^{2}H stable isotope analysis of human tooth enamel: a new tool to extract forensic information from human remains?

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GOALS

- Determine the feasibility of obtaining ^{2}H stable isotope abundance data from ground human tooth enamel procured from archaeological and modern teeth samples using continuous-flow isotope ratio mass spectrometry (IRMS) coupled on-line to a high-temperature conversion elemental analyzer (TC/EA.
- Evaluate the usefulness of the resulting data for determining human provenance in an archaeological and forensic setting.

INTRODUCTION

Stable isotope analysis of biogenic tissues such as tooth enamel and bone mineral has become a well recognized and increasingly important method for determining provenance of human remains, and has been used successfully in bio-archaeological studies as well as forensic investigations.[1,2] Both ^{18}O and ^{2}H stable isotopes are well established proxies as environmental indicators of climate (temperature) and source water and are therefore considered reliable indicators of geographic life trajectories of animals and humans.[3] While analysis of ^{2}H signatures in human hair, fingernails, and bone collagen is currently used to determine human provenance, i.e. geographic origin and identify possible migration patterns, studies involving the analysis of ^{2}H in tooth enamel appear to be nonexistent in the scientific literature. The apparent lack of research in this area may be associated with the following considerations: (1) Some of the hydroxyl ions in mineral calcium hydroxylapatite, $Ca_{10}(PO_4)_6(OH)_2$, by forming bio-apatite carbonate ions at a rate of one CO_3^{2-} for two OH^-; consequently, there might be not enough hydrogen present for ^{2}H stable isotope analysis.[4,5] (2) There exists the possibility for hydrogen exchange to render measured δ^2H-values meaningless even though the latter might be unlikely given the high pK_a-value of the OH^- ion.

In this study, feasibility to extract ^{2}H stable isotope abundance data from tooth enamel has been shown in principle. Ground tooth enamel was analysed by continuous-flow isotope ratio mass spectrometry (IRMS) coupled on-line to a high-temperature conversion elemental analyzer (TC/EA). An array of archaeological and modern teeth was analyzed under different experimental conditions, and results of this proof-of-concept study are presented.

MATERIALS AND METHODS

Tooth samples

Eleven second or third permanent molars from people of North African, Scottish, and Italian origins were all mechanically cleaned to remove any surface contamination and subjected to one of the following pre-treatment protocols.[6,7] *Protocol B:* Approx. 35 – 40 mg of tooth enamel was sampled using a dental drill with a diamond tipped drill bit and the collected powder was first washed with 1.5% NaOCl solution (40 µl/mg sample for 30 minutes) followed by 3 rinses with deionised water. The powder was subsequently washed with 0.1 M acetic acid (40 ml/mg for 10 minutes) followed by 3 rinses with deionised water and drying in an evacuated desiccator over phosphorus pentoxide (P_4O_{10}) as drying agent. *Protocol S:* The entire tooth was washed as described under Protocol B and subsequently sampled using a dental drill with a diamond tipped drill bit to yield a fine enamel powder, which was stored in an evacuated desiccator over phosphorus pentoxide as drying agent.

Bulk ^{2}H isotope analysis by TC/EA-IRMS

The working reference gas, H_2 (BOC, Guilford, Surrey, UK) was calibrated against VSMOW using the international calibration material VSMOW (δ^2H_{VSMOW} = 0 ‰) (IAEA, Vienna, Austria) and checked against the international reference materials (RMs) SLAP and GISP. The H^{3+} factor was determined on reference H_2 gas pulses of different signal size and was found to be 4.92 ‰/nA. A batch analysis typically comprised 10 samples run in triplicate, preceded and followed by a set of RMs (IAEA-CH-7, (δ^2H_{VSMOW} = -100.3 ‰; IAEA, Vienna, Austria). Batch runs also comprised an in-house standard as acquisition quality control, sodium benzoate (δ^2H_{VSMOW} = -118.6 ± 1.3 ‰; Iso-Analytical, Crewe, UK). Precision of measurement as monitored by the RM's and lab standards was ± 1.2 ‰ or better. Each batch was also preceded and followed by a blank capsule for blank correction. Measured δ^2H-values were two-end-point normalised to the VSMOW scale using IAEA-CH7 (δ^2H_{VSMOW} = -100.3 ‰) and coumarin (δ^2H_{VSMOW} = +62.6 ± 2.4 ‰; Iso-Analytical, Crewe, UK) in analogy to the internationally accepted VSMOW/SLAP scale normalization method.

RESULTS

- Analysis of ^{2}H isotopic composition of ground tooth enamel is possible but its low content of hydroxyl groups needed to be compensated for by the use of sample amounts of at least 1 mg per silver capsule (cf. Figure 1).
- Adding carbon in the form of graphite to ground enamel samples did not increase H_2 yield (cf. Figure 2).
- Exchange experiments carried out on powdered tooth enamel did not show any indication of significant hydrogen exchange as could be expected (see Table 1).
- Variation in cleaning protocols did not affect the observed δ^2H-values of tooth enamel (see Table 2). Observed differences were not statistically significant ($p = 0.13$).
- Enamel from molars of different geographic origins yielded the same δ^2H value of -111.3 ± 2.3 ‰ on average (sample preparation protocol B).
- The apparent dynamic range (= absolute range / 1σ) in δ^2H-values from tooth samples amounted to 4 ‰ while the corresponding range for source water at known points of origin was 40 ‰.

Figure 1: Size and shape of H_2 peak (at 94.3 s) evolved from 0.2mg of ground tooth enamel (top panel) or 1.0 mg of ground tooth enamel (bottom panel).

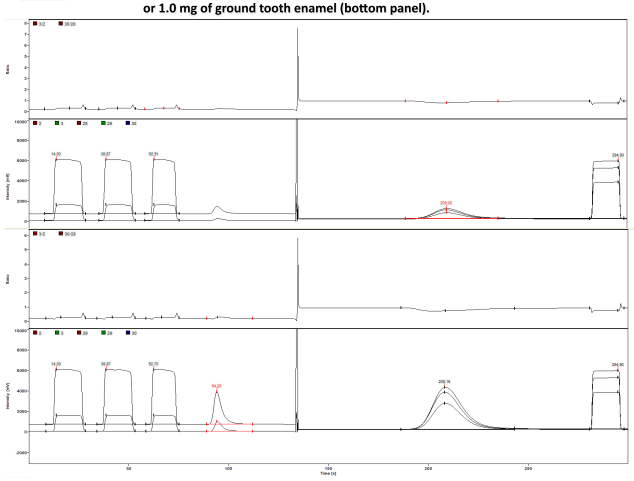

Table 1: Comparison of δ^2H values of tooth bio-apatite after H exchange experiment.

Sample	δ^2H [‰] enamel measured	$\Delta\delta^2$H [‰] (LTW-CCW)	δ^2H [‰] of waters used for H exchange
CLB3M_LTW*	-108.3 ± 1.9 ‰		
CLB3M_CCW*	-110.6 ± 1.1 ‰	2.3	
LTW			-54.1 ‰
CCW		87.8	-141.9 ‰

* Samples were prepared according to protocol S.

CONCLUSIONS

- It is possible to measure ^{2}H isotopic composition of the hydroxyl fraction of tooth enamel by TC/EA-IRMS.
- Given the ratio of proposed proxy range over source range of 0.1 the ^{2}H signature of tooth enamel is not deemed to be an appropriate proxy for provenance.
- However, the seemingly fixed nature of ^{2}H abundance in tooth enamel may hold information of interest to researchers in the field biogenic minerals.

Figure 2: Size and shape of H_2 peak (at 95.1 s) evolved from 1.5mg of ground tooth enamel in the absence (top panel) or in the presence of ground graphite (bottom panel).

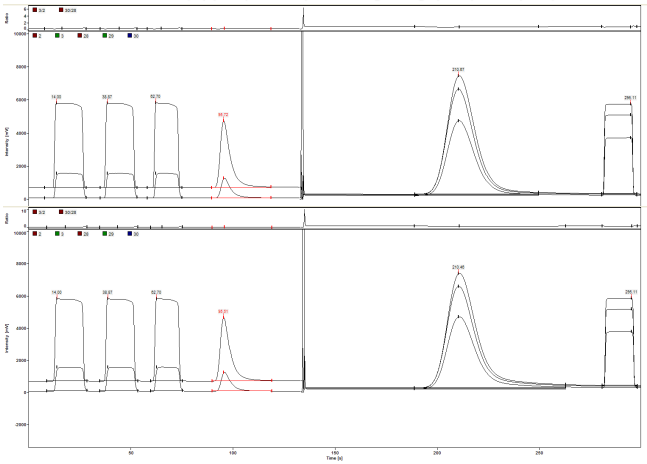

Table 2: Comparison of δ^2H values of tooth bio-apatite depending on sample treatment protocol.

Sample	Country of origin	δ^2H [‰], no pre-treatment	δ^2H [‰], pre-treatment B	δ^2H [‰], pre-treatment B plus graphite	δ^2H [‰], pre-treatment S
SC2M[a]	France	-106.5 ± 3.4	-115.0*	-115.9*	
SC3M[a]	France	-105.6 ± 3.7	-115.3*	-113.8*	
CLB1_2M[b]	UK		-110.4 ± 1.6		
CLB3_2M[b]	UK				-107.9 ± 1.1
CLB4_2M[b]	UK		-112.7 ± 0.8		
RM_2M[a]	Italy				-111.4 ± 0.3
IM_3M[a]	North Africa		-110.5 ± 0.4		-111.7 ± 0.5
HFK_3M[b]	Kent, UK		-111.7 ± 1.7		-106.5 ± 0.8
CLM1_2M[b]	Not known		-111.3 ± 1.1		-108.6 ± 0.5
CLM3_2M[b]	Not known		-108.3 ± 0.5		-106.8 ± 0.5
CLM4_2M[b]	Not known		-109.7 ± 0.5		-108.5 ± 1.0

[a] Archaeological sample; [b] modern sample.
* Samples for which ± 1σ values are not given were only analyzed once due to limited sample amount.

REFERENCES: [1] Schwarcz HP, Walker PL. Characterization of a murder victim using stable isotopic analyses. *Am J Phys Anthropol* 2006;129:160. [2] Meier-Augenstein W, Fraser I. Forensic isotope analysis leads to identification of a mutilated murder victim. *Science & Justice* 2008;48:153-9. [3] Sponheimer M, Lee-Thorp JA. Oxygen isotopes in enamel carbonate and their ecological significance. *J Archaeol Sci* 1999;26:723-8. [4] Wopenka B, Pasteris JD. A mineralogical perspective on the apatite in bone. *Mater Sci Eng , C* 2005;25:131-43. [5] Chakraborty S, Bag S, Pal S, Mukherjee AK. Structural and microstructural characterization of bioapatites and synthetic hydroxyapatite using X-ray powder diffraction and Fourier transform infrared techniques. *J Appl Crystallogr* 2006;39:385-90. [6] Lee-Thorp JA. On Isotopes and Old Bones. *Archaeometry* 2008;50:925-50. [7] Lee-Thorp J, Manning L, Sponheimer M. Problems and prospects for carbon isotope analysis of very small samples of fossil tooth enamel. *Bulletin de la Societe Geologique de France* 1997;168:767-73.

ACKNOWLEDGEMENTS: SCRI gratefully acknowledges the financial support by the Scottish Government Rural and Environment Research and Analysis Directorate (RERAD).