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The interpretability of stable hydrogen isotopes in modern herbivore tooth enamel

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Abstract

Stable hydrogen isotopes (δD values) in animal organic tissues such as collagen and keratin typically correlate with local meteoric water, but little is known about δD in tooth enamel apatite. In this study, we analyzed comminuted tooth enamel for oxygen isotopes ($\delta^{18}O$) of the CO_3 component and for δD of bulk enamel. We find positive correlations between enamel δD and $\delta^{18}O$ ($R^2 = 0.70$) and between enamel δD and local precipitation δD ($R^2 = 0.53$). However, the slopes of these relationships are much shallower (less variation in tooth enamel δD) than expected from studies of other tissues. Based on mass spectrometric peak areas, H_2 contents for enamel are 2–5 times higher than expected from chemical compositions, and we interpret as much as 50–90% of measured hydrogen from tooth enamel may be adsorbed water derived from laboratory water vapor. We tested this hypothesis by equilibrating tooth enamel with very high and very low δD values of water vapor, then exposing to laboratory air for different periods of time ranging from minutes to 8 hours. These experiments show that the apparent δD value of enamel converges to a nearly constant δD value in 1 to 2 hours. The large amount of adsorbed water and rapid approach to equilibrium will make it difficult to infer provenance from δD measurements alone, or to reproduce measured tooth enamel δD values among laboratories with different water vapor compositions. Heating at 70 °C for 48 hours in air does not remove adsorbed hydrogen, but does reduce δD values by c. 10‰ compared to unheated samples. Differences in δD values for heated vs. unheated enamel may reflect either a different, temperature-dependent partition coefficient between adsorbed water (on apatite) and water vapor, exchange of structural H at elevated temperatures, or subtle changes to crystal structure, such as loss of structural H.

Keywords: Hydrogen isotopes; Tooth enamel; Biogenic; Apatite; Labile hydrogen

1. INTRODUCTION

Stable hydrogen isotopes (δD values) from animal organic tissues have been widely used to track migratory patterns, diet type, trophic level, and climate (Estep and Dabrowski, 1980; Cormie et al., 1994; Hobson et al., 1999; Bearhop et al., 2003; Cryan et al., 2004; Bowen et al., 2005a; Leyden et al., 2006; Ehleringer et al., 2008; Reynard and Hedges, 2008; Hobson and Wassenaar, 2018; Sluis et al., 2019). However, the use of δD values within inorganic tissues, such as tooth enamel, has yet to be explored in greater depth (Holobinko et al., 2011). Some organic tissues do not always preserve well, and alternate tissues can in principle prove beneficial. Tooth enamel oxygen (and carbon) isotopes can be used in ecological and environmental studies of mammals (e.g., Clementz and Koch, 2001; Koch, 2007; Newsome et al., 2010), so by anal-

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ogy δD values in enamel might also serve similar scientific interests. Developing such a tooth enamel δD proxy requires addressing questions of analytical methods, especially any bias introduced by labile water (both exchangeable structural H and adsorbed water). For example, of the total H in modern hair (keratin) and bone collagen, 10–20% is exchangeable with atmospheric water vapor (Sharp et al., 2003; Cormie et al., 1994; Leyden et al., 2006). For some materials (e.g., chitin), nitration can remove exchangeable structural hydrogen (Epstein et al., 1976; Miller et al., 1988; Schimmelmann, 1991). In other materials where nitration is not feasible (e.g., keratins and collagen), the comparative equilibrium method (Wassenaar and Hobson, 2000) minimizes compositional bias from labile H.

Animal oxygen and hydrogen isotopes are linked to meteoric water, whose oxygen and hydrogen isotope compositions covary strongly across different localities on Earth to define the global meteoric water line (GMWL; $\delta D = \delta^{18}O \times 8 + 10‰$; e.g. Craig, 1961; Dansgaard, 1964; Rozanski et al., 1992). In terrestrial vertebrates, intake water, primarily from drinking and from water content of food, commonly results in strong correlations between measured $\delta^{18}O$ and δD, between measured δD and meteoric water δD, and between measured $\delta^{18}O$ and meteoric water $\delta^{18}O$ (Ayliffe and Chivas, 1990; Cormie et al., 1994; Koch, 1998; Kohn and Cerling, 2002; Sharp et al., 2003; Cryan et al., 2004; Leyden et al., 2006; Kohn and Dettman, 2007; O'Brien and Wooller, 2007; Ehleringer et al., 2008; Clementz, 2012; however see Pietsch et al., 2011, for counterexamples). Because of these correlations, if tooth enamel provides a reliable proxy for meteoric water δD, we expect strong correlations for tooth enamel δD with tooth enamel $\delta^{18}O$, with a slope approaching the meteoric water line (i.e. c. 8), and with meteoric water δD with a slope approaching 1. We also expect isotopic offsets between enamel δD and meteoric water δD reflecting fractionation between body water vs. local water compositions, and between body water and tooth enamel mineral (e.g., see Cormie et al., 1994).

If enamel δD values are to be related to climate, it is critical to first determine if tooth enamel δD and $\delta^{18}O$ values correlate. Despite correlations in δD and $\delta^{18}O$ values found in other animal substrates, a study of 3 archeological and 2 modern human teeth showed a weak correlation between tooth enamel δD and $\delta^{18}O$ values, and between tooth enamel δD and local water δD values (Holobinko et al., 2011). Because of the potential for exchangeable hydrogen, Holobinko et al. (2011) tested whether δD values could be altered by equilibrating sample aliquots for a single tooth with evaporatively enriched- and depleted-waters over a 4-day timespan. Tooth enamel compositions from enriched- and depleted-water equilibrations were similar, which was interpreted to mean that tooth enamel H isotopes are essentially immune to re-equilibration. As we discuss below, there may be other explanations for their observations.

In this study, we characterized the relationship between herbivore tooth enamel δD with respect to $\delta^{18}O$ values of enamel and δD values of local water; we also measured total H contents in comparison with other reference materials. With these data, we addressed the following questions:

(1) Does an expanded tooth enamel dataset over a larger isotopic range for local water compositions and for different herbivore taxa show a stronger correlation than Holobinko et al. (2011) observed for human tooth enamel? We pretreated bulk tooth enamel following standard procedures for stable isotope analysis (Koch et al., 1997) and measured its δD and $\delta^{18}O$ values. The resulting isotope compositions represent the combined contributions of hydrogen-bearing components of tooth enamel, plus any adsorbed water.
(2) Does adsorbed water contribute a significant amount of H to tooth enamel analyses? We compared measured H contents of tooth enamel (as calibrated against the H content of NBS-30 biotite), with the expected stoichiometric H contents (based on published chemical compositions of tooth enamel; Driessens and Verbeeck, 1990).
(3) What are the timescales of any adsorbed water uptake? We conducted a series of timed experiments with enamel that had been equilibrated with both high (c. +60‰) and low (c. −300‰) δD water to determine time-scales of labile water equilibration.
(4) Can heating remove adsorbed water and resolve original biogenic tooth enamel compositions? We analyzed enamel samples that had been heated to estimate the effect of water removal due to heating.

2. TOOTH ENAMEL FORMATION AND ISOTOPES

Several systematics to tooth enamel formation and isotopes are relevant to our data and interpretation. Tooth enamel consists of c. 98% bioapatite with an approximate composition of $(Ca_{4.5}[(PO_4)_{2.7}(HPO_4)_{0.2}(CO_3)_{0.3}](OH)_{0.5})$ (Driessens and Verbeeck, 1990). Hydrogen in enamel bioapatite occurs in two distinct components: c. 30% as HPO_4 (c. 0.04 wt% H in bulk enamel) and c. 70% as OH (c. 0.10 wt% H in bulk enamel; Driessens and Verbeeck, 1990). Oxygen in enamel bioapatite occurs in four different components: c. 89% as PO_4 (as PO_4 or HPO_4), 7% as CO_3, and 4% as OH (Driessens and Verbeeck, 1990). Enamel also contains small amounts of H-bearing organic compounds: protein (c. 0.03 wt%, or c. 0.003 wt% H in bulk enamel; Goldberg and Septier, 2002) and phospholipids (c. 0.5 wt %, or c. 0.05 wt% H in bulk enamel; Farah et al., 2010). Thus, the dominant reservoirs of H in chemically untreated enamel are from bioapatite (72%) and phospholipids (26%).

Teeth progressively mineralize from the occlusal (chewing) surface towards the root. Because enamel is not remodeled after its formation and because the temperature of precipitation is constant in mammals, enamel preserves an isotopic record of an animal's body water compositions over its period of growth. Differences in mineralization rate and overall tooth size between taxa result in wide variations in the amount of time recorded within a single tooth

(Passey and Cerling, 2002; Kohn, 2004; Trayler and Kohn, 2017), typically ranging from a few months to 1–2 years. Enamel $\delta^{18}O$ values correlate with local water compositions (Koch, 1998; MacFadden, 2000; Kohn and Cerling, 2002; Kohn and Dettman, 2007; Clementz, 2012), which are controlled by precipitation sources and temperature (Dansgaard, 1964; Rozanski et al., 1992). Because local water compositions vary seasonally (generally low $\delta^{18}O$ in winter and high $\delta^{18}O$ values in summer), O isotopic zoning within enamel can serve as a proxy for climate seasonality (Fricke and O'Neil, 1996; Kohn, 1996; Zanazzi et al., 2007). Because δD and $\delta^{18}O$ correlate along the GMWL, and because tooth enamel $\delta^{18}O$ values track seasonality, in principle, reconstructions of climate and ecology might alternatively employ enamel δD values.

3. SAMPLES AND METHODS

3.1. Specimens and samples

In our initial analysis, we selected 12 modern specimens of herbivore teeth (Table 1). Taxonomically, these specimens included: *Castor canadensis* (beaver), *Bos taurus* (cattle), *Bison bison* (bison), *Equus ferus caballus* (horse), *Nanger granti* (Grant's gazelle), *Oryx beisa* (oryx), *Capra hircus* (goat), and *Cervus elaphus* (elk). These specimens were collected opportunistically over a period of c. 25 years, and had been stored in the Boise State Stable Isotope Laboratory for 10 years excepting *Bison bison* from Santa Catalina Island, which was stored for 5 years.

We collected sub-samples along the growth axis of each tooth, typically every 1–2 mm using a Dremel™ rotary tool and a 0.5 mm dental drill bit. For large herbivores, enamel is sufficiently thick that we are confident we sampled only enamel. However, for beaver (*Castor canadensis*), enamel is thin, and we may possibly have contaminated enamel with small amounts of underlying protein-rich dentine. Powdered samples were pretreated as per Koch et al. (1997), first with H_2O_2 to remove organic material, then with a 1 M Ca acetate – acetic acid buffer solution to remove labile carbonates. Powdered samples were dried at 30 °C prior to analysis. Note that Holobinko et al. (2011) observed no significant hydrogen isotopic differences using two different pretreatment methods. Oxygen isotope compositions for our 4 samples DREW, NEPGO, M-00-49, and M-00-59 were previously published (Trayler and Kohn, 2017). Here we report new δD values for these samples, as well as δD and $\delta^{18}O$ (from CO_3) values for the remaining 8 teeth. For completeness, we report $\delta^{13}C$ values (see Supplementary Material), although we do not make comparisons to δD or $\delta^{18}O$.

In later analyses, we tested for exchangeable/adsorbed hydrogen by analyzing bulk enamel from 4 modern tooth specimens (2 *Bison bison* and 2 *Bos taurus*; BM1-Y, BM3-C, BOSM2-B, and UWB), a synthetic apatite (NIST-2910), and a powdered fossil bone (KBS; Table 1). The fossil bone powder derives from a large long-bone fragment of a taxonomically unknown mammal from the Juntura Formation (c. 10.6 Ma; Kohn and Fremd, 2008), central Oregon. Enamel was removed along the entire length of each tooth (from occlusal surface to root) using a Dremel™ rotary tool and a 0.5 mm dental drill bit, and was again pretreated to remove any organics and labile carbonates (Koch et al., 1997). For consistency, NIST-2910 and KBS were also pretreated prior to analysis.

3.2. Analytical methods

Stable oxygen isotopic compositions of the carbonate component of teeth (Tables 2 and A1) were measured by dissolving 1.5–2.0 mg of powdered pretreated enamel in supersaturated H_3PO_4. The evolved CO_2 was measured

Table 1
ID number, species and tooth type, and source location.

ID number	Taxa/Tooth or Sample type	Source/Location
BC	*Bison bison*/M3	Santa Catalina, CA, USA
BTM	*Castor canadensis*/I	Canyon City, CO, USA
COW2	*Bos taurus*/M2	Juntura, OR, USA
DREW	*Equus ferus caballus*/M3	Drewsey, OR, USA
EEC	*Equus ferus caballus*/M3	El Criado, Argentina
GBM2	*Equus ferus caballus*/M2	Gran Barranca, Argentina
GGK	*Nanger granti*/M2	Sibiloi National Park, Kenya
M-00-49	*Cervus elaphus*/M3	Yellowstone, WY, USA
M-00-59	*Cervus elaphus*/M3	Yellowstone, WY, USA
NEPGO	*Capra hircus*/M3	Pokhara, Nepal
O2120	*Oryx beisa*/M2	Sibiloi National Park, Kenya
UWB-1	*Bos taurus*/M3	Univ of Wisconsin, WI, USA
Bulk specimens		
BM1-Y	*Bison bison*/M1	Yellowstone, WY, USA
BM3-C	*Bison bison*/M3	Santa Catalina, CA, USA
BOSM2-B	*Bos taurus*/M2	Brazil (specific locality unknown)
UWB-2	*Bos taurus*/M3	University of Wisconsin, WI, USA
NIST 2910	hydroxylapatite standard	NIST
KBS	fossil bone powder	Juntura, OR, USA

Table 2
Modern tooth mean, minimum, and maximum δD and $\delta^{18}O$ values (±2σ and 2 s.e.), mean sample and H masses, and local water δD and $\delta^{18}O$ for each sample location.

ID Number	n	Mean δD (‰, VSMOW)	2σ	2 s.e.	max δD*	min δD*	Difference	Mean mass (mg)	Mean H mass (mg)	δD(water)†	Mean $\delta^{18}O$ (‰, VSMOW)	2σ	2 s.e.	max $\delta^{18}O$	min $\delta^{18}O$	Difference	$\delta^{18}O$(water)†
BC	11	−131.4	5.1	1.5	−120.7	−135.7	15.0	2.4	0.019	−48.0	28.2	1.8	0.3	29.4	26.9	2.5	−7.3
BTM	9	−131.6	5.3	1.8	−123.6	−139.2	15.6	1.2	0.019	−52.0	19.2	2.0	0.3	20.5	17.5	3.0	−7.8
COW2	11	−150.2	4.2	1.3	−144.3	−157.9	13.6	1.4	0.011	−103.0	21.2	3.8	0.7	24.5	19.1	5.4	−14.1
DREW	30	−152.2	4.2	0.8	−145.4	−160.5	15.2	1.3	0.016	−103.0	20.1	4.7	0.4	24.7	16.9	7.8	−14.1
EEC	12	−130.4	3.4	1.0	−123.6	−133.3	9.6	1.8	0.018	−86.0	24.8	3.4	0.5	28.8	23.5	5.4	−12.0
GBM2	21	−142.0	5.7	1.3	−132.1	−148.9	16.8	1.4	0.010	−86.0	25.1	4.7	0.5	30.5	19.2	11.3	−12.0
GGK	8	−130.7	4.4	1.6	−124.6	−135.2	10.6	2.2	0.018	22.0	33.0	1.6	0.3	33.8	31.4	2.4	1.5
M-00-49	8	−164.6	4.6	0.4	−162.8	−165.9	3.0	1.6	0.021	−140.0	15.2	1.9	0.3	16.8	14.1	2.7	−18.8
M-00-59	7	−163.7	1.1	1.6	−159.4	−173.9	14.5	1.9	0.032	−140.0	14.5	1.6	0.4	16.4	13.5	2.9	−18.8
NEPGO	20	−149.8	4.5	1.0	−137.0	−155.3	18.3	2.2	0.023	−85.0	21.6	2.2	0.2	23.2	19.6	3.6	−11.9
O2120	12	−129.5	4.4	1.3	−117.5	−134.0	16.5	1.4	0.015	22.0	35.6	1.2	0.2	36.3	34.6	1.7	1.5
UWB-1	13	−147.7	1.8	0.5	−144.2	−151.4	7.2	1.9	0.015	−50.0	20.2	5.0	0.7	23.3	15.8	7.5	−7.5
BM1-Y		−140.6	36.4	9.4	−102.1	−163.5	61.3	1.8	0.014	-140.0	N.A.‡	N.A.	N.A.	N.A.	N.A.	N.A.	−18.8
BM3-C		−130.9	42.4	11.0	−93.5	−181.0	87.5	1.8	0.007	−48.0	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	−7.3
BOSM2-B		−126.9	49.4	12.7	−66.0	−177.5	111.5	2.2	0.012	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
UWB-2		−144.0	37.5	9.7	−88.8	−168.1	79.3	1.4	0.010	−50.0	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	−7.5
NIST 2910		−140.0	64.6	16.7	−38.3	−201.4	163.1	1.7	0.011	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
KBS		−124.0	59.9	15.5	–22.6	−158.2	135.6	2.1	0.008	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

† Isotope values derived from the Online Isotopes in Precipitation Calculator (Bowen, 2018) except GGK (Johnson et al., 1991).
‡ N.A. = not applicable.
* Each minimum and maximum value represents an extreme of measurements on a zoned, serial sectioned tooth.

for oxygen and carbon isotopes using a ThermoFisher GasBench II in-line with a Thermo Delta V Plus mass spectrometer housed at the Stable Isotope Laboratory at Boise State University. Each sample set was standardized to VPDB using eight to nine aliquots of the calcite standards NBS-18 ($\delta^{18}O = -23.2‰$, VPDB) and NBS-19 ($\delta^{18}O = -2.2‰$, VPDB). Enamel $\delta^{18}O$ values expressed in VPDB were converted to VSMOW using the following equation (Coplen, 1988):

$$\delta^{18}O_c(VSMOW) = 1.03091 \times \delta^{18}O_c(VPDB) + 30.91 \quad (1)$$

Five to six NIST-120c ($\delta^{18}O = +28.5‰$, VSMOW and $\delta^{13}C = -6.55‰$, VPDB; Kohn et al., 2015) aliquots were prepared using the same cleaning pretreatment methods and analyzed with each sample set as a check standard. Analytical reproducibility for oxygen isotopes was: NIST-120c = ±0.83‰ (2σ); NBS-18 = ±0.66‰; and NBS-19 = ±0.46‰. Reproducibility for carbon isotopes was: NIST-120c = ±0.43‰ (2σ); NBS-18 = ±0.40‰, and NBS-19 = ±0.40‰. Reproducibility of sample weights was ±0.002 mg.

Stable hydrogen isotope compositions (Tables 2, A1, and A2) were measured by combusting 1–2 mg of powder in silver capsules using a ThermoFisher TC/EA coupled with the same Thermo Delta V Plus mass spectrometer. For standardization, we included 10 aliquots of biotite standard NBS-30 ($\delta D = -65.7‰$, VSMOW) with each analytical run; for time-series samples we increased the number of biotite aliquots to 26 and included 26 aliquots of caribou hoof standard (CBS; $\delta D = -157‰$, VSMOW; Soto et al., 2017) to evaluate predicted vs. measured H contents. Reproducibility for stable hydrogen isotopes was ±4.8‰ (2σ) for NBS-30 and ±4.4‰ for CBS. Importantly this technique cannot distinguish hydrogen from different enamel components (i.e., OH, HPO_4, phospholipid, protein) plus extraneous H, so our results should be viewed as the bulk H isotope composition of enamel plus any contamination. While our pretreatment should have removed all organic components from teeth, we cannot rule out the possibility that some fraction of H was derived from phospholipid. This possibility does not change our interpretations. All $\delta^{18}O$ and δD values (Tables 2, A1, and A2) are reported relative to VSMOW.

We compared sample weights of the 12 sub-sampled teeth to the mass spectrometrically measured ^1H–^1H peak area (Table A1) to test whether measured δD values reflect solely structural hydrogen, or a combination of structural and adsorbed hydrogen. If measured H inferred from measurements exceeds the amount expected from published chemical compositions, adsorbed water must contribute to total H. That is, on a plot of measured H content (based on mass spectrometer sample peak area) vs. sample weight, the difference in slope between measured vs. expected H contents provides an estimate of the amount of adsorbed water per mg of sample. To determine water contents from mass spectrometer measurements we calibrated the signal intensity of the enamel H_2 peak to that of NBS-30 (biotite), assuming a H_2 content of 0.0041 wt% (Qi et al., 2017). For the data presented here, the mass fraction of H equaled the ^1H–^1H peak area times 0.0004.

3.3. Time-series experimental methods

Because we found evidence for significant adsorbed water contamination, we performed equilibration experiments with depleted-water ($\delta D = -300.4 \pm 2.2‰$; VSMOW) and enriched-water ($\delta D = +62.0 \pm 1.7‰$) to better understand equilibration timescales between adsorbed water and ambient water vapor. Water compositions for D-depleted- and D-enriched-waters (Table A2) were measured using a fourth generation Los Gatos Research Liquid Water Isotope Analyzer (LWIA) at the Stable Isotope Laboratory at Boise State University.

For time series analysis of bulk powders (Sample IDs in Table 1, data in Table A2), multiple aliquots of each material were split into open silver capsules and placed in large airtight containers with small open containers of either D-depleted- or enriched-water for 48 hours at 20 ± 0.5 °C. Bulk samples were removed, exposed to ambient laboratory conditions for 0, 1, 2, 4, and 8 hours (which are notated as: t_0, t_1, t_2, t_4, and t_8, respectively) then δD values were measured. Experiments with ^{18}O and ^2H-depleted water were conducted on May 2, 2018 and ^{18}O and ^2H-enriched water experiments were conducted on May 20, 2018. The silver capsules were crimped just prior to analysis, transferred into the TC/EA zero blank autosampler, and the autosampler was flushed with UHP He for 3 minutes before analysis. Because of the delay between removing each sample from its equilibration chamber and actual analysis, each atmospheric exposure time should be assumed to be about 15 minutes longer than the nominal time we report. This has minimal effect on exposure times ≥ 1 hour but may be significant for "0" time experiments. We assume laboratory water vapor is in equilibrium with local water, which has a mean δD value of −111‰ for Boise State University (Tappa et al., 2016). Alternative assumptions about water vapor composition do not change our interpretations.

To test whether adsorbed H could be completely removed simply by heating, sample aliquots were placed in a 70 ± 1 °C oven (exposed to laboratory air) for 48 hours on May 25, 2018. Previous thermogravimetric analysis, coupled with IR spectroscopy (Holcomb and Young, 1980), shows that adsorbed water is rapidly removed during continuous heating at 8 °C/min to 100 °C. However, small changes to structural CO_3 content also occur by 100 °C. A temperature of 70 °C balances the loss of water against the potential loss of CO_3 and structural changes to tooth enamel mineral. Samples were then removed and exposed to laboratory air for 0, 1, 2, 4, and 8 hours and δD values were measured. Again, capsule crimping and sample loading times increased these times by c. 15 minutes.

3.4. Water isotope compositions

Local water δD values were estimated from the Online Isotope Precipitation Calculator (Bowen and Revenaugh, 2003; Bowen, 2018) except for Boise, Idaho and Kenya, where local waters were analyzed directly (Idaho: Tappa et al., 2016; c. −111‰; Kenya: Johnson et al., 1991; c. +22‰).

4. RESULTS

4.1. Stable hydrogen and oxygen isotope compositions

From the 12 sub-sectioned teeth, 2 *Cervus elaphus* teeth—M-00-49 and M-00-59— from Yellowstone, WY had the lowest mean δD and $\delta^{18}O$ values of about −160‰ and +15‰, respectively, whereas *Oryx gazelle* (O2120) and *Nanger granti* (GGK) from Kenya had the highest δD and $\delta^{18}O$ values of about −135‰ and +34‰, respectively (Fig. 1; Table 2). *Castor canadensis* from Cañon City, CO (BTM), and *Equus ferus caballus* from Argentina (EEC) both have anomalously high δD values (c. −132‰), which are comparable to *Oryx gazelle* (O2120) and *Nanger granti* (GGK); BC (*Bison bison*) also falls slightly above the regression line for all data (Fig. 1; Table 2).

For regressing mean isotope data for δD_{enamel} and $\delta^{18}O_{enamel}$ values, we used all data except specimen BTM (*Castor canadensis*). Semiaquatic mammals often have systematically lower body water isotope compositions (Clementz and Koch, 2001), and also beaver enamel is very thin, raising the possibility that small amounts of protein-rich dentine might have contaminated "enamel". The resulting regression is:

$$\delta D(‰) = 1.70 \pm 0.19\left(\delta^{18}O\right) - 185 \pm 5‰ \quad (R^2 = 0.70) \qquad (2)$$

A slope of 1.7 is considerably shallower than the GMWL slope (c. 8.0).

The lowest tooth enamel δD values from Yellowstone, WY, correspond with the lowest local water δD values (−140‰), while the highest tooth enamel δD values from Kenya correspond with the highest local water δD values (+22‰; Fig. 2; Table 2). A regression of δD_{enamel} vs. $\delta D_{local\ water}$ values yields:

$$\delta D_{enamel} = 0.19 \pm 0.03\left(\delta D_{precipitation}\right) - 131 \pm 3‰ \quad (R^2 = 0.53) \qquad (3)$$

The slope of this regression is considerably less than 1.0, which implies a small dependence of measured enamel compositions on original source water composition.

4.2. Labile hydrogen

Using NBS-30 biotite as a standard for hydrogen content, a regression of tooth enamel hydrogen content vs. sample mass yields an average hydrogen content of 0.97 ± 0.06 wt% (data in Table A1). A representative hydrogen content for tooth enamel bioapatite (Driessens and Verbeeck, 1990) is 0.14 wt% (or 0.19 wt% including phospholipids). Thus, most hydrogen in our analyses must be derived from another source. In contrast, similar measurements and calculations for keratin (CBS) show that the

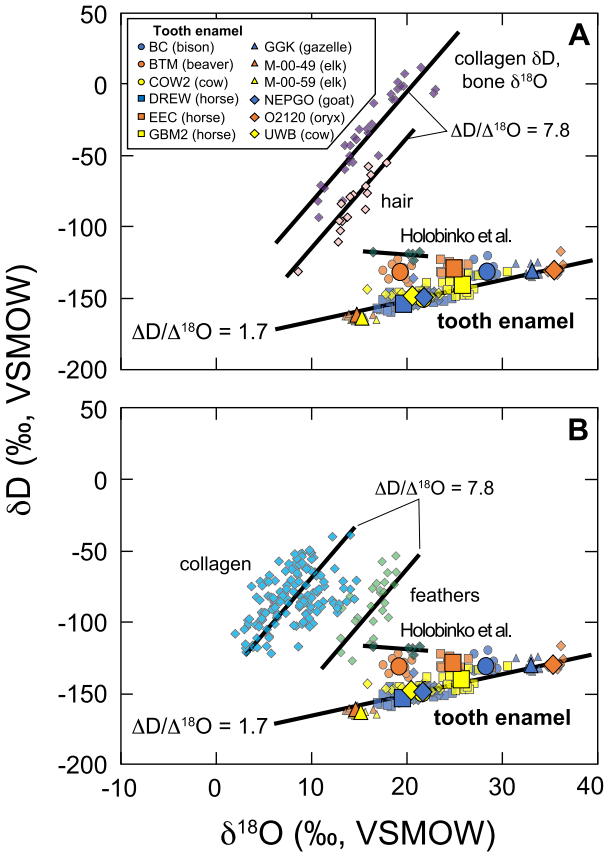

Fig. 1. δD vs. $\delta^{18}O$ for tooth enamel and previously published data for other organic materials, showing much shallower slope for tooth enamel. The slope of 7.8 for collagen, feathers, and collagen plus bone is for illustration, but indistinguishable within uncertainty for all 3 datasets. (A) Comparison with human hair (Bowen et al., 2009) and with deer collagen δD plus bone $\delta^{18}O$ values (Cormie et al., 1994). (B) Comparison with goat plus sheep collagen (Kirsanow et al., 2008) and with feathers (Hobson et al., 2004). Large symbols show averages; standard errors on averages are smaller than symbol size.

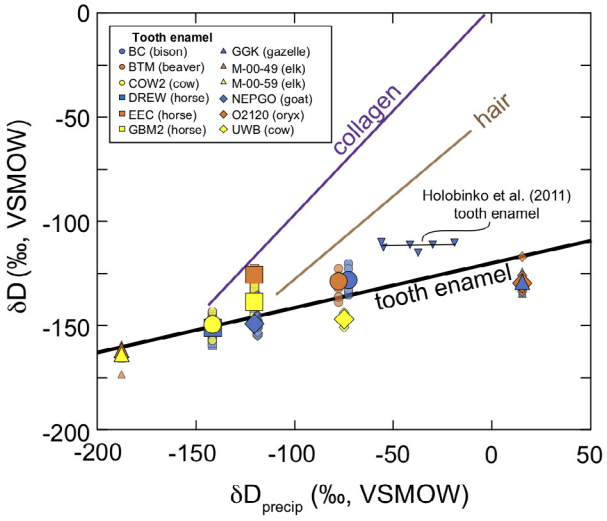

Fig. 2. δD values of tooth enamel (this study), human hair (Bowen et al., 2009), and deer collagen (Cormie et al., 1994) vs. local water δD (Johnson et al., 1991; Bowen and Revenaugh, 2003; Bowen, 2018) showing much shallower slope for teeth. Data from Holobinko et al. (2011) are displaced to higher δD, likely reflecting higher δD of adsorbed water. Large symbols show averages.

measured slope of hydrogen content vs. sample mass for hoof keratin is the same as the predicted slope (Fig. 3). That is, any labile hydrogen in keratin does not increase the total H content, but rather exchanges isotopes with ambient water vapor (Wassenaar and Hobson, 2003).

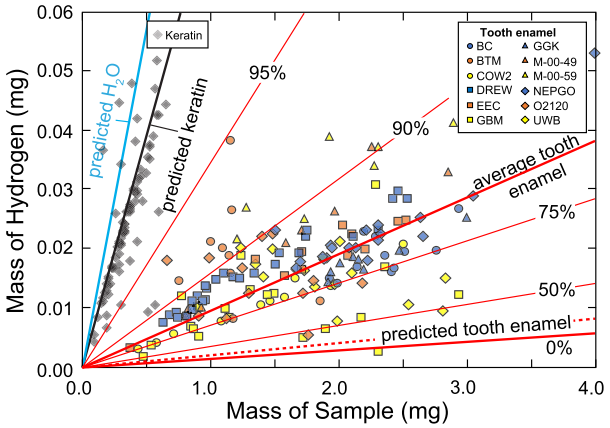

Fig. 3. Calculated mass of hydrogen and hydrogen peak area vs. mass of sample for tooth enamel and caribou hoof standard (keratin). Thick lines show expected mass correlations between sample weight and H yield for water (blue), keratin (black), enamel apatite (solid red), enamel apatite + protein + phospholipid (dashed red), and overall correlation for enamel (solid red). Thin lines show contours for different percentages of H contamination. Enamel data are highly scattered, but generally imply that more H was analyzed than would be present in enamel alone, likely because adsorbed water contributes roughly 50–90% of analyzed H. Keratin data show better correspondence between measured and expected masses. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.3. Equilibration experiments

For equilibration experiments at room temperature, all tooth enamel samples show anomalous hydrogen isotope compositions for the t_0 experiment (Fig. 4), with higher δD for samples equilibrated with D-enriched water, and lower δD for samples equilibrated with D-depleted water. All compositions shift towards an intermediate composition after 1 hour of exposure to laboratory air, then gradually converge with increasing time to a common composition, similar to local water (Fig. 4). The absolute values of the t_0 data range widely, in part reflecting the timing of analysis after removal. For example, for the D-enriched experiments, the first analyses collected within the t_0 set (BM3-C and BOS-M2-B) show much higher δD values than the next 4 analyses in that set (Fig. 4). Samples subjected to heating for 48 hours show an increase or decrease of 10-30‰ with time and converge on a slightly lower δD value as unheated samples (Fig. 5).

5. DISCUSSION

5.1. Hydrogen isotopes in tooth enamel

A positive correlation between tooth enamel stable hydrogen vs. oxygen isotope compositions (Fig. 1; Table 2) is expected because of the direct dependence of isotope compositions in animals on meteoric water δD and $\delta^{18}O$ values. Tooth enamel $\delta^{18}O$ values are a function of consumed waters (Luz and Kolodny, 1985; Bryant and Froelich, 1995; Kohn, 1996), directly from surface water and from plant material, which generally correlate with meteoric waters (Koch, 1998; MacFadden, 2000; Kohn and Cerling, 2002; Kohn and Dettman, 2007). Hair and collagen also demonstrate a correlation between δD and $\delta^{18}O$

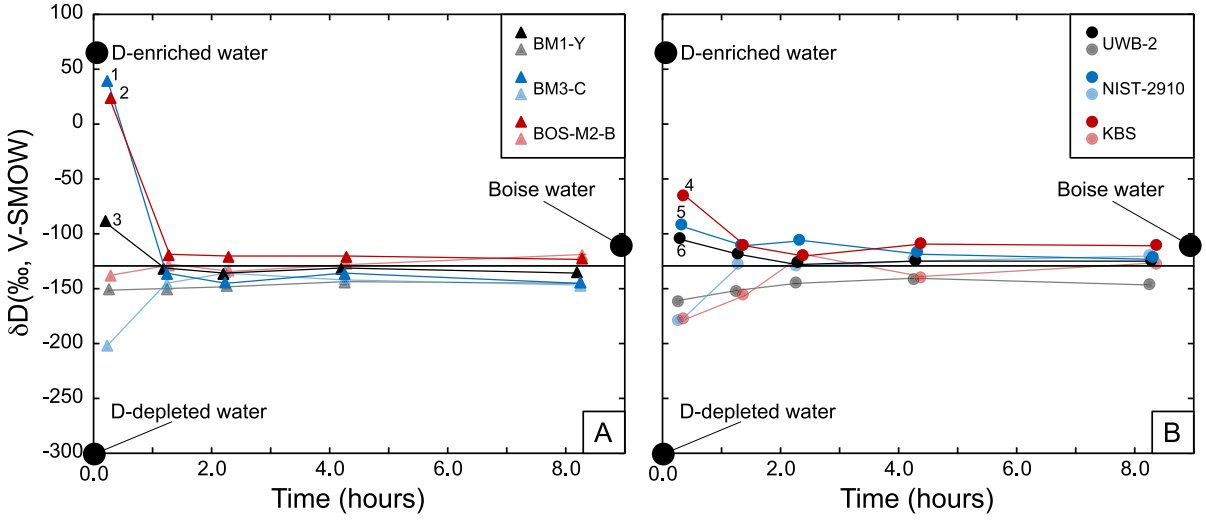

Fig. 4. Results of enamel-water exchange experiments. Darker vs. lighter symbols are for data from D-enriched vs. -depleted experiments. Enamel δD values show rapid changes on timescales of minutes to hours. Symbols are offset slightly along x-axis for visibility. Horizontal lines are mean values of final compositions. Large dots show mean isotope composition of local water in Boise (δD = −111‰), D-enriched water (+62‰), and D-depleted water (-300‰). (A, B) Subsets of data separated for clarity. Numbers next to 0-time experiments with ^{18}O-enriched water show sequence of analysis. Similarity of final compositions for most of the ^{18}O-enriched vs. -depleted experiments indicates a close approach to equilibrium.

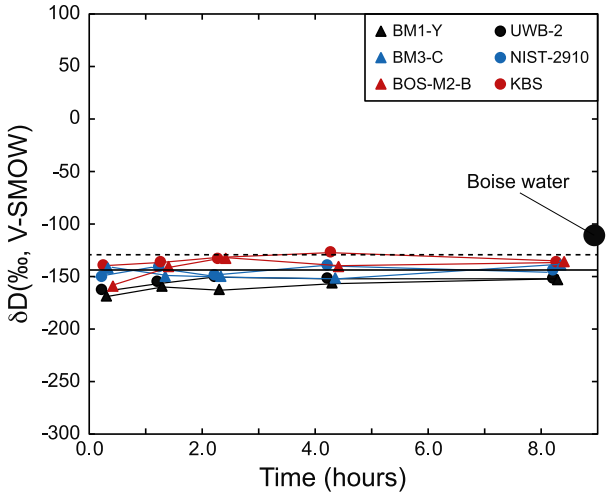

Fig. 5. Enamel-water exchange experiments at 70 °C show little isotopic variation. Dashed line is average composition of unheated samples after 8 hours (see Fig. 4). Small differences in final compositions compared to initial compositions (nearly all increase through time) and to experiments at room temperature (lower δD values) may reflect a combination of temperature-dependent isotopic partitioning, exchange of H isotopes with structural H, or a change in H-content of enamel mineral.

values (Cormie et al., 1994; Reynard and Hedges, 2008; Kirsanow et al., 2008; Sluis et al., 2019; Topalov et al., 2019), but, unlike tooth enamel, many of these correlations have slopes more similar to that of the GMWL (i.e. 8.0; Fig. 1). Some δD and $\delta^{18}O$ correlations within individual teeth (e.g. BTM and BC) are weaker than others (e.g. DREW and GBM2). The source of these differences is unclear, although we note that the dataset of Holobinko et al. (2011) similarly shows no resolvable correlation. In our view, focusing on the dataset as a whole is more useful than individual intra-tooth trends.

Outlier data (high δD) for *Castor canadensis* (BTM) may reflect inadvertent sampling of dentine. *Castor canadensis* has thin enamel, and dentine is rich in collagen, which has higher δD and lower $\delta^{18}O$ values (Fig. 1). Although H_2O_2 pretreatment should remove this contaminant, a small amount of residual collagen (2–4 wt%) would shift compositions towards collagen data (Fig. 1). Because collagen has such a large H/O ratio, such contamination would affect δD far more than $\delta^{18}O$. In contrast to our interpretation of *Castor canadensis* data, sample EEC (horse), also shows elevated δD values (Figs. 1 and 2). However, horse enamel is relatively thick and not especially prone to dentine contamination during sampling. Overall, we find no single explanation for elevated δD values for both BTM and EEC.

5.2. Adsorbed hydrogen

For enamel, the regression of hydrogen content vs. sample mass shows that only c. 10–50% of measured hydrogen can be ascribed to enamel hydrogen from apatite and phospholipids. The differences between δD values for samples that have been exposed to water with either very high or low δD values suggest that the source of excess hydrogen is from adsorbed water. If so, the data of Holobinko et al. (2011) should have been offset to higher δD values than ours because their data were measured in a geographic region with higher local water δD values (c. -50‰; Dundee, UK; Bowen and Revenaugh, 2003; Bowen, 2018). Assuming a 50–90% contribution from local water vapor, their data should plot approximately 30–55‰ higher than ours (Bowen and Revenaugh, 2003; Bowen, 2018). Relative to our regression, their data plot approximately 25‰ higher (Fig. 2), in general concordance with expectations.

In contrast to enamel, keratin shows no evidence for additional, adsorbed hydrogen, as data for animal hooves (CBS) show the expected trend for hydrogen vs. sample weight (Fig. 3). These organic substrates do contain exchangeable hydrogen (Epstein et al., 1976; Schimmelmann, 1991; Cormie et al., 1994), but in the context of adsorbed water, no reevaluation of isotope compositions is required.

5.3. Equilibration experiments

With increasing duration of exposure, the time-series samples approach a steady-state δD value that is approximately 0–40‰ lower than local water. The final composition for all analyses is likely governed by sample-specific biogenic and adsorbed water compositions that reflect equilibration partitioning between ambient water vapor and adsorbed water. Because we do not know the compositions of either biogenic or adsorbed components independently, we cannot infer any fractions quantitatively. However, first order reaction kinetics would predict that compositions would evolve towards a common value, rapidly at first, and more slowly later. The t_0 experiments show quite different hydrogen isotope compositions compared to final compositions, while t_1 experiments are more similar to final hydrogen isotope compositions, although they are still resolvably different (Fig. 4; Table A2). The t_2, t_4, and t_8 experiments show nearly indistinguishable hydrogen isotope compositions. It is difficult to determine original biogenic compositions from powdered tooth enamel with good resolution using our (conventional) methods because (a) extremely rapid changes occur to composition, (b) the amount of structural H that is hosted in the tooth enamel is small, and (c) the compositions of water vapor in different laboratories are different. Analysis of un-powdered enamel might show less dependence on adsorbed water, but because of small amounts of included proteins and phospholipids, compositions would not completely reflect tooth enamel apatite.

In some organic substrates, full equilibration between exchangeable (not adsorbed) hydrogen and water vapor also occurs in hours. For example, it takes 1–2 hours for hydrogen in keratin from butterfly wings and quail feathers to equilibrate with water vapor (Wassenaar and Hobson, 2000), although exchangeable hydrogen in keratin from horse hair that is subjected to isotopically unusual water reaches an invariant composition in c. 4 days (Bowen et al., 2005b). Tooth enamel responds more rapidly than

keratin, probably because excess adsorbed water dominates the exchange rather than structurally bound OH and HPO_4. The highest and lowest δD values for tooth enamel correspond with geographic regions with the highest and lowest values of local water δD (Fig. 1). Inasmuch as most of these teeth were stored in our laboratory for 10 years, exchange of H on the hydroxyl site of enamel apatite must be at least partially immune to exchange with ambient water vapor, otherwise compositions would be indistinguishable.

5.4. Heating experiments

Tooth enamel δD values measured for t_0 experiments were lower than values for samples that were equilibrated for 1–8 hours at ambient temperatures (Fig. 5; Table A2). However, low δD values of tooth enamel for the t_0 experiment probably do not reflect removal of adsorbed water, but rather a larger temperature-dependent fractionation. That is, adsorbed water contents are similar for heated vs. unheated samples (Fig. A1), but there is apparently greater discrimination at higher temperatures. This behavior might prove useful for modeling the temperature-dependence of adsorbed water isotopes. More adsorbed water might be removed if temperatures were increased, but thermogravimetric analysis shows changes to tooth enamel chemistry by 100 °C (Holcomb and Young, 1980). As with D-depleted and -enriched water experiments, equilibration with laboratory water vapor must have been closely approached within 1–2 hours (Fig. 4; Table A2). The reasons that isotope compositions for heated samples converge to a different composition than unheated samples remain elusive. Possibly with longer equilibration times, these differences would disappear. Alternatively, heating even at 70 °C might cause subtle changes to the internal or surface structure of bioapatite, permanently changing its isotopic fractionation with respect to ambient water vapor.

6. CONCLUSIONS

We observe positive correlations between tooth enamel δD and $\delta^{18}O$ values ($R^2 = 0.70$) and between tooth enamel δD and local precipitation δD values ($R^2 = 0.53$). These correlations imply that environmental water δD values might be recoverable. However, compared to expectations of analysis of $OH + HPO_4$ analysis alone, the slopes of these correlations are much lower and the ratio of H contents and sample weights are much higher. These observations suggest a large component of labile hydrogen that we interpret to be adsorbed water. Time series experiments for unheated and heated samples show rapid exchange with ambient water vapor within 1–2 hours, and also imply a temperature dependence to the isotope composition of the adsorbed water. Rapid equilibration and large amounts of adsorbed hydrogen mean that the original biogenic compositions of tooth enamel apatite can be recovered only imprecisely using conventional methods. Any intercomparisons of compositions among laboratories should account for geographic variations in local water composition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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APPENDIX A. SUPPLEMENTARY MATERIAL

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gca.2019.11.013.

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