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Pica 8: Refining dietary reconstruction through amino acid $\delta^{13}C$ analysis of tendon collagen and hair keratin

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ABSTRACT

Stable isotope analysis of archaeological human remains is routinely applied to explore dietary habits and mobility patterns. The isotope information pertaining to the period prior to death may help in identifying locals and non-locals, especially when investigating individuals from the same funerary context but believed to have been highly mobile across the landscape.

Based on the variety of the funerary goods in graves and what it is believed their diets comprised, it is thought that both local and non-local individuals were buried at the inland funerary site of Pica 8 (northern Chile, Late Intermediate Period, ~1050–500 BP); however, uncertainties over the dietary intakes and mobility histories of these individuals still persist. The aim of this study is to refine the dietary characterization of a subset of Pica 8 individuals by increasing the temporal resolution of their dietary reconstructions, specifically throughout the last period of their life, and by identifying the multiple sources of food in their overall diets. This is achieved by analysing the amino acid carbon isotope composition of hair keratin and, for the first time, that of tendon collagen.

This study proposes a new method for identifying the predominant food source (terrestrial or marine) in a mixed diet using phenylalanine, valine and leucine $\delta^{13}C$ values measured in collagenous tissues. Herein, tendon is proven to be an ideal tissue for isotopically characterising the final year of an individual's life. Our results show that individuals previously identified as non-locals, based on long-term food consumption, had in reality abandoned their original dietary habits typical of distant regions many months before death, and hence had presumably relocated to the locality of Pica.

1. Introduction

Stable isotope analysis is routinely applied to archaeological human remains for characterising dietary habits and mobility patterns of past populations (Makarewicz and Sealy, 2015). In recent years, attention has focussed on the reconstruction of the life histories of individual identities in past societies (Knapp and van Dommelen, 2008). The reconstruction of an individual's life at different points over their life course is achieved by isotopically analysing multiple tissues (skeletal and non-skeletal) from the same individual, which have differential deposition times and/or turnover rates (Lynnerup, 2007). In particular, the analysis of soft tissues, that have fast remodelling rates, gives information pertaining to the last year/months of life (Lamb, 2015). Moreover, hair retains an unaltered isotope signal locked into the keratins when the tissue was growing (Petzke et al., 2010). This isotope information pertaining to the period prior to death is important when investigating individuals from the same funerary context but believed to have been highly mobile across the landscape, or having had access to long-distance resources. Individuals identified as non-local because of their diet, based on stable isotope compositions averaged over several years (from, for instance, bone collagen) may, in reality, have been consuming locally accessible resources and been part of the local community for a considerable period of time before death.

At the inland funerary site of Pica 8 in northern Chile (Late Intermediate Period, ~1050–500 BP), uncertainties over dietary intakes and mobility histories of the buried individuals still persist, especially pertaining to the last period of their life. Previous archaeological and biomolecular studies (Núñez, 1984; Petruzzelli et al., 2014; Santana-Sagredo et al., 2015a) have suggested that the individuals buried at Pica may have had different geographical

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origins and/or a high degree of mobility. Núñez (1984), who first excavated the cemetery, identified the presence of non-local items among the funerary inclusions in the graves (e.g. pottery, textiles, bird feathers, foods). These were thought to have been imported from either the eastern Andes, Altiplano, Azapa valley, or from the coast. Stable isotope analyses of bone and tooth enamel have identified three major dietary related groups of people, consisting of: (1) individuals relying mainly on marine resources, complemented by some maize, (2) individuals relying mainly on maize, complemented by marine resources, and (3) individuals consuming predominantly C_3 terrestrial resources (Santana-Sagredo et al., 2015a).

Based on the variety of grave goods and diets, it can be argued that the people buried at Pica 8 represent a combination of local and non-local individuals who were involved in inter-regional exchange of foodstuffs and exotic objects, and/or sedentary individuals who benefited from having access to a broad range of resources and maintained dietary habits distinctive of their place of origin. Despite the paucity of information surrounding the placement of the burials in the cemetery, there is some evidence (i.e. broad distribution of funerary goods and diets) that the Pica 8 cemetery was divided into ten sectors, A to J (Núñez, 1984; Santana-Sagredo et al., 2015a; Zlatar, 1984), where people with common geographic origins, socio-economic status, and/or cultural traits may have been interred.

In light of new radiocarbon dates of paired human and camelid tissues and estimated ^{14}C offsets, Santana-Sagredo et al. (2017) have reconsidered the original dietary interpretation (Santana-Sagredo et al., 2015a) of the Pica individuals, proposing the consumption of C_4 crops fertilised with seabird guano as a major cause for the high $\delta^{15}N$ values (>20‰) measured in bone collagen, rather than the direct consumption of marine resources. Andean archaeological and ethnohistoric records (Covey, 2000; Denevan, 2001; Julien, 1985; Marcus et al., 1999) report that guano was traditionally mined and marine fish were procured and dried by coastal communities and transported to the highlands via llama caravans. Estimating the proportion of guano-fertilised maize and marine fishes in the diet of the Pica individuals is not straightforward since the practice of fertilising maize with guano increases the plant $\delta^{15}N$ values to as much as 20‰, but does not affect $\delta^{13}C$ values (Szpak et al., 2012a,b). As a result, consumption of high-trophic level marine resources (fish and mammals) and guano-fertilised C_4 crops may generate similar ranges of bulk $\delta^{13}C$ and $\delta^{15}N$ values in human tissues (Szpak et al., 2012b). Single amino acid carbon isotope analysis may help in identifying these diverse sources (marine, terrestrial) of food macronutrients (protein, carbohydrate, lipid) by comparing the $\delta^{13}C$ values of essential and non-essential amino acids, given that different metabolic pathways are involved in the processes of assimilation or synthesis of the amino acids making up whole proteins (Newsholme et al., 2011; Reeds, 2000), and that guano is not affecting the carbon stable isotope compositions of plant tissues (Szpak et al., 2012a).

The aim of the present study is to refine the dietary characterization of a subset of Pica 8 individuals by increasing the temporal resolution of palaeodietary reconstructions, specifically throughout the last period of their life, and by identifying the different sources of food (marine and terrestrial) that likely comprised their mixed dietary intakes. This is achieved by analysing the amino acid carbon isotope composition of hair keratin and, for the first time, that of tendon collagen. Tendon has the potential to be an ideal substrate for characterising the period leading to/close to the time of death, considering that (1) collagen is significantly more abundant in tendons (~677 + 57 nmol/g wet tissue) than in the dermis (~335 + 64 nmol/g wt), bone (~307 + 71 nmol/g wt) or skeletal muscle (~59 + 17 nmol/g wt) (Kjaer et al., 2005), and that (2) the rate of collagen turnover in tendon (Babraj et al., 2005; Miller et al., 2005) is more rapid than in bone (Hedges et al., 2007).

2. The cemetery of Pica 8

Pica 8 is located approximately 80 km inland, at circa 1300 m of elevation, on the plain of the *Pampa del Tamarugal* (northern Chile, Fig. 1) (Jayne et al., 2016). This funerary site comprises 254 burials (Núñez, 1984) and dates to the Late Intermediate Period (~1050–500 BP), based on ceramic seriation and radiocarbon dating (Núñez, 1976; Santana-Sagredo et al., 2017; Uribe et al., 2007). The Pica oasis was part of a complex system of communities situated in the Tarapacá region between the Río Camiña in the north and the Río Loa in the south, and covered an altitudinal transect from the coast to the Precordillera (~2500 masl) (Uribe et al., 2007).

During this period, the Pica-Tarapacá oases were connected to each other by a complex network of routes and campsites, which allowed long-distance trade of resources and exotic objects between the coast and the highlands, via llama caravans (Briones et al., 2005; Pomeroy, 2013). In this arid region, inter-regional redistribution of surpluses between ecologically different zones was crucial, especially in times of shortage of staple resources (Núñez and Dillehay, 1979; Zori and Brant, 2012). Competition over the control of this trade network likely generated disputes between the local elites. The fact that artefacts related to conflict (found as

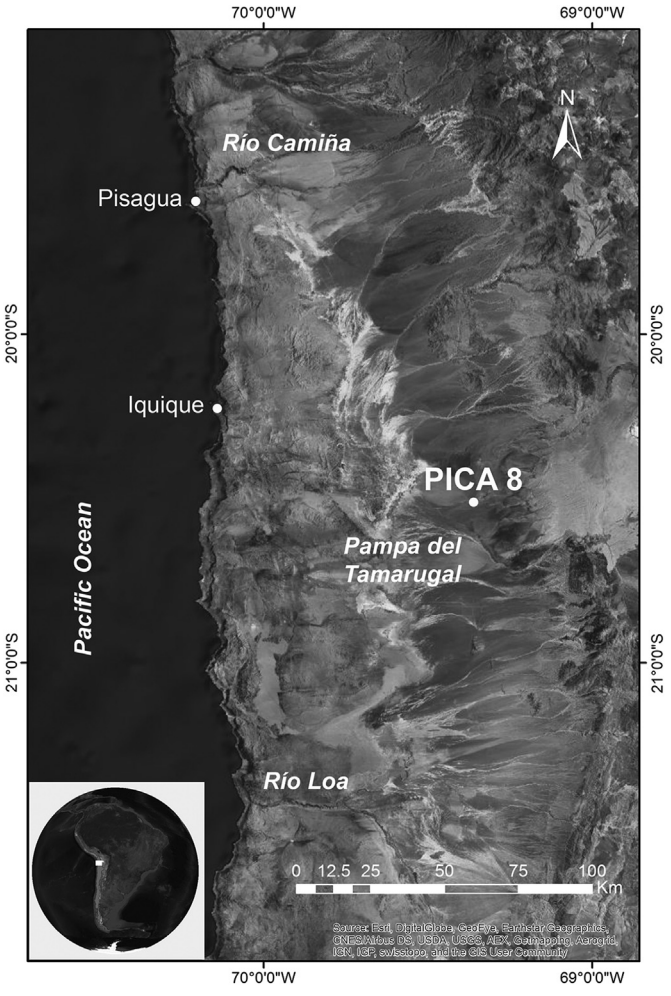

Fig. 1. Map of northern Chile showing the location of the Pica 8 site.