Appendix 4: Stable Isotope C/N/S-Analyses: a Report on Negative Results

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In prehistoric communities, food choices were influenced by cultural preferences, social, technological, and economic conditions as well as by the availability of floral and faunal resources. Changes or variations in dietary practices may reflect significant cultural, economic, technological, social, or environmental factors. Therefore, a crucial aspect of the micro-perspective of the *Household and Death in Ba`ja Project* was to identify the isotopic signatures of the ancient diets of the Ba`ja residents. The analysis aimed to provide insights into the subsistence basis and mobility of the population by examining stable isotope ratios of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulphur ($\delta^{34}S$) to complement insights gained from other evidence, such as groundstone and chert tools (see Gebel forthcoming; Purschwitz forthcoming), as well as from archaeological remains of animals and plants (see Neef forthcoming; Öğüt forthcoming; Prust and Pöllath forthcoming).

As only skeletal remains from Area C were available for analysis, the goal of identifying differences based on households had to be postponed. Nevertheless, given the notable variations in burial customs (single/double/collective; with and without abundant grave goods, *etc*.) in Area C, dietary patterns might have provided interesting data to determine whether this differentiation was also reflected in daily life or if it was solely for display during burial ceremonies (for a similar integrative approach, see Benz *et al.* 2016). The substantial number of infant remains also gave us hope of obtaining information about weaning practices.

At the beginning of 2020, bone samples from 16 human skeletal remains from various contexts from the new excavations (2016-2019; Gebel *et al.* 2017, 2019, 2020), along with three samples from the collective burial CG1 (Loc. C10:152) excavated in 2005 (Gebel *et al.* 2006), were dispatched to the Department of Physical Anthropology at the Institute of Forensic Medicine, University of Bern (Table 1). We decided to begin with a subset of samples to assess the overall collagen preservation and its suitability for further analysis using MS (mass spectrometric) measurements. Unfortunately, due to the poor preservation of this initial sample set, a second attempt was made using the remaining *pars petrosae* (Table 2). Regrettably, none of the samples had enough collagen preservation to continue with the analysis. This information is presented here to prevent any future sampling of these bones.

Material

Since different designations were used, the samples from spring and autumn 2020 are presented in two different tables (Tables 1-2).

Table 1 List of samples from Ba`ja for analyses of stable isotopes. Ind=Individuum, f=female, m=male, yrs=years, mths=months, prox=proxima, dist=distal, LV=lumbar vertebra, Dig=Digitus, frgm./frgms.=fragment/s.

Year	Room	Locus	ID	Age	Sex	Samples	Weight (g)
BJ16	CR35	Loc. C10:405	Ind I	6-9 mths	f	Rib left	0.57
		Loc. C10:405	Ind II	3-4 yrs	indet.	Rib left	0.59
		Loc. C10:408.8		adult	m	Prox and dist phalanx	2.14
BJ18	CR36.1	Loc. C1:46		8 yrs ± 24 mths	f	Frgmts. femur left	0.68
BJ19	CR6	Loc. CR6:23a		3 yrs ± 12 mths	indet.	Fragm. of rib left	0.35
		Loc. CR6:23b		1.5-2 yrs	indet.	Frgm. of rib left	0.26
		Loc. CR6:40		0 yrs ± 2 mths	indet.	4th Rib right	0.2
		Loc. CR6:48		7 yrs ± 24 mths	indet.	Fragments of rib left	0.7
	DR19	Loc. DR19:110		0 yrs ± 2 mths	indet.	Rib right	0.1

	CR5	Loc. CR5:49A		1.5-2 yrs	indet.	10th Rib right	0.14
		Loc. CR5:53		4 yrs ± 12 mths	indet.	Frgmts of rib	0.26
		Loc. CR5:54		1-2 yrs	indet.	Fragment of rib left	0.35
	CR17	Loc. CR17:133	No 110	0 yrs ± 2 mths	indet.	2 Bows of LV	0.17
		Loc. CR17:133	infant beneath western wall	6 mths	indet.	2 Frgms. of rib	0.2
		CR17:127b	Nos 14, 18, 19	2 yrs ± 8 mths	f (aDNA)	Frgm. of skull	0.25
		Loc. CR17:133	No 100	adolescent	indet.	Frgm. of skull	0.8
BJ05	CR35	Loc. C10:152 F	No 74 Ind V	14-17 yrs	m>f	Prox phalanx, right hand dig III	1
		Loc. C10:152 F	No 80 Ind IV	young adult	m	Dist phalanx, right hand dig III	2.3
		Loc. C10:152 H	No 92 Ind VI	young adult	m>f	Prox phalanx, right hand dig III	2.1

Table 2 List of samples from Ba`ja for analyses of stable isotopes. ID=Identification; Ind=Individuum; indet.=unidentified; f=female; yrs=years.

ID Jena	ID Locus	Age	Sex	Samples	Weight (g)
BJ007B	Loc. DR110:19	neonatus	indet.	pars petrosa left	1.9
BJ008A	Loc. CR6:40	neonatus	indet.	pars petrosa right	1.9
BJ008B	Loc. CR6:40	neonatus	indet.	pars petrosa left	1.7
BJ009A	Loc. CR6:48	infans II	indet.	pars petrosa right	1.3
BJ010A	Loc. CR6:23a	infans Ib	indet.	pars petrosa right	1.9
BJ010B	Loc. CR6:23a	infans Ia	indet.	pars petrosa left	3.1
BJ012A	Loc. CR5:54	infans Ia	indet.	pars petrosa right	2.1
BJ012B	Loc. CR5:54	infans Ia	indet.	pars petrosa left	2.4
BJ014A	Loc. CR28.2:122/123	infans I	indet.	pars petrosa left	0.47
BJ015A	Loc. CR28.2:122/123	infans I	indet.	pars petrosa left	0.08
BJ016A	Loc. CR17:130, No 21	matur	indet.	pars petrosa left	0.4
BJ017A	Loc. CR17:133, No 101	indet.	indet.	pars petrosa right	2
BJ018A	Loc. CR17:133, No 110	neonatus	indet.	pars petrosa right	1.5
BJ018B	Loc. CR17:133, No 110	neonatus	indet.	pars petrosa left	1.9
BJ019A	Loc. CR17:133, No 100	adolescent	indet.	pars petrosa right	0.4
BJ019B	Loc. CR17:133, No 100	adolescent	Indet.	pars petrosa left	0.75
BJ020A	Loc. CR17:127b, No 18 [NB: belongs to No 13 det. by aDNA]	infans I	f (aDNA)	pars petrosa right	6.3
BJ021A	CR17, Burial CG11, No 102	non-adult	indet.	pars petrosa left	1.8
BJ023A	CR17, Burial CG11, infant beneath western wall	infans Ia	indet.	pars petrosa right	1.4
BJ023B	CR17, Burial CG11, infant beneath western wall	infans Ia	indet.	pars petrosa left	1.4
BJ024A	CR17 Burial CG11	adult	f	pars petrosa right	2.18
BJ024B	CR17 Burial CG11	adult	f	pars petrosa left	2.3
BJ026A	CR28.2.:122/123	infans I	indet.	pars petrosa right	0.8
BJ027A	CR28.2.:122/123	infans I	Indet.	pars petrosa right	0.86
BAJ001.A	Loc. C10:405 Ind I	infans Ia	f (a-DNA)	pars petrosa right	0.48
BAJ002.A	Loc. C10:405, Ind II	infans Ib	indet.	pars petrosa right	0.9
BAJ003.A	Loc. C10:408.8	young adult	male	tooth 27	1
BAJ004.A	Loc. C1:46	infans II (8+/-2 yrs)	f (?)	pars petrosa left	3.8
BAJ005.A	Loc. *CR17:117* [2018], No 12	adolescent	indet.	tooth 37	1

Methods

For the planned analyses of the stable isotopes $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ we took samples from the phalanx and pars petrosal fragments. Because of the small amount and the poor preservation of the bone fragments, we prioritised samples which weight 1g or more for the first extraction (n=16) (Tables 3-4). If we had the *pars petrosae* from the left and right side of one individual, only one of them was used for extraction. The collagen extraction followed modified procedures of Ambrose (1990, 1993) and Longin (1971; see also DeNiro 1985; van Klinken 1999; Nehlich and Richards 2009): After cleaning with distilled water, all samples were pulverised. Then, 500 mg ± 3 mg of bone powder were demineralised with 10 ml of 1M hydrochloric acid (HCl) for 20 min. at room temperature. The solution was washed until neutral (pH ~ 6-7). 10 ml of 0.125 M of sodium hydroxide (NaOH) were added and left for incubation at room temperature for 20 h. The solution was then washed until neutral and 10 ml of 0.001 M HCl were added. The samples were placed in a water bath for incubation at 90 °C (10-17 h). The solubilised collagen was filtered (VitraPOR filter-funnel, porosity 16-40 µm) and lyophilised at 0.42 mbar for a minimum of 48 h. None of the extracted samples yielded any collagen residues after lyophilisation. Therefore, we decided to terminate the following MS measurements and waive to mention the potential following methods.

Results

Table 3 Yield of collagen from the bone samples. Bone powder and weight of collagen are given in g, the yield of the collagen concentration in % of the amount of bone powder used for extraction. Ind = Individuum; f =female; m=male; y=years.

Year	Room	Locus	ID	Age	Sex	bone powder (g)	Collagen weight (g)	Collagen yield (%)
BJ18	CR35	Loc. C10:408.8		adult	m	0.5002	/	/
BJ05	CR35	Loc. C10:152 F	No 74 Ind V	14-17 yrs	m>f	0.5002	/	/
BJ05	CR35	Loc. C10:152 F	No 80 Ind IV	young adult	m	0.5002	/	/
BJ05	CR35	Loc. C10:152 H	No 92 Ind VI	young adult	m>f	0.5002	/	/

Table 4 Yield of collagen from the bone samples. Bone powder and weight of collagen are given in g, the yield of the collagen concentration in % of the amount of bone powder used for extraction. ID= Identification; Ind = Individuum; nd = not determined; f=female

ID Jena	Locus	Age	Sex	bone powder (g)	Collagen weight (g)	Collagen yield (%)
BJ007B	Loc. DR110:19	neonatus	indet.	0.5002	/	/
BJ008A	Loc. CR6:40	neonatus	Indet.	0.4997	/	/
BJ009A	Loc. CR6:48	infans II	indet.	0.5003	/	/
BJ010B	Loc. CR6:23a	infans Ib	indet.	0.4999	/	/
BJ012B	Loc. CR5:54	infans Ia	indet.	0.5000	/	/
BJ017A	CR 17, N° 101	indet.	indet.	0.5000	/	/
BJ018B	CR 17, N° 110	neonatus	indet.	0.4998	/	/
BJ020A	CR17, N° 18 [NB: belongs to No 13]	infans I	f (aDNA)	0.5001	/	/
BJ021A	CR17, N° 102	non-adult	indet.	0.4999	/	/
BJ023A	CR17 Infant beneath western wall	infans Ia	indet.	0.5000	/	/
BJ024B	CR17	Adult	F	0.4998	/	/
BAJ004.A	L. C1:46	Infans II	f?	0.5001	/	/

Discussion

Similar to other studies (*e.g.*, Lösch *et al.* 2006; Wang *et al.* 2023) from this broader geographical region and period, we evidence bad preservation of proteins, respectively collagen within the bones. However, at least nearly 50% of the specimens from PPN Nevalı

Çori, Southeast Anatolia, contained collagen of sufficient quality for subsequent MS measurements. None of the 47 samples from PPN Jericho, Southern Levant, contained collagen, which fulfilled the quality criteria, like in our study here.
For future studies, we recommend careful sampling procedures for similar contexts (region, climate and/or period), with regard to skeletal elements and handling after excavation. In general, skeletal elements with thick compacta and of sufficient weight should be sampled to increase the chances of extracting enough collagen. In our opinion, storage of the specimen at stable, dry, and cool temperature conditions after the excavation should also be guaranteed.

Conclusion

We conclude that the collagen preservation is neither sufficient for further extractions of these samples nor for extractions of the remaining sample set. We decided to preserve the anyhow poorly preserved bone samples. For future stable isotope ratio investigations, we recommend sampling of more bone material – preferably of *pars petrosae* or other skeletal parts with thick compacta –, and storage in stable, dry, and cool environments.

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