Difference in Death? A Lost Neolithic Inhumation Cemetery with Britain's Earliest Case of Rickets, at Balevullin, Western Scotland

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Supplementary information: methods employed in stable isotope analysis

METHODS

Strontium Isotope analysis of enamel

The enamel sample was transferred to the clean laboratories at NIGL where strontium was extracted according to established laboratory procedures (Evans *et al.* 2006). Briefly, the enamel chip was leached and ultrasonicated in sequential acetone and deionised water washes, spiked with ^{84}Sr, dissolved in Teflon-distilled 8 M HNO_3, and converted to chloride. Strontium was extracted using cation exchange chromatography. The strontium isotope ratio ($^{87}Sr/^{86}Sr$) and concentration (ppm) were measured by Thermo Triton multicollector thermal ionisation mass spectrometry. $^{87}Sr/^{86}Sr$ values were normalised relative to measurements of the international strontium standard, NBS 987. External precision for $^{87}Sr/^{86}Sr$ over the period of analysis was ±0.001% (1σ). Procedural laboratory blanks were negligible relative to the amount of strontium extracted from the sample.

Oxygen and carbon isotope analysis of enamel

The enamel surface was removed using a diamond dental burr and discarded. Samples of powdered enamel (~5–15 mg) were produced for oxygen isotope analysis of both the carbonate and phosphate fractions. At the University of Bradford, the sample was treated following a procedure modified after Sponheimer (1999). To remove organic matter and exogenous carbonate, it was treated with 1.7% NaOCl solution for 30 minutes, rinsed with de-ionised water, treated with 0.1 M acetic acid for ≤10 min, and then rinsed once more. After freeze-drying, the sample was weighed into a septa-capped vial and loaded into a Finnigan Gasbench II. Oxygen and carbon isotope ratios were measured in a Thermo Delta V Advantage mass spectrometer connected to the Gasbench II. The enamel value was measured in duplicate and normalised relative to internal and international standards. The values in Table 2 (main text) are the resulting mean values, and the analytical error, based on the reproducibility of the standards, was ±0.2‰ (1sd) or better for both $\delta^{18}O_{carb}$ and $\delta^{13}C_{carb}$.

At NIGL, oxygen isotope ratios were produced for both the enamel phosphate and enamel carbonate fractions following the procedures published by Chenery *et al.* (2010; 2012). For $\delta^{18}O_{phos}$, the enamel sample was converted to silver phosphate and measured by isotope ratio mass spectrometry (TC/EA-CFIRMS) with a ThermoFinnigan thermal conversion elemental analyser gas chromatography (GC) column coupled to a ThermoFinnigan DeltaplusXL via a ConFlo III continuous flow interface. The sample was measured in triplicate, corrected for non-linearity and drift, and converted to the VSMOW scale against NBS120C calibrated against the certified reference material NBS127 using conventional fluorination techniques. Reproducibility for NBS120C was ±0.32‰ (2σ, n=32). A batch control standard (ACC-1, Aldrich) converted to Ag_3PO_4 had an analytical precision of ±0.12‰ (2σ, n=5).

For enamel carbonate, $\delta^{18}O_{carb}$ and $\delta^{13}C_{carb}$ values were measured using a GV Isoprime duel inlet mass spectrometer. In-house carbonate reference material (KCM) was calibrated against NBS19 reference material to normalise the $\delta^{18}O_c$ values to the VPDB scale. The reproducibility of KCM was ±0.06‰ (1σ; n=9). The resultant $\delta^{18}O_{carb}$ values were converted to the SMOW scale using Coplen (1988).

Carbon and nitrogen isotope analysis of dentine and bone

Collagen was extracted from dentine and rib bone. The bone was prepared by cleaning the surfaces by air abrasion. One half of the bisected tooth was prepared by first removing enamel, circumpulpal dentine, and the outer surface of the tooth root. The tooth was embedded in plaster, sectioned, and an approximate age assigned to each dentine segment following Method 1 in Beaumont *et al.* (2013). Sixteen sections were obtained from the premolar dentine formed between the ages of 3.5 and 14.5 years (AlQahtani 2009); each section thus contained dentine formed over a 6–9 month period. Bone and dentine collagen were prepared using the modified Longin method (Brown *et al.* 1988; O'Connell & Hedges 1999). No filtration was carried out on the dentine samples. Collagen samples were combusted in a Thermo Flash EA 1112 and the separated N_2 and CO_2 was introduced to a Delta plus XL via a Conflo III interface which can measure small (0.5 mg) samples. Samples were measured in duplicate, and laboratory (fish gelatin and bovine liver) and international (IAEA 600, CH6, CH7, N1 and N2) standards were interspersed throughout the run. The analytical error was ± 0.2‰ (1σ) or better for both $\delta^{13}C$ and $\delta^{15}N$ and all samples met accepted quality control parameters for identifying good quality collagen (van Klinken 1999). Mean values are presented in Tables 2 and 3 (main text).