

Stable Isotopes and Archaeology in Central Chile: Methodological Insights and Interpretative Problems for Dietary Reconstruction

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ABSTRACT This paper discusses the problems faced when making interpretations of human stable isotope values due to the various explanatory alternatives that arise when reading archaeological data. These interpretative issues are analysed and discussed using the isotopic results for approximately 100 human individuals from archaeological contexts spanning from 5000 BC to 1540 AD in central Chile, supported by data for more than 50 plant and animal samples to establish a local C₃ and C₄ baseline. A number of assumptions are frequently used to establish the bridge between isotopic results in human tissues and their corresponding diets. The problem is that different assumptions lead to different dietary reconstructions. Past feeding experiments on herbivores, pigs, rats and mice give different results, so we need to be cautious when applying these models to human isotope data. One specific problem concerns estimates of % C₄ from collagen and apatite data, a very important issue when looking for evidence of maize in archaeological contexts, which was one of the major objectives we had in the isotopic analyses of archaeological specimens in central Chile. We conclude that the opportunity for estimating the actual percentage of C₄ foods in human diets is limited, since a specific apatite fractionation value for humans cannot be experimentally determined, while maize consumption is underrepresented in bone collagen. This may be addressed in our study by sampling more specimens of wild gramineae to establish baseline plant values, more humans that could have had a low maize intake, and more Archaic period individuals when there was certainly no maize in the region. Copyright © 2009 John Wiley & Sons, Ltd.

Key words: stable isotope analyses; archaeology of central Chile; fractionation models; prehistoric human diet

Introduction

The experience of using stable isotope data for reconstructing dietary patterns of pre-Hispanic populations in central Chile has alerted us to the

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difficulties in applying different models of isotopic fractionation to human samples, especially for bone apatite. In this paper our objective is to discuss the problems which we faced when making interpretations of the isotopic signals due to the different explanatory alternatives that arise when reading the data. These interpretative issues are analysed and discussed using the isotopic results on approximately 100 individuals, from archaeological contexts spanning from 5000 BC to 1540 AD in a small region (Figure 1; Table 1) (Falabella *et al.*, 2007, 2008). Fifty-one plant and animal samples were also analysed to establish a local C_3 and C_4 baseline. All samples were prepared and analysed using established techniques, including removal of potential carbonate contaminants, evaluation of collagen degradation, and reliability and precision of the mass

spectrometer data. This work, done at the University of South Florida, used the same procedures as for many other projects in South America (e.g. Gil *et al.*, 2006a,b; Tykot *et al.*, 2006). Further details on the collagen and apatite sample preparation, stable isotope analysis and reliability testing are available in our earlier publications.

Many years ago the well-known sentence 'you are what you eat' inspired in the work of DeNiro and Epstein (1976) became the paradigm for dietary interpretation based on isotopic analysis (see Tykot, 2006, for a history of stable isotope studies). It stated that the chemical composition of bones is determined by the foods we eat, and that there is a linear relationship between the isotopic composition of consumers' tissues and their diet. Later, 'you are what you eat + 5‰' (van

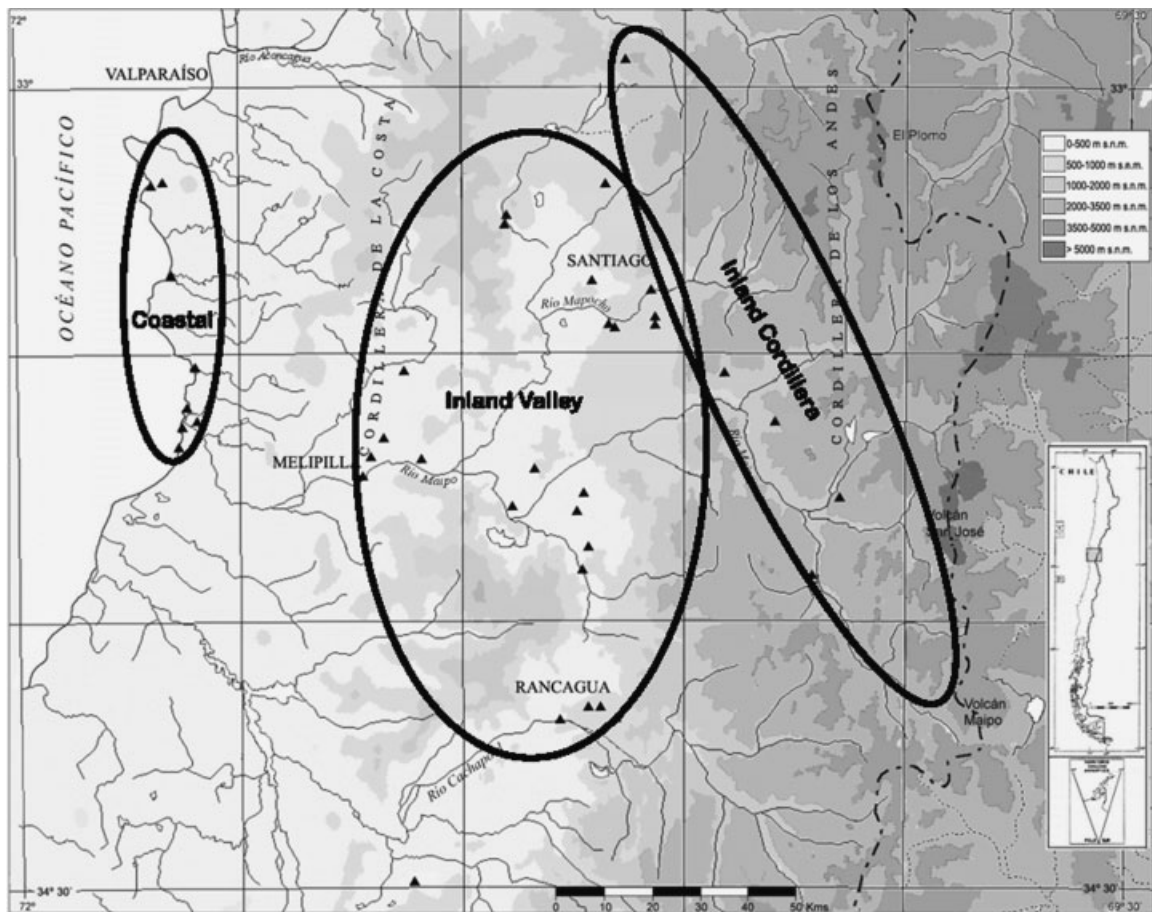


Figure 1. Map showing archaeological sites in central Chile with skeletal remains isotopically analysed in this study.

Table 1a. Isotopic values for samples analysed from inland sites

Context ¹	Chronology	Archaeological site	Sex ²	Region	$\delta^{13}\text{C}_{\text{COL}}\%$	$\delta^{15}\text{N}\%$	$\delta^{13}\text{C}_{\text{AP}}\%$	$\delta^{13}\text{C}_{\text{AP-COL}}$	
Archaic	5000–3000 BC	Alero Queltehues	nd	Cordillera	–17.9	3.9	–9.4	8.5	
	3000–300 BC	Los Hornos	M	Cordillera	–17.7*		–11.1	6.6	
CAI	3000–300 BC	Alero La Paloma	F	Cordillera	–19.2	7.6	–12.5	6.7	
	200 BC–200 AD	Lenka Franulic	M	Valley	–20.9*		–11.9	9.0	
	200 BC–200 AD	Valle Verde	F	Valley	–20.1	5.5	–10.0	10.1	
	200 BC–200 AD	Valle Verde	F	Valley	–19.8	4.3	–12.8	7.0	
	200 BC–200 AD	Valle Verde	nd	Valley	–20.3	3.8	–13.5	6.8	
	200 BC–200 AD	Valle Verde	nd	Valley	–20.3	4.1			
	200 BC–200 AD	Valle Verde	F	Valley	–20.2	4.1	–12.3	7.9	
	200 BC–200 AD	Valle Verde	M	Valley	–19.6	5.2	–12.7	6.9	
Other	60 BC–1035 AD	La Batea 1	F	Cordillera	–19.7	3.8	–13.5	6.2	
	680–900 AD	Chacayes	nd	Cordillera	–18.6	7.6	–10.3	8.3	
Bato	660–900 AD	Paso Agrícola	M	Valley	–19.7	6.3	–10.4	9.3	
	575–766 AD	Hospital 8-9	nd	Valley	–17.0	5.8	–8.8	8.2	
Llolleo	350–1000 AD	El Mercurio	F	Valley	–17.6	4.9	–5.6	12.0	
	350–1000 AD	El Mercurio	M	Valley	#	#	–7.7		
	350–1000 AD	El Mercurio	M	Valley	#	#	–9.6		
	350–1000 AD	El Mercurio	F	Valley	#	#	–9.8		
	350–1000 AD	El Mercurio	M	Valley	#	#	–9.1		
	350–1000 AD	El Mercurio	F	Valley	#	#	–9.3		
	350–1000 AD	El Mercurio	F	Valley	#	#	–9.7		
	350–1000 AD	El Mercurio	F	Valley	–16.1	5.0	–10.3	5.8	
	350–1000 AD	El Mercurio	F	Valley	#	#	–9.6		
	350–1000 AD	El Mercurio	F	Valley	#	#	–9.7		
	350–1000 AD	Villa Virginia	M	Valley	–13.6	5.7	–8.0	5.6	
	350–1000 AD	Alto Jahuel	F	Valley	–13.2	6.2	–6.8	6.4	
	350–1000 AD	Cond. Los Llanos	F	Valley	–12.8	5.7	–8.1	4.7	
	350–1000 AD	Las Pataguas	F	Valley	–12.8	7.5	–8.3	4.5	
	350–1000 AD	Las Pataguas	M	Valley	–14.0	6.9	–9.2	4.8	
	350–1000 AD	Las Coloradas	nd	Valley	–13.9	6.4	–7.21	6.7	
	350–1000 AD	Las Coloradas	nd	Valley	#	#	–6.6		
	350–1000 AD	Las Coloradas	nd	Valley	–14.7	5.1	–7.4	7.3	
	350–1000 AD	La Granja	F	Valley	–14.0	5.6	–7.5	6.5	
	350–1000 AD	Country Club	nd	Valley	–13.7	5.0	–8.0	5.7	
	Aconcagua	350–1000 AD	Lonquén	M	Valley	–12.7	5.7	–7.3	5.4
		1000–1450 AD	Pomaire	M	Valley	–10.3	7.5	–5.5	4.8
		1000–1450 AD	María Pinto	F	Valley	–12.7	6.7	–8.1	4.6
		1000–1450 AD	María Pinto	F	Valley	–12.4	7.1	–7.6	4.8
		1000–1450 AD	María Pinto	F	Valley	–12.0	6.3	–6.9	5.1
		1000–1450 AD	María Pinto	M	Valley	#	7.6	–7.1	
		1000–1450 AD	María Pinto	M	Valley	–12.2	7.5	–7.3	4.9
1000–1450 AD		María Pinto	M	Valley	–10.7	8.1	–7.5	3.2	
1000–1450 AD		El Bajo Melipilla	nd	Valley	–10.4	7.5	–6.5	3.9	
1000–1450 AD		Chiñigüe 2	nd	Valley	–12.5	5.0	–6.2	6.3	
1000–1450 AD		Las Mercedes	F	Valley	–12.0	5.8	–6.3	5.7	
1000–1450 AD		Las Mercedes	M	Valley	–10.8	6.6	–6.1	4.7	
1000–1450 AD		Valle Chicauma	F	Valley	#	#	–6.1		
1000–1450 AD		Valle Chicauma	F	Valley	#	#	–5.7		
1000–1450 AD		Valle Chicauma	F	Valley	–11.6	7.6	–5.8	5.8	
1000–1450 AD		Valle Chicauma	M	Valley	–11.8	5.7	–5.9	5.9	
1000–1450 AD		Valle Chicauma	M	Valley	–10.6	7.4	–5.7	4.9	
1000–1450 AD		Valle Chicauma	M	Valley	–11.4	8.1	–6.7	4.7	
1000–1450 AD		El Almendral	F	Valley	–12.8	7.3	–7.7	5.1	
1000–1450 AD		Carrascal 3	M	Valley	–11.9	5.7	–7.5	4.4	
1000–1450 AD		Carrascal 3	M	Valley	–11.7	5.6	–6.4	5.3	
Inca	1000–1450 AD	Chacayes	M	Cordillera	–11.3	5.6	–6.7	4.6	
	1450–1540 AD	Villa Galilea	M	Valley	–13.5	8.5	–8.1	5.4	
	1450–1540 AD	Villa Galilea	nd	Valley	–13.5	6.6	–8.6	4.9	
	1450–1540 AD	Línea 5 Metro	nd	Valley	–12.8	5.8	–7.5	5.3	

(Continues)

Table 1a. (Continued)

Context ¹	Chronology	Archaeological site	Sex ²	Region	$\delta^{13}\text{C}_{\text{COL}}\%$	$\delta^{15}\text{N}\%$	$\delta^{13}\text{C}_{\text{AP}}\%$	$\delta^{13}\text{C}_{\text{AP-COL}}$
	1450–1540 AD	Línea 5 Metro	F	Valley	–12.8	4.8	–6.2	6.6
	1450–1540 AD	Línea 5 Metro	F	Valley	–12.3	5.4	–7.5	4.8
	1450–1540 AD	Línea 5 Metro	M	Valley	–13.1	4.8	–6.5	6.6
	1450–1540 AD	Carrascal 1	nd	Valley	–15.3	5.5	–7.7	7.6
	1450–1540 AD	Carrascal 1	M	Valley	–12.9	5.9	–6.9	6.0
	1450–1540 AD	Las Tinajas Quil.	M	Valley	–11.6	6.5	–7.5	4.1
	1450–1540 AD	Las Tinajas Quil.	F	Valley	–14.7	5.9	–8.0	6.7
	1450–1540 AD	Las Tinajas Quil.	M	Valley	#	#	–6.7	
	1450–1540 AD	Lenka Franulic	F	Valley	–13.4	5.7	–7.7	5.7
	1450–1540 AD	Nos	F	Valley	–10.5	7.0	–5.6	4.9
	1450–1540 AD	Nos	M	Valley	#	#	–9.3	
	1450–1540 AD	Nos	nd	Valley	–14.4	5.8	–6.7	7.7
	1450–1540 AD	Nos	F	Valley	–13.4	4.4	–7.7	5.7
	1450–1540 AD	Nos	F	Valley	–13.0	6.4	–7.2	5.8

¹CAI = Comunidades Alfareras Iniciales; Other = other or uncertain cultural group.

²nd, not determined; F, female; M, male.

* $\delta^{13}\text{C}$ value from AMS analysis.

Sample with no or unreliable collagen yield.

Table 1b. Isotopic values for samples analysed from coastal sites

Context	Chronology	Archaeological site	Sex	Region	$\delta^{13}\text{C}_{\text{COL}}\%$	$\delta^{15}\text{N}\%$	$\delta^{13}\text{C}_{\text{AP}}\%$	$\delta^{13}\text{C}_{\text{AP-COL}}$
Archaic	3000–300 BC	Laguna El Peral-C	M	coast	–19.4	9.0	–11.5	7.9
	3000–300 BC	Laguna El Peral-C	F	coast	–19.8	7.0	–11.2	8.6
Bato	200–1000 AD	Cancha de Golf 1	M	coast	–19.0	9.0	–12.1	6.9
	200–1000 AD	Cancha de Golf 1	F	coast	–17.5	11.3	–9.7	7.8
	200–1000 AD	Cancha de Golf 1	M	coast	–17.4	11.2	–9.7	7.7
	200–1000 AD	Cancha de Golf 1	M	coast	–16.6	9.4	–8.3	8.3
	200–1000 AD	El Trébol SE 11	F	coast	–17.4	9.8	–11.3	6.1
	200–1000 AD	El Trébol SE 11	F	coast	–19.9	7.1	–11.5	8.4
	200–1000 AD	El Trébol SE 11	F	coast	–18.7	8.2	–13.6	5.1
	200–1000 AD	El Trébol SE 11	M	coast	–17.7	10.9	–11.1	6.6
	200–1000 AD	Arévalo 2	M	coast	–17.8	11.7	–10.4	7.4
Llolleo	350–1000 AD	Laguna El Peral-C	F	coast	–15.3	9.6	–9.7	5.6
	350–1000 AD	Laguna El Peral-C	F	coast	–13.9	10.7	–8.2	5.7
	350–1000 AD	Laguna El Peral-C	M	coast	–13.5	12.6	–8.7	4.8
	350–1000 AD	Laguna El Peral-C	F	coast	–14.8	11.3	–10.0	4.8
	350–1000 AD	Laguna El Peral-C	M	coast	–15.6	9.8	–10.1	5.5
	350–1000 AD	Tejas Verdes 4	nd	coast	–15.6	10.5	–10.8	4.8
	350–1000 AD	Tejas Verdes 5	M	coast	–14.7	7.7	–8.9	5.8
	350–1000 AD	Los Puquios	F	coast	–16.2	12.3	–10.1	6.1
Aconcagua	1000–1450 AD	Las Brisas 10-14	M	coast	–13.6	8.4	–6.7	6.9
	1000–1450 AD	Las Brisas 10-14	F	coast	–14.3	8.5	–6.3	8.0
	1000–1450 AD	Las Brisas 10-14	M	coast	–13.5	8.1	–7.5	6.0
	1000–1450 AD	Las Brisas 10-14	M	coast	–12.4	11.2	–6.2	6.2
	1000–1450 AD	Las Brisas 10-14	F	coast	–12.3	10.3	–7.7	4.6
	1000–1450 AD	Las Brisas 10-14	F	coast	–17.3	14.3	–12.7	4.6
	1000–1450 AD	Tejas Verdes 5	F	coast	–13.2	8.2	–7.6	5.6
	1000–1450 AD	Santo Domingo 1	M	coast	–10.7	11.6	–5.2	5.5

der Merwe & Vogel, 1978; Ambrose *et al.*, 1997) recognised adjustments for isotopic fractionation in collagen carbon.

Early on in isotope studies, several experiments were conducted on different animal species,

allowing the recognition of a complex web of processes in the metabolism of living creatures, humans included (Krueger & Sullivan, 1984). It was recognised that carbon could be read for diet based on two models. In the *linear mixing* model,

now applied mainly to apatite carbon, all the carbon atoms from dietary proteins, lipids and carbohydrates are incorporated in the animal tissue in the same way. The *macronutrient routing* model instead recognises that protein in the diet is selectively incorporated into protein tissue (Chisholm *et al.*, 1982); therefore consumers' collagen should be a good predictor of dietary protein, and apatite of dietary lipids and carbohydrates as well as protein (Ambrose & Norr, 1993; Tieszen & Fagre, 1993).

This specificity in animal tissues brought to the fore the relevance of incorporating apatite analysis together with collagen, for a better interpretation of the carbon component of diets using isotopic analyses. Nitrogen is found only in bone collagen, but is extremely useful in estimating the trophic level of the foods consumed, and in differentiating between diets based on agriculture *vs.* those based on hunting and/or fishing activities. Nitrogen isotope analysis of faunal and soil samples may also indicate whether manure fertilisation was practised.

In the last 15 years, several studies have contributed to acknowledging the complexities of food webs, how nutrients are incorporated in organisms, and how carbon, nitrogen and oxygen isotopes in different animal tissues may be used to infer different components of past diets. Among these factors are: (a) that our collagen is not 'what we eat + 5‰' when dietary protein, lipids and carbohydrates have different $\delta^{13}\text{C}$ values; (b) ruminant herbivores are not *the* model for apatite fractionation for all animals; (c) $\delta^{13}\text{C}_{\text{AP}}$ values render the best estimation for the whole diet. Feeding experiments in large and small herbivores (Krueger & Sullivan, 1984; Passey *et al.*, 2005), pigs (Howland *et al.*, 2003), rats (Ambrose & Norr, 1993; Jim *et al.*, 2006) and mice (Tieszen & Fagre, 1993) give different results, and are telling us to be cautious when applying these models to human beings. Are we closer to small herbivores, pigs or rats?

Models used in archaeological studies

Archaeological studies in South America have incorporated isotopic information into the

reconstruction of past societies and have relied on different models to estimate $\text{C}_3\%$, $\text{C}_4\%$, and marine versus terrestrial components of diets (e.g. Burger & van der Merwe, 1990; Barberena, 2002; Tykot & Staller, 2002; Borrero & Barberena, 2006; Finucane *et al.*, 2006; Falabella *et al.*, 2008; Gil *et al.*, 2006, 2009). A number of assumptions are frequently used to establish the bridge between isotopic values in human tissues and their corresponding diets. The problem is that different assumptions lead to different dietary reconstructions. In the following, we shall make reference only to those assumptions used for bone tissues as a background for our discussion.

Collagen

Traditionally there has been a consensus over the fractionation between diet and bone collagen, considered to be +5.1‰. In contrast to this, feeding experiments have recognised that there is variation according to the protein and non-protein C_3 *vs.* C_4 components in the diet. Jim *et al.* (2006) quantified the carbon isotopic composition of whole diet, protein, non-protein and bone collagen for animals raised on controlled diets, and found that only in mono-isotopic diets did the model 'you are what you eat + 5‰' hold true (4.9–5.6‰ for C_3 diets and 4.2–4.5‰ for C_4 diets), confirming the results of previous experiments (Ambrose & Norr, 1993). For C_3 protein and C_4 non-protein diets, the offset between diet and bone collagen is 2.0–2.3‰. In C_4 protein and C_3 non-protein diets, the offset increases to 9.6–10.2‰. This produces an under- or over-estimation of the amount of C_4 by as much as 45%, and since maize only has about 10% protein, it should be underrepresented in bone collagen (Ambrose & Norr, 1993).

Another situation to be considered is the protein intake in human diets. Chisholm *et al.* (1982) stated that $\delta^{13}\text{C}$ from collagen comes only from the protein portion of the diet. However, it has been demonstrated that the composition of bone in human diets with low protein intake also represents the non-protein components and comes closer to the linear mixing model (Schwarcz, 2000).

Apatite

In contrast to what happens with collagen, diet-apatite does not vary when diet is not mono-isotopic, therefore confirming the linear mixing model for apatite (Ambrose & Norr, 1993). Data from controlled diet experiments confirm that the relationship between $\delta^{13}\text{C}$ in apatite and $\delta^{13}\text{C}$ of the whole diet is more robust and predictable than apatite-dietary energy, or than collagen-protein and collagen-whole diet (Kellner & Schoeninger, 2007). But diet-bone apatite spacing is not as straightforward as diet-bone collagen because of different shifts found for different animal species (Passey *et al.*, 2005). An overall range of diet-bone apatite values from +8‰ to +14‰ is listed here:

Diet = bone apatite + 12‰ to 14‰ for herbivores (Krueger & Sullivan, 1984; Passey *et al.*, 2005)

Diet = bone apatite + 8‰ to 9‰ for carnivores (Krueger & Sullivan, 1984; Lee-Thorp *et al.*, 1989)

Diet = bone apatite + 9.5‰ for rats (Ambrose & Norr, 1993)

Diet = bone apatite + 9.1‰ for mice (Tieszen & Fagre, 1993)

Diet = bone apatite + 10‰ for carnivores (Bocherens, 2000)

Diet = bone apatite + 8.7‰ to 13.3‰ for pigs (Howland *et al.*, 2003; Passey *et al.*, 2005)

Diet = bone apatite + 12.8‰ for rabbits (Passey *et al.*, 2005)

Since there are no experiments like these with humans, different models have been selected for human bones in the archaeological literature. Ruminant herbivores are not an appropriate model for human vegetarian diets because humans are non-methanogenic (Crutzen *et al.*, 1986). In recent archaeological publications, there has been a tendency to use the +9.5 model following the works of Ambrose and Norr (1993) and Tieszen and Fagre (1993) (e.g. Emerson, 2005; Kosiba *et al.*, 2007). Notwithstanding, Harrison and Katzenberg (2003) found the best fit for whole diet in humans with a value of +12‰ (reflecting true diet), and Prowse *et al.* (2004) with a value of +13‰.

Collagen-apatite spacing

The offset between $\delta^{13}\text{C}_{\text{COL}}$ and $\delta^{13}\text{C}_{\text{AP}}$ also gives dietary information. Their spacing depends

on how collagen and apatite carbon vary isotopically and why they do so. It has been used to infer the proportions of protein *vs.* carbohydrates/lipids in the diet. Obviously, this spacing will differ according to the diet-apatite model that is applied in each case, and varies according to the C_3 and C_4 components of the diet. Table 2 shows examples for two common models used.

For human archaeological bone samples, Harrison and Katzenberg (2003) found a larger offset in maize agriculturalists whose diet included C_3 -fed animals than in foragers with C_3 plants and marine foods in their diet. There is also a trophic-level effect on collagen-apatite spacing: herbivores and human vegetarians should show greater spacing (6.8‰) than omnivores, and omnivores (5.2‰) greater than carnivores (4.3‰) (Lee-Thorp *et al.*, 1989). This seems to be valid for high-protein diets, when enough meat is consumed. But when protein intake is low, carbohydrates are used to synthesise some necessary amino acids, and collagen carbon will approach the whole dietary carbon isotope values. The effects of protein on whole diet isotopic representation are complex and carbon isotope enriched resources can also be obscured in low-protein diets (Hedges, 2004).

Percentage contribution of dietary staples

There are several different calculations to establish the percentage contribution of different food staples in a diet. Variations are mainly due to: (a) the difference in carbon isotopic values between a theoretically pure C_4 *vs.* a pure C_3 consumer, which is used to establish the 'conversion' baseline (a difference of 14‰, 15‰ or other); (b) the fractionation factor used for diet-bone; and (c) the component of the diet inferred from the tissue.

Table 2. Two common collagen-apatite spacing models for C_3/C_4 dietary components

C_3 vs. C_4 components in diet	+12 model	+9.5 model
Mono-isotopic diets	7.0‰	4.5‰
C_3 protein/ C_4 total diet	>7.0‰	>4.5‰
C_4 protein/ C_3 total diet	<7.0‰	<4.5‰

Many studies draw conclusions by simple correspondences, or with general statements (more C_3 , less C_4 , or *vice versa*), while others apply mathematical models or equations in analysing diets. Among the latter, some are linear and some are non-linear (e.g. Little & Little, 1997; Newsome *et al.*, 2004). Software packages have also been developed to perform these calculations, such as IsoSource (Newsome *et al.*, 2004). With the proliferation of isotopic studies, it is becoming increasingly evident that simple models do not fit all types of data. Differences according to feeding behaviour, metabolism and body mass have been explored. But there are other differences, such as why enamel values are so distant from bone values of the same individuals in experiments with pigs, or the different regression lines needed to fit data from several regions according to C_3 , C_4 or marine diets (Kellner & Schoeninger, 2007), which are alerting us to the complexities of reconstructing diet from isotopic data.

The interpretation of isotopic signals in central Chile

As archaeologists, we would like to rely on straightforward models, but the complexities of metabolism and digestive processes alert us against this. We should not rely on single values, but cross-check the information provided by $\delta^{13}C_{COL}$, $\delta^{13}C_{AP}$ and $\delta^{15}N$. In this scenario, fractionation models and especially apatite signals are of great importance.

'Endpoints' of dietary resources in central Chile

We directly tested a large number of plants and fauna that could have been consumed in pre-Hispanic Chile. The average isotopic endpoint of an entirely terrestrial vegetarian C_3 diet (corrected for the industrial effect) falls between -25.4% (non-gramineae C_3 plants), and -20.8% (C_3 gramineae), while the C_4 maize endpoint is -9.7% . This means a 15.7% to 11.1% spacing to model a conversion table in order to estimate the percentage of maize in diets in this region.

According to our data, a diet based on guanaco (*Lama guanicoe*) meat fed exclusively on C_3 resources approximates $\delta^{13}C_{COL}$ values of $-21.5 \pm 0.7\%$, whereas a diet based on guanaco meat partially fed on maize can reach a value of -16% (Table 3).

The main variation among C_3 plants is gramineae *vs.* other plant fruits and seeds: wild C_3 fruits ($-25.4 \pm 2.7\%$) have similar values to domesticated C_3 plants ($-25.1 \pm 1.6\%$), while a wild gramineae tested shows a value of -20.8% . The only C_4 plant analysed is maize which yielded a value of -9.7% . According to the biogeographical and climatic characteristics of central Chile, there should be no C_4 wild resources in this region and we do not expect CAM plants to have been a major food source (Falabella *et al.*, 2008).

Human results

We have analysed 100 human adults from different regions and pre-Hispanic time periods in central Chile (Tables 1 and 4). For this discussion, we select only those individuals who most likely did not consume marine foods, mainly those from inland sites as suggested by zooarchaeological evidence (Figure 1). According to chronological and cultural information, the samples are grouped as follows:

- (a) 5000–300 BC: Archaic mobile hunter-gatherers whose subsistence was based on wild resources.
- (b) 200 BC–200 AD: Comunidades Alfareras Iniciales (CAI) or Early Ceramic communities, which were sedentary quinoa horticulturalists. No maize has been found to date, either in these archaeological contexts or during this time period.
- (c) 200–1000 AD: known locally as Alfarero Temprano (PAT) or Early Ceramic Period, there were different contemporary groups that differed in settlement system, subsistence practices and cultural traditions. Among these, the so-called Bato people continued with subsistence and settlement practices quite similar to Archaic groups, although incorporating horticulture to some extent.

Table 3. Expected isotopic dietary values for each category of local resources

Time period	Resource	n	Range $\delta^{13}\text{C}$ ‰	Range dietary value $\delta^{13}\text{C}$ ‰	Average $\delta^{13}\text{C}$ ‰	Average dietary value $\delta^{13}\text{C}$ ‰	SD	Average $\delta^{15}\text{N}$ ‰	Average dietary value $\delta^{15}\text{N}$ ‰	SD
Pre-Hispanic	Lake fauna – littoral	2	-23.4 to -22.3	-25.4 to -24.3	-22.9	-24.9	0.8	7.1	10.1	2.6
Pre-Hispanic	Lake fauna – inland	1			-16.0	-18.0		2.1	5.1	
Pre-Hispanic	Marine mammal	1			-11.7	-13.7		20.2	23.2	
Contemporary	Fish – carnivorous	3	-13.6 to -11.4	-14.1 to -11.9	-12.8	-13.3	1.2	18.2	21.2	0.3
Contemporary	Fish – herbivorous	1			-15.5	-16.0		16.0	19.0	
Contemporary	Fish – estuarine	1			-14.9	-15.4		12.7	15.7	
Contemporary	Marine mollusks	4	-17.2 to -13.2	-15.7 to -11.7	-15.3	-13.8	1.7	16.0	19.0	1.5
Pre-Hispanic/pre-1000 AD	<i>Lama guanicoe</i>	4	-20.2 to -18.6	-22.2 to -20.6	-19.5	-21.5	0.7	4.6	7.6	0.6
Pre-Hispanic/post-1000 AD	<i>Lama guanicoe</i>	11	-19.7 to -14.0	-21.7 to -16.0	-17.9	-19.9	2.0	5.9	8.9	0.9
Contemporary	Gramineae	1			-22.3	-20.8				
Contemporary	Wild fruits	13	-30.5 to -21.0	-29.0 to -19.5	-26.9	-25.4	2.7			
Contemporary	<i>Zea mays</i>	1			-11.2	-9.7				
Contemporary	Domesticated C ₃	6	-29.3 to -24.7	-27.8 to -23.2	-26.6	-25.1	1.6			
Contemporary	Marine algae	2	-17.3 to -14.0	-15.8 to -12.5	-15.7	-14.2	2.3			

Calculated from the samples' $\delta^{13}\text{C}$ values, +1.5‰ carbon industrial effect added to contemporary samples, -2‰ subtracted from bone samples to estimate animal flesh, and +3‰ added to $\delta^{15}\text{N}$ for trophic-level effect. Lake fauna: *Myocastor coipus*, *Caudiverbera caudiverbera*. Marine mammal: *Otaria* sp. Fish: *Cilus gilberti*, *Aplodactylus punctatus*, *Trachurus symmetricus*, *Merluccius gayi*, *Microgogonia turnieri*. Marine mollusks (flesh): *Tegula atra*, *Choromytilus chorus*, *Mesodesma donacium*, *Concholepas concholepas*. Terrestrial mammal: *Lama guanicoe*. Gramineae: *Nasella chilensis* (Trin). Wild fruits: *Puya* sp., *Peumus boldus*, *Cryptocaria alba*, *Bromus setifolius*, *Brodiaea porrifolia*, *Schinus latifolius*, *Jubaea chilensis*, *Prosopis chilensis*, *Luma apiculata*, *Madia sativa*, *Aristotelia chilensis*. Domesticated C₃: *Solanum maglia*, *Lagenaria* sp., *Solanum tuberosum*, *Chenopodium quinoa*, *Cucurbita* sp., *Phaseolus* sp. Domesticated C₄: *Zea mays*. Marine algae: *Durvillea antarctica*, *Porphyra columbina*. See Falabella et al. (2007: 9, Table 1) for details on the isotopic values of these resources.

Llolleo contexts are associated with sedentary horticulturalists that relied on cultigens (maize, quinoa, beans and squash) for staple foods. In both contexts there is evidence of guanaco hunting and consumption as the main dietary meat source. Other groups are less known and inhabited the Andes cordillera.

- (d) 1000–1450 AD: known locally as Intermedio Tardío (PIT) or Late Intermediate Period, with only one cultural system known as Aconcagua in the region. It represents a major change in the cultural sequence, with an increase in the evidence of maize through macrobotanical remains, and a new pattern of guanaco management.
- (e) 1450–1540 AD: known locally as Tardío (PT) or Late Period, it was a very short span of time characterised by Inca occupation of the region.

Prior to 200 AD (Archaic and CAI samples), for which there is no archaeological macrobotanical evidence of maize or other C_4 edible plants in this region, diet could have been mono-isotopic, based entirely on C_3 resources. The human collagen values from these samples are as expected (Sanhueza & Falabella, 1999–2000; Cornejo & Sanhueza, 2003): when applying a fractionation factor of +5.1‰, the CAI average $\delta^{13}C_{COL}$ value of $-20.1‰$ represents an average isotopic diet of $-25.2‰$, a very good match with the isotopic values of local C_3 plants (Table 3). The average $\delta^{13}C_{COL}$ value for Archaic period individuals is $-18.6‰$. The variations and enrichment of some of these individuals do not follow a pattern, and could be explained by mobility and access to regions other than inland central Chile (Sanhueza & Falabella, in press). The CAI $\delta^{15}N$ values are very low ($4.5 \pm 0.7‰$), suggesting a diet focused on plant foods; therefore we would expect the values for humans to be similar to those for the guanacos. The pre-1000 AD guanacos tested show an average $\delta^{13}C_{COL}$ value of $-19.5 \pm 0.7‰$ (isotopic diet = $-24.6‰$) in their bones, which is coherent with the inclusion of C_3 plants and some gramineae in the diet, and matches closely with the CAI human samples.

The average $\delta^{13}C_{AP}$ values are $-12.2 \pm 1.2‰$ for CAI samples and $-11 \pm 1.6‰$ for the Archaic

period samples (Table 4). Since protein and bulk diet are probably isotopically similar in these groups, we should expect a 'linear fit' between the collagen and apatite models. The 'best fit' is rendered with a fractionation of +13‰ in apatite (Table 4), far away from the +9.5‰ used in many archaeological interpretations. A +9.5‰ offset for CAI apatite ($-21.7‰$) would represent a more enriched diet than that rendered with collagen. The effect of eating aquatic fauna or terrestrial carbon isotope enriched fauna leads to the contrary result, since it would enrich the protein fraction. An alternative explanation to make the +9.5‰ model feasible would be that most of the protein was obtained from protein-rich plants such as *Chenopodium quinoa* ($\delta^{13}C = -26.1‰$), and non-protein components from much more enriched C_3 plants, a situation which is unlikely considering the isotopic values available for edible fruits that are as depleted in carbon, such as *Chenopodium quinoa*. Complementary information comes from collagen-apatite spacing. The Archaic and CAI samples have a $\delta^{13}C_{COL-AP}$ difference of 7.7‰ and 7.9‰ respectively, explaining the best fit obtained with a +13 fractionation value. These values are coincident with the greater spacing expected for herbivores and vegetarians compared with omnivores, so we could be encountering a trophic-level effect in these samples.

The isotope values for Bato are slightly enriched, 0.3‰ more in apatite than in collagen, which might be explained by some maize intake. In this slightly isotopically mixed diet (non-protein $C_3 + C_4$; protein C_3), collagen fractionation might be less than 5‰ in order to represent the whole diet, but with only one sample we cannot explore further interpretations.

The carbon isotope values for Llolleo, a context with clear evidence of maize from macrobotanical remains (Planella & Tagle, 2004), are increasingly enriched in both collagen and apatite, coherent with an increasing dietary importance of this resource. If collagen carbon were an adequate isotopic representation of protein in diet, we would expect Llolleo bone $\delta^{13}C_{COL}$ less 5.1‰ to approximate pre-1000 AD guanaco meat values ($-22.2‰$ to $-20.6‰$). This is not the case, since the estimated diet from $\delta^{13}C_{COL}$ is ca. $-18.7‰$, coinciding with the

Table 4. Averaged results of inland samples grouped by context

Context	Chronology	n	$\delta^{13}C_{COL}$ ‰	SD	$\delta^{15}N$ ‰	SD	$\delta^{13}C_{AP}$ ‰	SD	$\delta^{13}C_{COL-AP}$ ‰	Collagen +5.1	Apatite +9.5	Apatite +10	Apatite +11	Apatite +12	Apatite +13
Inland samples										diet	diet	diet	diet	diet	diet
Archaic	5000–300 BC	3	-18.6	0.9	5.8	2.6	-11.0	1.6	7.7	-23.7	-20.5	-21.0	-22.0	-23.0	-24.0
	200 BC–200 AD	7	-20.1	0.3	4.5	0.7	-12.2	1.2	7.9	-25.2	-21.7	-22.2	-23.2	-24.2	-25.2
Other	600–900 AD	3	-19.3	0.6	5.9	1.9	-11.4	1.8	7.9	-24.4	-20.9	-21.4	-22.4	-23.4	-24.4
	575–766 AD	1	-17.0		5.8		-8.8		8.2	-22.1	-18.3	-18.8	-19.8	-20.8	-21.8
Llolleo	El Mercurio	10	-16.9	1.1	5.0	0.1	-9.0	1.4	7.8	-22.0	-18.5	-19.0	-20.0	-21.0	-22.0
	Llolleo other sites	10	-13.6	0.6	6.0	0.8	-7.7	0.8	5.9	-18.7	-17.2	-17.7	-18.7	-19.7	-20.7
Aconcagua	1000–1450 AD	19	-11.6	0.8	6.8	1.0	-6.6	0.8	5.0	-16.7	-16.1	-16.6	-17.6	-18.6	-19.6
Inca	1450–1540 AD	17	-13.1	1.2	5.9	1.0	-7.4	0.9	5.8	-18.2	-16.9	-17.4	-18.4	-19.4	-20.4

Numbers in bold show the 'best fit' between inferred collagen isotopic diet and apatite isotopic diet.

experiments that show deviations from the +5‰ value in mixed C₃-C₄ diets or a diet with low meat/protein intake. In this case, $\delta^{13}C_{COL}$ values would represent more closely the non-protein component of the diet. The $\delta^{15}N$ mean value of 6‰ supports this last possibility. It also shows that the +13 model for apatite carbon fractionation, valid for previously discussed samples, looks less likely since the $\delta^{13}C_{AP}$ value of -7.7‰ would represent an estimated $\delta^{13}C_{DIET}$ of ca. -20.7‰, more depleted than the estimated diet for the same samples through collagen. With a +9.5 offset, the estimated whole-diet $\delta^{13}C$ is -17.2‰, which is much more coherent with the collagen data and with the archaeological faunal and botanical evidence (Sanhueza *et al.*, 2003).

The collagen-apatite spacing for Llolleo samples averages 5.9‰. If the +13 model for apatite-whole diet is used, the inference is that Llolleo people consumed C₄ protein and C₃ whole diet, which considering all the available evidence is not likely. There is no evidence of marine intake and no known enriched terrestrial fauna during this time period. The +9.5 model represents C₃ protein and C₄ whole diet, which is more coherent and fits the expectation for omnivores.

The Aconcagua samples show the most enriched carbon values of all the data-sets, with a $\delta^{13}C_{COL}$ average of -11.6 and a $\delta^{13}C_{AP}$ average of -6.6. In these contexts, macrobotanical evidence shows an increase in size and variety of maize remains, which supports the idea of maize being a staple food (Planella, 2005). Comparing the Llolleo and Aconcagua data-sets, the collagen values are increased more than the apatite values, suggesting that the greater change is in dietary protein rather than straight C₄ plants (maize). The possible interpretations of this would include that more C₄ resources were being consumed by animals (guanaco) that humans ate, rather than by humans directly, or that aquatic foods with low $\delta^{15}N$ were being consumed.

Issues and interpretation

In our geographical region of study, several factors could be introducing noise into carbon isotope values, especially in the differential

enrichment of collagen *vs.* apatite, and thus how we estimate dietary components. These include:

- (a) *Marine resources.* Central Chile is a narrow territory with *ca.* 100–150 km maximum distance from the central valley to the coast. This geographical condition might lead us to think that it is plausible for inland human groups to have access to marine resources through several mechanisms (seasonal movements, exchange, etc.). None the less, there is very scarce archaeofaunal evidence (fish bones, mollusk shells, marine mammal bones) of seafood in inland assemblages. The archaeological data are supported by very low $\delta^{15}\text{N}$ values (Table 3). But we cannot rule out the consumption of algae: there is no hard evidence to be found of algae in middens, and marine plants, although enriched in carbon isotope values, will not raise collagen nitrogen values significantly.
- (b) *Inland aquatic resources.* In this territory there are many rivers, lakes and lagoons, whose associated plants and fauna need more isotopic analyses and further consideration of their potential contribution to human diet. Freshwater algae which can enrich carbon isotopic values, such as *Nostoc* spp. (Tieszen & Chapman, 1992: 415), grow on the margins of small streams and in lakes in the Andes mountains in central Chile (Pereira *et al.*, 2000). Although there is no information about people eating *Nostoc* in our study region, this algae is eaten by the Aymara people in the altiplano of northern Chile where it is abundant in lakes, and considered a good foodstuff.
- (c) *Guanacos.* Available archaeofaunal data for *Lama* sp. in central Chile suggests that, prior to Inca contact, all camelid bones correspond to *Lama guanicoe* (guanaco), a wild native species (Becker, 1993). Being wild implies feeding on wild resources which, as previously discussed for our region, are mainly C_3 grasses. The four pre-Hispanic guanacos from pre-1000 AD archaeological contexts analysed in this region yielded an average $\delta^{13}\text{C}$ of $-19.5\text{‰} \pm 0.7$, showing a C_3 diet (Table 3). This scenario works well with archaeological inferences for guanaco man-

agement in Archaic, Bato and Llolleo contexts, when animals were hunted and only the more profitable parts were transported back to domestic sites (Becker, 1995–1996). For later Aconcagua groups, the faunal evidence from inland valley sites near Santiago has led Becker (1994) to hold the hypothesis that guanacos were not only being hunted, but that some were partly tamed and held near the houses where people lived. Since maize was grown in small plots near the houses, it is highly probable that if taming were the case, guanacos would have been fed some maize and that their isotopic carbon content would be enriched. We tested 11 post-1000 AD guanacos from Aconcagua archaeological sites. Two of them came from sites located on the coast and the cordillera, probably temporary occupations. Both have $\delta^{13}\text{C}_{\text{COL}}$ isotope values similar to pre-1000 AD fauna (-19.7‰ and -19.3‰). The other nine came from sedentary inland valley sites. Among these, seven are noticeably more enriched than the pre-1000 AD average, ranging in $\delta^{13}\text{C}_{\text{COL}}$ from -18.8 to -14‰ , signalling a definite C_4 input into their diet and also significant variation among the guanaco tested. The 11 post-1000 AD Aconcagua guanacos yielded an average $\delta^{13}\text{C}$ of $-17.9 \pm 0.7\text{‰}$. The consequence for human diet is that eating guanaco meat means C_4 -enriched protein. In this scenario we would expect that human Aconcagua samples would be more enriched in collagen relative to previous groups. In fact this is what our results have shown. Comparing Llolleo with Aconcagua, there is a 2‰ increase in collagen *vs.* a 1‰ increase in apatite.

For Aconcagua human samples, the collagen-apatite spacing averages 5.0‰. This value is very near to a mono-isotopic (C_4 in this case) and omnivorous diet. An offset of *ca.* 4.5‰ between $\delta^{13}\text{C}_{\text{COL}}$ and whole diet (following Ambrose & Norr, 1993, and Jim *et al.*, 2006, for mono-isotopic C_4 diets) represents an isotopic diet of -16.1‰ , a perfect match with the estimated whole diet from apatite $+9.5\text{‰}$ in our study.

One last problem we address concerns estimating % C_4 from collagen and apatite data,

a very important issue when looking for evidence of maize in archaeological contexts. This was one of the major objectives we had in conducting isotopic analyses of human remains in central Chile. To estimate these percentages, isotopic endpoints representing 100% C₃ and 100% C₄ diets must be established. If we take the values of C₃ resources as a baseline for 100% C₃ and the value for *Zea mays* as 100% C₄, the estimated $\delta^{13}\text{C}$ values for these consumers' collagen are *ca.* -20.4‰ and -4.2‰ respectively. Since there is a hypothetical range of C₃ plant values that can move this baseline by as much as $\pm 5.0\text{‰}$, depending on which plants were actually consumed, the estimated percentage of C₄ in the diet could be lower by up to 10% or higher by as much as 20%.

When considering apatite results, we not only need an accurate estimate of the 0% C₄ value, but an adequate fractionation factor to estimate whole diet from bone samples. As discussed above, we are uncertain as to which fractionation model best fits the real diet for humans with no or very little C₄ components. We have calculated that estimating C₄ intake with different models makes a difference of as much as 17% (Falabella *et al.*, 2008). This can make a big difference when trying to recognise the initial introduction of maize. As a result, the ability of apatite to indicate small quantities of maize consumed is hampered because in the case of maize introduction, collagen will still not be changed, and we expect that C₄ plant consumption will only be 'seen' in apatite. In the samples analysed, for inland sites with next-to-no chance of seafood in the diet, or the existence of other C₄ plants, we have only four individuals whose values could show early consumption of maize ($\delta^{13}\text{C}_{\text{AP}}$ values from the site of El Mercurio suggest low C₄ intake, but since no collagen could be measured, we cannot compare). Three of the samples have uncertain cultural assignment, but chronologically are contemporaneous with maize horticulturalists (context = Other in Table 1a). Their collagen and apatite values are quite similar to the Archaic and CAI samples; therefore we could also interpret their diet as 100% C₃. The Bato sample is certainly more enriched in apatite than in collagen relative to CAI, and might be a good example of low quantities of maize consumed.

Conclusions

It is clear that the reconstruction of ancient diets is a complex yet worthwhile endeavour. Interpreting stable isotope values for collagen is straightforward for the protein component of mono-isotopic diets, with the collagen value estimated as *ca.* $+5\text{‰}$ relative to diet. But interpreting collagen values is more problematic in mixed C₃–C₄ diets, because of metabolic processes that can incorporate carbon from different sources into the production of amino acids.

A specific apatite fractionation value for humans, according to our results, is still uncertain, supporting the variability seen in publications where some authors interpret human data with $+13\text{‰}$, others with $+12\text{‰}$, and some as low as $+9.5\text{‰}$. Could it be possible that different fractionations work for different types of diet? As shown above, if we apply the same $+13\text{‰}$ fractionation for diet-apatite to periods when C₄ plants were progressively consumed (Llolleo, Aconcagua, Inca), the percentage of dietary C₄ (maize) calculated is lower than that estimated from collagen. This trend is contrary to what we would expect, since the incorporation of maize in the diet would be signalled initially and preferentially in apatite, unless maize was only grown to feed animals which were then consumed. Following the $+9.5\text{‰}$ model, a considerable amount of non-protein C₄ resources must have been consumed in periods for which there is absolutely no evidence of maize in our region. None the less, the $+9.5$ model works well with maize consumers (Llolleo, Aconcagua, and Inca).

Estimating the actual percentage of C₄ foods in the diet is difficult. Bone collagen carbon isotope values are dominated by dietary protein, while interpretation of both collagen and apatite values are also limited by the wide range of plant $\delta^{13}\text{C}$ values affecting the determination of the real C₃ baseline. At a minimum, isotopic values are needed for the specific plants likely to have been consumed in a particular area in order to make semi-quantitative estimates. Macrobotanical remains and phytolith studies would help to identify these plants.

Knowledge of local isotopic ecology is also important when trying to understand small dietary changes. In our research, we have been

able to test the hypothesis of more C₄-rich meat intake by the Aconcagua people, previously stated only as a possibility (Falabella *et al.*, 2007, 2008). Analysing more archaeological guanacos from the contexts where a partial C₄ diet was suspected allowed us to understand the reason behind the differential enrichment between collagen and apatite. After working through the isotopic data we have assembled for central Chile, we support the idea that many authors have stated, that no single value *per se* can render an adequate reconstruction of past diets. Pooling together both collagen and apatite carbon isotope data, looking at the offset between them, and adding nitrogen isotope results is definitely the best way forward.

Lastly, there are still several pending problems and questions to deal with:

- We cannot totally rule out diagenetic problems, especially for bone apatite.
- A major concern that we cannot solve at the moment is the contribution of aquatic resources. There is scarce archaeological evidence, but there are many lakes, lagoons and inland rivers rich in fauna and vegetation.
- We need to sample more humans that could have had a low maize intake, and more Archaic period individuals when there was certainly no maize in the region.
- We need to sample more specimens of wild gramineae since they can show more enriched C₃ resources.

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