.4 shows the $\delta^{13}C$ values of apatite samples next to those of tooth enamel samples. A boxplot is a graphical summary of the values of a group of numbers. The upper and lower portions of the box represent the upper and lower quartiles of a variable, while the horizontal line dividing the box represents the median of the sample. The vertical lines at the top and bottom extend to the minimum and maximum data points of the sample (Landau and Everett 2004).

Tooth enamel samples yielded more positive results than respective bone apatite samples for both provinces. In particular, the individual, SJ2 (see Table 5.2), demonstrated $\delta^{13}C$ enamel values that were 3.8‰ more positive than their corresponding bone apatite values. These results may indicate that diet in the earlier part of a prehistoric

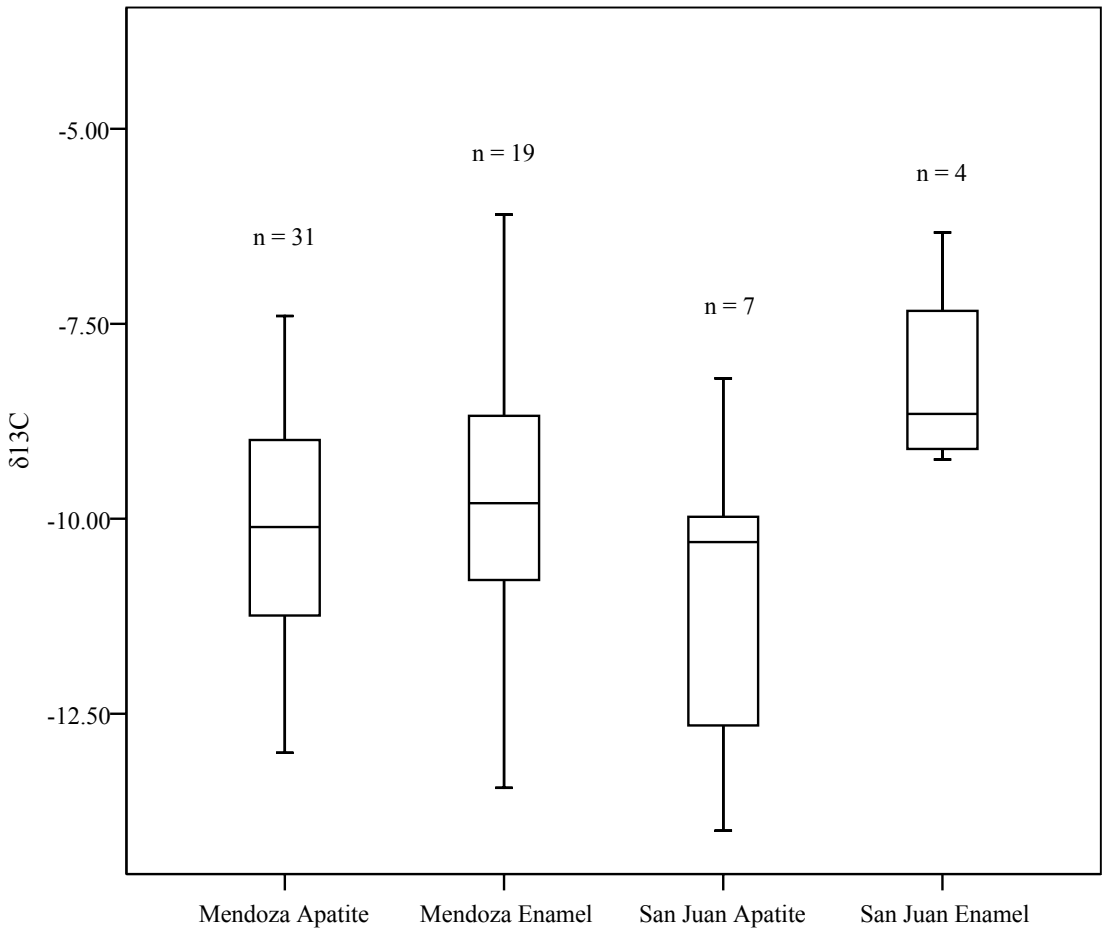

Figure 5.4. δ13C values of apatite and tooth enamel samples

Argentinean's life included more maize than that in adulthood. Furthermore, the means of the San Juan apatite and tooth enamel datasets are different, and the difference is statistically significant at the .05 level (t = -2.503; df = 9; $t_{critical}$ = 2.262; p = .034). The means of the Mendoza apatite and tooth enamel data also vary, but the difference is not statistically significant at the .05 level (t = -.748; df = 47; $t_{critical}$ = 2.012; p = .458). This suggests that, while San Juan individuals consumed different diets during their juvenile and adult lives, Mendoza individuals did not.

Individuals AF-2025, AF-503, and ENT-3 (see Table 5.2) expressed unusual $\delta^{13}C$ apatite versus $\delta^{13}C$ enamel values. Typical tooth enamel values are slightly more positive than those of bone apatite, as trophic level has a significant effect on those teeth formed prior to weaning. However these three individuals possessed more negative $\delta^{13}C$ enamel values. The lower values suggest far less intake of C_4 resources at the time of enamel formation. These individuals might be affecting the means of the Mendoza dataset. The disparity in values of these individuals might have been caused by differences in juvenile versus adult diets, changing dietary patterns as adults, or perhaps even migration to an agricultural area after the age of tooth formation (Tykot 2004). Unfortunately, the sample size, particularly for San Juan tooth enamel (n=4), was small so further analysis is certainly warranted.

Human Soft Tissue Sample Results

One major benefit offered by this study was the opportunity to learn more about the use of soft tissue materials for isotopic analysis. Unlike the hard tissue materials previously discussed, soft tissues have a more rapid turnover rate. Their almost constant regeneration allows a more thorough reconstruction of diet in the Argentine Cuyo.

This thesis seeks to track dietary transitions over a long time span, as well as short, seasonal changes using human scalp hair samples. A project performed in the fall of 2004 focused on the use of sequential segments of scalp hair samples from naturally mummified individuals from prehistoric San Juan to examine the possibility of detecting short-term dietary changes. This analysis allows one to examine the ways that humans

have modified their environments, by utilizing a foreign crop, and the ways these changes in the natural ecology might have, in turn, affected those populations.

Table 2.1 (see page 28) displays some of the cultural changes that are thought to have taken place in the province of San Juan. This chronology was constructed using both ceramic and faunal analyses (Gil, personal communication, 2004). Over time, populations are thought to have transitioned from hunting and gathering, to farming, and eventually to agropastoralism before being incorporated into the Inca Empire. The hair samples come from eight individuals representing a wide temporal span from 4070 to 590 BP, and therefore a number of cultural periods of the Cuyo. Although the project concentrated primarily on laboratory methods, the analysis also provided some interesting results.

Hair samples

Table 5.3 displays the $\delta^{15}N$ values obtained from the stable isotope analysis of hair samples. The first column shows the number of sequential samples, with each segment, e.g., Hair 1, representing a two-month period. Accounting for precision of mass spectrometric analysis, nearly all individuals demonstrate short-term dietary variation. It should be noted that less than one year's worth of hair samples exists for many of the individuals (e.g., SJ9); thus all seasonal variation could not be included in this analysis. Cultural factors, such as a spring growing season or summer harvest, might not be represented. Individuals are arranged in an increasing chronological order from left to right. Nitrogen values, which may indicate quantity of aquatic resources or mobility in altitude (Mulvaney 1993; Schoeninger et al. 1985), remain fairly consistent throughout

Table 5.3. $\delta^{15}N$ values for hair segments

	SJ7-ENT2 4070 BP	SJ6-ENT8 2000 BP	SJ8-ENT5 2000 BP	SJ9-ENT1 1300 BP	SJ5-ENT2 880 BP	SJ2 800 BP	SJ10-ENT1 600 BP	SJ3-ENT3 590 BP
Hair 1	10.2	9.0	9.0	10.0	10.3	10.6	9.6	9.2
Hair 2	9.7	9.5	*	9.0	10.3	10.2	9.0	9.7
Hair 3	9.4	9.6	7.5	9.2	8.7	10.4	8.7	9.7
Hair 4		9.3	7.8	9.6	9.1	9.6	8.6	9.4
Hair 5		8.8	8.6	9.6	9.3		8.0	9.6
Hair 6				9.5			8.7	
Hair 7				9.1			9.4	
Hair 8				9.5			9.6	
Mean	9.7	9.2	8.2	9.5	9.6	10.2	8.9	9.5
Range	0.8	0.8	1.6	1.0	1.6	0.9	1.6	0.5
SD	0.4	0.3	0.7	0.3	0.7	0.4	0.6	0.2

*Results not available due to a mechanical error in mass spectrometric analysis

all time periods analyzed. This is unsurprising due to the distance of the nearest ocean (across the Andes). This information mimics that obtained from the bone collagen samples, with an accepted standard offset of approximately 1‰ between collagen samples of hair and bone (O'Connell et al. 2001), due to the recycling of other body tissues into hair cells.

Student's *t*-tests (Table 5.4) comparing the standard deviations of $\delta^{15}N$ in the hair samples reveal that Individuals SJ8, SJ5, and SJ3 have higher variation of $\delta^{15}N$ than the analytically determined range for the dataset. Cultural factors may be viewed as a reason some members of the populations demonstrated higher variability than others. While many of these inland occupants are from sites very near to river systems, it is not thought that the prehistoric people of the area depended heavily on fish or shellfish for their diets, with the majority of associated faunal remains at sites consisting of terrestrial resources (Gil 1997; Gil et al. 2005).

Table 5.4. Student's *t*-test results for SD of $\delta^{15}N$ hair segments

Individual	Time[a]	SD[b]	t	p	CI_{lower}[c]	CI_{upper}
SJ7-ENT2	4070	.40	.73	.49	-.11	.21
SJ6-ENT8	2000	.30	2.20	.06	-.01	.31
SJ8-ENT5	2000	.70	-3.67	**.01**	-.41	-.09
SJ9-ENT1	1300	.30	2.20	.06	-.01	.31
SJ5-ENT2	880	.70	-3.67	**.01**	-.41	-.09
SJ2	800	.40	.73	.49	-.11	.21
SJ10-ENT1	600	.60	-2.20	.06	-.31	.01
SJ3-ENT3	590	.20	3.67	**.01**	.09	.41

[a]years BP
[b]Standard Deviation (SD)
[c]Confidence intervals (CI) represent 95 percent
Note: values in bold typeface are significant at the $\alpha \leq .05$

Next, Table 5.5 shows $\delta^{13}C$ results of the analyses. The mean values are given for all hair samples tested for each individual, but do not show an overall trend towards increasing amounts of maize in the human diet. Thus, the mean $\delta^{13}C$ values obtained from hair analysis are not in accordance with the hypothesis that groups increasingly adopted maize agricultural practices throughout the prehistoric period. However, an examination of the standard deviations of these values, shown in the bottom row, may clarify this discrepancy. Observe the markedly high standard deviations in individuals SJ8 and SJ9. Individual SJ8 is from ca. 2000 BP and associated with the period when the transition to agriculture is thought to have taken place. Individual SJ9 is from a period of another dietary transition, to agropastoralism. The high values, such as –14.2‰ for SJ8 and –16.5‰ for SJ9, could be outliers, but they may indicate actual dietary change.

One-way independent sample Student's *t*-tests (Table 5.6) of the standard deviations from the San Juan individuals' hair samples suggest that Individuals SJ7 and SJ6 do not demonstrate dietary variation as compared to the other individuals tested.

Table 5.5. $\delta^{13}C$ values for hair segments

	SJ7-ENT2 4070 BP	SJ6-ENT8 2000 BP	SJ8-ENT5 2000 BP	SJ9-ENT1 1300 BP	SJ5-ENT2 880 BP	SJ2 800 BP	SJ10-ENT1 600 BP	SJ3-ENT3 590 BP
Hair 1	-16.8	-19.4	-14.2	-10.7	-15.0	-14.9	-13.4	-15.3
Hair 2	-16.7	-20.1	*	-12.9	-15.2	-13.4	-12.7	-16.2
Hair 3	-17.3	-20.6	-19.4	-13.4	-15.5	-13.5	-13.6	-16.9
Hair 4		-20.8	-20.0	-16.5	-15.2	-14.4	-13.2	-16.6
Hair 5		-20.2	-19.8	-10.6	-17.1		-13.9	-15.6
Hair 6				-11.0			-15.1	
Hair 7				-10.3			-16.6	
Hair 8				-10.0			-15.6	
Mean	-16.9	-20.2	-18.4	-11.9	-15.6	-14.1	-14.3	-16.1
Range	0.6	1.4	5.8	6.5	2.1	1.5	3.9	1.6
SD	0.3	0.6	2.8	2.2	0.9	0.7	1.4	0.7

*Results not available due to a mechanical error in mass spectrometric analysis

However, Individuals SJ8 and SJ9 demonstrate greater standard deviations than the established range of variation for this dataset. Thus, it could be argued that heightened dietary variability began around 2000 BP and decreased after 1300 BP.

Furthermore, it seems there was variability within diets of the San Juan population during the time period of 2000 BP. Individuals SJ6 and SJ8 are both from the same time period and, while the results of the Student's-*t* indicate high variability for Individual SJ8, Individual SJ6 does not show as much dietary variation. This difference might be attributed to cultural factors; again, 2000 BP has been suggested as the time at which agriculture began in San Juan (Gil 2003). Perhaps some individuals were still eating highly varied diets, while others began to increasingly consume maize products at the expense of other foods. This information may indicate that the times before, during, or after a dietary a transition were particularly inconsistent, with seasonal patterns eventually steadying out over long periods.

Table 5.6. Student's *t*-test results for SD of $\delta^{13}C$ hair segments

Individual	Time[a]	SD[b]	t	p	CI_{lower}[c]	CI_{upper}
SJ7-ENT2	4070	.30	2.91	**.02**	.17	1.63
SJ6-ENT8	2000	.60	1.94	.09	-.13	1.33
SJ8-ENT5	2000	2.80	-5.17	**.00**	-2.33	-.87
SJ9-ENT1	1300	2.20	-3.23	**.01**	-1.73	-.27
SJ5-ENT2	880	.90	.97	.37	-.43	1.03
SJ2	800	.70	1.62	.15	-.23	1.23
SJ10-ENT1	600	1.40	-.65	.54	-.93	.53
SJ3-ENT3	590	.70	1.62	.15	-.23	1.23

[a]years BP
[b]Standard Deviation (SD)
[c]Confidence intervals (CI) represent 95 percent
Note: values in bold typeface are significant at the $\alpha \leq .05$

The information given here suggests that diets during times of transition were more irregular, based upon the Student's *t*-test of the standard deviations of sequential hair samples. These results can be explained in a variety of ways. For example, some researchers (e.g., Price and Gebauer 1995) argue that populations consciously began to produce food in satisfactory ecological systems as a preemptive way of dissolving potential risks of dietary stress in future times of need.

Alternatively, authors such as Gilbert (1985) and Keene (1985) suggest that environmental or nutritional deficiencies necessitated the adoption of alternative means of food acquisition. Some anthropologists (e.g., Larsen 1995, 2000; Smith et al. 1984) point to detrimental changes in health that correspond to the adoption of maize agriculture, such as anemia and osteoporosis. It has even been noted (Steckel et al. 2002a) that agricultural societies were the first to fall victim to the health epidemics brought on by the invasion of the Spanish. This might have been due to weakened immune systems brought on by inadequate diets, thereby making groups such as the Inca

easy prey for European disease vectors. Further, some members of the Argentine study population demonstrated existence of nutritional stress indicators, such as dental caries, that can be viewed in relation to the dietary shifts that may have been a cultural adaptation to the environment of the Cuyo.

Admittedly, the small number of samples analyzed in this portion of the study requires much more work before any firm declaration can be made about dietary transitions and seasonal variation. It would be particularly worthwhile to analyze individuals from the periods surrounding 2000 and 1300 BP to document whether heightened variation actually occurred, and when, if at all, it began and eventually decreased. This method of analysis could address many of the current hypotheses regarding the *motivation* for the transition to agriculture. Osteological studies would be useful to examine the effects of dietary transitions and to document any physical evidence of malnutrition. Additional testing of soft tissue samples has the potential to shed light on these topics.

Skin/Muscle tissue

Sampling for skin and muscle tissues presented unique difficulties including uncertainty of tissue type, depth of tissue sample, and variable preservation levels. Nevertheless, procedures were developed to prepare samples as best as possible under the given circumstances; the primary benefit of this portion of the study was intended to be the assessment of laboratory procedures. Lipids were removed using a defatting solution and samples were frozen using liquid nitrogen to facilitate powdering and thus homogenization of various layers of soft tissue.

$\delta^{13}C$ values ranged from –20.3 to –11.6‰ (Table 5.7). Figure 5.5 displays a boxplot comparing $\delta^{13}C$ values for hair samples (left) to $\delta^{13}C$ values for skin samples (right); outliers represent cases ≥1.5 times the interquartile range. This plot shows that, with the exception of Individuals 1 and 4 for which there are no data for hair samples, the values for the hair and skin samples are generally congruent. The contrary cases are Individuals 5, 6, and 7, where the values for the skin samples are outside the range of those for the hair samples.

A greater range (11.0‰) was given by the $\delta^{15}N$ values, with a minimum value of 11.8‰ and a maximum value of 22.8‰ (Table 5.7). Figure 5.6 compares $\delta^{15}N$ values for hair samples (left) to $\delta^{15}N$ values for skin samples (right); outliers represent cases ≥1.5 times the interquartile range. This plot shows that, with the exception of Individuals 1 and 4 for which there are no data for hair samples, the values for the skin samples are far outside the range of those for the hair samples displayed in Table 5.3.

Short-term dietary variations could greatly affect flesh values, given their rapid regeneration, but the significance of this irregularity is not yet understood. The larger ranges of variation may indicate extremely frequent changes in diet, or they may be regarded as erroneous due to the issues discussed above and in further detail in Chapter Three. Thus, it is suggested that a much larger population size and further evaluation of laboratory procedures, to be able to clearly determine tissue types (e.g. skin or muscle), are necessary before any dietary inferences can be made on the basis of flesh analysis.

Table 5.7. Stable isotope analysis results of skin/muscle tissues

Museum Sample #	USF Lab #	Years BP	$\delta^{13}C$	$\delta^{15}N$	Site
SJ1-ENT7	7103	7900-4200	-16.4	11.8	Gruta Morrillos
SJ2	7107	800	-14.4	13.5	Calingasta
SJ3-ENT3	7383	590	-15.8	13.3	Punta del Barro
SJ4-ENT2	7141	640	-15.3	13.1	Angualasto
SJ5-ENT2	7144	880	-17.2	12.7	C Calvario
SJ6-ENT8	7147	2000	-18.0	22.8	Gruta 1 Morrillos
SJ7-ENT2	7149	4070	-20.3	15.0	Gruta 1 Morrillos-Morrillos
SJ8-ENT5	7154	2000	-18.2	13.2	Gruta 1 Morrillos- Ansilta
SJ9-ENT1	7155	1400-1200	-11.6	12.3	Hilario
SJ10-ENT1	7158	600	-14.1	12.0	Angualasto
Mean			-16.1	14.0	
Range			8.7	11.0	
SD			2.5	3.2	

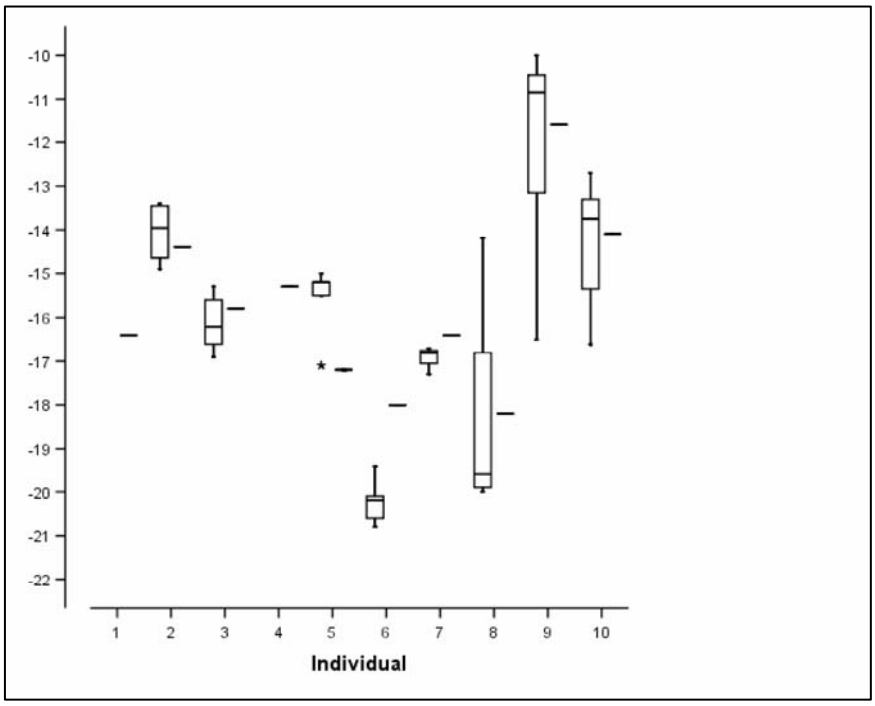

Figure 5.5. $\delta^{13}C$ values for hair (left) vs. skin (right) samples

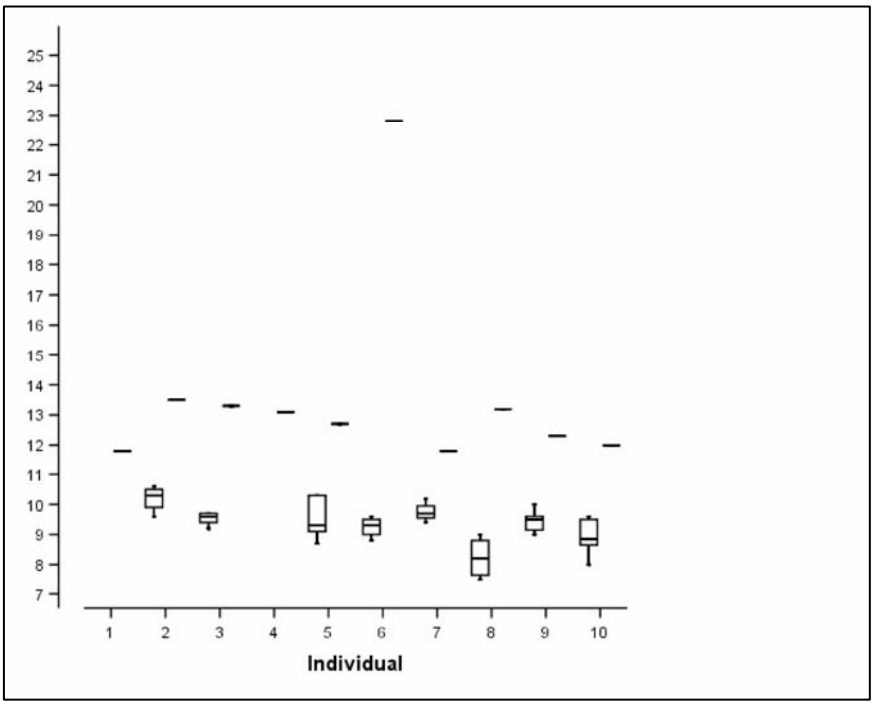

Figure 5.6. $\delta^{15}N$ values for hair (left) vs. skin (right) samples

Spatial Comparison

It has been previously suggested that the samples from San Juan and Mendoza represent two distinct populations that practiced different food procurement strategies throughout the majority of prehistory (Gil 2003). To test this hypothesis, inter-population relationships were examined in relation to space and time dimensions to determine whether the two groups separated dietary practices and how these customs may have changed over time. The results were divided by province (Table 5.8). Means of $\delta^{13}C$ values obtained from bone collagen (-14.7‰) and tooth enamel samples (-8.2‰) from San Juan were higher than those of Mendoza (-15.9 and -9.8‰, respectively). The average of bone apatite samples from Mendoza (-10.2‰) was slightly higher than that of San Juan (-11.1‰); however, the difference between the means is not statistically

Table 5.8. Comparison by province of human bone and tooth samples

	Mendoza			San Juan		
	N	Mean	SD	N	Mean	SD
Tissue*						
Bone Collagen						
δ13C	29	-15.9‰	1.5	7	-14.7‰	2.0
δ15N	29	10.3‰	1.3	7	9.7‰	0.8
Bone Apatite	31	-10.2‰	1.6	7	-11.1‰	2.0
Tooth Enamel	20	-9.8‰	2.2	4	-8.2‰	1.3

*All results given are for $\delta^{13}C$ values unless otherwise stated

significant at the .05 level (t = -1.1337; df = 36; $t_{critical}$ = 2.021; p = .190). The reasons for the similarities between the two groups are difficult to postulate given the relatively small sample size of San Juan samples. Additionally, this information includes samples from all analyzed periods, so temporal differences may be affecting values as well.

Temporal Comparison

Figure 5.7 displays $\delta^{13}C$ values of the three hard tissue types as they vary by time. San Juan and Mendoza individuals have been grouped together to allow for a larger sample size. Bone collagen and bone apatite samples, indicative of protein portions and whole diets respectively, seem to increase throughout the long period of 6050 to 50 BP; however, ANOVA shows that dietary change was not significant at the .05 level for either bone collagen (f = .656; df = 20,1; $f_{critical}$ = 248.013; p = .767) or bone apatite (f = -3.282; df = 21,1; $f_{critical}$ = 248.310; p = .128). Additionally, values of tooth enamel samples, which represent diet during time of tooth formation, seem to have remained fairly stable during all periods considered. A Student's t-test was used to

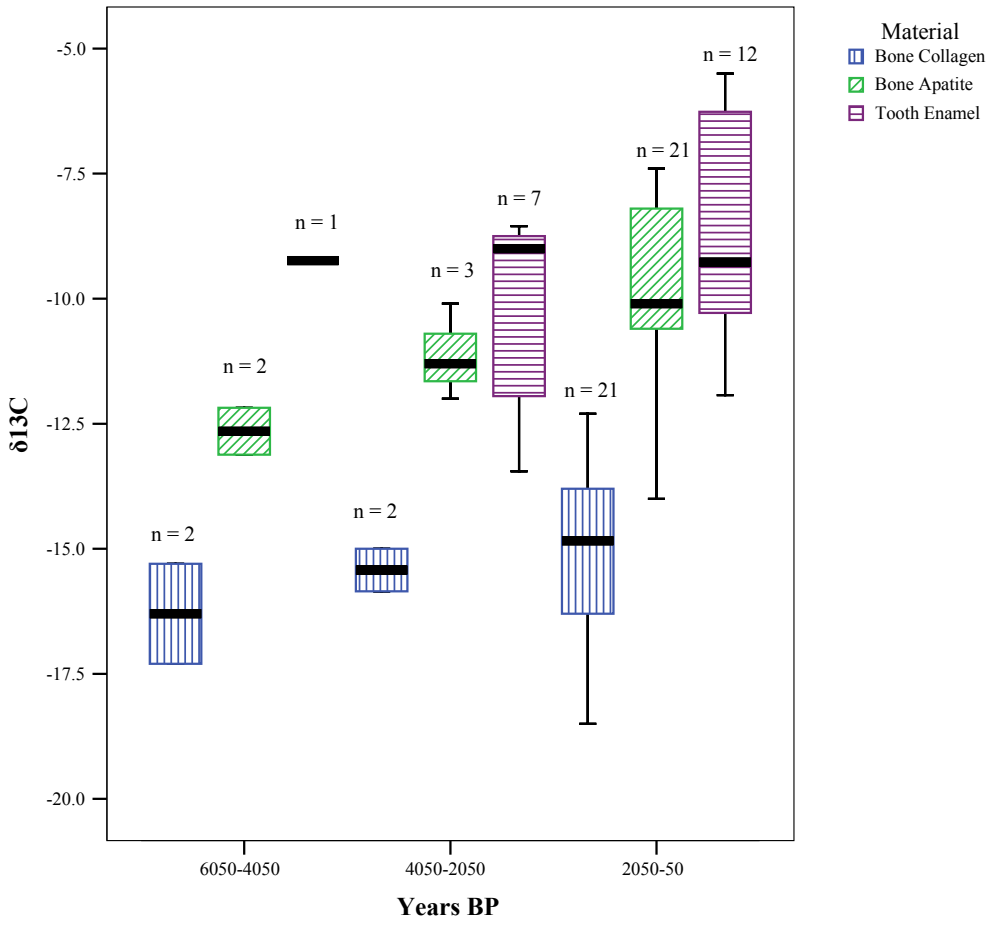

Figure 5.7. $\delta^{13}C$ results for both provinces vary by period

evaluate change between the two later time periods of 4050-2050 BP and 2050-50 BP, because the dataset for the time period of 6050-4050 consisted of only one tooth enamel result. The difference between the means of the two tooth enamel datasets is not significant at the .05 level (t = -1.644; df = 17; $t_{critical}$ = 1.740; p = .119).

At this time, dietary change does not seem to have been great enough to significantly affect $\delta^{13}C$ values. A criticism of this analysis is the uneven distribution of sample sizes throughout various periods. Unfortunately, the sample was heavily skewed towards later individuals, with a total number of 54 bone collagen, bone apatite, and tooth enamel $\delta^{13}C$ results from the period of 2050-50 BP. Twelve samples have been given for

the period of 4050-2050 BP, and only five from 6050 to 4050 BP. Thus, a much larger number of samples from the earlier periods should be analyzed to examine more accurately issues of temporal distribution.

Discussion

The analysis of floral and faunal resource samples produced expected results, with the low $\delta^{13}C$ values of all tested C_3 plants and wild herbivorous animals considered standard. Likewise, the maize samples that were analyzed produced $\delta^{13}C$ values within the expected norm. The samples of domesticated llama suggest that these animals consumed considerable amounts of C_4 plants, most likely maize, during their lives.

These results can be viewed in relation to the human samples, as the isotopic ratios of dietary resources affect the body tissues, including bone collagen, bone apatite, and tooth enamel, of their consumers. $\delta^{13}C$ values obtained from both hard and soft tissue samples did not support the hypothesis that individuals from prehistoric San Juan more commonly consumed maize, or animals that ate maize, than the residents of Mendoza. While these two geographic areas may differ within the archaeological record, their dietary patterns might have been more similar than previously thought. However, the small number of San Juan individuals that were tested leaves further analysis desirable.

Individuals tested from several Mendoza sites have average $\delta^{13}C$ values reflecting a 25-30 percent dependence on C_4 products in their whole diet, as indicated by bone apatite analysis, while the 5.8‰ difference between collagen and apatite values supports the interpretation that plant food was of greater dietary significance than animal products.

Considering the diverse times and contexts represented by the individuals tested, the 5‰ range in carbon isotope values among the individuals tested is not unexpected and may be partly correlated with increasing maize dependence over time.

The $\delta^{15}N$ values do not imply that large amounts of aquatic resources were consumed in either province, but the range of variation merits further investigation. $\delta^{15}N$ can vary by both altitude and dietary intake. The dramatic altitudes found in the central Andes might have affected the $\delta^{15}N$ values of resource samples and the people who consumed them. This could also be a signal of aquatic resource consumption or a combination of both. The analysis of freshwater fish and additional human bone collagen samples might shed light on this issue.

Most importantly, it appears that maize could have been a significant part of human diets in 2000 BP as hypothesized by Gil (2003). It is possible that the people of the prehistoric Argentine Cuyo adopted maize agriculture much earlier than was previously thought, and possibly as early as 6050 BP. This evidence in more in line with that of other areas of South America, such as coastal Ecuador, where Tykot and Staller (2002) used stable isotope analysis to argue that domesticated maize was an important part of human diet as early as 4000 BP. Additionally, Pearsall and colleagues (2004) have demonstrated the antiquity of maize throughout Ecuador by analyzing residues left on stone tools from the Real Alto site. The previous hypothesis of maize diets first occurring at 2000 BP is later than the domestication date suggested by Benz (2001) at 5400 BP.

For San Juan, a much smaller number of individuals have been tested, such that, while there appears to be different carbon isotope averages when compared with the

Mendoza sites, the differences are not statistically significant. The hypothesis (Gil 2003) of a chronological shift towards greater maize dependence over time is not supported by the current study, as demonstrated by the analysis that did not show these individuals to be statistically different from those of later time periods.

Chapter Six: Conclusion

The introduction of maize to prehistoric diets is of fundamental importance to understanding the evolution and development of agriculture in the New World (Tieszen and Fagre 1993). As previously stated, this introduction of a new food resource has been the subject of investigation for some time, yet there remains a lack of information about the history of plant domestication in the southern Andes, where there existed Andean cultures with somewhat less emphasis on agriculture (Gil 2003; Isbell 1997). Information on ceramic typology, lithic analysis, and settlement patterns dominates New World archaeology; what is known about the history of maize in Argentina has been discovered mainly through traditional archaeological techniques, in the few instances when archaeologists have been fortunate enough to come across well preserved floral and faunal remains. A lack of proper excavation techniques, such as flotation and other systematic botanical recovery methods, may further distort some of the existing data regarding prehistoric diets (Keene 1985; Núñez Regueiro 1978). The few reports that do exist have been published as a sort of "gray literature," and are often difficult to obtain for archaeologists who are not directly involved in the specific project or who do not speak English (Pearsall 1992).

Because South America is so geographically and culturally diverse, maize cultivation models cannot often be applied to various regions; thus, there exists a need to establish a clear chronology for the central west portion of Argentina, a unique area that once existed at the edge of the Inca Empire (Pearsall 1994). This disparity of information can be addressed by isotopic analysis of consumer tissues, which provides an invaluable record of dietary change when isotopically different foods are introduced into a diet

(Fogel and Tuross 2003; Schoeninger and Schurr 1994; Schwarcz and Schoeninger 1991).

It is important to consider the cultural setting of maize agriculture, as well as the crop itself. Differences in settlement patterns, material cultures, and subsistence adaptations exist among all maize-based agricultural societies (Pearsall 1994; Schoeninger and Schurr 1994). Gremillion (1996) and Gil (2003) assert that the issue of crop adaptation can produce insights into issues such as those mentioned above. This project, with the analysis of multiple tissues, may assist other researchers working in South America who contribute to a more thorough explanation of the history of agriculture and human dietary adaptations throughout the New World.

Considerations for Isotopic Studies

Results of this study indicate that the faunal and floral resource species that were analyzed provide a suitable dataset for examining the use of dietary elements in the prehistoric Argentine Cuyo. While $\delta^{15}N$ values show relatively high variation when compared to corresponding $\delta^{13}C$ values, this may be linked to the dramatic altitudes of the Andes mountain range, rather than diet alone.

The analysis of human tissues does not support hypotheses regarding temporal and spatial variation in central western Argentina. $\delta^{13}C$ values from both provinces indicate the presence of C_4 plants in human diets and while a transition from foraging to farming is indicated in the archaeological record, the timing of this transition is still unclear. Further analysis may yield greater understanding of the dietary transition that

was previously thought to have taken place around 2000 BP (Gil 2003), but now seems much earlier.

While the effects of dietary transitions are still not completely understood, the analysis of human scalp hair samples shows significant seasonal variation in diet. Still, the analysis of a much larger sample of individuals is necessary before addressing hypotheses regarding the transition to agriculture. Radiographic studies on the mummified individuals may allow for a more thorough examination of the effects of dietary transition.

Scholarly and Educational Significance

The combination of analyses of multiple tissues has allowed for the reconstruction of a dietary life history of well-preserved individuals from the Argentine Cuyo, while advancing knowledge and expertise in archaeological science. The results obtained affect South American archaeologists and researchers practicing stable isotope analysis, while addressing research topics and areas that have been previously overlooked. The information given here adds knowledge to the fields of prehistoric diet reconstruction, New World archaeology, bone chemistry, and archaeological science. This knowledge can be used to enhance theories on cultural adaptation, seasonal dietary variation, foraging versus agricultural diets and more.

This project can be used to construct a preliminary chronology for the adoption of maize agriculture in the areas currently known as San Juan and Mendoza, Argentina. The results obtained add to the scientific database of knowledge on the ability of stable isotope analysis to model prehistoric diets. The experimental soft tissue analysis adds to

the understanding of laboratory procedures and provides essential information regarding seasonal variation in diet. The transition from foraging to farming has been linked with alterations in social organization, economic systems, politics, religion, and even disease (Larsen 2002; Schoeninger and Schurr 1994). By analysis of isotopic ratios in a quantitative manner, researchers can begin to thoroughly address these issues, and gain valuable insight into the human past.

Applied Perspectives

Gilbert (1985:339) notes that "the relationship of diet to the continued survival and perpetuation of a species is fundamental to understanding that species' interaction with its environment." This sentiment hints at the question of *why* do this type of research. The University of South Florida's Department of Anthropology engages in an applied approach, with an emphasis on assisting modern day populations. The more that is learned about the beginnings of human disease, the better the human condition can itself be understood. Diseases like anemia and osteoporosis may be vitally linked to the transition to agriculture. Information about the origins of these ailments used in conjunction with data obtained from stable isotope analysis can help researchers better understand, and possibly even treat or prevent, disease in populations that are struggling with these ailments today. In particular, information garnered from the soft tissue analysis portion in this study indicates dietary instability during times of transition. Further research has been planned that may link dietary variability to weakened immune systems and disease susceptability prior to Spanish contact in the New World.

Additionally, scientists faced with problems of destruction and deforestation of the American rainforests are beginning to examine past food production systems (Bruhns 1994). Bray (2000) argues that archaeologists are in the position to make unique contributions to the topic of sustainable methods of tropical land use by exploring previous land management schemes and educating the public about both the benefits and shortcomings of such practices.

Future Directions

This study is but a small portion of a much larger project attempting to reconstruct prehistoric lifeways in the Argentine Cuyo. Researchers at the Museo de Historia, interested in dietary adaptations and socioeconomic systems prior to European contact, are performing ongoing archaeological examinations, including faunal, macrobotanical and paleoclimatic analyses. Morphometric examination of human remains and dental pathologies is in process, and lithic and ceramic analyses are ongoing.

The Laboratory for Archaeological Science at the University of South Florida continues isotopic studies of osteological materials, while researching funding opportunities for more detailed and extensive analyses of human materials from Argentina.

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Appendix A. Correspondence of Museo de Historia to USF sample numbers per individual

	Sample Type					
Museum Sample #	Collagen	Apatite	Enamel	Hair*	Skin	Muscle
12	7623	8198	-	-	-	-
2038	6217	6218	-	-	-	-
AF-1082	6212	6213	-	-	-	-
AF-1083	7624	8199	8196	-	-	-
AF-13894	7352	7353	-	-	-	-
AF-2000	7619	8191	8192	-	-	-
AF-2018	7354	7355	7356	-	-	-
AF-2019	7349	7350	7351	-	-	-
AF-2020	7358	7359	7357	-	-	-
AF-2021	7618	8189	8190	-	-	-
AF-2022	6194	-	6196	-	-	-
AF-2025	7333	7334	7332	-	-	-
AF-2036	6206	6207	6208	-	-	-
AF-2072	7622	8197	8194	-	-	-
AF-500	6222	6223	-	-	-	-
AF-503	6203	6204	6205	-	-	-
AF-505	6197	6198	-	-	-	-
AF-508	6209	6210	-	-	-	-
AF-510	7331	7330	7329	-	-	-
AF-673	7335	7336	7337	-	-	-
AF-681	7360	7361	7362	-	-	-
AF-8	7625	-	-	-	-	-
AF-828	7621	8195	-	-	-	-
AF-830	7620	8193	-	-	-	-
CS-10001	6199	6200	-	-	-	-
ENT-2	6226	6227	6228	-	-	-
ENT-3	7342	7343	7341	-	-	-
GIRA-27	-	6225	-	-	-	-
GIRA-70	-	6202	-	-	-	-
GIRA-71	6201	-	-	-	-	-
GIRA-831	-	7364	-	-	-	-
JP/J4	7347	7348	7346	-	-	-
JP-1155	6219	6220	6221	-	-	-
JP-1352	7340	7339	7338	-	-	-
MGA-1	6214	6215	6216	-	-	-

R-V-1 (SJ)	7371	-	-	-	-	-
R-V-2 (SJ)	7372	-	-	-	-	-
R-V-3 (SJ)	7373	-	-	-	-	-
R-V-4 (SJ)	7374	-	-	-	-	-
R-V-5 (SJ)	7375	-	-	-	-	-
R-V-6 (SJ)	7376	-	-	-	-	-
R-V-7 (SJ)	7377	-	-	-	-	-
R-V-8 (SJ)	7378	-	-	-	-	-
R-V-9 (SJ)	7379	-	-	-	-	-
SJ1-ENT7	7381	7104	-	-	7103	-
SJ2	7382	7106	7108	7105.1-.4	-	7107
SJ3-ENT3	7111	7110	7112	7109.1-.5	-	7383
SJ4-ENT2	7384	7142	-	-	7141	-
SJ5-ENT2	-	-	7143	7145.1-.5	7144	-
SJ6-ENT8	7385	7380	-	7146.1-.5	-	7147
SJ7-ENT2	7152	7151	7150	7148.1-.3	-	7149
SJ8-ENT5	-	-	-	7153.1-.5	-	7154
SJ9-ENT1	-	-	-	7156.1-.8	7155	-
SJ10-ENT1	7386	7159	-	7157.1-.8	7158	-

*Series of numbers indicating sequential 2 centimeter samples, with .1 being closest to scalp

Appendix B. Results of stable isotope analysis for all samples

Museo Sample #	USF #	Material	δ13C	δ15N	Species	Years BP	Sex	Age	Site
12	8198	Apatite	-11.3		Homo sapiens	3850		Adult	MZ; Caverna de las Brujas
2038	6217	Collagen	-18.8	6.4	Homo sapiens		F	35-49 yrs	MZ; El Desecho
2038	6218	Apatite	-11.2		Homo sapiens		F	35-49 yrs	MZ; El Desecho
AF-1082	6212	Collagen	-16.5	12.9	Homo sapiens		F	35-49 yrs	MZ; Agua del toro
AF-1082	6213	Apatite	-11.3		Homo sapiens		F	35-49 yrs	MZ; Agua del toro
AF-1083	8196	Enamel	-9.8		Homo sapiens	100			MZ; Arbolito 8
AF-1083	8199	Apatite	-10.6		Homo sapiens	100			MZ; Arbolito 6
AF-13894	7352	Collagen	-15.0	9.8	Homo sapiens	2300		Perinatal?	MZ; Gruta del Indio
AF-13894	7353	Apatite	-10.1		Homo sapiens	2300		Perinatal?	MZ; Gruta del Indio
AF-2000	8191	Apatite	-7.4		Homo sapiens	580			MZ; C Negro del Escorial
AF-2000	8192	Enamel	-5.5		Homo sapiens	580			MZ; C Negro del Escorial
AF-2018	7354	Collagen	-14.3	11.5	Homo sapiens	1700-1400			MZ; Canada Seca
AF-2018	7355	Apatite	-9.8		Homo sapiens	1700-1400			MZ; Canada Seca
AF-2018	7356	Enamel	-9.0		Homo sapiens	1700-1400			MZ; Canada Seca
AF-2019	7349	Collagen	-14.5	10.4	Homo sapiens	1700-1400			MZ; Canada Seca
AF-2019	7350	Apatite	-10.1		Homo sapiens	1700-1400			MZ; Canada Seca
AF-2019	7351	Enamel	-8.8		Homo sapiens	1700-1400			MZ; Canada Seca
AF-2020	7357	Enamel	-8.7		Homo sapiens	1700-1400			MZ; Canada Seca
AF-2020	7358	Collagen	-14.3	11.3	Homo sapiens	1700-1400			MZ; Canada Seca
AF-2020	7359	Apatite	-9.5		Homo sapiens	1700-1400			MZ; Canada Seca
AF-2021	8190	Enamel	-6.1		Homo sapiens	510		Infant	MZ; Gruta del Indio
AF-2022	6194	Collagen	-18.5	10.5	Homo sapiens	1200	M	30-45 yrs	MZ; Ojo de Agua
AF-2022	6196	Enamel	-11.9		Homo sapiens	1200	M	15-18 yrs	MZ; Ojo de Agua
AF-2025	7332	Enamel	-10.8		Homo sapiens	200			MZ; Tierras Blancas
AF-2025	7333	Collagen	-15.5	9.5	Homo sapiens	200			MZ; Tierras Blancas

AF-2025	7334	Apatite	-8.2		Homo sapiens	200			MZ; Tierras Blancas
AF-2036	6206	Collagen	-17.5	9.7	Homo sapiens		F	16-20 yrs	MZ; India embarzada
AF-2036	6207	Apatite	-10.1		Homo sapiens		F	16-20 yrs	MZ; India embarzada
AF-2036	6208	Enamel	-10.8		Homo sapiens		F	16-20 yrs	MZ; India embarzada
AF-2072	8194	Enamel	-6.2		Homo sapiens	970	F	15-18 yrs	MZ; Las Ramadas
AF-2072	8197	Apatite	-7.6		Homo sapiens	970	F	15-18 yrs	MZ; Las Ramadas
AF-500	6222	Collagen	-13.5	10.3	Homo sapiens	1760	M	>50 yrs	MZ; Rincon del Atuel
AF-500	6223	Apatite	-8.1		Homo sapiens	1760	M	>50 yrs	MZ; Rincon del Atuel
AF-503	6203	Collagen	-13.8	9.4	Homo sapiens	1760	M	34-45 yrs	MZ; RA-1
AF-503	6204	Apatite	-7.9		Homo sapiens	1760	M	34-45 yrs	MZ; RA-1
AF-503	6205	Enamel	-9.9		Homo sapiens	1760	M	34-45 yrs	MZ; RA-1
AF-505	6197	Collagen	-16.0	11.9	Homo sapiens		M	45-50 yrs	MZ; La Matancilla
AF-505	6198	Apatite	-10.1		Homo sapiens		M	45-50 yrs	MZ; La Matancilla
AF-508	6209	Collagen	-17.9	10.8	Homo sapiens		M	38-49 yrs	MZ; Cerro Mesa
AF-508	6210	Apatite	-12.2		Homo sapiens		M	38-49 yrs	MZ; Cerro Mesa
AF-510	7329	Enamel	-12.7		Homo sapiens	300-200			MZ; Cerro Mesa
AF-510	7330	Apatite	-13.0		Homo sapiens	300-200			MZ; Cerro Mesa
AF-510	7331	Collagen	-17.9	10.9	Homo sapiens	300-200			MZ; Cerro Mesa
AF-673	7335	Collagen	-17.2	10.2	Homo sapiens				MZ; El Manzano
AF-673	7336	Apatite	-12.5		Homo sapiens				MZ; El Manzano
AF-673	7337	Enamel	-12.8		Homo sapiens			Adult	MZ; El Manzano
AF-681	7360	Collagen	-15.6	8.7	Homo sapiens	2000			MZ; Medano Puesto Diaz
AF-681	7361	Apatite	-10.2		Homo sapiens	2000			MZ; Medano Puesto Diaz
AF-681	7362	Enamel	-10.7		Homo sapiens	2000			MZ; Medano Puesto Diaz
AF-828	8195	Apatite	-7.6		Homo sapiens	580	F	30-49 yrs	MZ; Gruta del Indio
AF-830	8193	Apatite	-12.0		Homo sapiens	3860			MZ; Gruta del Indio
CS-10001	6199	Collagen	-15.7	11.6	Homo sapiens		M	30-45 yrs	MZ; Canada Seca
CS-10001	6200	Apatite	-9.0		Homo sapiens		M	30-45 yrs	MZ; Canada Seca

ENT-2	6226	Collagen	-14.9	11.7	Homo sapiens	400	F		MZ; Capiz Alto
ENT-2	6227	Apatite	-10.6		Homo sapiens	400	F		MZ; Capiz Alto
ENT-2	6228	Enamel	-9.6		Homo sapiens	400	F		MZ; Capiz Alto
ENT-3	7341	Enamel	-10.2		Homo sapiens				MZ; El Chacay
ENT-3	7342	Collagen	-16.2	7.9	Homo sapiens				MZ; El Chacay
ENT-3	7343	Apatite	-9.2		Homo sapiens				MZ; El Chacay
GIRA-27	6225	Apatite	-11.9		Homo sapiens			Adult	MZ; Gruta del Indio
GIRA-70	6202	Apatite	-9.8		Homo sapiens			Adult	MZ; Gruta del Indio
GIRA-71	6201	Collagen	-14.0	10.8	Homo sapiens			Adult	MZ; Gruta del Indio
GIRA-831	7364	Apatite	-10.5		Homo sapiens				MZ; Gruta del Indio
JP/J4	7346	Enamel	-13.5		Homo sapiens	2100-1700			MZ; Jaime Prats
JP/J4	7347	Collagen	-17.4	9.8	Homo sapiens	2100-1700			MZ; Jaime Prats
JP/J4	7348	Apatite	-13.5		Homo sapiens	2100-1700			MZ; Jaime Prats
JP-1155	6219	Collagen	-16.8	10.6	Homo sapiens	2100-1700	F	20-26 yrs	MZ; Jaime Prats
JP-1155	6220	Apatite	-10.2		Homo sapiens	2100-1700	F	20-26 yrs	MZ; Jaime Prats
JP-1155	6221	Enamel	-8.6		Homo sapiens	2100-1700	F	20-26 yrs	MZ; Jaime Prats
JP-1352	7338	Enamel	-11.2		Homo sapiens	2100-1700			MZ; Jaime Prats
JP-1352	7339	Apatite	-10.6		Homo sapiens	2100-1700			MZ; Jaime Prats
JP-1352	7340	Collagen	-16.3	9.9	Homo sapiens	2100-1700	F?		MZ; Jaime Prats
MGA-1	6214	Collagen	-14.2	10.9	Homo sapiens				MZ; RQ-1
MGA-1	6215	Apatite	-8.9		Homo sapiens				MZ; RQ-1
MGA-1	6216	Enamel	-8.7		Homo sapiens				MZ; RQ-1
R-A-1	5905	Apatite	-10.7		Lama guanicoe				
R-A-1	6170	Collagen	-19.0	4.3	Lama guanicoe				
R-A-1	7368	Faunal	-14.2	9.0	Lama guanicoe				SJ; Angualasto
R-A-10	5913	Apatite	-8.9		Lama guanicoe				
R-A-10	6179	Collagen	-18.8	4.3	Lama guanicoe				
R-A-11	5914	Apatite	-11.5		Pterocnemia pennata				

R-A-11	6180	Collagen	-21.0	4.9	Pterocnemia pennata		
R-A-2	5906	Apatite	-6.8		Lama guanicoe		
R-A-2	6171	Collagen	-14.7	5.0	Lama guanicoe		
R-A-2	7369	Faunal	-18.3	6.7	Rhea americana		SJ; Angualasto
R-A-3	5907	Apatite	-11.1		Lama guanicoe		
R-A-3	6172	Collagen	-19.4	4.6	Lama guanicoe		
R-A-3	7370	Collagen	-18.1	5.6	Lama guanicoe		SJ; Morrillos
R-A-4	6173	Enamel	-9.1		Lama guanicoe		
R-A-5	5908	Apatite	-11.5		Cholephaga melanoptera		
R-A-5	6174	Collagen	-22.0	4.1	Cholephaga melanoptera		
R-A-6	5909	Apatite	-11.8		Rhea americana		
R-A-6	6175	Collagen	-20.0	5.7	Rhea americana		
R-A-7	5910	Apatite	-12.1		Pterocnemia pennata		
R-A-7	6176	Collagen	-20.6	4.6	Pterocnemia pennata		
R-A-8	5911	Apatite	-9.1		Lagidium viscacia		
R-A-8	6177	Collagen	-19.3	3.7	Lagidium viscacia		
R-A-9	5912	Apatite	-11.1		Chaetophractus villosus		
R-A-9	6178	Collagen	-17.7	5.6	Chaetophractus villosus		
R-V-1	6181	Botanical	-9.7	3.4	Zea mays		
R-V-10	6190	Botanical	-20.8		Geoffroea decorticans		
R-V-11	6191	Botanical	-24.9	11.6	Prosopis sp.		
R-V-13	6193	Botanical	-24.4	1.6	Schinus polygamus		
R-V-2	6182	Botanical	-9.6	3.9	Zea mays		
R-V-3	6183	Botanical	-23.2	13.1	Cucurbita maxima		
R-V-4	6184	Botanical	-25.4		Lagenaria sp.		
R-V-5	6185	Botanical	-27.6	6.9	Chenopodium sp.	2200	MZ; GrN 5398
R-V-6	6186	Botanical	-23.9		Prosopis sp.		
R-V-6	7376	Botanical	-24.3	7.0	Cucurbita maxima		SJ; Calingasta

R-V-7	6187	Botanical	-25.4	1.6	Cassia arnottiana			
R-V-8	6188	Botanical	-24.0	5.5	Phaseolus vulgaris			
R-V-9	6189	Botanical	-20.2	14.0	Geoffroea decorticans			
R-V-9	7379	Botanical	-24.2	9.8	Cucurbita maxima			SJ; Iglesia
SJ10-ENT1	7157.1	Hair	-13.4	9.6	Homo sapiens	600	F	SJ; Angualasto
SJ10-ENT1	7157.2	Hair	-12.7	9.0	Homo sapiens	600	F	SJ; Angualasto
SJ10-ENT1	7157.3	Hair	-13.6	8.6	Homo sapiens	600	F	SJ; Angualasto
SJ10-ENT1	7157.4	Hair	-13.2	8.6	Homo sapiens	600	F	SJ; Angualasto
SJ10-ENT1	7157.5	Hair	-13.9	8.0	Homo sapiens	600	F	SJ; Angualasto
SJ10-ENT1	7157.6	Hair	-15.1	8.7	Homo sapiens	600	F	SJ; Angualasto
SJ10-ENT1	7157.7	Hair	-16.6	9.4	Homo sapiens	600	F	SJ; Angualasto
SJ10-ENT1	7157.8	Hair	-15.6	9.6	Homo sapiens	600	F	SJ; Angualasto
SJ10-ENT1	7158	Skin	-14.1	12.0	Homo sapiens	600	F	SJ; Angualasto
SJ10-ENT1	7159	Apatite	-8.2		Homo sapiens	600	F	SJ; Angualasto
SJ10-ENT1	7386	Collagen	-12.3	9.9	Homo sapiens	600	F	SJ; Angualasto
SJ1-ENT7	7103	Skin	-16.4	11.8	Homo sapiens	7900-4200	F	SJ; Gruta Morrillos
SJ1-ENT7	7104	Apatite	-13.1		Homo sapiens	7900-4200	F	SJ; Gruta Morrillos
SJ1-ENT7	7381	Collagen	-17.3	9.7	Homo sapiens	7900-4200	F	SJ; Morrillos Gruta 1(F)
SJ2	7105.1	Hair	-14.9	10.6	Homo sapiens	800		SJ; Calingasta
SJ2	7105.2	Hair	-13.4	10.2	Homo sapiens	800		SJ; Calingasta
SJ2	7105.3	Hair	-13.5	10.4	Homo sapiens	800		SJ; Calingasta
SJ2	7105.4	Hair	-14.4	9.6	Homo sapiens	800		SJ; Calingasta
SJ2	7106	Apatite	-10.1		Homo sapiens	800		SJ; Calingasta
SJ2	7107	Skin	-14.4	13.5	Homo sapiens	800		SJ; Calingasta
SJ2	7108	Enamel	-6.3		Homo sapiens	800		SJ; Calingasta
SJ2	7382	Collagen	-13.8	9.5	Homo sapiens	800		SJ; Calingasta
SJ3-ENT3	7109.1	Hair	-15.3	9.2	Homo sapiens	590		SJ; Punta del Barro
SJ3-ENT3	7109.2	Hair	-16.2	9.7	Homo sapiens	590		SJ; Punta del Barro

SJ3-ENT3	7109.3	Hair	-16.9	9.7	Homo sapiens	590			SJ; Punta del Barro
SJ3-ENT3	7109.4	Hair	-16.6	9.4	Homo sapiens	590			SJ; Punta del Barro
SJ3-ENT3	7109.5	Hair	-15.6	9.6	Homo sapiens	590			SJ; Punta del Barro
SJ3-ENT3	7110	Apatite	-9.8		Homo sapiens	590			SJ; Punta del Barro
SJ3-ENT3	7111	Collagen	-13.3	9.5	Homo sapiens	590			SJ; Punta del Barro
SJ3-ENT3	7112	Enamel	-9.0		Homo sapiens	590			SJ; Punta del Barro
SJ3-ENT3	7383	Skin	-16.3	11.6	Homo sapiens	590			SJ; Punta del Barro
SJ4-ENT2	7141	Skin	-15.3	13.1	Homo sapiens	640			SJ; Angualasto
SJ4-ENT2	7142	Apatite	-10.3		Homo sapiens	640			SJ; Angualasto
SJ4-ENT2	7384	Collagen	-13.8	10.1	Homo sapiens	640			SJ; Angualasto
SJ5-ENT2	7143	Enamel	-8.3		Homo sapiens	880			SJ; C Calvario
SJ5-ENT2	7144	Skin	-18.0	14.0	Homo sapiens	880			SJ; C Calvario
SJ5-ENT2	7145.1	Hair	-15.0	10.3	Homo sapiens	880			SJ; C Calvario
SJ5-ENT2	7145.2	Hair	-15.2	10.3	Homo sapiens	880			SJ; C Calvario
SJ5-ENT2	7145.3	Hair	-15.5	8.7	Homo sapiens	880			SJ; C Calvario
SJ5-ENT2	7145.4	Hair	-15.2	9.1	Homo sapiens	880			SJ; C Calvario
SJ5-ENT2	7145.5	Hair	-17.1	9.3	Homo sapiens	880			SJ; C Calvario
SJ6-ENT8	7146.1	Hair	-19.4	9.0	Homo sapiens	2000	F	Adult	SJ; Gruta 1 Morrillos- Ansilta
SJ6-ENT8	7146.2	Hair	-20.1	9.5	Homo sapiens	2000	F	Adult	SJ; Gruta 1 Morrillos- Ansilta
SJ6-ENT8	7146.3	Hair	-20.6	9.6	Homo sapiens	2000	F	Adult	SJ; Gruta 1 Morrillos- Ansilta
SJ6-ENT8	7146.4	Hair	-20.8	9.3	Homo sapiens	2000	F	Adult	SJ; Gruta 1 Morrillos- Ansilta
SJ6-ENT8	7146.5	Hair	-20.2	8.8	Homo sapiens	2000	F	Adult	SJ; Gruta 1 Morrillos- Ansilta
SJ6-ENT8	7147	Skin	-19.8	17.4	Homo sapiens	2000	F	Adult	SJ; Gruta 1 Morrillos
SJ6-ENT8	7380	Apatite	-14.0		Homo sapiens	2000			SJ; Gruta 1 Morrillos- Ansilta
SJ6-ENT8	7385	Collagen	-17.3	8.1	Homo sapiens	2000			SJ; Gruta 1 Morrillos- Ansilta
SJ7-ENT2	7148.1	Hair	-16.8	10.2	Homo sapiens	4070	M	Adult	SJ; Gruta 1 Morrillos-Morrillos
SJ7-ENT2	7148.2	Hair	-16.7	9.7	Homo sapiens	4070	M	Adult	SJ; Gruta 1 Morrillos-Morrillos
SJ7-ENT2	7148.3	Hair	-17.3	9.4	Homo sapiens	4070	M	Adult	SJ; Gruta 1 Morrillos-Morrillos

SJ7-ENT2	7149	Skin	-20.9	16.7	Homo sapiens	4070	M	Adult	SJ; Gruta 1 Morrillos-Morrillos
SJ7-ENT2	7150	Enamel	-9.2		Homo sapiens	4070	M	Adult	SJ; Gruta 1 Morrillos-Morrillos
SJ7-ENT2	7151	Apatite	-12.2		Homo sapiens	4070	M	Adult	SJ; Gruta 1 Morrillos-Morrillos
SJ7-ENT2	7152	Collagen	-15.3	10.8	Homo sapiens	4070	M	Adult	SJ; Gruta 1 Morrillos-Morrillos
SJ8-ENT5	7153.1	Hair	-14.2	9.0	Homo sapiens	2000	M		SJ; Gruta 1 Morrillos- Ansilta
SJ8-ENT5	7153.2	Hair	-	-	Homo sapiens	2000	M		SJ; Gruta 1 Morrillos- Ansilta
SJ8-ENT5	7153.3	Hair	-19.4	7.4	Homo sapiens	2000	M		SJ; Gruta 1 Morrillos- Ansilta
SJ8-ENT5	7153.4	Hair	-20.0	7.8	Homo sapiens	2000	M		SJ; Gruta 1 Morrillos- Ansilta
SJ8-ENT5	7153.5	Hair	-19.8	8.6	Homo sapiens	2000	M		SJ; Gruta 1 Morrillos- Ansilta
SJ8-ENT5	7154	Skin	-17.6	12.2	Homo sapiens	2000	M		SJ; Gruta 1 Morrillos- Ansilta
SJ9-ENT1	7155	Skin	-11.6	12.3	Homo sapiens	1400-1200	F	Adult	SJ; Hilario
SJ9-ENT1	7156.1	Hair	-10.7	10.0	Homo sapiens	1400-1200	F	Adult	SJ; Hilario
SJ9-ENT1	7156.2	Hair	-12.9	9.0	Homo sapiens	1400-1200	F	Adult	SJ; Hilario
SJ9-ENT1	7156.3	Hair	-13.4	9.2	Homo sapiens	1400-1200	F	Adult	SJ; Hilario
SJ9-ENT1	7156.4	Hair	-16.5	9.6	Homo sapiens	1400-1200	F	Adult	SJ; Hilario
SJ9-ENT1	7156.5	Hair	-10.6	9.6	Homo sapiens	1400-1200	F	Adult	SJ; Hilario
SJ9-ENT1	7156.6	Hair	-11.0	9.5	Homo sapiens	1400-1200	F	Adult	SJ; Hilario
SJ9-ENT1	7156.7	Hair	-10.3	9.1	Homo sapiens	1400-1200	F	Adult	SJ; Hilario
SJ9-ENT1	7156.8	Hair	-10.0	9.5	Homo sapiens	1400-1200	F	Adult	SJ; Hilario

Appendix C. Bone Apatite Sample Processing Form

Name of Sample Series _____ Lab Worker_____

Fill in the date for each item below. Use a separate sheet for each batch of samples processed.

_____ 1. Select approximately 1 gram of whole bone.
_____ 2. Clean bone physically and then ultrasonically to remove dirt and adherent materials. Use brushes and dental tools as necessary.
_____ 3. Dry bone in drying oven.
_____ 4. Pulverize bone using ball mill or use drill to produce bone powder.
_____ 5. Pass bone powder through mesh screen stack to separate size fractions (if necessary).
_____ 6. Weigh out approximately 10 mg of bone powder into a 1.5 ml centrifuge tube. Label with USF number.
_____ 7. Add 1 ml of 2% bleach solution to remove collagen, bacterial proteins, humates. Let stand for 72 hours.
_____ 8. Centrifuge the sample and pour off bleach solution using pipette if necessary to remove solution without losing sample. Replace with distilled water. Repeat 4 times.
_____ 9. Dry bone powder in drying oven. Weigh sample.
_____ 10. Pre-treat bone with 1 ml of 1 M acetic acid/sodium acetate buffer solution for 24 hours.
_____ 11. Centrifuge the sample, pour off acetic acid/sodium acetate solution using pipette if necessary to remove solution without losing sample. Replace with distilled water. Repeat 4 times.
_____ 12. Dry bone powder in drying oven. Weigh sample.

USF #	Lab/Museum #	Initial Weight	Weight after bleach	Weight after acetic acid	Run Weight

Appendix D. Tooth Enamel Sample Processing Form

Name of Sample Series _____ Lab Worker_____

Fill in the date for each item below. Use a separate sheet for each batch of samples processed.

_____ 1. Select tooth sample.
_____ 2. Clean tooth physically and then ultrasonically to remove dirt and adherent materials. Use brushes and dental tools as necessary.
_____ 3. Dry tooth in drying oven.
_____ 4. Drill enamel powder from tooth surface being careful *not to reach dentin*.
_____ 5. Weigh out approximately 10 mg of enamel powder into a 1.5 ml centrifuge tube. Label with USF number.
_____ 6. Add 1 ml of 2% bleach solution to remove collagen, bacterial proteins, humates. Let stand for 24 hours.
_____ 7. Centrifuge the sample and pour off bleach solution using pipette if necessary to remove solution without losing sample. Replace with distilled water. Repeat 4 times.
_____ 8. Dry enamel powder in drying oven. Weigh sample.
_____ 9. Pre-treat enamel with 1 ml of 1 M acetic acid/sodium acetate buffer solution for 24 hours.
_____ 10. Centrifuge the sample, pour off acetic acid/sodium acetate solution using pipette if necessary to remove solution without losing sample. Replace with distilled water. Repeat 4 times.
_____ 11. Dry bone powder in drying oven. Weigh sample.

USF #	Lab/Museum #	Initial Weight	Weight after bleach	Weight after acetic acid	Run Weight

Appendix E. Bone Collagen Sample Processing Form

Name of Sample Series _____ Lab Worker_____

Fill in the date for each item below. Use a separate sheet for each batch of samples processed.

_____ 1. Select approximately 1 gram of whole bone and put in labeled jar with lid.
_____ 2. Clean bone physically and then ultrasonically to remove dirt and adherent materials. Use brushes and dental tools as necessary.
_____ 3. If bone was treated with preservative, rinse or soak in acetone.
_____ 4. Dry bone in drying oven. Weigh sample.
_____ 5. Soak in 50 mls 0.1 M NaOH (4g per liter) for 24 hours to remove humic acids.
_____ 6. Pour off NaOH and rinse thoroughly with distilled water. Add 50 mls 2% HCl (200 mls concentrated HCL, 3.6 liters distilled water) to remove bone mineral.
_____ 7. Cut or pulverize bone into smaller pieces as necessary.
_____ 8. Replace HCl solution with fresh acid after 24 hours.
_____ 9. Replace HCl again after another 24 hours. Repeat as necessary until bone is totally demineralized.
_____ 10. Pour off HCl acid solution and rinse thoroughly with distilled water. Add 50 mls 0.1 M NaOH (4 g per liter) and soak 24 hours to remove humic acids.
_____ 11. Pour off NaOH solution and rinse thoroughly with distilled water. Add 50 mls defatting solution (2:1:0.8 mixture of methanol, chloroform and distilled water), and soak 24 hours to remove fat content.
_____ 12. Pour off defatting solution into waste jars; rinse extra thoroughly. Transfer samples to 2-dram vials, and label with indelible marker.
_____ 13. Oven-dry samples.
_____ 14. Weigh samples and calculate collagen yield.

USF #	Lab/Museum#	Initial Weight	Final Weight	% Yield	Run Weight

Appendix F. Hair Sample Processing Form

Name of sample series _____ Lab worker responsible_____

Fill in the date for each item below. Use a separate sheet for each batch of samples processed.

____ 1. Put on latex gloves and a hair net or ball cap
____ 2. Clean several large test tubes using distilled water
____ 3. Clean a fairly large beaker by rinsing it with acetone and then with distilled water
____ 4. Select a hair sample
____ 5. Line up approximately 15 strands of hair (record number of strands)
____ 6. Cut into 2 cm sections along the shaft; distilled water may be used if necessary to dampen the hair for easier handling
____ 7. Label the test tube with sample identifier (.1,.2,.3, etc. of their lab number)
____ 8. Place the hair sample in the test tube
____ 9. Fill the test tube with distilled water
____ 10. Place the test tube upright in the large beaker, place it in the sonication unit
____ 11. Repeat steps 4 through 10 until all the samples are prepared
____ 12. Fill the sonication unit to a level below the top of the test tubes with distilled water
____ 13. Sonicate the samples for 15 minutes at room temperature
____ 14. Remove the samples from the sonication unit
____ 15. Remove a test tube and carefully pour out the liquid while keeping the hair inside the tube
____ 16. Put on a mask and fill the test tube with a 2:1 v/v mixture of methanol and chloroform
____ 17. Place the filled tube upright in a large beaker. When the beaker is full, place it in a sonication unit
____ 18. Repeat steps 13 through 17 for the rest of the samples
____ 19. Ensure the sonication unit is filled to a level below the top of the test tubes with distilled water
____ 20. Sonicate the samples for 15 minutes at room temperature
____ 21. Replicate the methanol/chloroform cleansing by repeating steps 15 through 20 (two total methanol:chloroform rinses)
____ 22. Remove a test tube and carefully pour out the methanol/chloroform mixture while keeping the hair inside the tube
____ 23. Fill the tube with distilled water.
____ 24. Place the tube upright in a large beaker. When the beaker is full, place it in the sonication unit
____ 25. Repeat steps 22 through 24 until all samples are prepared
____ 26. Fill the sonication unit to a level below the top of the test tubes with distilled water
____ 27. Sonicate the samples for 15 minutes at room temperature
____ 28. Remove the samples from the sonication unit
____ 29. Remove each test tube and carefully pour out the distilled water while keeping the hair inside the tube. Place the tubes upright in a large beaker
____ 30. Put the samples in a safe area with circulating air, such as a chemical hood
____ 31. Allow the samples to air dry for at least 48 hours
____ 32. Examine microscopically to ensure cleanliness