**Isotopic evidence of diet and mobility from the Megalithic Burials of the Rego da Murta Dolmens (Portugal)**

**Anna J. Waterman,Alexandra Figueiredo, Cláudio Monteiro, Daniel Alves, Augusto Ferreira, Andrew D. Somerville, Robert H. Tykot and David W. Peate**

**Supplementary Material**

**Detailed Methods**

**87Sr/86Sr ratios**

Samples were processed in two batches, in 2011 and in 2021. The enamel surfaces of the teeth were first cleaned with acetone and surface enamel was removed to decrease possible diagenetic contamination (Budd et al., 2000; Price et al., 2002; Wright, 2005). A Dremel tool with Dremel 5/64-inch diamond wheel point was used to produce and collect 4–10 mg of enamel powder.

All chemical processing of the samples was conducted in the University of Iowa Department of Earth and Environmental Sciences’ clean laboratory. All enamel samples were precleaned in 5% acetic acid for 48 hours, then triple rinsed. Soils were put through a fine mesh sieve and then were leached in 1 M NH4NO3 for 48 hours and then centrifuged at 3000 ppm for 15 minutes. Supernatant was pipetted into new centrifuged tubes and evaporated (Willmes et al., 2014). Water samples were filtered and evaporated. Next all samples were dissolved in 1 mL of 3M HNO3. Strontium was isolated with Eichrom Sr-spec ion-exchange resins using standard procedures (Waight et al., 2002).

The 87Sr/86Sr ratios were measured using a Nu Plasma HR multi-collector inductively-coupled-plasma mass-spectrometer (MC-ICP-MS) at the University of Illinois at Urbana-Champaign. Samples were alternately run with standards using a sample-standard-bracketing measurement protocol (Rehkämper et al., 2004). Ratios were normalized to a NIST 987 international standard value of 0.710268 (NIST Inorganic Crystal Structure Database, Levin, 2018) which had a reproducibility of ±0.000042 (2 s.d.; n= 25) for the samples processed in 2011, and ±0.000036 (2 s.d.; n= 36) for the 2021 samples. The local bioavailable 87Sr/86Sr composition was calculated by taking two standard deviations from the mean of the 87Sr/86Sr ratios for small fauna and soil leachate and water samples (Table S3).

**Stable isotopesδ13C, δ15N and δ18O**

The stable isotopic data were produced at two laboratory facilities. The 2011 samples were analysed at the Laboratory for Archaeological Science at the University of South Florida. Here collagen and apatite data were obtained using the techniques described in Tykot (2006). To remove non-biogenic carbonates that may have leached into the bone, all bone apatite was pretreated with a buffered acetic acid (CH3COOH) solution. The samples were analysed for carbon, nitrogen, and oxygen isotopes using a CHN analyser coupled with a Finnigan MAT Delta Plus stable isotope ratio mass spectrometer using continuous flow for the bone collagen, and a Finnigan MAT Delta Plus instrument using a Kiel III device with 100% phosphoric acid (H3PO4) at 90° C for the bone apatite.

The preparation of the 2021 samples was completed at the Paleoecology Laboratory of Iowa State University. Here collagen was extracted using a procedure similar to the ‘whole bone’ method described by Sealy and colleagues (2014) in which bone fragments (*c.* 5 × 5 mm diameter) were demineralized at room temperature in 0.25 M hydrochloric acid. After demineralization, collagen pseudomorphs were rinsed with ultrapure water. Next humic acids and base-soluble contaminants were removed through a six-hour soak in 0.125 M sodium hydroxide. Collagen pseudomorphs were then solubilized in a weak acid (pH 3 HCl) at 85⁰ C for 24 hours, filtered through an Ezee-filter TM separator (60–90 µm), and then lyophilized using a benchtop freeze dryer. For bone carbonate, samples were prepared using procedures similar to those of Koch and colleagues (1997). Here, powdered bone was removed using a Dremel drill with a diamond-tipped engraving bit. Samples were next sieved and reacted with 2% sodium hypochlorite for 24 hours. Afterwards samples were rinsed to neutrality with ultrapure water, reacted for 24 hours with 0.1 M acetic acid (buffered with calcium acetate to pH 5), and rinsed again to neutrality before drying for 24 hours. The mass spectrometry of prepared samples was conducted in the Stable Isotope Laboratory of the Department of Geological and Atmospheric Sciences at Iowa State University. Collagen samples were analysed for carbon and nitrogen isotopes using a Costech Elemental Analyser coupled to a ThermoFinnigan Delta Plus XL mass spectrometer. Along with visual analyses and collagen yield percentages, C:N ratios of the analysed gases were calculated to determine the preservation of collagen and the reliability of the isotope results. Carbon and oxygen isotope ratios in bone apatite were analysed with a Gas Bench with a CombiPAL autosampler coupled to the ThermoFinnigan Delta Plus XL mass spectrometer.

Reference standards USGS-62, USGS-42, and Acetanilide (laboratory standard) were used for isotopic corrections for collagen, and for apatite, reference standards NBS-18, IAEA 603 were employed for isotopic corrections, and to assign the data to the appropriate isotopic scale. Corrections used a regression method and isotope results are reported in delta notation (‰). Percent concentration (%) was calculated using the peak area of the sample. The combined uncertainty (analytical uncertainty and average correction factor) for δ13Cco is ±0.10‰ (VPDB) and for *δ*15N is ±0.11‰ (Air) for the Iowa State University (ISU) laboratory samples, and 0.10‰ for the *δ*13Cco sample data and 0.2‰ for the δ15N data produced at the University of South Florida (USF). The analytical uncertainty for *δ*13Cap is ±0.05‰ (VPDB) and for *δ*18O is ±0.10‰ (VPDB) at ISU and 0.10‰ (VPDB) for the *δ*13Cap and *δ*18O at USF. All carbon, nitrogen, and oxygen ratios are reported using the standard delta (*δ*) notation and calibrated to an international standard with the formula: *δ* = [(*R*sample/ *R*standard) - 1) × 1000]( Tykot, 2006).

**Supplementary Figures and Tables**

***A white paper with black text and red line

Description automatically generated***

**Figure S1**. Results of Grubbs' Outlier Test for δ18O.

**Table S1**. Stable and radiogenic isotopic data for humans from Rego da Murta I and II.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Site** |  | **Sample** | **Individual** | **Sr** | **Lab #** | **d13C** | **d15N** | **C:N** | **% Yield** | **Lab #** | **d13C** | **d18O** |
| Rego da Murta I | 2011 | RMI 110 | Adult | 0.713916 | 15588 | -19.8 | 8.8 | 3.2 | 2.3 | 15607 | -14.3 | -1.7 |
| Rego da Murta I | 2011 | RMI 299 | Adult | 0.719651 | 15589 | -19.9 | 8.9 | 3.3 | 6.3 | 15608 | -11.6 | -1.7 |
| Rego da Murta I | 2011 | RMI 26 | Adult | 0.711895 | 15590 |  |  |  | 0.0 | 15609 | -12.8 | -2.7 |
| Rego da Murta I | 2011 | RM1 279 | Adult | 0.711571 |  |  |  |  |  |  |  |  |
| Rego da Murta I | 2011 | RMI 267 | Adult | 0.713633 | 15591 | -19.5 | 9.2 | 3.4 | 3.7 | 15610 | -9.3 | -1.6 |
| Rego da Murta I | 2011 | RMI 405 | Adult | 0.712485 | 15592 | -19.5 | 9.2 | 3.4 | 0.3 | 15611 | -13.4 | -2.2 |
| Rego da Murta I | 2011 | RMI 674 | Adult | 0.711613 |  |  |  |  |  |  |  |  |
| Rego da Murta I | 2011 | RMI 384 | Adult | 0.711013 |  |  |  |  |  |  |  |  |
| Rego da Murta I | 2011 | RMI 325 | Adult | 0.713089 |  |  |  |  |  |  |  |  |
| Rego da Murta I | 2011 | RMI 305 | Adult | 0.719811 |  |  |  |  |  |  |  |  |
|  |  |  | ave | 0.713868 |  | -19.7 | 9.0 |  |  |  | -12.3 | -2.0 |
|  |  |  | sd | 0.003227 |  | 0.2 | 0.2 |  |  |  | 1.9 | 0.5 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| Rego da Murta II | 2011 | RMII 163 | Adult | 0.716429 | 15593 | -19.5 | 9.2 | 3.4 | 0.3 | 15612 | -14.0 | -2.3 |
| Rego da Murta II | 2011 | RMII 1000 | Adult | 0.711713 | 15594 |  |  |  | 0.0 | 15613 | -11.9 | -2.1 |
| Rego da Murta II | 2011 | RMII 823 | Adult | 0.713396 | 15595 | -19.5 | 9.2 | 3.4 | 5.4 | 15614 | -12.3 | -1.8 |
| Rego da Murta II | 2011 | RMII 802 | Adult | 0.712033 | 15596 | -20.2 | 8.5 | 3.3 | 2.5 | 15615 | -13.8 | -1.8 |
| Rego da Murta II | 2011 | RMII 631 | Adult | 0.715928 | 15597 | -19.8 | 10.8 | 3.3 | 1.6 | 15616 | -11.5 | -2.4 |
| Rego da Murta II | 2011 | RMII 1003 | Adult | 0.712122 | 15598 | -19.9 | 10.9 | 3.3 | 4.1 | 15617 | -12.5 | -1.3 |
| Rego da Murta II | 2011 | RM1 668 | Adult | 0.714104 |  |  |  |  |  |  |  |  |
| Rego da Murta II | 2011 | RMII 624 | Adult | 0.717985 | 15599 | -20.0 | 9.2 | 3.2 | 6.4 | 15618 | -11.1 | 0.1 |
| Rego da Murta II | 2011 | RMII 5 | Adult | 0.712344 | 15600 | -20.2 | 9.3 | 3.3 | 6.7 | 15619 | -14.2 | -1.7 |
| Rego da Murta II | 2011 | RMII 1005 | Adult | 0.710758 | 15601 | -20.2 | 9.5 | 3.3 | 1.0 | 15620 | -13.6 | -1.9 |
| Rego da Murta II | 2011 | RMII 807 | Adult | 0.715177 | 15602 | -20.2 | 9.3 | 3.3 | 1.1 | 15621 | -14.0 | -2.3 |
| Rego da Murta II | 2011 | RMII 685 | Adolescent | 0.713254 | 15603 | -20.6 | 9.1 | 3.3 | 0.8 | 15622 | -14.3 | -1.7 |
| Rego da Murta II | 2011 | RMII 690 | Adolescent | 0.713594 | 15604 | -20.3 | 8.7 | 3.2 | 2.0 | 15623 | -14.5 | -1.6 |
| Rego da Murta II | 2011 | RMII 1001 | Adolescent | 0.712021 | 15605 | -20.0 | 7.3 | 3.3 | 0.5 | 15624 | -13.9 | -1.9 |
| Rego da Murta II | 2011 | RMII 640 | Child | 0.711519 | 15606 | -21.9 | 7.4 | 3.3 | 4.4 | 15625 | -12.8 | -1.9 |
|  |  |  |  | 0.713492 |  | -20.2 | 9.1 |  |  |  | -13.2 | -1.8 |
|  |  |  |  | 0.002071 |  | 0.6 | 1.0 |  |  |  | 1.1 | 0.6 |

**Table S2**. Stable isotopic data for fauna.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Site** |  | **Sample** | **Individual** | **Lab #** | **d13C** | **d15N** | **C:N** | **% Yield** | **Lab #** | **d13C** | **d18O** |
| Rego da Murta I | 2021 | RM 1 F5 | rabbit |  |  |  |  |  | PEL-0492 | -12.1 | -2.6 |
| Rego da Murta I | 2021 | RM1 F 7 | rabbit |  |  |  |  |  | PEL-0493 | -13.6 | -2.4 |
| Rego da Murta I | 2021 | RM1 F 8 | rabbit | PEL-0494 | -22.1 | 4.0 | 3.4 | 1.7 | PEL-0494 | -12.5 | -2.7 |
| Rego da Murta II | 2021 | RM 2 F10.1 | rabbit | PEL-0495 | -21.9 | 4.8 | 4.3 | 0.4 | PEL-0495 | -11.8 | -2.5 |
| Rego da Murta II | 2021 | RM 2 F13 | ovicaprid | PEL-0496 | -21.1 | 5.4 | 3.4 | 3.2 | PEL-0496 | -11.9 | -2.6 |
| Rego da Murta II | 2021 | RM 2 F17 | deer | PEL-0498 | -21.7 | 4.9 | 3.4 | 3 | PEL-0498 | -11.1 | -2.8 |
|  |  |  |  | ave | -21.7 | 4.8 |  |  |  | -12.2 | -2.6 |
|  |  |  |  | sd | 0.4 | 0.6 |  |  |  | 0.8 | 0.1 |

**Table S3**. Calculated local 87Sr/86Sr ratio range using small fauna and environmental samples.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Site** | **Sample** | **Year** | **Material** | **Sample type** | **87Sr/86Sr** | **SE** |
| RdM I | RM 5.21 | 2021 | Oryctolagus (rabbit) | Enamel | 0.71119 | 0.000029 |
| RdM I | RM 6.21 | 2021 | Oryctolagus (rabbit) | Enamel | 0.71241 | 0.000029 |
| RdM I | RM 7.21 | 2021 | Oryctolagus (rabbit) | Enamel | 0.71264 | 0.000029 |
| RdM I | RM 8.21 | 2021 | Oryctolagus (rabbit) | Enamel | 0.71194 | 0.000029 |
| RdM I | RM 9.21 | 2021 | Oryctolagus (rabbit) | Enamel | 0.71089 | 0.000029 |
| RdM II | RM F9 | 2011 | Oryctolagus (rabbit) | Enamel/dentine | 0.71185 | 0.000004 |
| RdM II | RM F10 | 2011 | Oryctolagus (rabbit) | Enamel/dentine | 0.71106 | 0.000004 |
| RdM II | RM10.21 | 2021 | Oryctolagus (rabbit) | Enamel | 0.71114 | 0.000029 |
| RdM II | RM 11.21 | 2021 | Oryctolagus (rabbit) | Enamel | 0.71164 | 0.000029 |
| RdM II | RM 12.21 | 2021 | Oryctolagus (rabbit) | Enamel | 0.71076 | 0.000029 |
| RdM II | RM F1 | 2011 | Rodentia (small rodent) | Enamel/dentine | 0.71180 | 0.000004 |
| RdM II | RM F6 | 2011 | Rodentia (small rodent) | Enamel/dentine | 0.71242 | 0.000004 |
| RdM II | RM F7 | 2011 | Rodentia (small rodent) | Enamel/dentine | 0.71148 | 0.000004 |
| RdM Relvas | RM S2 | 2021 | Soil | Soil Leachate | 0.70808 | 0.000029 |
| RdM I | RM S6 | 2021 | Soil | Soil Leachate | 0.71241 | 0.000029 |
| RdM Relvas | RM W1 | 2021 | Water | Surface Water | 0.71375 | 0.000029 |
| RdM Castelo | RM W2 | 2021 | Water | Surface Water | 0.71178 | 0.000029 |
| Ponta Fonta | RM W3 | 2021 | Water | Surface Water | 0.71420 | 0.000029 |
|  |  |  |  | ave | 0.71175 |  |
|  |  |  |  | 2sd | 0.00250 |  |
|  |  |  |  | Local Range | 0.0714 | to 0.709 |

**Table S4**. 87Sr/86Sr isotope ratios of fauna, soil leachate and water from Rego da Murta I and II and the surrounding area.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Site** | **Sample** | **Year** | **Material** | **Sample type** | **87Sr/86Sr** | **SE** |
| RdM I | RM 1.21 | 2021 | Cervus (deer) | Enamel | 0.71951 | 0.000029 |
| RdM I | RM 2.21 | 2021 | Ovis/Capra (sheep or goat) | Enamel | 0.71444 | 0.000029 |
| RdM I | RM 3.21 | 2021 | Ovis/Capra (sheep or goat) | Enamel | 0.71117 | 0.000029 |
| RdM I | RM 4.21 | 2021 | Cervus (deer) | Enamel | 0.71930 | 0.000029 |
| RdM I | RM 5.21 | 2021 | Oryctolagus (rabbit) | Enamel | 0.71119 | 0.000029 |
| RdM I | RM 6.21 | 2021 | Oryctolagus (rabbit) | Enamel | 0.71241 | 0.000029 |
| RdM I | RM 7.21 | 2021 | Oryctolagus (rabbit) | Enamel | 0.71264 | 0.000029 |
| RdM I | RM 8.21 | 2021 | Oryctolagus (rabbit) | Enamel | 0.71194 | 0.000029 |
| RdM I | RM 9.21 | 2021 | Oryctolagus (rabbit) | Enamel | 0.71089 | 0.000029 |
| RdM II | RM F2 | 2011 | Equus (horse) | Enamel | 0.71291 | 0.000004 |
| RdM II | RM F3 | 2011 | Bos (cow) | Enamel | 0.71354 | 0.000004 |
| RdM II | RM F5 | 2011 | Sus (pig) | Enamel | 0.71396 | 0.000004 |
| RdM II | RM 13.21 | 2021 | Ovis/Capra (sheep or goat) | Enamel | 0.71315 | 0.000029 |
| RdM II | RM 14.21 | 2021 | Cervus (deer) | Enamel | 0.71012 | 0.000029 |
| RdM II | RM 15.21 | 2021 | Cervus (deer) | Enamel | 0.71137 | 0.000029 |
| RdM II | RM 16.21 | 2021 | Bos (cow) | Enamel | 0.71517 | 0.000029 |
| RdM II | RM 17.21 | 2021 | Cervus (deer) | Enamel | 0.71385 | 0.000029 |
| RdM II | RM 18.21 | 2021 | Ovis/Capra (sheep or goat) | Enamel | 0.71375 | 0.000029 |
| RdM II | RM F9 | 2011 | Oryctolagus (rabbit) | Enamel | 0.71185 | 0.000004 |
| RdM II | RM F10 | 2011 | Oryctolagus (rabbit) | Enamel | 0.71106 | 0.000004 |
| RdM II | RM10.21 | 2021 | Oryctolagus (rabbit) | Enamel | 0.71114 | 0.000029 |
| RdM II | RM 11.21 | 2021 | Oryctolagus (rabbit) | Enamel | 0.71164 | 0.000029 |
| RdM II | RM 12.21 | 2021 | Oryctolagus (rabbit) | Enamel | 0.71076 | 0.000029 |
| RdM II | RM F1 | 2011 | Rodentia (small rodent) | Enamel/dentine | 0.71180 | 0.000004 |
| RdM II | RM F6 | 2011 | Rodentia (small rodent) | Enamel/dentine | 0.71242 | 0.000004 |
| RdM II | RM F7 | 2011 | Rodentia (small rodent) | Enamel/dentine | 0.71148 | 0.000004 |
| RdM Relvas | RM S2 | 2021 | Soil | Soil Leachate | 0.70808 | 0.000029 |
| RdM I | RM S6 | 2021 | Soil | Soil Leachate | 0.71241 | 0.000029 |
| RdM Relvas | RM W1 | 2021 | Water | Surface Water | 0.71375 | 0.000029 |
| RdM Castelo | RM W2 | 2021 | Water | Surface Water | 0.71178 | 0.000029 |
| Ponta Fonta | RM W3 | 2021 | Water | Surface Water | 0.71420 | 0.000029 |
|  |  |  |  | ave | 0.71270 |  |
|  |  |  |  | sd | 0.00231 |  |

**References**

Budd, P., Montgomery, J., Barriero, B. & Thomas, R.G. 2000. Differential Diagenesis of Strontium in Archaeological Human Dental Tissues. *Applied Geochemistry*, 15: 687–94. <https://doi.org/10.1016/S0883-2927(99)00069-4>

Koch, P.L., Tuross, N. & Fogel, M.L. 1997. The Effects of Sample Treatment and Diagenesis on the Isotopic Integrity of Carbonate in Biogenic Hydroxylapatite. Journal of Archaeological Science, 24: 417–29. <https://doi.org/10.1006/jasc.1996.0126>

Levin, I, 2018. NIST Inorganic Crystal Structure Database (ICSD). National Institute of Standards and Technology [online] [accessed 2 December 2024]. Available at: <https://doi.org/10.18434/M32147>

Price T.D., Burton J.H. & Bentley R.A. 2002. The Characterization of Biologically Available Strontium Isotope Ratios for the Study OF Prehistoric Migration. Archaeometry, 44: 117–35. <https://doi.org/10.1111/1475-4754.00047>

Rehkämper, M., Wombacher, F. & Aggarwal, J.K. 2004. Stable Isotope Analysis by Multiple Collector ICP-MS. In: P.A. de Groot, ed. *Handbook of Stable Isotope Analytical Techniques 1*. Amsterdam: Elsevier, pp. 692–725. <https://doi.org/10.1016/B978-044451114-0/50033-8>

Sealy, J., Johnson, M., Richards, M. & Nehlich, O. 2014. Comparison of Two Methods of Extracting Bone Collagen for Stable Carbon and Nitrogen Isotope Analysis: Comparing Whole Bone Demineralization with Gelatinization and Ultrafiltration. Journal of Archaeological Science, 47: 64–69. <https://doi.org/10.1016/j.jas.2014.04.011>

Tykot, R.H. 2006. Isotope Analyses and the Histories of Maize. In: J.E. Staller, R.H. Tykot & B.F. Benz, eds. Histories of Maize: Multidisciplinary Approaches to the Prehistory, Linguistics, Biogeography, Domestication, and Evolution of Maize. New York: Routledge, pp. 131–42. <https://doi.org/10.4324/9781315427331>

Waight, T.E., Baker, J.A. & Peate, D.W. 2002. Sr Isotope Ratio Measurements by Double Focusing MC-ICPMS: Techniques, Observations and Pitfalls. *International Journal of Mass Spectrometry*, 221: 229–44. <https://doi.org/10.1016/S1387-3806(02)01016-3>

Willmes, M., McMorrow, L., Kinsley, L., Armstrong, R., Aubert, M., Eggins, S. et al. 2014. The IRHUM (Isotopic Reconstruction of Human Migration) Database – Bioavailable Strontium Isotope Ratios for Geochemical Fingerprinting in France. *Earth System Science Data*, 6: 117–22. <https://doi.org/10.5194/essd-6-117-2014>

Wright, L.E. 2005. Identifying immigrants to Tikal, Guatemala: defining local variability in strontium isotope ratios of human tooth enamel. *Journal of Archaeological Science*, *32*: 555-566. <https://doi.org/10.1016/j.jas.2004.11.011>