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An isotopic exploration of diet, climate, mobility in prehistoric human remains from Seulo (Sardinia)

Report presented to Dr. Robin Skeates on stable isotopic analyses from **Is Bituleris, su Cannisoni, Longu Fresu.** Research carried out in collaboration with Dr. J.F. Beckett, for osteological determinations and sampling; with Dr. T.C. O'Connell (University of Cambridge), Mr. E. Goddard and Dr. David Hollander (Paleolab, University of South Florida), for mass-spectrometry analyses; under general project supervision by Dr. R. Skeates, with funding from the Autonomous Region of Sardinia, within the project 'Alimentazione e società alle origini della civiltà nuragica', CRP_661

February 2013

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Summary

Materials: the sample population

For this study nine individuals were sampled (Table 1), of which two infants, one juvenile, and six adults, from three different sites near Seulo: Is Bituleris, su Cannisoni and Longu Fresu. Sex estimation was not possible due to the conditions of the specimens, nor was a better age estimate. Samples come from contexts without clear stratigraphy, but have associated radiocarbon dates that place them in the Middle Bronze Age, around the mid-2nd millennium BC (Is Bituleris and su Cannisoni) and in the Late Neolithic (Longu Fresu). The number and conditions of the sample population were from the outset key limiting factors for solid and reliable interpretation, but the results were sought as exploratory record of isotopic values in a context that is both temporally and spatially poorly known from this perspective.

Methods

Ca. 1 g of bone was selected per individual, cleaned from soil or concretions, ultrasonicated and dried. Preparation (Tykot 2004) is a variation of one (Ambrose 1990) of different common procedures: after soaking the sample ~24 h in 50 ml of 0.1 mol/L NaOH aq. to remove humic acid contaminants, collagen was extracted by soaking in 50 ml of 2% HCl aq. in two or more ~24-h baths, based on need. Whenever appropriate, samples were cut into smaller pieces to help the solution permeate the tissue. After demineralization, another ~24 h in NaOH removed further contaminants that were not exposed to reaction when the bone was whole. Demineralization is visible from the coloring of the solution and often from the bubbles on the surface. Samples were then soaked for ~24 h in 50 ml of a methanol-chloroform-distilled water solution (proportion 2:1:0.8) to remove lipids. Samples were rinsed at every step involving the change of reagent. The extracted material, consisting of pellets, was dried overnight in vials at ~65°C. Preparation of the samples was carried out at the Laboratorio di Termodinamica dei Complessi Equilibri in Soluzione, Dept. of Inorganic Chemistry, University of Cagliari (Sardinia, Italy). Analytes were run in triplicates, and analyzed as well in continuous-flow mode, using a Costech elemental analyser coupled to a Finnigan MAT253 mass spectrometer, with precision better than ±0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$, located at the Godwin Laboratory, Department of Earth Sciences, University of Cambridge, UK.

To isolate the apatite, 10 mg of bone powder are treated with a ~72-hour bath in sodium hypochlorite to dissolve the organic portion; non-biogenic carbonate is removed by soaking the sample for ~4 hours in a 1M buffered acetic acid/sodium acetate solution (Koch et al. 1997; Berna et al. 2004). Bone apatite is less accurate and reliable if compared to collagen and particularly to tooth enamel, because of the risk of re-crystallization of exogenous carbonates leaked from the soil matrix into the bone, which may not be removed completely (Lee-Thorp & van der Merwe 1991; Nielsen-Marsh & Hedges 2000a). However, the reliability of bone apatite has been recently reassessed, showing that depending on the environmental conditions of the matrix, crystallization may in some cases obscure, in others favor the preservation of isotopic signal in carbonate (Lee-Thorp & Sponheimer 2003). The protocol used and the assessment of the sample integrity through the yields (Nielsen-Marsh & Hedges 2000b) measured after each preparation treatment, besides the range of isotopic values themselves, can be used as a proxy for reliability. The apatite powder was then analyzed by mass spectrometry at the Paleolab, Department of Marine Science, University of South Florida, St. Petersburg campus. Measurement of standard materials insures the analytical reliability of the results. Precision (2σ) is better than ±0.04‰ for $\delta^{13}C$ and ±0.06‰ for $\delta^{18}O$ (Tykot 2004: 436-438).

Preservation of bone tissues

Collagen preservation was highly inconsistent, likely due to different microenvironments in different parts of the caves and a differentiated taphonomic history for the different specimens. In fact, collagen yields (table 1) range from 0.0% to 20.0%, that is from no preservation to virtually complete. Despite high variation, the specimens from Is Bituleris appear on average in worse (histological) conditions, with a collagen yield average of 6.0 ± 6.1%, whereas the average yield between the two specimens from Su Cannisoni is 14.8 ± 7.4%; the single human sample from Longu Fresu had a yield of 10.5%. C:N ratios, the main parameter to assess preservation (DeNiro 1985; Ambrose 1993), were within the norm in a remarkably consistent way across the sites (3.2), reflecting a genuine collagen.

Apatite yields (table 1) were among the lowest recorded in Sardinia: ranging from 13.6% (the specimen from Longu Fresu) to 47.6%; averages are 25.3 ± 13.5% at Is Bituleris, and 35.7 ± 10.7% at Su Cannisoni, a difference that reflects collagen preservation; 13.6% the single sample from Longu Fresu. A picture of mineral dissolution parallel to collagen degradation might be reflected in the chart that plots collagen and apatite yields (Fig. 1): with the exclusion of the sample from Is Bituleris pertaining to the infant (inv. #377 = UniCa 623/632), the remaining values appear to some degree linearly correlated. This might signify that in general collagen loss exposed apatite to dissolution possibly also with the acidity of decomposition, since a calcareous environment should be alkaline. Sample #38 from Longu Fresu (UniCa #630/639) instead shows remarkably low apatite yield vs a good collagen yield; in this case, the pattern would indicate a highly carbonatic environment, saturated with exogenous carbonate, favored the formation of a coating of crystals on the bone, which protected the core from further exposure to diagenetic agents, causing collagen to preserve better. In fact, a thick layer of calcareous encrustations was present all around the bone (Fig. 2). This could have led to cast a doubt on the isotopic values of the remaining apatite, since the dissolution of the sample in the sodium acetate/ acetic acid may have saturated it, so that no more apatite dissolved despite being as well non-biogenic; however, the sample did not provide a reliable result due to instrumental problems (signal too low). The same risk, however, is also present for the rest of the samples, especially those with a low yield; unfortunately, such a small number of samples does not allow to observe more reliable trends, and distinguish the apparent visual correlation also between δ^{18}O and yield from the effect of breastfeeding and growth physiology in subadult individuals as are the two with unusually enriched δ^{18}O values (see discussion below). It must be noted, though, that precisely the preservation of the breastfeeding signal as expected may in itself be an indication that values retained their genuine, biogenic isotopic ratios.

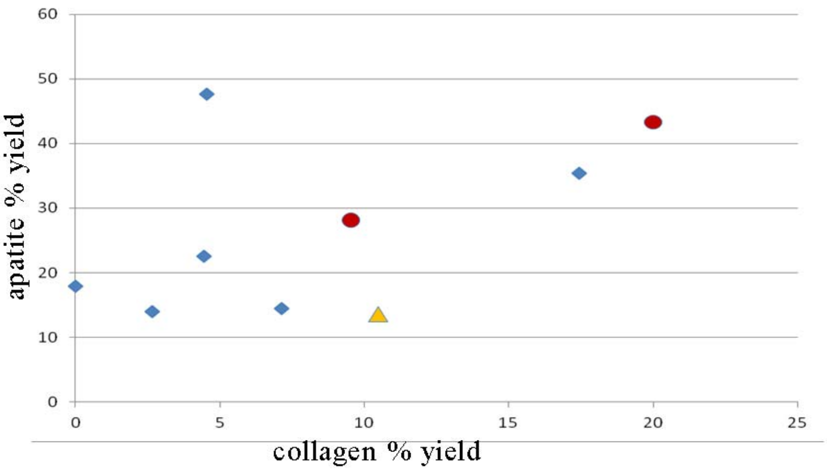

Figure 1. Scatterplot of collagen and apatite yields, in percentages; ♦= Is Bituleris, ●= Su Cannisoni, ▲= Longu Fresu.

Figure 2. Sampled incomplete fibula from Longu Fresu; the coat of calcareous deposits is clearly visible all around the bone.

Results and interpretation

Table 1. Is Bituleris, Su Cannisoni, Longu Fresu; all isotopic values.

UniCa#						**Subgroup**		**Collagen**		**Apatite**		
Coll.	**Apat.**	**# ind.**	**% collagen yield**	**C:N ratio**	**% apatite yield**	**site**	**Age class**	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{13}C$**	**$\delta^{18}O$**	**$\delta^{13}C_{col\text{-}apa}$**
622	631	375	17.44	3.2	35.38	**BIT**	juvenile	-19.6	8.2	-10.5	-2.1	***-9.1***
623	632	377	4.53	3.2	47.62	**BIT**	infant	-18.6	9.6	-14.6	-1.9	***-4.0***
624	633	239	2.66	3.2	13.95	**BIT**	adult	-19.9	7.5			
625	634	240	0.00	3.2	17.94	**BIT**	adult			-15.4	-3.9	
626	635	241	4.44	3.2	22.59	**BIT**	adult	-19.4	7.9	-14.1	-3.7	***-5.3***
627	636	242	7.13	3.2	14.40	**BIT**	adult	-19.7	8.0			
	Avg.		**6.0±6.1**	**3.2**	**25.3±13.5**			**-19.5±0.5**	**8.2±0.8**	**-13.7±2.1**	**-2.9±1.0**	**-6.1±2.6**
628	637	3	20.01	3.2	43.29	**CAN**	adult	-19.8	7.9	-15.0	-3.5	***-4.8***
629	638	52	9.53	3.2	28.13	**CAN**	infant	-19.7	8.2	-12.1	-3.4	***-7.6***
	Avg.		**14.8±7.4**	**3.2**	**35.7±10.7**			**-19.7±0.0**	**8.1±0.2**	**-13.6±2.0**	**-3.5±0.0**	**-6.2±2.0**
630	639	38	10.48	3.2	13.61	**LFR**	adult	-19.9	7.9			

Diet: the origin of proteins

Dietary protein is preferentially routed towards the synthesis of new protein in the tissues, so that collagen, if diets are fairly balanced, reflects mainly the protein portion of the diet. Without faunal samples, the conclusions based on human values are by necessity an approximation. In fact, several sources of ecosystem-wide variation, including climatic parameters as precipitation (Amundson et al. 2003; Schwarcz et al. 1999; Gröcke et al. 1997; Austin & Sala 1999 among others), but also geology, acidity of soils, geomorphology (Garten 1993; Murphy & Bowman 2006), cultivation and land management practices as manuring or fires (Bogaard et al. 2007; Cook 2001), can affect the isotopic composition of soils, and from soils the composition of plants and animals, so that the whole food chain depends on its baseline. Since this is lacking from the materials sampled for stable isotopic analyses, it is impossible to evaluate the relationship of humans with the consumed sources of protein. This must be added to the general weakness of a very small population sample, represented by only six, two and one individual respectively for the three sites. What seems assured (Table 1; Fig. 3) is that there is no contribution from either marine resources, which is expected due to cultural reasons and also geographic distance from the seashore, nor from C4 plants, which is also expected on cultural grounds, since there is evidence for substantial use of C4 plants only in the Iron Age, and for the Bronze Age only in the

Po Valley (Tafuri et al. 2009). This because neither δ^{13}C is compatible with enriched values linked to C4 plants and seafood, nor δ^{15}N is enriched as aquatic ecosystems would. All data so far collected on Sardinian human remains spanning from the Late Neolithic through the Middle Ages do not show any significant presence of C4 plants. So, all protein sources derive from a C3 plant ecosystem, which are the majority of plants in temperate environmental areas, with some imported C4 crops appearing only late in Mediterranean subsistence, such as millet, sorghum, and much later maize and sugarcane, among others.

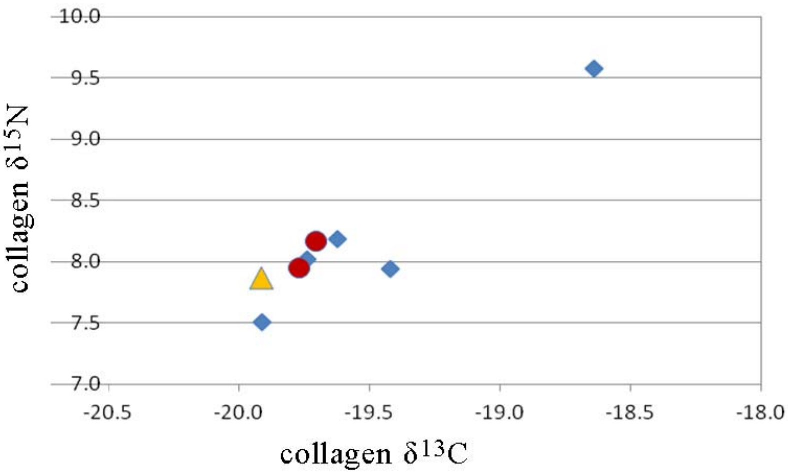

Figure 3. Scatterplot of collagen δ^{15}N vs. collagen δ^{13}C for individuals sampled from the three sites near Seulo. ♦= Is Bituleris, ●= Su Cannisoni, ▲= Longu Fresu. Evident the tight cluster of values irrespectively from the site/ phase (outlier from Is Bituleris is infant, with breastfeeding trophic level effect).

As most Sardinian and Mediterranean values between Neolithic and the end of the Bronze Age, δ^{13}C values at all three sites near Seulo correspond to firmly terrestrial C3 ecosystem, ranging from -19.9 to -19.4, with the addition of one value, -18.6‰, which being associated with an infant likely reflects the physiology of breastfeeding. δ^{15}N values are instead considered as the best detector of trophic level, although as anticipated without faunal remains it remains impossible to assess whether any given value is due to enrichment/depletion from human diet, or from the faunal, ecosystemic baseline. There is no isotopic trace of freshwater fish in the diet, either; which could have been a resource as it was in historic times, given how close several streams are, and especially the Flumendosa river.

Keeping this in mind, one correction has been applied to all the values available so far for Sardinia, based on mean annual precipitation at each specific locality (Lai 2008). This enhances comparability, but cannot address all the other sources of variation. With this limitation, based on the graph in Figure 4, it seems that a high and a low animal protein consumption are equally probable, with higher probability for an intermediate consumption, compared with all other observations. It must be underlined that the whole cloud of observations, however, is contained within a range of variation likely to reflect a diet, as concerns proteins, in great portion carnivorous. In fact, groups showing different values if faunal specimens are not considered appear to have a similar consumption when the interval with the consumed domesticated species is observed. Moreover, values from the three contexts in Seulo, despite their different chronology, show a tight cluster, suggesting that if climate didn't change significantly altering the baseline, the overall diet, in its animal vs vegetal protein proportions, may have remained virtually identical, pointing to a higher likelihood of continuity than variation in subsistence practices.

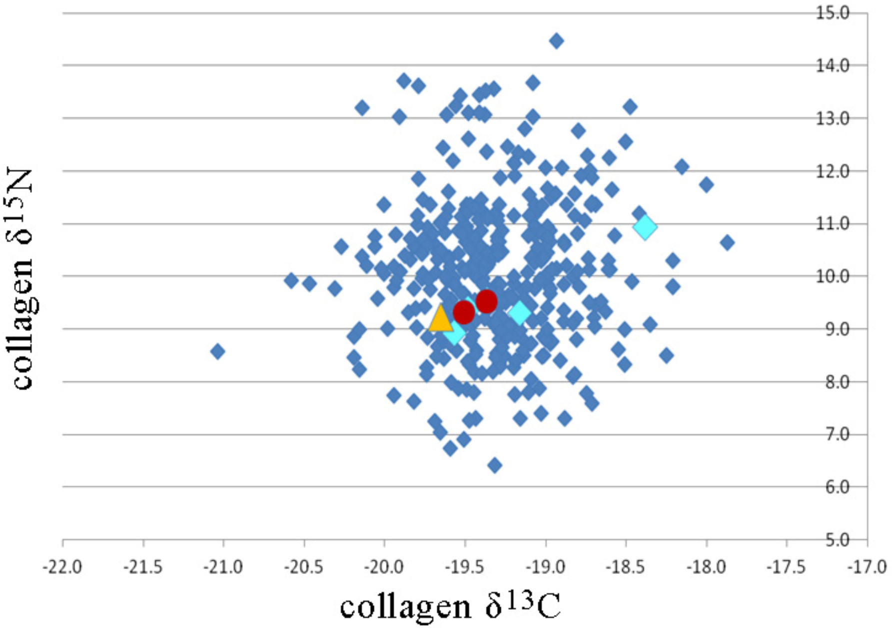

Figure 4. Scatterplot of collagen δ^{15}N vs. collagen δ^{13}C for all Sardinian sites analyzed until now. δ^{15}N values are corrected based on mean annual precipitation, δ^{13}C values based on mean annual temperature; ◆= Is Bituleris, ●= Su Cannisoni, ▲ = Longu Fresu, ◆ = all other sites. The tight cluster of values irrespectively from the site/ phase is evident (outlier from Is Bituleris is infant, with breastfeeding effect).

Diet: the origin of carbohydrates and lipids

Bone apatite is the tissue that has been shown to reflect best, as does cholesterol, the whole diet of an individual. This means that the richer in protein a diet is, the more apatite and collagen values should correlate; the poorer in protein, the less collagen and apatite values will be correlated. To mask this theoretical correlation, however, are all the sources of isotopic variation that have been already mentioned, and most of which are often impossible to quantify at any given site or archaeological assemblage.

Instead of the raw δ^{13}C it is common to use the spacing between apatite δ^{13}C and collagen δ^{13}C (Table 1; Fig. 5), which is a number less dependent on ecosystem-wide shifts, since it is a difference instead of an absolute value. Only five individuals out of nine had both collagen and apatite results to obtain the spacing. Of these, three have values likely affected by growth physiology: juvenile BIT-375 and infant CAN-52 (already ~5 years of age) show a large spacing possibly due to enrichment δ^{13}C during late childhood and adolescence, after depleted values during breastfeeding (Wright & Schwarcz 1998); infant BIT-377, the same that showed the highest δ^{15}N, also shows the smallest spacing, as expected due to its carnivorous diet rich in animal lipids (by suckling her/his mother's secretion). The remaining two individuals are adults most likely to reflect the group's average, normal diet; their small spacing might indicate a diet rich in animal fats and products in general, but due to δ^{15}N values not particularly enriched this may not be the case; if values were actually comparable with the rest of the record, coupling fairly low δ^{15}N and such a small spacing could be due to a diet rich in protein from ruminants that eat a considerable amount of legumes in their diet. If so, we can imagine their use in grazing legume fields after harvest, and/or feeding on wild legumes and/or being fed legume hayes and pods. Also a moderate consumption of milk coupled with a diet generally poor in animal protein could possibly account for part of this variation. In any case, without a faunal baseline, this is almost a speculative exercise.

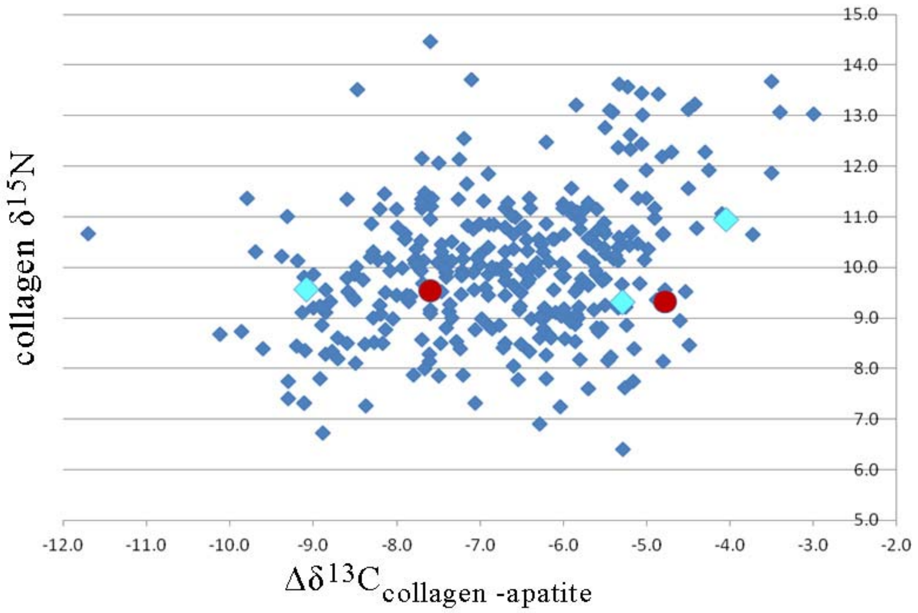

Figure 5. Scatterplot of collagen δ^{15}N vs. $\Delta\delta^{13}$Capatite-collagen for all Sardinian sites analyzed so far. δ^{15}N values are corrected based on mean annual precipitation; ♦= Is Bituleris, ●= Su Cannisoni, ♦ = all other sites. The two individuals on left are a juvenile and an older child, the one on right, with higher δ^{15}N, is an infant.

δ^{18}O values: climate and mobility

δ^{18}O values, which reflecting to a large degree meteoric water ingested through drinking are a proxy for climatic conditions and possibly mobility (Koch 1998; Kohn 1996; Kohn & Cerling 2002), do not show any significant difference among individuals nor groups (Table 1). The only two individuals who do differ, are the two subadults, one infant and one juvenile, for which the physiology of breastfeeding and development during adolescence could have affected values – although the juvenile should not necessarily show enrichment, but possibly rather depletion. The conditions of the remains unfortunately prevents a more accurate estimation of age, making impossible a reliable interpretation of isotopic variation related to different phases of life.

Data from the two remaining adults overlap largely with most values from the 2nd millennium BC in Sardinia already known (Fig. 6), pointing to a climate intermediate between drier/warmer phases in the 3rd millennium and cooler and rainier phases interspersed in the record of the preceding millennia.

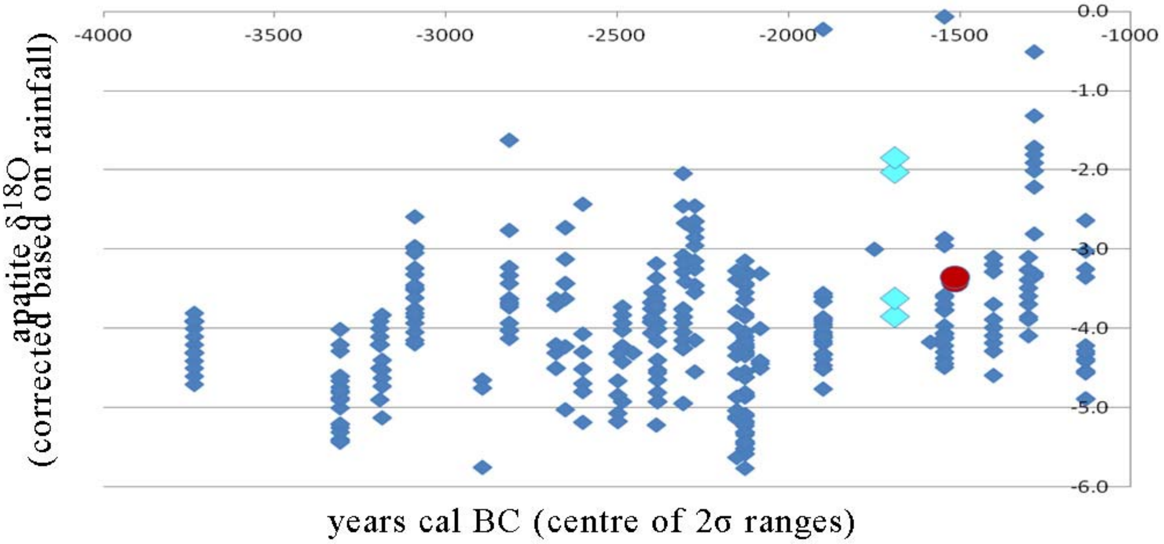

Figure 6. Scatterplot of apatite δ^{18}O vs. AMS dates (intermediate value between extremes in 2σ calibrated range). δ^{18}O values are corrected using mean annual precipitation to account for geographic variation. ◆= Is Bituleris, ● = Su Cannisoni, ◆ = all other sites.

For none of the above isotopic values any exploration was possible of sex nor of age as factors in dietary or mobility differences, due to the highly fragmentary conditions of the bone specimens, which did not allow any estimates. Moreover, there is no evidence for mobility based on fairly similar δ^{18}O values; of course such a small sample population may not be representative.

Conclusions

Summarizing the knowledge acquired on the individuals sampled, the following conclusions can be drawn safely:

- there was no substantial consumption of foods of aquatic origin, whether marine – as expected – or freshwater;
- there was no consumption of C4 plants; millet, recorded in Northern Italy in the Early/Middle Bronze Age, was not a staple near Seulo, in the Neolithic nor in the Bronze Age.

Conclusions within the realm of possibility and probability are the following:

- considering the lack of a faunal isotopic baseline, intermediate $\delta^{15}N$ values only indicate that animal protein consumption is likely to have been sufficient, but any further assessment is impossible;
- with all the caution due to the many potential sources of variation, and to the small number of samples, it is probable that overall proportion of animal protein in the diet was fairly consistent between Neolithic and Bronze Age, across the three sites.
- While some doubts remain on the reliability of the $\delta^{18}O$ values (as recorded at Is Bituleris and su Cannisoni), if they were genuine, they would reflect a Bronze Age Climate, probably somewhat dry and warm, intermediate between drier/warmer and rainier/cooler periods as documented in the 3rd millennium BC.

Financial note

Funding was provided with their inclusion in the project 'Alimentazione e società alle origini della civiltà nuragica', CRP_661, Borse Giovani ricercatori (Sardinian Regional Law n.7/7 aug. 2007) by the Autonomous Region of Sardinia, Centro di Programmazione (Centre for Planning).

Acknowledgments

- **Sardinian Autonomous Region**, Centre for Planning, for funding the fellowship and the project 'Alimentazione e società alle origini della civiltà nuragica', CRP_661, and to J. Robb, T. O'Connell and G. Tanda for their support in the application process.
- **Robin Skeates**, for his availability to let me be involved with this project.
- **Ethan Goddard, David Hollander, Tamsin O'Connell** and **Catherine Kneale** for collaboration in isotopic analyses.
- **Valeria Nurchi** and **Guido Crisponi,** for allowing me to use the laboratory they direct (Inorganic Chemistry, University of Cagliari), as a guest for the duration of the project; **Antonio Rescigno** and **Carla Maria Calò** for granting use of their labs when necessary.
- **Miriam Crespo, Ioanna Lachowich, Leonardo Toso,** for help and assistance in the chemistry lab
- My mother **Marina Melis**, my wife **Sharon Watson,** for constant support and help.

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