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Research Article

New Data on Food Consumption in Pre-Hispanic Populations from Northwest Argentina (ca. 1000–1550 A.D.): The Contribution of Carbon and Nitrogen Isotopic Composition of Human Bones

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We present data on carbon and nitrogen isotopic composition of human bones from Tolombón (Calchaqui Valley, Salta) and Esquina de Huajra (Quebrada de Humahuaca, Jujuy) sites located in Northwest Argentina (NWA). Both are complex archaeological residential settlements ascribed to the Regional Development Period (ca. 900–1430 A.D.), the Inca Period (ca. 1430–1536 A.D.), and the Early Colonial Period (ca. 1536–1600 A.D.). Twelve samples of human bones were collected and analyzed, including remains from individuals of both sexes and different ages at death. We also present the carbon and nitrogen isotopic composition of modern plants from nearby areas in order to start building an isotopic ecology of the area and compile available information on food consumption from different lines of evidence. The isotopic results obtained reveal the consumption of C_4 plants, which for the area are maize and amaranth, combined with animal proteins. The integration of these results with the broader database was useful to discuss the political and economical implications of the findings, especially in the context of this area under the Inca domination.

1. Introduction

The analyses of carbon and nitrogen stable isotopes were introduced to archaeology in the middle 1970s and have been used worldwide to assess human and animal diets of archaeological populations [1–5]. Their main potential is that they allow direct access to the average diet of an individual's life time before death which for bone samples is expected to reflect the last 7 to 10 years; while for hair samples, the value is expected to reflect a shorter time span [6, pages 137-138], complementing or broadening the interpretations made from traditional archaeological data, such as plant macro- and microremains, faunal remains, artifacts for food processing, or the osteological analysis of nutritional pathologies [7].

In the Andean area, the analyses of carbon and nitrogen isotopes have been used to assess the political implications of food consumption and distribution [8–10], the mobility and subsistence models of pre-Hispanic societies [11–13] or, the study of gendered food consumption in domestic contexts [14].

Following this line, we intend to approach the food consumption profiles of individuals from the archaeological sites of Tolombón (Calchaqui Valley, Salta) and Esquina de Huajra (Quebrada de Humahuaca, Jujuy) located in NWA (Figure 1). The occupation of these sites encompasses a time span characterized by rapid social changes including a period of hostile conflict between communities and the annexation of the area to the Inca Empire, which probably affected these communities lifestyles—including which food was consumed. The Inca Empire (or *Tawantinsuyu* in Quechua) was acknowledged by considering maize as a staple, not only for daily consumption but also for *chicha* making, a traditional alcoholic beverage made from fermented maize which was consumed in feasts and celebrations [15–17]. Nevertheless, as recent isotopic studies demonstrate, maize was a staple in the Andes well before the Inca domination [18–21] but

the increase of maize production seen in certain areas can be related to the empire's strategy of negotiation with local polities. In this regard, maize overproduction could have been used to support *Tawantinsuyu*'s populations, including the production of *chicha* for daily use and for sharing in festivities sponsored by the state [10].

What these studies suggest is that subsistence and diet were a complex matter that surely involved the interlocking of cultural predispositions on what was right to eat, the ecological settings, and the economic and political contexts that changed through time, especially as societies underwent rapid alterations in their social lives. In this regard, we intend to evaluate as many lines of evidence as possible to assess food consumption in two nuclear archaeological areas of NWA from ca. 1000 to 1550 A.D., including the results of recently performed analyses of carbon and nitrogen isotopic composition of human bone. Although we recognize that the sample is small in relation to the time span considered, these are the first results for theses core areas of archaeological development in NWA. In addition, we will consider information from vegetable macro- and microremains, written records, isotopic analysis on human and animal bones, and isotopic composition of edible plants from the area, including those published by other scholars as well as those recently performed in the context of our research agenda. As Tykot [3] mentions, where the consumed food might have included C_4 or CAM plants other than maize, as in our case, it is important to test archaeological faunal remains and other sources of information such as ethnohistoric records to interpret the isotope results.

The theoretical and methodological background for carbon and nitrogen isotope studies in ecology and archaeology in particular have been revised at length in previous literature [19–23]. For the purpose of the present study, it is worth noting that plants that form the basis of the trophic network take up carbon from the atmosphere and fix it in their tissue through photosynthesis, following three different metabolic ways: C_3 (calvin), C_4 (hatch-slack) and CAM (crassulacean acid metabolism). C_3 plants take less atmospheric $^{13}CO_2$ and thus present values of ^{13}C that range from −21‰ to −26‰. Instead, C_4 plants take up more atmospheric $^{13}CO_2$ and their tissues are rich in this element, thus giving ^{13}C values between −7‰ and −12‰ [3]. CAM plants switch between C_3 and C_4 depending on their actual location and environmental circumstances [3], their values ranging from −10‰ to −14‰ if they grow by night following a CAM cycle or from −24‰ to −30‰ if they grow via a C_3 pathway [24]. Generally speaking, it is assumed that an individual living on C_3 resources will show a $\delta^{13}C$ value of −21.5‰ on collagen, while an individual living on C_4 resources will show a $\delta^{13}C$ value of −7.5‰ on collagen. Isotopic fractionation (i.e., changes in the isotopic ratio due to biosynthetic chemical reactions) between diet and values reported in tissues are estimated in the order of 5‰ for bone collagen and from 9.5‰ to 12‰ for bone apatite [6].

Moreover, research suggests that bone collagen mainly derives from diet proteins and that bone apatite reflects all macronutrients included in the diets (i.e., lipids, carbohydrates, and proteins not used in the animal's own protein synthesis) [25, 26]. Thus, when protein and energy (i.e., carbohydrates and lipids) in an individual's diet derive from resources with different isotopic proportions, collagen may not signal the total isotopic composition of the diet but only its protein portion. Some researchers suggested that the whole diet may be inferred from the apatite bone isotopic composition or from the offset between $\delta^{13}C$ on collagen and $\delta^{13}C$ on apatite (i.e., $\Delta^{13}C_{CO\text{-}AP}$) [19, 20]. However, Kellner and Schoeninger [25] have recently suggested that most single measures of $\delta^{13}C$ either for collagen or apatite fall short of expectations for diet reconstruction. They suggest that the value of $\Delta^{13}C_{CO\text{-}AP}$ is not specific to any combination of protein or to the whole diet. Instead, by plotting $\delta^{13}C_{CO}$ and $\delta^{13}C_{AP}$ values from organisms with different known diets, they presented a model of three regression lines which provides a way of distinguishing between diets with C_3 protein and those with C_4 protein. Thus, according to the authors, the increase in $\delta^{13}C_{AP}$ in the three regression lines largely represents the increase of $\delta^{13}C$ values of diet energy (i.e., carbohydrate and lipid except in diets with excessively high protein levels). Taking this into account, we will plot data from $\delta^{13}C$ on collagen and apatite to assess the possible source of protein included in the diet of the individuals analyzed.

The atmosphere is nitrogen's biggest reservoir, and its stable isotope (^{15}N) is present as a gas (N_2). Plants capture nitrogen in two different ways. Some of them do it through special nitrogen fixing bacteria attached to their roots that convert N_2 into forms that can be used by the plant, leading to little fractionation, which means that these plants have N values similar to that of the atmosphere (0‰). Other plants, known as nonfixing, capture N through the decomposition of organic matter present in their habitats. Generally, these plants possess significantly more positive values. The median value for fixing plants is +1‰, with a typical range of −2‰ to +2‰; whilst in the case of the nonfixing, it is +3‰, with a typical range of 0‰ to +6‰ [2, 24].

It has been noted that organisms increase their ^{15}N content in 3 or 4‰ along the trophic chain, both for terrestrial and marine resources. For example, terrestrial mammals have ^{15}N mean values of about 5.7‰ while marine mammals have mean values of 15.6‰ [26]. Nitrogen has proved to be important for investigating topics like weaning or differentiating consumption of marine resources [6], and, unlike carbon, all nitrogen present in bone collagen derives from dietary proteins, representing trophic-level effects in protein intake associated with organisms' positions in food webs [21, 22]. However, several studies have also discussed how geographical and ecological factors such as aridity, humidity, cold, and latitude affect the values of ^{15}N recorded in different tissues. Particularly, a negative relation has been observed between water availability and the values obtained from the collagen of herbivore bones [27]. Research performed by Amundson et al. [28] noted that in drier ecosystems there seems to be a greater loss of nitrogen via lixiviation and its transformation (nitrification, denitrification, and volatility of ammonia) which would lead to the enrichment of ^{15}N in the remaining nitrogen in the system. This fact implies

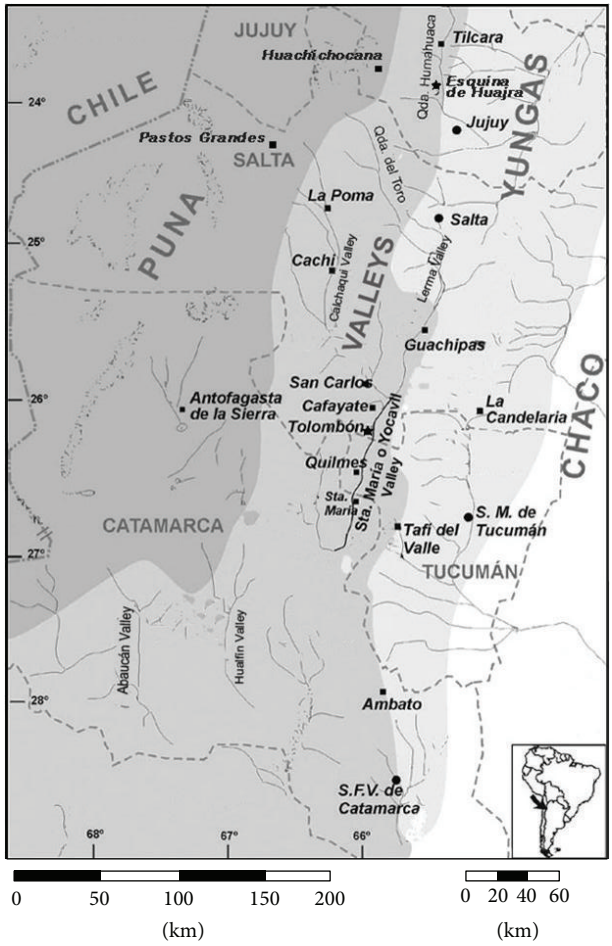

Figure 1: Northwest Argentina: main geographical areas and archaeological sites mentioned in the text. Sites comprised in this study are marked with a star symbol.

that human tissues with enriched δ^{15}N could not reflect the consumption of animal protein, but the consumption of plants with enriched values [29].

2. Materials and Methods

2.1. The Study Area. The analyzed material came from the geographical area located in the northwestern corner of Argentina (Figure 1), specifically from two of its main valleys: Calchaquí Valley in Salta Province and Quebrada de Humahuaca in Jujuy Province. The former is characterized by a semiarid high altitude climate, as winds are depleted in humidity since they cross the mountain range. Mean annual precipitation is about 200 mm in Cafayate and rains are markedly distributed, as they occur more often in the western slopes of the valley during the summer months (December to March) which determines a lower water use due to evapotranspiration [6]. However, there exists a relatively prolonged period free of frosts which is beneficial for agricultural production [30].

The Quebrada de Humahuaca is a deep narrow valley or ravine extending along 120 km and presenting two different climates, semi-arid and arid, according to the variation of precipitations [31]. Mountainous relief influences the total annual precipitations, causing the geographical distribution of rains to be markedly irregular. For example, in the southern sector of the area, mean precipitation values of 391 mm and 199 mm were recorded for Volcán and Tumbaya, respectively, while in Abra Pampa (northern sector) the mean value recorded is 282 mm [31]. Although rains are torrential and are concentrated from November to March, they do not compensate for the marked water stress present in the area [31].

In a general sense, NWA is a geographical region defined by the presence of the Andean mountain range, running in a NS direction, as well as several intermountain valleys, *quebradas*, and a high altitude area (more than 3,500 masl) called *puna* that sustained the development of interconnected societies along its temporal occupation. The most important valleys and *quebradas* from North to South are Quebrada de Humahuaca, Quebrada del Toro, Calchaquí Valley, Santa María or Yokavil Valley, Cajón Valley, and Hualfín Valley (Figure 1). The human occupation in the *puna* areas was concentrated in certain oasis such as Antofagasta de la Sierra in Catamarca Province, Huachichocana, Inca Cueva and Rinconada in Jujuy Province, and Pastos Grandes in Salta Province.

Although NWA has been occupied for more than 10.000 years B.C., when the first mobile hunter-gatherers established in the area, we are interested in the four to five centuries before the Spanish conquest, a time span comprising important economic, political, demographic, and climatic changes that radically shaped the trajectories of these communities. From about 900 A.D., in some places of NWA, communities started to cluster in nucleated settlements, many of them located on landforms with difficult access, such as the slopes or tops of hills and small plateaus. Agricultural intensification played a key role in the development of these societies, which cleared vast extensions of land and built agricultural structures (e.g., irrigation systems, terraces) in Calchaquí Valley [32, 33], Quebrada de Humahuaca [34], and Antofagasta de la Sierra [35]. Different ceramic styles emerged within specific geographical areas like *Santamariano* style in Calchaquí and Yocavil Valleys and *Belén* style in Hualfín and Abaucán Valleys [36] (Figure 2).

While the traditional view of these societies posited that they were organized into chiefdoms that hierarchically ranked settlements levels [37–39], other scholars suggest that communities from the Regional Development Period (RDP, ca. 900–1450 A.D.) were organized into corporative or communal structures with flattened or no social hierarchies at all [40–43]. In this sense, regionalization of ceramic styles and material culture in general points to the existence of political fragmentation, which can also be seen in the replacement of previous dispersed hamlets for key nucleated settlements located in defensive locations. This scenario possibly resulted from the presence of interregional conflict, evidenced in the appearance of war related paraphernalia (e.g., weapons, rock art scenes, and defensive architecture) [44, 45] and violent trauma on human bones [46].

Staple goods in communities from NWA temperate valleys included maize (*Zea mays*), potatoes (*Solanum spp.*),

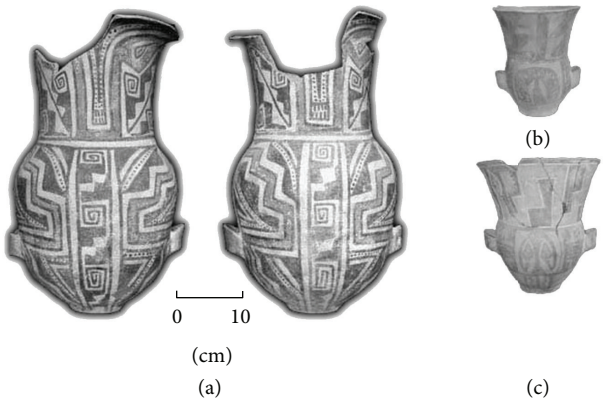

Figure 2: Examples of *Santamariano* and *Belén* pottery styles. (a) *Santamariano*-style urn [56]. (b) *Belén*-style urn from Las Mojarras [57]. (c) *Belén*-style urn from Famablasto [57].

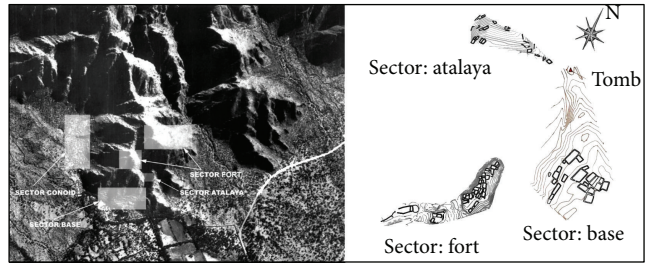

Figure 3: Aerial photograph showing the different sectors of Tolombón and their architectural remains.

grains such as quinoa (*Chenopodium quinoa*) and *kiwicha* (*Amaranthus caudatus*), squashes (*Cucurbita spp.*), peppers such as *ají* (*Capsicum baccatum*), salt, and beans (*Phaseolus spp.*). Outstandingly, apart from maize, *kiwicha* (*A. caudatus*) is the only C_4 plant in the western hemisphere consumed by Precolumbian human populations. Besides, they relied on seasonal resources such as algarroba (*Prosopis nigra* or *Prosopis alba*) and *chañar* (*Geoffroea decorticans*) and meat from camelids and micromammals, among others [47–53].

2.2. The Archaeological Sites. Tolombón is a complex archaeological settlement located in Salta Province near the modern town of Cafayate (Figure 1). Although mentioned in the archaeological literature as early as 1894 by Ten Kate, it was later studied by different archaeological research teams [53]. The settlement consists of architectural remains built on the slopes of a hill, a pattern shared with other sites from the Yocavil Valley (e.g., Rincón Chico, Cerro Pintado, Fuerte Quemado, and Quilmes). Research conducted by Dr. Williams [54, 55] revealed the distribution of the architectural remains in the hill, covering 35 ha, in the following way: (1) a fort located at the top of the hill, a residential area on the slope of the hill, and possibly a public sector; (2) a residential sector located at the base of the hill and another one on the southeastern side of the hill spatially segregated and with agricultural structures between them; (3) more than 17 tombs located on the southeastern side of the hill; (4) staggered structures on the southern side of the hill, over the residential area [55] (Figure 3).

Radiocarbon dating allows ascribing the occupation of the site to the years 1200 to 1600 A.D. (Table 1). The excavation of one of the residential enclosures located in the base sector (Building 6) yielded a number of cultural materials, including metal artifacts and debris, ceramic remains, lithic instruments and by-products, vegetables and faunal remains, and a hearth in the centre of the enclosure. The analysis of these materials allowed for the interpretation of this enclosure as a domestic space where quotidian activities were carried out [55]. Local ceramic styles are abundant in this enclosure (i.e., *Santamariano*, *Famabalasto negro grabado,* and *Belén* styles), while foreign material is scarce, including only a minor percentage of Incaic pottery style. Many undecorated fragments show fire marks, possibly related to their use as cooking vessels [58]. The vegetable macroremains found consisted of five different races or variants of maize (*Colorado*, *Chaucha*, *Socorro*, *Amarillo Chico,* and *Pisingallo*); several caryopses were burnt, suggesting that they had probably been cooked [50] (Table 2).

Faunal remains found in this enclosure were divided into three components. The first one (stratigraphic levels 7 to 10) is mainly composed of camelids (*Lama* sp., *Lama glama* and *Lama vicuña*), although fox (*Lycalopex* sp.), *Chinchillidae,* and bird bones were identified. The second component (stratigraphic levels 3 to 6) presents a variety of resources, including fox (*Lycalopex* sp.), *armadillo* (*Dasypodidae*), *viscacha* (*Chinchillidae*), weasels (*Mustelidae*), ostrich egg shells, and camelids (*Lama* sp.; *Lama glama* and *Lama vicuña*). In the third component (stratigraphic levels 1 and 2) the only osseous remains found were from camelids (*Lama* sp.). In general, faunal remains from this enclosure show the predominant consumption of camelids with a mortality profile of young individuals in two of the three defined components. Additionally, remains of an Andean deer (*Hippocamelus antisensis,* commonly known as *taruca*) were found. Anthropic modifications, like cut marks or thermal alterations, were recorded, suggesting the possible performance of human activities such as slaughter and cooking [55].

During one of the field seasons in Tolombón, a burial place which had been disturbed was discovered in one of the ravines leading to the fort. The tomb was located under a big rock which stood on the sides of a ditch forming a kind of natural roof of 2 m wide and 2.6 m deep, 0.9 m tall in one extreme and 0.4 m in the other. Although the human remains were highly fragmented, osteological analysis allowed determining that at least six individuals were buried, including both adults and subadults [60]. From this funerary context, five individuals were sampled for isotopic analysis (see details in Table 3) using bones that could be clearly associated to an individual.

Esquina de Huajra is located 45 km from the modern city of San Salvador de Jujuy in Tumbaya Department, Quebrada de Humahuaca (Figure 1). The site was built on the slopes of a landform elevated 90 m above the valley level which extends close to the alluvial plain of Río Grande. In 2001, a

Table 1: Radiocarbon dating for Tolombón and Esquina de Huajra.

Site	Code	Material	Chronology B.P.	Cal. A.D. 2 sigmas	Reference
Tolombón	GX-29252	Charcoal	720 ± 60	1221-1400	[55]
	GX-29251	Charcoal	500 ± 60	1327-1612	
	Beta 168672	Charcoal	440 ± 50	1410-1520	
	GX-29663	Charcoal	350 ± 60	1441-1793	
	Beta 171425	Charcoal	460 ± 60	1400-1515	
	Beta 171426	Charcoal	440 ± 60	1405-1525	
Esquina de Huajra	Beta 193319	Charcoal	340 ± 55	1455-1796	[59]
	Beta 206919	Charcoal	280 ± 50	1496-1952	
	AA88375	Charcoal	393 ± 82	1400-1664	
	UGA 16200	Human bone	550 ± 40	1318-1463	
	GX 32577	Human bone	450 ± 60	1419-1627	
	GX 32576	Human bone	320 ± 50	1460-1799	

Table 2: Vegetable macro remains possibly used as food in Tolombón.

Family	Species	Plant or reproductive structure	Reference
Poaceae	*Zea mays*	Fruit (caryopsis)	[50]
Fabaceae	*Prosopis* sp.	Seeds and fruits (pods)	
Fabaceae	*Prosopis ferox*	Fruits (pods)	

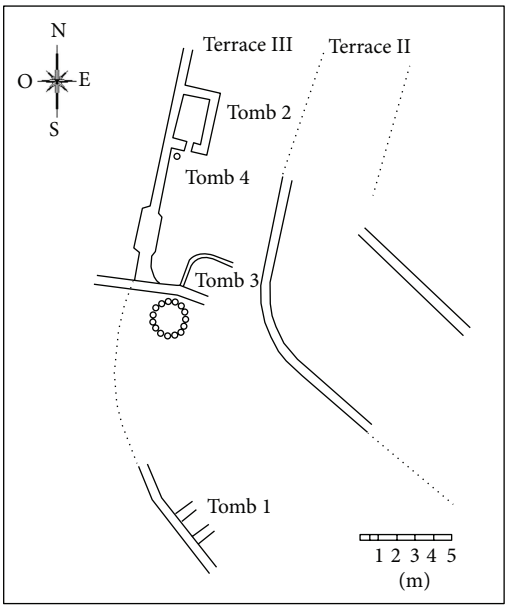

Figure 4: Plan of the tombs from Esquina de Huajra.

salvage project was conducted at the site and six tombs were found, along with other archaeological materials [61]. The tombs were located in what was called Terrace III, and five of them were excavated (tombs 1, 2, 3, 4, and 6) (Figure 4). The laboratory analysis of the human bone remains allowed establishing the presence of at least 18 individuals, comprising adults of both sexes and unsexed subadults of different ages at death [61].

Radiocarbon analyses postulate an occupation span ranging from 1400 A.D. onwards (Table 1). This would imply that the site was occupied (and possibly built) in a late stage of the Inca Period, continuing this occupation in the initial stage of the Early Colonial Period, although the real control of the territory by the Spaniards was not exerted until 1600 A.D. approximately [59]. This is consistent both with the findings of Inca material culture in the tombs and the absence of Spanish objects in the site. The analyses of the materials recovered suggest the performance of different activities in the sectors of the site. In Terrace I, a possible patio was excavated, which contained a hearth with carbon lenses, ceramic and bone fragments, a stone grinder, red pigment, and several ceramic remains. This occupation floor also contained abundant animal remains, two bone instruments, and a metal instrument [61, 62]. The ceramic assemblage contains some of the typical Inca forms present in the provinces of the Empire [63]: footed *ollas*, small plates, plates and *aríbalos* possibly related to *chicha* elaboration and consumption [59] and nonlocal ceramics (i.e., *Chicha/Yavi, Inca Pacajes,* and *Casabindo Pintado*). The lithic material obtained points to the performance of reduction activities directed to obtaining base forms [61]. Faunal remains analyzed by Mengoni Goñalons [64, 65] came from this domestic context and correspond to members of the Camelidae family, including llama (*Lama glama*), vicuña (*Lama vicugna*), and guanaco (*Lama guanicoe*). As in Tolombón, remains from Cervidae were found, including a specimen of *taruca* (*Hippocamelus antinensis*), as well as rodents (undetermined) and birds (*Cairina moschata*).

In turn, Terrace II probably functioned as a circulation path. More than 1,000 ceramic fragments were found, half of them undecorated and corresponding to medium and large vessels and *ollas*. A few fragments were from the body of five small vessels, and also bowl fragments were recovered. Nonlocal pottery fragments corresponded to *Chicha/Yavi* bowls and six polished bowls [61]. As far as we know, most of the surface of Terrace III seems to have been used mainly as a funerary space [59], as six tombs containing the remains of several individuals were found and analyzed. Five of the six detected tombs could be excavated, and six samples of human bone were collected for isotopic analysis, including adult

Table 3: Carbon and nitrogen results for human bone samples.

Lab Code	Site/sample code	Chronology	Sex/age	Bone	δ^{13}C‰ Apatite	δ^{13}C‰ Collagen	δ^{15}N‰	$\Delta^{13}C_{CO\text{-}AP}$	C/N ratio
UGA 16200	Esquina de Huajra T1G2MS	550 ± 40 AP	Male Adult	Left humerus fragment	−4.8	−9.63	6.7	4.83	2.66
UGA 2087	Esquina de Huajra T1G1MS		Male Adult	Left humerus fragment	−6.2	−11.0	12.6	4.8	2.7
Geochron 32577	Esquina de Huajra T2 I4	450 ± 60 AP	Indeterminate 8 ± 2 years	Left radius fragment	−6.5	−11.1	9.7	4.6	Not performed
UGA 2088	Esquina de Huajra T2I1		Indeterminate 6 ± 2 years	Right tibia fragment	−5.2	−11.3	11.7	6.1	2.68
Geochron 32576	Esquina de Huajra T3I1	320 ± 50 AP	Female 40 ± 5 years	Left femur fragment	−6	−11.3	12.2	5.3	Not performed
UGA 2089	Esquina de Huajra T4I1		Indeterminate 7 ± 2 years	Right femur fragment	−6.6	−12.0	11.3	5.4	2.72
UGA 2090	Esquina de Huajra T6I1		Possibly Female 17–21 years	Right tibia fragment	−6.7	−11.5	12.1	4.8	7.95
Geochron 32578	Tolombón T4C1L1		Indeterminate Indeterminate	Left humerus fragment	−4.0	−9.30	9.0	5.3	Not performed
UGA 16199	Tolombón T4C2L1		Indeterminate Indeterminate	Left humerus fragment	−4.8	−9.03	3.2	4.23	2.66
UGA 16201	Tolombón T4C1L1		Indeterminate Indeterminate	Left humerus fragment	−5.0	−9.63	11.1	4.63	2.7
UGA 2085	Tolombón T4C4L1		Indeterminate Indeterminate	Right humerus fragment	−4.7	−9.9	10.1	5.2	3.05
UGA 2086	Tolombón T4L5		Indeterminate Indeterminate	Left humerus fragment	−4.9	−9.3	11.7	4.4	2.74

individuals from both sexes and unsexed subadults from different ages as described in Table 3. These bone elements were selected so as to have at least one sample from each tomb.

2.3. The Methods. The isotopic analyses were carried out in different laboratories, implying that, although results are reported relative to the same standard (VPDB for δ^{13}C and Air for δ^{15}N) some caution must be taken when comparing and combining data. A first set of human bone samples was sent to the Center for Applied Isotope of the University of Georgia (CAIS) and a second set to Geochron. The bone was cleaned with wire brush and washed using ultrasonic bath. After cleaning, the dried bone was gently crushed into small fragments. The crushed bone was treated with 1 N acetic acid to remove any secondary carbonates at room temperature with periodic evacuation of CO_2 for 24 hours. The residue was filtered and rinsed with deionized water to remove any remains of acetic acid. Then, the sample was dried overnight at 60°C. The dried sample was evacuated in the flask and treated with diluted HCl at 4°C to recover CO_2 from bioapatite. The carbon dioxide was cryogenically purified and collected for stable isotope ratio analysis. The acid solution was collected and left overnight at 4°C. Then the solution was filtered on fiberglass filter, and precipitate was rinsed with deionized water. The precipitate was boiled in deionized water (pH = 3) for 6 hours to dissolve collagen and leave humic substances in the precipitate. The collagen solution was then filtered and dried out to isolate pure collagen. The dried collagen was combusted at 575°C in an evacuated/sealed Pyrex ampoule in the presence of CuO. The carbon dioxide and nitrogen were cryogenically separated and purified and collected in the flasks for analyses. The δ^{13}C sample was measured with respect to PDB, with an error of less than 0.1‰; the δ^{15}N sample was measured with respect to atmospheric air nitrogen, with an error of less than 0.2‰. At CAIS, stable isotope ratio analyses were conducted on Finnigan MAT252 mass spectrometer. The C/N ratio was measured using Perkin-Elmer 2400 CHN analyzer. At Geochron, a VG Micromass gas source stable isotope ratio mass spectrometer was used.

Additionally, modern plants from Calchaquí Valley were isotopically characterized. They consisted of seeds from quinoa, amaranth, squashes, and maize collected from a farm located in Animaná (Salta). No industrial fertilizers were used in order to avoid any potential nitrogen enrichment [66]. Samples were raw and frozen-dried and ground prior to characterization of δ^{13}C and δ^{15}N. These samples were sent to the Instituto Nacional de Geología Isotópica of the Universidad de Buenos Aires. The specimens were washed by ultrasonic bath, dried for 24 hours at 60°C, and crushed into 1 mm fragments. Three mg of each sample was weighed in tin capsules and processed in a Carlo Erba EA1108 elemental analyzer coupled to an isotope ratio mass spectrometer of continuous flow interfaces (Thermo Scientific Delta V Advantage ConFlo IV).

Modern plant samples were corrected for the Suess Effect; that is, the reduction by at least 1.5‰ of δ^{13}C values in the atmosphere as a consequence of the use of fossil fuels during the recent industrial period. It is, therefore, necessary to correct these samples by adding 1.5‰ at the moment of using them to reconstruct paleodiets and to plot them along archaeological data [25].

Table 4: δ^{13}C and δ^{15}N for modern plants in the Calchaquí area. Carbon results were corrected for postindustrial enrichment.

Code lab	Material	δ^{13}C	δ^{15}N	C/N ratio
27626	Quinoa	−22.7	9	21.9
27627	Gourd	−21.5	7.1	11.4
27628	Squash	−24.5	6.1	16.7
27629	Sweet gourd	−26	8.2	11.3
27630	Red maize	−10	4.2	44.9
27631	*Kiwicha*	−10.8	14	18.7

We also developed a database of published data on carbon and nitrogen isotopic composition of archaeological and modern plants, including results from Argentina [67] and Perú [68]. Isotopic data from archaeological camelid bones were taken from Mengoni Goñalons [64, 65] and included four samples for Tolombón and five samples for Esquina de Huajra. Only data containing both carbon and nitrogen information were considered, and median values were presented for each type of data (i.e., maize, camelid, etc.). Carbon isotopic results for the modern plant samples were corrected for postindustrial enrichment of atmospheric ^{12}C, in order to make these data comparable with archaeological llama and human δ^{13}C results.

3. Results

The isotopic results for the Tolombón and Esquina de Huajra human bone samples are summarized in Table 3. The results for analyzed food samples are summarized in Table 4. To compare the values, we first designed a graphic plotting δ^{13}C and δ^{15}N values from modern and archaeological plants and fauna against human bone samples for Esquina de Huajra (Figure 5) and Tolombón (Figure 6).

If we first consider the values for $\delta^{13}C_{col}$, it is observed that results both for Esquina de Huajra and Tolombón are comfortably included in the range of C_4 plant group (i.e., maize, amaranth). Interestingly, camelid diet from both sites is no far from that of humans, showing only subtle differences. As mentioned above, collagen reflects the protein portion of the diet consumed in the last 5 to 7 years of life. In this regard, the results obtained for both sites show that the ingested proteins came from a C_4 source mainly. Moreover, data from carbon apatite suggest that energy also came from a C_4 source, as we shall discuss later.

In the case of nitrogen (δ^{15}N), the value reflects directly the kinds of protein incorporated to the diet, which may be animal, vegetal, leguminous or marine, in relation to the position of an organism in a food web. Assuming a roughly 3‰ enrichment between food source and consumer, the δ^{15}N results obtained would signal the consumption of organisms with N values between 4‰ and 8‰ of δ^{15}N in their diets, agreeing with faunal references (camelids only)

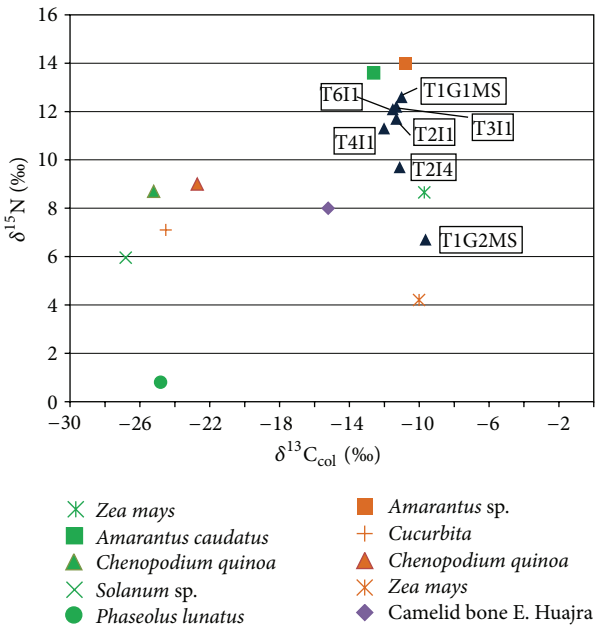

Figure 5: Plot of modern plants from database (green markers), modern plants from the current study (dark orange markers), and archaeological fauna against human bone samples from Esquina de Huajra.

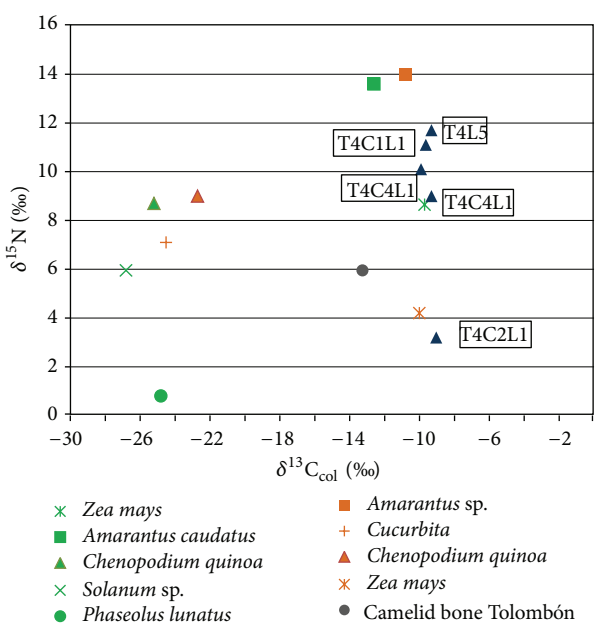

Figure 6: Plot of modern plants from database (green markers), modern plants from the current study (dark orange markers), and archaeological fauna against human bone samples from Tolombón.

from both sites. There is an individual from Tolombón (T4C2L1) with a very low value of δ^{15}N (3.2‰) that is difficult to interpret and a person from Esquina de Huajra (T1G2MS) that shows the lowest δ^{15}N value of the sample from this site (6.2‰), although not as low as the one from Tolombón.

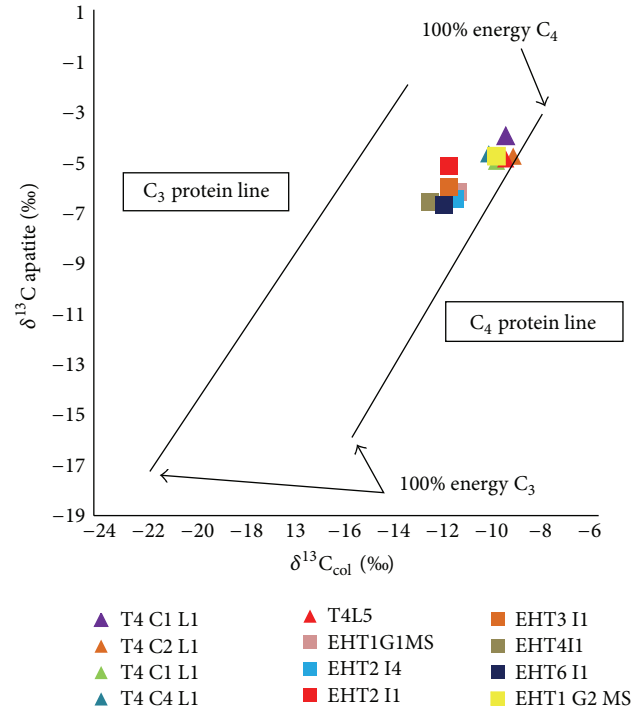

Figure 7: Data from this study contrasted to Kellner and Schoeninger [25] regression lines.

Remarkably, values of δ^{15}N for human bone show a higher dispersion than δ^{13}C values, as nitrogen values for reference food sources do.

We contrasted our data to those provided by Kellner and Schoeninger [25], who have questioned the utility of the difference between the value of carbon on collagen and apatite ($\Delta^{13}C_{CO\text{-}AP}$) to assess the whole diet of an individual, and have indicated the necessity of considering the photosynthetic path in which protein and energy resources were separately inscribed. What we can see from the following plot (Figure 7) is that both subsets are form discrete clusters, except for one individual from Esquina de Huajra (T1G2MS) which is fully included into Tolombón's cluster. Both clusters are located over the 100‰ C_4 energy line, although Esquina de Huajra's individuals are slightly closer to the C_3 energy line.

4. Discussion

The samples' range in δ^{13}C encompasses much of the values found in C_4 resources available for animal and plant data presented here and in other studies. Interestingly, camelid diet is quite similar to that of humans. Isotopic data from camelid bone from Esquina de Huajra suggest that their diet was quite homogeneous and that they would correspond to locally raised herds or to animals that lived freely in low altitude grasslands [64]. The diet of camelids from Tolombón is more variable, suggesting that they obtained food from different ecological zones. Some of them, especially larger animals like *llamas*, were raised in lower areas (less than 4000 masl) while smaller animals, like *vicuñas*, might

have been brought from the Highlands (close to or over to 4,000 masl) [64]. In spite of these differences, human diets from both sites seem quite homogeneous, at least for carbon values, as they cluster tightly. This would imply that diets from both adults and subadults and both males and females were quite similar; moreover, they came primarily from a C_4 source, as we can see in Figures 4 and 5.

In contrast, $\delta^{15}N$ values for human bone are more varied, ranging from 3‰ to almost 13‰ for the samples considered, which was also noted for camelid bones from both sites [64] and, as we can see in this study, modern vegetal resources show higher dispersion in nitrogen values. As we previously discussed, it is possible that in arid or semi-arid environments as the one we are dealing with, plants respond physiologically to water stress by showing higher nitrogen values, which would prevent or at least warn about interpreting results *vis-à-vis* protein consumption. In this regard, it is important to highlight that our sample has a tendency towards protein consumption both in carbon and nitrogen values.

Judging from the data presented, the origin of all dietary sources (protein and energy) came mainly from C_4 resources. It is interesting to consider that some written documents of the Colonial era (after 1540 A.D. approximately) point to a wider diet, quoting Governor of Tucumán, Don Mercado de Villacorta: "*the calchaquíes* [are] *more supplied than others, as they do not content with only maize, but wheat and barley and legumes and potatoes and quinoa and algarroba* ..." [51]. Regarding the consumption of the latter, this resource seems to have been especially important as several written sources mention quarrels over its exploitation. For example, Torreblanca mentions that "... *the* quilmes *and other nations,* [in] *a year of famine, (...) had no resources and were going to perish if they did not become friends with the* pacciocas*, who had abundance and were owners of San Carlos, where a large quantity of algarroba existed, they made peace and the enemies' towns were depopulated to pick up the algarroba* ..." [51]. The archaeological record of the regions points to a similar direction, as maize remains were found in Molinos I, La Paya, and El Churcal as well as gourd, squash, and beans in La Paya and El Churcal [69, 70]. In Valdéz site, besides corn grains and cobs, remains of *Chenopodium*, tubers (*Solanum*), beans, and peppers were also found [47]. In the northern sector of Calchaqui Valley, D'Altroy and coauthors [47] mention the existence of maize, quinoa, and tubers in both Potrero de Payogasta and Cortaderas Bajo sites. In Los Graneros site, Tarragó and González [71] mention the presence of maize, beans, gourd, and algarroba macro remains.

The source of C_4 energy and protein could have been provided by maize, amaranth, or animals that, in turn, consumed C_4 plants (as mentioned, camelid isotopic data points to this direction) [64, 65]. The overconsumption of maize in American pre-Hispanic populations has been traditionally linked to the presence of iron deficiency anemia, which, in turn, was linked to the appearance of porotic lesions in the skull (i.e., porotic hyperostosis) and the orbits (i.e., cribra orbitalia) [72]. The absence of porotic lesions in relation to diets with a high component of maize was already noted [73, 74]. Osteological markers of nutritional stress in the population of Esquina de Huajra are present in a low percentage (only one male adult showed mild signs of porotic hyperostosis [73]), and although the preservation of the remains from Tolombón analyzed here prevents a thorough examination in this respect, the study of a collection of nine skulls from this site carried out by one of the authors [75] did not show positive results for the presence of porotic lesions in the skull or orbits. This observation may indicate two possibilities: whether the link between lesions traditionally associated with iron deficiency anemia in relation to high maize consumption is incorrect, as recently published studies suggest [76, 77], or populations may have achieved an adequate balance between maize consumption and other resources (e.g., grasses, legumes, tubers, and meat) that possibly counteracted the negative effects of the sole consumption of maize. As one of the reviewers of this paper suggested, the values obtained for $\delta^{13}C$ on apatite and collagen, although signaling the importance of C_4 plants consumption, leave room for the consumption of other resources as well.

Our results on carbon and nitrogen isotopes for modern amaranth do not allow us to rule out the consumption of this plant, even though no archaeological findings of this cultivar were made in the area of study. Medina et al. [78] mention that Amaranthaceae pollen remains found in some archaeological sites from Córdoba (Argentinean Central area) may correspond to the species *Amaranthus caudatus*. Babot [79] mentions the finding of amaranth remains in Peñas Chicas and Punta de la Peña (Antofagasta de la Sierra, Catamarca), El Remate and Cueva de los Corrales (Tucumán, Argentina), and Los Viscos (Belén, Catamarca), but, as far as we are concerned, no remains of this plant have been found in Quebrada de Humahuaca nor in Calchaqui Valley. The $\delta^{15}N$ value reported here for this pseudocereal is substantially enriched and similar to the results presented by Turner et al. [68]; it is also slightly higher than nitrogen values obtained for the human bone samples in our study, requiring further efforts in isotopically characterizing this cultivar.

5. Conclusions

This study has provided the first data on paleodiet of small scale complex societies from Northwest Argentina through analyzing the variation of carbon and nitrogen isotopes in a sample of individuals from archaeological sites of temperate valleys. Some advance has been made in starting to construct an isotopic ecology of the area. Although the sample is small, both for human bones and modern plants, the contribution lies in the originality of the results, especially for a nuclear area of archaeological research in NW Argentina.

In this regard, our results serve to posit the importance of C_4 plants for the diet, which could include maize and amaranth, before the arrival of the Inca Empire to the region, as Tolombón results show an important component of C_4 plants in the individuals' diet. Apparently, this importance continued in the Inca period, as results from Esquina de Huajra reveal a high consumption of C_4 plants. Research in Central Chile reports the first enriched $\delta^{13}C$ on collagen and apatite from 1800 B.P. in Llolleo populations, signaling the importance of maize for these communities [11]. Interestingly,

during the Inca period, δ^{13}C values decrease both in collagen (mean δ^{13}C–13.1‰) and apatite (mean δ^{13}C 5.9‰), which do not coincide with expectations regarding the archaeological and bioarchaeological record [11]. In a study that compared isotopic information from *puna*, valleys, and *quebradas* from NWA, Killian Galván and Samec [80] did not find a temporal tendency towards more enriched δ^{13}C, although this was expected from previous research which emphasized that the consumption of maize would have progressively increased through time. Also, the research conducted by Gil et al. [67] with samples from Mendoza (Central Western Argentina) has not allowed for the postulation of a clear tendency towards increasing maize consumption in this region of South America.

Different results were reported from Peruvian populations, where maize consumption seems to have been intensified during the Inca domination. Burger et al. [8] report that, although maize was consumed with different intensity in farmer communities (Waman Wain), in communities with elite members (Jauja), and in Machu Picchu, with retainers serving the Inca nobility (*yanaconas*), it seems to have been a staple. This information not only supports the politics fostered by the Incas of replacing local cultivars but also the idea of intensification and expansion of agricultural lands under their dominion [8]. In a similar vein, Hastorf and Johannessen [10] argued that during the Wanka III period (Inca) in Mantaro Valley, stable isotopes results suggest that maize consumption was increased but probably in the form of *chicha* drinking in connection to feasts sponsored by the state. However, Finucane et al. [9] indicate that maize was a staple well before the Inca Period in Conchopata and even suggest that it might have been the resource that supported one of the first urban development of the Peruvian Sierra and its associated social complexity.

This study, therefore, illustrates the potential of using multiple lines of evidence, despite noted limitations in sample size, for interpreting food consumption in prehistory. In this regard, the archaeological record of the study areas shows a variety of resources not entirely reflected in the isotopic values obtained for the human bone samples. Thus, this implies improving the studies on the isotopic ecology of the areas, considering resources not yet included (e.g., peppers, tubers, *algarroba*, *chañar*, and micromammals). Finally, we consider it imperative to develop a model for the contribution of carbon and nitrogen to the protein and nonprotein portions of bones in cases of mixed diets.

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