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Exploring the potential of human bone and teeth collagen from Prehistoric Cyprus for isotopic analysis

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ABSTRACT

This pilot study attempts to document the potential of Prehistoric human bone and teeth collagen from Cyprus (9th-2nd mill. BC), for isotopic analysis and palaeodietary reconstruction. We sampled archaeological human skeletons and some faunal remains coming from six sites located in different locations, with different burial modes. The analysis of carbon and nitrogen elemental compositions and stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$), indicate an extremely poor preservation of collagen, probably in relationship with burying conditions. Although very few individuals were successfully analysed, stable isotope data from this study allow a discussion on different protein food resources intake by humans in comparison with some other published data in the Near East (Greece, Cyprus, Turkey) from the Neolithic to the Bronze Age. These diachronic data provide documentations for future studies, including palaeodietary and environmental field research.

1. Introduction

In the Eastern Mediterranean and the Near East, most of the C and N stable isotope studies have been conducted on the Neolithic period (Lange-Badré and Le Mort, 1998; Lösch et al., 2006; Papathanasiou, 2003; Richards et al., 2003). Some Bronze Age materials (e.g. Triantaphyllou et al., 2008; Vika, 2009, 2015) and materials from more recent periods (e.g. Byzantine; Bourbou and Richards, 2007) has also been analysed, mainly in Greece. These local studies mainly indicate the lack of marine resources in human diet even close to the sea, and give some information regarding herding practices and plant consumption. In contrast, studies have rarely been conducted on Cypriot materials whatever the chronological period. The heterogeneity of collagen preservation is also highlighted, and collagen is often difficult to extract, especially in the arid areas of the Near East (Lange-Badré and Le Mort, 1998; Papathanasiou, 2003). In the case of Cyprus there are some data from Neolithic sites but a lack of isotopic and palaeodietary data for Chalcolithic and Bronze Age populations is in contrast to the greater range of archaeological data available. This initial bioarchaeological study aims to contribute towards understanding ancient diet from Prehistoric Cyprus by using bone and teeth collagen extraction and elemental and stable isotope (carbon and nitrogen) analyses. This research focuses on (1) the evaluation of the organic preservation (collagen reliability for stable isotope analysis) in human bone and teeth, and (2) if the previous step is successful, the evaluation of human protein intake in comparison with contextual archaeological data, in order to discuss some hypotheses in regard to human palaeodiets on Cyprus.

2. Sites and contexts

Our research focused on six Neolithic-Chalcolithic and Bronze Age sites of Cyprus: Souskiou-*Laona* settlement (Souskiou-*Laona* Operation A, SL_S Op. A; Peltenburg et al., 2006; Lorentz, 2016) and cemetery (Souskiou-*Laona* Operation C, SL_C Op. C; Crewe et al., 2005; Lorentz, 2016; ca. 3000 BCE cal., Paphos district, Chalcolithic), Kissonerga-*Mylouthkia* (KMyl; Peltenburg, 2003; 8600–3500 BCE cal., Paphos district, Neolithic-Chalcolithic), Kalavasos-*Kokkinoya* (KKok; Clarke, 2007; ca. 4000 BCE, Larnaca district, Chalcolithic), Psematismenos-*Trelloukkas* (PseTre; Georgiou et al., 2011; Lorentz, 2016; Larnaca district, Early

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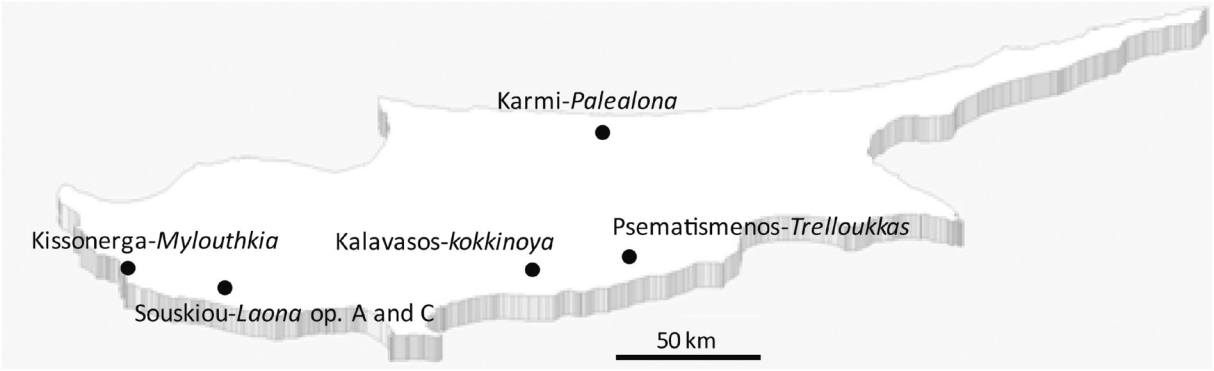

Fig. 1. Location of the archaeological sites studied.
(Map prepared by G. Iannone).

Bronze Age), and Karmi-*Palealona* (KPal; Webb et al., 2009; Frankel and Webb, 2004; Kyrenia district, Early-Middle Bronze Age) (Fig.1). These sites are located in different locations and show different burial modes. The data provided by the geological map of Cyprus (Devillers, 2005; Cohen et al., 2012) indicate a significant lithological and sedimentological variability for each site studied. PseTre and Kalavasos-Kokkinoya are located at the limit between the Cretaceous, Upper Pliocene and Quaternary deposits (South coast of Cyprus). Kissonerga-Mylouthkia site is located in the Quaternary deposits (West coast of Cyprus). Souskiou-Laona site is between the Triassic-Lower Cretaceous and Quaternary deposits (West coast of Cyprus), and Karmi-Palealona in the Middle-Upper Miocene and Pleistocene deposits (North coast of Cyprus).

Zooarchaeological studies performed on several Cypriot sites furnished first information on subsistence strategies. As a general observation, the hunting still an important food supply until the Chalcolithic period and then decreases through time (Croft, 1991). Hunting is focused on fallow deer (*Dama mesopotamica*) which was, on certain sites, the main meat supply fraction (ca. 70%) compared to herded species as sheep and goat for Neolithic and Chalcolithic periods (Croft, 1991). Similarly to deer, pig also largely contributed to subsistence while cattle is absent as its husbandry was probably inefficient in the island (Knapp, 2013). At the end of the Chalcolithic, hunting and consumption of deer decrease considerably (ca. 41%; Croft, 1991). Animal exploitation changed during the Bronze Age with a decrease of deer hunting, an increase of caprine and a new investment on cattle breeding (Spigelman, 2006; Knapp, 2013). On the Bronze Age site of Marki Alonia (in central Cyprus) animal resources are dominated by caprines, cattle and then deer (Croft, 2006). Even if caprine remains are numerically more important, cattle should have supplied the main meat and milk resources (Croft, 2006). On the site of Nitovikla caprines constitute > 90% of the faunal remains (Spigelman, 2006). The intensification of caprines exploitation goes with a diversification of animal use, i.e. for meat, milk and other secondary products according to the sites (Spigelman, 2006).

Some Several archaeobotanical studies have been performed on different Prehistoric and Bronze Age sites in Cyprus (e.g. Colledge, 1989; Thiebault, 2003; Willcox, 2003; Lentini, 2009; Lucas, 2014). These studies indicate the presence of cultivated C_3 plant species from the Neolithic period onward, such as wheat, barley, oats and legumes. For the Chalcolithic, the study conducted at Souskiou-*Laona* (Lucas, 2014) indicate the presence cultivated cereals (emmer wheat and hulled barley), fruit trees (fig in great quantity, pistachio and grape), and lentil (only one remain). For the Early Bronze Age period, the work conducted on the site of Pyrgos-*Mavroraki* provides some data on plant species cultivated consisting of different kinds of wheat, barley and legumes, as well as on other species as olive, grape, and officinal plants (Lentini, 2009). Legumes species are diversified, and we note the presence of beans (*Vicia faba*), chickpeas (*Cicer arietinum*), lentils (*Lens culinaris*), peas (*Pisum sativum*), and bitter vetch (*Vicia ervilia*) for example (Lentini, 2009).

3. Materials and methods

The archaeological materials include adult human bone and teeth and some contemporary animal remains from the Chalcolithic and Early Bronze Age sites mentioned above (Table 1). Sampling was performed on each archaeological site as follows: one human bone fragment and one human tooth from Souskiou-*Laona* settlement (Souskiou-*Laona* Operation A), twelve human bone fragments and one human tooth from Souskiou-*Laona* cemetery (Souskiou-*Laona* Operation C), two human bone fragments and one human tooth from Kalavasos-*Kokkinoya*, two human bone fragments and one human tooth (from a context with multiple commingled burials, may derive either from a single individual, or two different individuals) from Psematismenos-*Trelloukkas*, and one human bone fragment as well as one human tooth from Karmi-*Palealona*. The faunal remains (sheep/goat, pig, and deer) were sampled to characterize the isotopic signal range of known diet species (e.g. plant consumers), for each specific archaeological environment (e.g. Bösl et al., 2006; Goude and Fontugne, 2016). This sampling includes two bone fragments and one tooth from Kissonerga-*Mylouthkia*, and one bone fragment and one tooth from Psematismenos-*Trelloukkas*. In total, twenty three human and five animal samples were selected (bone, $n = 21$ and teeth, $n = 7$) to be further studied after visual examination at the relevant excavation field centres and at the Archaeological Sciences Laboratory of STARC at CyI. We selected bone with a thick cortex (diaphysis of long bones) and roots of teeth, in order to get enough material for the collagen extraction. Moreover, as archaeobotanical remains were not available for these sites, modern botanical materials was considered in our study (carob tree, pod and seed; $n = 6$); this botanical sample has been carried out on freely growing tree from Kissonerga, close to the archaeological site. No information on possible modern manuring is available; however, modern manuring is normally recognizable due to very low $\delta^{15}N$ of synthetic products (Kendall et al., 2007) which is not the case for our samples (Table 1). Bone and teeth collagen were extracted following Longin's (1971) method, modified by Bocherens (1992) (see details in Goude, 2007). $\delta^{13}C$ and $\delta^{15}N$ denote the ratio of the heavy isotope (^{13}C, ^{15}N) to the light isotope (^{12}C, ^{14}N) in a sample relative to international standards (IAEA), and are expressed in ‰. Percentages of C and N and isotopic ratios are measured from 1.0 mg of freeze-dried collagen and analysed with EA-IRMS (Iso-Analytical Ltd., UK). As a first step, the preservation of collagen is verified according to different criteria: collagen yield > 10 mg/g (van Klinken, 1999; Dobberstein et al., 2009), carbon content > 30 weight % (wt%) and nitrogen content greater or equal to 10 wt% (Ambrose, 1990) and C/N ratios between 2.9 and 3.6 (DeNiro, 1985). Then, stable isotope ($\delta^{13}C$, $\delta^{15}N$) measurements are performed on human and animal bone collagen in order to evaluate protein intake