^{2}H Stable Isotope Analysis of Human Tooth Dentine: A Pilot Study

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Introduction

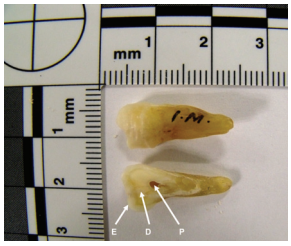
Stable isotope analysis of biogenic tissues such as tooth enamel and bone, and hair has become a well recognized and commonly implemented technique for determining provenance of human remains in bioarchaeological studies and, more recently, to facilitate the forensic identification of unidentified human remains (Lee-Thorp 2008; Meier-Augenstein and Fraser 2008). Both ^{18}O and ^{2}H stable isotope signatures 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 (Hobson *et al*. 2004; Schwarcz and Walker 2006). Similarly, δ^{13}C and δ^{15}N data have distinguished dietary preferences in ancient human populations, and have been used to qualify ^{2}H and ^{18}O geolocational data that is consistent with more than one location (Meier-Augenstein and Fraser 2008; Rauch et al. 2007). While studies focusing on the isotopic analysis of mammalian tooth dentine have emerged in the literature, few if any studies have systematically investigated the multi-isotopic signatures in human tooth dentine (Dupras and Tocheri 2007). Since δ^{18}O values obtained from tooth enamel of late-erupting molars are a source of information on geographic origin of an individual during adolescence when crown formation takes place, we hypothesized that potentially valuable information can be obtained from the stable isotopic composition of human crown dentine related to geographic provenance and dietary intake.

In this study we measured isotopic abundance of ^{2}H and ^{13}C in human dentine collagen obtained from archaeological and modern teeth using continuous-flow isotope ratio mass spectrometry (IRMS) and compared these findings with the ^{18}O and ^{13}C isotopic composition of the corresponding tooth enamel.

Materials and Methods

Tooth samples

Seven second or third permanent molars from people of different geographic provenance were all mechanically cleaned to remove any surface contamination (Henton *et al*. 2010). Crown enamel and mantle dentine samples were collected using a dental drill with a diamond tipped drill bit. Powdered enamel was cleaned and subjected to acid digest as previously described (Henton *et al*. 2010; Holobinko *et al*. 2011). Powdered primary dentine was demineralised for 72 h in 0.5 N HCl. Alkali-soluble contaminants were removed in 0.1 M NaOH for 24 h and residual lipids were dissolved in a 2:1:0.8 mixture of methanol, chloroform and water for 24 h. The collagen pellet isolated after repeated washes with deionized water and centrifugation was freeze-dried to yield an amorphous white to yellowish material.



Vertically sectioned 3rd molar showing enamel (E), dentine (D) and pulp cavity (P).

Bulk ^{2}H, ^{18}O, ^{13}C, ^{15}N isotope analysis

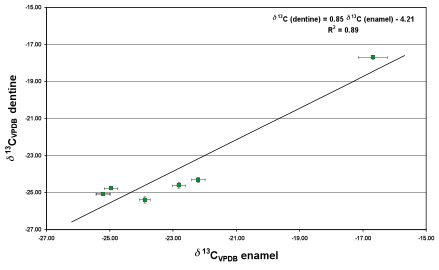
Stable isotope analysis of the various tissue samples by Continuous Flow – Isotope Ratio Mass Spectrometry (CF-IRMS) was carried out as described elsewhere (Henton *et al*. 2010; Holobinko *et al*. 2011; Landwehr *et al*. 2011). To determine 'true' dentine collagen δ^{2}H values, i.e. δ^{2}H values of non-exchangeable H, all dentine collagen samples were subjected to a two-stage H exchange procedure described in detail in Landwehr *et al*. 2011, based on the protocol reported by Bowen *et al*. 2005. All measured stable isotope data were properly scale normalized to VPDB or VSMOW using two-point end-member correction.

Results and Discussion

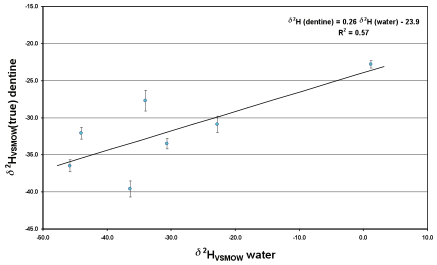
Comparing enamel δ^{13}C values with dentine collagen δ^{13}C values, both adjusted for trophic level isotopic shift, showed a consistent difference with dentine collagen δ^{13}C values being on balance slightly more negative than corresponding enamel δ^{13}C values. As to whether this difference is a reflection solely due to correspondingly minute changes in diet or is a reflection of diet change in conjunction with a very slow remodeling rate, it is not possible to answer this question conclusively on the basis of the currently available data.

Dentine collagen δ^{2}H values covered a range of 16.8 ‰ while the range for corresponding source water δ^{2}H was 47.0 ‰. The slope of the linear regression line was 0.26, which is less than that reported for the correlation between hair δ^{2}H values and corresponding source water δ^{2}H values. One possible explanation for this finding could be a slow remodeling rate of primary dentine. For example, human hair is formed at a 10-30 times faster rate than human nails and this seems to be reflected by the fact that published correlation slopes for $\Delta\delta^{2}$H (hair) / $\Delta\delta^{2}$H (water) are higher than the slope for $\Delta\delta^{2}$H (nails) / $\Delta\delta^{2}$H (water).

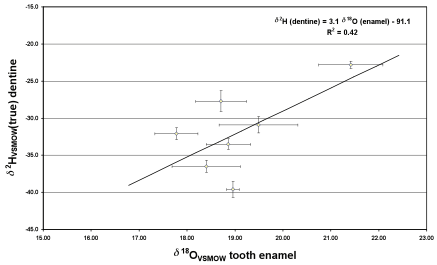
Analysis of dentine collagen δ^{2}H values with the matrix independent variable enamel δ^{18}O, known to be well correlated with source water δ^{18}O values, yielded a positive correlation between these two variables.



Correlation plot of dentine δ^{13}C values vs. enamel δ^{13}C values.



Correlation plot of dentine δ^{2}H values vs. water δ^{2}H values.



Correlation plot of dentine δ^{2}H values vs. enamel δ^{18}O values.

Conclusions

- The positive correlation between collagen δ^{2}H values of primary dentine with δ^{2}H values for source water provides the first indication that collagen δ^{2}H values of primary dentine may serve as a proxy for geographic provenance.
- The positive correlation of collagen δ^{2}H values from primary dentine with enamel δ^{18}O values supports the findings that primary dentine collagen δ^{2}H values are linked to the isotopic composition of source water.
- The observed difference of about only 15% on average between corresponding enamel δ^{13}C values and primary dentine collagen δ^{13}C value could be interpreted as an indication that in healthy teeth the remodeling rate of primary dentine collagen is relatively slow. In support of this interpretation further corroborative data would be require from more recently formed tissues.

Our preliminary findings suggest that multi-isotope signatures of human tooth dentine but in particular the information locked into ^{2}H isotopic composition of dentine collagen may improve the quality or accuracy of a quantifiable dietary and geographical life trajectory of an as yet unidentified individual.

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