Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) from North West Argentina: paleodietary implications

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Abstract

Presented here are the first results of an ongoing research project on stable isotopic analysis of human bone samples from six sites located at Jujuy province, NW Argentina. The region was inhabited by hunter gatherers as early as 10,000 years ago (Archaic Period). Sometime between 500 BC-AD 1600, peoples from the region became agricultural-pastoralists. The prehistoric development of these societies was severed in 1596 by the Spanish Conquest. A diversity of environmental as well as chronological differences led the prehistoric societies of the region to adopt and display a series of adaptive responses. Active exchange of products was a common fact. The aforementioned circumstances raise expectations concerning the existence of possible variations in the actual consumption of available alimentary items in Puna (3000 to 4700 m asl), as well as in Quebrada de Humahuaca (2000 to 2800 m asl). The obtained $\delta^{13}C$ and $\delta^{15}N$ values on both apatite and collagen show elevated consumption of C4 resources in Quebrada de Humahuaca since the onset of the Formative Period (*ca*. 500 BC). Puna samples, on the other hand, show a sustained, long lasting chronological emphasis on camelid production and consumption as well as other C3 resources exploited. We conclude that, in spite of the considerable interactions and trading that took place between these two regions in prehistoric times, they were not strong enough to erase the environmental influences that determined the existence of distinctive stable isotope patterns related to diet (and composition of meals) between Quebrada de Humahuaca and Puna peoples.

Introduction

The Jujuy province, in NW Argentina, has political borders with Bolivia and Chile. The area was inhabited during prehistoric times (10,000 BC-AD 1596). A marked environmental diversity made possible the emergence of a noticeable spectrum of subsistence strategies among the peoples from the province. Bioarchaeological, archaeofaunal as well as archaeobotanical records make it possible to postulate about both the hunting and the domestication of camelids, complemented by the consumption of numerous plant resources (tubers, roots, peppers, beans, gourds, *etc*.). Corn (*Zea mays*) was the most important edible C4 plant (Albeck, 2000; Lagiglia, 2001; Yacobaccio, 2001). The archaeological record shows the existence of widespread commercial networks, of both short- and long-distance, where sustained interaction and circulation of material goods and alimentary resources took place. These exchange dynamics were particularly intensified *ca*. 1200 BC and continued up to the end of the sixteenth century (Albeck, 1994, 2000; Nielsen, 2001).

This paper considers two main regions from Jujuy province that are characterised by marked environmental differences. The western section of Puna is a wide highland plateau that covers approximately some 3000 to 4700 m asl, occasionally interrupted by some streams, salt mines, and mountains (Yacobaccio *et al*., 2009). To the east at 2000 m asl is the Quebrada de Humahuaca, a long, extended valley paralleled by two geologic formations with a north-south orientation, through which the Rio Grande runs (Figure 1). Food acquisition in Puna was mostly based upon hunting and gathering (Archaic period *ca.* 10,000-600 BC). Later, its food production involved the raising and herding of camelids, particularly llama (*Lama glama*), activity mostly occurring in the meadows and grasslands at the flat bottoms of riverine basins (Albeck, 2000, 2007). In the most favorable zones in this region, it was possible to develop economies based on the cultivation of a few microthermic Andean plant species with a C3 photosynthetic pathway (*quinoa,* potato, *yam and ulluco*). Corn (C4) cultivation was limited to the existence of very localised microclimates, such as those observed in the Casabindo-Doncellas region (Otonello and Krapovickas, 1973; Albeck, 2007) (Figure 1). Unlike Puna, in Quebrada de Humahuaca the climatic conditions are favorable for agriculture, a fact that can be seen in the construction of extensive terraces for cultivation. The archaeological record so far recovered shows the adoption of agricultural systems since formative times (*ca.* 600 BC to AD 600), with a variety of cultigens, corn (*Zea mays*) being the predominant species (Albeck, 2000; Lagiglia, 2001).

The reconstructions of subsistence systems adopted by pre-Hispanic populations in the province are mainly based on the analysis of the archaeological, archaeobotanical, and zooarchaeological records. Regarding stable isotope signaling, while a moderate number of bibliographic references exists for regional fauna (Fernández and Panarello, 1999-2001; Fernández *et al*., 1999; Yaccobaccio *et al*., 2009), the paper from Olivera and Yacobaccio (1999-2001) constitutes the sole reference on human paleodietary inference so far proposed for Jujuy.

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Key words: bioarchaeology, NW Argentina, paleodiet, stable isotopes, subsistence economies.

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Analyses based upon stable isotopic ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) in human bone tissues have been used in numerous archaeological contexts in the last three decades (*e.g.* Ambrose and Katzenberg, 2000) and constitute a resource of great methodological value to estimate the ratios of alimentary resources, particularly the relations of consumption of C3 and C4 plants. Note that in this study isotopic rates are expressed in parts per thousand or per mil (‰). Furthermore, values are calculated as $\delta‰=(Rsample/Rstandard-1)x1000$, where R is the relationship between the most heavy and the lighter isotope (Ambrose, 1990, 1993). Such estimates are based on the fact that isotopic composition of tissues is a constant function of the diet. In this way, individuals from a given population are largely what they habitually eat, according to the rate of physiologic renewal of the tissue being analysed. Laboratory studies show that in bone samples, which include both organic (*i.e.* collagen) as well as inorganic (*i.e.* apatite) components, the bone collagen reflects mainly the protein diet, while the bone apatite reflects the average of all the macromolecules, or total diet. In this paper we explore the existence of paleodietary trends as suggested by a new set of stable isotope values from prehistoric bone samples from Quebrada de Humahuaca and Puna. Additionally, quantitative frames of reference are provided in order to locate, compare, discuss and interpret stable isotopic values obtained from new analytic processes in the future. According to what is known from archaeology, paleobotany, and zooarchaeology, when compared to those from Puna, individuals from Quebrada de Humahuaca will possess isotopic values mostly linked to corn consumption. Furthermore, according to the available evidence on secular changes in dietary adaptation, the isotopic values related with the consumption of edible C4 plants during Agricultural-Pastoralist Late Developments will be much more marked than those obtained in Archaic and formative individuals.

Materials and Methods

Nine adult individuals from five pre-Hispanic sites in Jujuy province were selected to perform this study (Table 1). Given the fact that some of the values published in the paper by Olivera and Yacobaccio (2001) were also obtained from bone tissue, and that there is consistency with the variables we analysed, some values

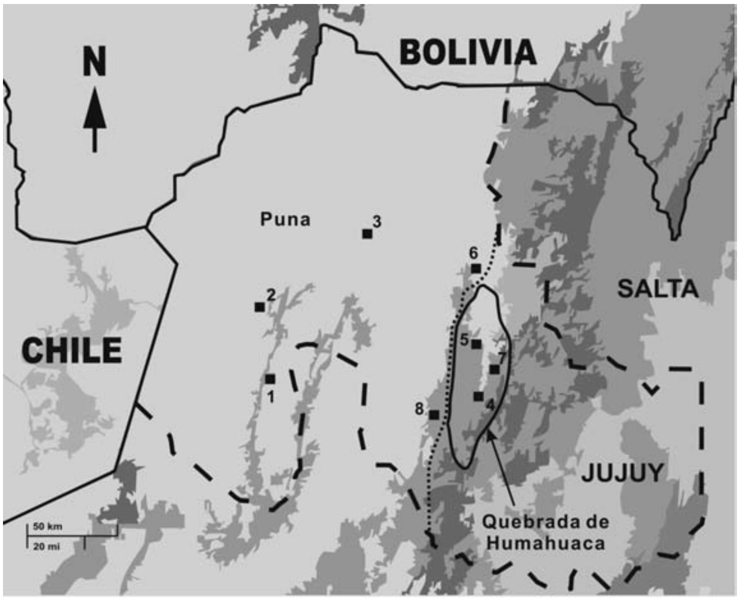

Figure 1. Regions and archaeological sites considered: 1) Morro Ciénago Chico; 2) Hornillos 3; 3) Doncellas; 4) Tilcara (Til1, Til20, Til43); 5) Amarillos and Yacoraite; 6) Quebrada de la Cueva; 7) La Huerta; 8) Huachichocana.

Table 1. Bone isotopic values by archaeological site, region and chronology.

Code	Site	Region	δ^{13}Cap	δ^{13}Ccol	δ^{15}Ncol	Chronology	Reference
LHR	La Huerta	Quebrada	-8.2	-11.1	9.3	Late Period	Olivera and Yacobaccio (2001)
QC1	Qda de la Cueva	Puna	-11.6	-12.7	8.9	Formative Period	Olivera and Yacobaccio (2001)
QC2	Qda de la Cueva	Puna	-12.9	-13.1	9.6	Formative Period	Olivera and Yacobaccio (2001)
Hor3	Hornillos 3	Puna	-12.1	-17.0	12.1	Late Period	Olivera and Yacobaccio (2001)
Hua1	Huachichocana	Puna	-8.6	-13.1	8.1	Late Period	Olivera and Yacobaccio (2001)
Hua2	Huachichocana	Puna	-8.7	-17.1	10.6	Archaic Period	Olivera and Yacobaccio (2001)
Hua3	Huachichocana	Puna	-3.6	-15.7	10.5	Archaic Period	Olivera and Yacobaccio (2001)
MCCH1	Morro Ciénago Chico	Puna	-13.1	-17.8	11.1	Formative Period	Olivera and Yacobaccio (2001)
Am1	Los Amarillos	Quebrada	-6.4	-11.8	9.9	Late Period	This work
Yac1	Yacoraite	Quebrada	-6.6	-12.0	10.2	Late Period	This work
Yac2	Yacoraite	Quebrada	-5.2	-10.9	10.2	Late Period	This work
Til20-1	Til 20 (Tilcara)	Quebrada	-2.9	-9.9	11.4	Formative Period	This work
Til20-2	Til 20 (Tilcara)	Quebrada	-2.6	-9.2*	10.4*	Formative Period	This work
Til43-1	Til 43 (Tilcara)	Quebrada	-5.4	-10.3	10.6	Late Period	This work
Til43-2	Til 43 (Tilcara)	Quebrada	-6.9	-11.6	10.1	Late Period	This work
Don1	Doncellas	Puna	-10.3	-15.5	11.4	Late Period	This work
Til1-1	Til 1 (Tilcara)	Quebrada	-5.6	-11.4	10.4	Late Period	This work
Mean Puna (n=8) -10.1		-15.2	10.3				
Mean Quebrada de Humahuaca (n=9) -5.5			-10.9	10.3			

*Bone values calculated using tooth values of the same individual.

published by Olivera and Yacobaccio are also used in this paper. The addition of data from Olivera and Yacobaccio (2001) contributes to the increase of available information for Puna, particularly in relation to earlier chronological periods. In all cases, information for adults with reliable contextual and chronologic association was selected in order to avoid possible trophic and/or chronologic distortions (Katzenberg *et al*., 1993). A total of seventeen individuals (n=17) are analysed here (Table 1).

Chemical treatment and isotopic measuring of bone samples were performed at the Laboratory for Archaeological Science, Department of Anthropology, University of South Florida (USF), directed by one of the authors (RHT). Bone collagen and apatite were extracted, prepared and analysed isotopically according to the recommendations by Ambrose (1990), Ambrose *et al.* (1997), Koch *et al.* (1997), Lee-Thorp (2000), and Tykot (2004, 2006), among others. Parameters for the observation of collagen preservation levels were determined during the process of sample preparation and isotopic analysis. This procedure included the analysis of the atomic relationship C:N (where C:N=%C/%Nx1.1667), whose acceptable ratio is 2.9-3.6 (Ambrose 1990, 1993; DeNiro, 1985), as a consequence of which lower or higher values to this ratio were ruled out. Values were obtained for bone isotopic rates for carbon [whose isotopic rate is expressed as δ^{13}C value difference between ^{13}C/^{12}C related with their proportions in the standard *Pee Dee Belemnite* (PDB) (Ambrose, 1990, 1993)] both in collagen and apatite ($\delta^{13}C_{col}$ and $\delta^{13}C_{ap}$), as well as for nitrogen [whose isotopic rate is expressed as δ^{15}N in relation to the difference ^{15}N/^{14}N in the air as standard (Ambrose, 1990, 1993)] ($\delta^{15}N_{col}$). In only one case (Til20-2), due to the lack of enough organic matrix in the bone, $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ correlative isotopic values were obtained from a dental root of the same individual. In accordance with the recommendations by Ambrose *et al*. (2003) and Schwarcz (1991), percentages of C4 consumption in diet [*i.e.* $\%C_4=(-25-(\delta_b-\Delta))/15 \times 100$, where -25‰ is the average δ^{13}C value of C3 plants, δ_b is the δ^{13}C value of collagen or apatite, Δ is the fractioning diet-collagen (5.1‰) or diet-apatite (9.4‰), and 15 is the difference between the average C3 and C4 (-10‰) plants (Ambrose *et al*., 2003)] were calculated from δ^{13}C values, estimating a precision of ±10% in collagen as well as in apatite. Given the size of the sample and the non-parametric behaviour of distributions, $\delta^{13}C_{col}$, $\delta^{13}C_{ap}$ and $\delta^{15}N_{col}$ values were compared within and between regions, as well as within and between periods using a Mann-Whitney test.

Results

The results of our research are synthetised in Tables 1, 2 and 3. In Table 1, there are detailed values for $\delta^{13}C_{col}$, $\delta^{13}C_{ap}$ and $\delta^{15}N_{col}$ variables of both original data and those taken from Olivera and Yacobaccio (2001), as well as mean values per region considered. The C:N ratios corresponding to our materials displayed acceptable values, a fact that let us rule out the presence of bias due to diagenetic processes (Ambrose, 1990; DeNiro, 1985). In Table 2, percentages of C4 resource consumption obtained from $\delta^{13}C_{ap}$ and $\delta^{13}C_{col}$ isotopic values for each individual case are presented according to Ambrose *et al*. (2003). Table 3 presents the results obtained through the application of the Mann-Whitney test on $\delta^{13}C_{ap}$, $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$, firstly between regions (Puna *vs* Quebrada), while Table 4 by chronology (formative and archaic developments *vs* late agricultural-pastoralist developments).

Discussion

The percentages of consumption of C4 resources corresponding to individuals from Puna (Figure 2) show an important dispersion and have estimates lower than 50% of consumption of C4 resources for both collagen and apatite (Table 2). This fact may be pointing at diversity in strategies of incorporation of C4 resources, co-assisted by spatial and chronological variations. For example, the existence of intra-regional environmental variations is a well known fact (Ottonello and Krapovickas, 1973; Albeck, 2000). Individual Hua3, characterised by a consumption of C4 resources for apatite superior to 75%, is the one with the largest distance in the general dispersion that characterises the Puna assemblage. According to Olivera and Yacobaccio (2001), based upon archaeobotanic evidence, this value, as well as the one reached by individual Hua2 might be the result of the consumption of CAM resources and not a reliable indicator of corn consumption. With regards to Quebrada de Humahuaca, and considering the precision

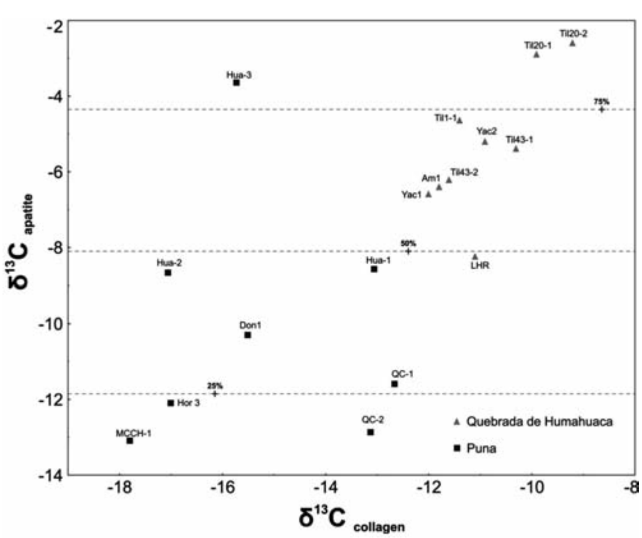

Figure 2. Scatterplots of δ^{13}Cap and δ^{13}Ccol values. The + symbol and unbroken horizontal lines reflect the estimated percentage of C4 consumption (Ambrose *et al.*, 2003).

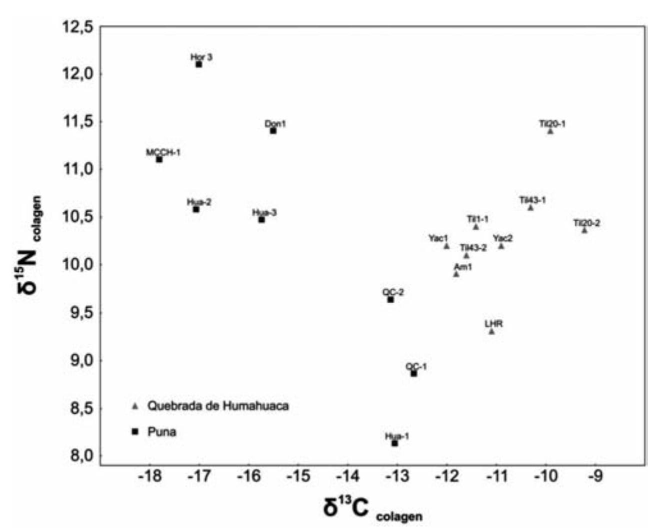

Figure 3. Scatterplot with δ^{13}C and δ^{15}N collagen values classified by regions.

(±10%) of the percentage calculation of consumption of C4 resources (Ambrose *et al*., 2003), it is possible to consider that all the sites from this region surpass the 50% mark (Table 2), and also distribute increasingly towards more positive $\delta^{13}C$ values. This condition could be due to the existence of a differential integration of C3 type resources among the represented populations. Nevertheless, such integration of resources in no case would have reached a level of intensity strong enough to disguise completely the emphasis on the consumption of C4 resources among the populations from the region.

In the Quebrada de Humahuaca set, two individuals from the Formative site SJTil 20 (AD 545-660) have noticeable values. In fact, these two individuals average $\delta^{13}C$ values of -9.6 and -2.8‰ for collagen and apatite respectively (Table 1, Figure 2). These values strongly point to the existence of a very high (>75%) average consumption of corn for the total diet (*i.e*. apatite). This high consumption of corn registered for the Formative site SJTil 20 could represent a case not necessarily comparable to the rest of the Formative settlements in the region, expressing a particularly local economic condition, instead of a regional one. In any case, a study with a larger number of individuals from different Formative sites is required in order to explore an explanation on more solid basis for this noticeable fact.

The comparison of $\delta^{13}C_{col}$ and $\delta^{13}C_{ap}$ values between Puna and Quebrada de Humahuaca using the Mann-Whitney test is significantly different (P<0.05) only for the Late Ceramic Period (Table 3). It is then possible to postulate for the cultural development in Late Ceramic Period, a larger relative consumption of corn in Quebrada de Humahuaca compared to that of Puna. The marked altitudinal gradient between these two regions caused more favorable conditions for the cultivation of C4 plants at lower altitudes (*i.e.* Quebrada de Humahuaca) compared with the Puna (Fernández and Panarello, 1999-2001; Yacobaccio *et al*., 2009). These environmental differences determined a preponderance in Puna of subsistence strategies based on herding of camelids, accompanied by a varied spectrum of C3 resources, while people from Quebrada de Humahuaca developed diets based mainly on the production of alimentary resources with a C4 photosynthetic pathway (*i.e. Zea mays*).

Isotopic values corresponding to the stage of agricultural-pastoralist intensification in Late Ceramic Period adjust well to the theoretical frame mentioned above. Results of the Mann-Whitney test differ according to the environment considered. In Puna there were no significant differences between individuals with different chronologies (Table 4, left columns). In Quebrada de Humahuaca, there are significant differences (P<0.05) between periods, the samples from Formative Period (SJTil20) being the ones that show the highest relative consumption of C4. However, when the probabilities of significant differences for the Mann-Whitney test for small samples (2*1 sided P) are recalculated, values are not significantly different. This difficulty in the identification of chronologic differences could be a consequence of the small number of observations between periods so far available. Broadly speaking, results point to the more favorable environmental conditions present at Quebrada de Humahuaca. Those conditions might have allowed the incorporation in Late Ceramic times of a larger variety of C3 resources. Meanwhile, in Puna sites, the incorporation of C4 resources in the diet would not be strong enough to be seen isotopically.

Variable $\delta^{15}N_{col}$ makes it possible to explore the differences between trophic levels (approx. 3.0 to 3.4‰ between levels), and because of this, it is usually employed as an estimator of relative consumption of animal resources (Ambrose, 2000; Ambrose and DeNiro, 1987; Schoeninger, 1985). The results of the Mann-Whitney test do not permit the identification of differences (P>0.05) between environments or between the two chronologies considered here (Tables 3 and 4). It is then possible to

Table 2. Estimation of the percentage of C4 plants consumed based on isotope fractions for bone apatite and collagen (Ambrose *et al*., 2003).

Site code	C4 apatite (%)	C4 collagen (%)	Region
LHR	49.1	58.7	Qda. de Humahuaca
QC 1	26.7	48.3	Qda. de Humahuaca
QC 2	18.2	45.1	Qda. de Humahuaca
Hor 3	23.3	19.3	Puna
Hua 1	46.8	45.7	Puna
Hua 2	46.2	19.0	Puna
Hua 3	79.7	27.8	Puna
MCCH 1	16.7	14.0	Puna
Lab-Am1	61.3	54.0	Qda. de Humahuaca
Lab-Yac1	60.0	52.7	Qda. de Humahuaca
Lab-Yac2	69.3	60.0	Qda. de Humahuaca
Lab-Til20-1	84.7	66.7	Qda. de Humahuaca
Lab-Til20-2	86.7	71.3	Qda. de Humahuaca
Lab-Til43-1	68.0	64.0	Qda. de Humahuaca
Lab-Til43-2	62.6	55.3	Qda. de Humahuaca
Lab-Don1	35.3	29.3	Puna
Lab-Til1-1	73.0	56.7	Qda. de Humahuaca

For explanation of site codes, see Table 1.

Table 3. Results of the Mann-Whitney test between regions, with separate time periods.

	Mann-Whitney U test – between regions (Arch+Form)		Mann-Whitney U test – between regions (Late Ceramic)	
	P level	2*1 sided P	P level	2*1 sided P
$\delta^{13}C_{col}$	0.0528	0.0952	0.0167*	0.0167*
$\delta^{13}N_{col}$	0.4386	0.5714	0.4250	0.5167
$\delta^{13}C_{ap}$	0.0528	0.0952	0.0167*	0.0167*

Table 4. Results of the Mann-Whitney test between time periods, with separate regions.

	Mann-Whitney U test – between periods (Puna)		Mann-Whitney U test – between periods (Quebrada)	
	P level	2*1 sided P	P level	2*1 sided P
$\delta^{13}C_{col}$	0.6547	0.7857	0.0404*	0.0556
$\delta^{13}N_{col}$	0.6547	0.7857	0.0404*	0.0556
$\delta^{13}C_{ap}$	0.6547	0.7857	0.0404*	0.0556

think that the practice of camelid herding would have not been discontinued in either of the two regions by the time agricultural-pastoralist intensification took place. Nevertheless, it is necessary to highlight the rank of observed values (8.1-12.1; Table 1 and Figure 3), which could be suggesting the existence of possible differential consumption of animal protein (camelids and other species), or the occurrence of periods of differential draughts in both regions (Ambrose, 1991).

Conclusions

Our conclusions must be considered particularly for their exploratory value, and it must be taken into account that the reaches and limitations imposed on them are directly related to the number of observations so far available inside the provincial borders. Results of our experience can be summarised as follows.

Differences among isotopic signals found in the set of selected individuals are due mainly to variations between environments.

Among pre-Hispanic populations from Quebrada de Humahuaca, it can be noted that the diet was strongly based on the consumption of C4 resources, particularly corn. The emphasis on the consumption of C3 resources shows a characteristic trait among Puna sites.

It is possible to consider that, even in times where the intensification of productive agricultural activities was broadened, the consumption of camelids and other alimentary resources was never abandoned and/or diminished.

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