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RESEARCH ARTICLE

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Stable isotope relationships between apatite phosphate ($\delta^{18}O$), structural carbonate ($\delta^{18}O$, $\delta^{13}C$), and collagen ($\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) in modern human dentine

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Rationale: The use of multi-isotopic analysis ($\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, and $\delta^{34}S$ values) of modern human body tissues for provenancing of unknown individuals in forensics is increasing. Tooth dentine develops during childhood and adolescence, therefore providing geographical information from that period of life. Tooth apatite $\delta^{18}O$ values are commonly used for the reconstruction of drinking water values, and H–C–N–S isotope ratios in collagen supply additional information about the composition of diet. We tested if dentine collagen $\delta^{2}H$ values provide similar information to apatite $\delta^{18}O$ values with a proof-of-concept study.

Methods: Tooth samples were taken from modern-day individuals born in different regions of the world. Apatite and collagen were prepared from dentine. Stable isotope analyses were performed on apatite phosphate oxygen ($\delta^{18}O_{phos}$); oxygen and carbon of the structural carbonate ($\delta^{18}O_{carb}$, $\delta^{13}C_{carb}$); and hydrogen, carbon, nitrogen, and sulfur of the collagen ($\delta^{2}H_{coll}$, $\delta^{13}C_{coll}$, $\delta^{15}N$, $\delta^{34}S$).

Results: $\delta^{18}O_{phos}$, $\delta^{18}O_{carb}$, and $\delta^{2}H_{coll}$ values are highly correlated in modern human dentine. There are significant relationships of $\delta^{18}O$ values in the apatite fraction and $\delta^{2}H$ values in the collagen fraction with local $\delta^{18}O$ and $\delta^{2}H$ precipitation values, respectively. Pearson correlation coefficients indicate no direct relationship between $\delta^{15}N$ values and the isotope ratios of any other element. Weak relationships exist between collagen $\delta^{34}S$ values and $\delta^{18}O_{carb}$ or $\delta^{18}O_{phos}$ values.

Conclusions: The highly significant correlation of $\delta^{18}O_{phos}$, $\delta^{18}O_{carb}$, and $\delta^{2}H_{coll}$ values in the modern human dentine implies that measurement of $\delta^{2}H$ values in collagen or $\delta^{18}O$ values in bioapatite will provide reliable information about the climate at the person's whereabouts.

1 | INTRODUCTION

Stable isotope analyses on different human tissues can be used for provenancing of unknown individuals. An overview of publications on the isotope analysis of skeletal remains from across the globe is given, for example, by Bartelink et al and Chesson et al.[1,2] By analyzing body tissues (hair, nail, bones, teeth), geographical information from childhood through death can be obtained.[3] Permanent teeth develop during childhood and youth,[4] hair and nails grow continuously during the last months before death, and bones may yield information over a time period from several years up to decades preceding the death of a person. The stable isotope ratios of the bioelements ($\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, and $\delta^{34}S$ values) in body

tissues are linked to the composition of an individual's diet and the origin of food and drink. In general, $\delta^{13}C$ values show the content of C4 or C3 plants or marine versus terrestrial sources in diet, $\delta^{15}N$ values show the amount of animal protein (meat, dairy products, fish), and $\delta^{34}S$ values hints at any marine influences (sea products, sea-spray effect, sediments of marine origin). $\delta^{2}H$ and $\delta^{18}O$ values predominantly contain information about the climatic conditions at the whereabouts of individuals, as the isotopic composition of regional meteoric water is strongly linked to annual air temperature.[5] Region-specific stable isotope ratios of the elements from food and drink are integrated into body tissues of living organisms.

Stable isotope data of human teeth may provide information as to where a person spent his or her childhood and adolescence. In view of the molecular level of dentine collagen, its amino acid composition is almost similar to that of bone collagen.[6] Calculated correction factors enable a direct comparison of the stable isotope ratios of hydrogen, carbon, nitrogen and sulfur of bone collagen with those of reference hair samples collected worldwide, allowing the prediction of the geographical origin of unidentified individuals.[3] For the prediction of a person's residence during childhood and youth, the H–C–N–S stable isotope ratios of tooth collagen might be applicable in the same way.

Tooth collagen is primarily abundant in the organic fraction of dentine. Due to its bone-like structure, dentine contains 70% calcium hydroxyl apatite, 20% organic material (containing 90% collagen), and 10% water.[7] In contrast, tooth enamel contains up to 95% calcium hydroxyl apatite and has a maximum collagen content of 0.4%.[8] The final structure of primary dentine and enamel develops during ontogenesis, and neither of them is subjected to any marked conversion during a person's lifetime.[9]

In addition to the stable isotopes in the organic fraction of the tooth, the ^{18}O content in tooth apatite can be used as a source of geographical information. $\delta^{18}O$ analyses can be performed on the apatite phosphate ($\delta^{18}O_{phos}$) or on the structural carbonate ($\delta^{18}O_{carb}$). Both measurements are directly connected to the $\delta^{18}O$ values in body water, and more than 50% of the oxygen in apatite is derived from water in food and drink.[10]

Hydrogen and oxygen isotope ratios in body tissues are linked to the $\delta^{18}O$ or $\delta^{2}H$ values of body water, which mainly stem from the consumed drinks and liquid components of food. The main water source for modern humans is tap water, and the $\delta^{18}O$ or $\delta^{2}H$ values of local tap water are connected to the local precipitation values and the climate.[11] The relationship of $\delta^{18}O$ values between apatite in enamel and drinking or tap water for humans is well established,[12-14] and for the $\delta^{18}O_{carb}$ and $\delta^{18}O_{phos}$ values in enamel of archaeological individuals, a strong correlation has been documented.[15]

Oxygen isotope analyses on apatite from bones and teeth have been primarily used in archaeology for the reconstruction of geographical provenance and migration behaviors of individuals. Studies on $\delta^{18}O$ values in tooth apatite of contemporary humans confirm their relationship to the oxygen isotope ratios of drinking water, tap water, or precipitation.[12-14,16-20] In addition, the $\delta^{2}H$ and $\delta^{18}O$ values of modern human hair and nails are connected to precipitation.[3,21-24] In human hair, 27% of the oxygen atoms and 36% of the hydrogen atoms are derived from drinking water.[25] As previously shown by the relationship between keratin $\delta^{2}H$ and bone collagen $\delta^{2}H$ values,[3] the same may be supposed for dentine collagen $\delta^{2}H$ values. Therefore, dentine collagen $\delta^{2}H$ values should be related to precipitation or drinking water $\delta^{2}H$ values and consequently may serve as a proxy for the climatic conditions in which an individual grew up.

There are several studies dealing with the relationships between the $\delta^{18}O_{phos}$, $\delta^{18}O_{carb}$, and $\delta^{2}H$ values in mammal teeth and bone samples[26-28] and archaeological human enamel samples,[15,29] but human dentine samples have been rarely investigated. There is one study about carbon and oxygen isotope spacing between tooth collagen and hydroxyapatite in human archaeological remains.[29] Furthermore, one study elucidated the collagen $\delta^{2}H$ and apatite $\delta^{18}O$ relationship in human bones,[30] but so far, no study has established any relationship between $\delta^{18}O_{phos}$ and $\delta^{18}O_{carb}$ values in modern human dentine.

This is the first study compiling basic relationships between $\delta^{18}O_{phos}$, $\delta^{18}O_{carb}$, and $\delta^{2}H_{coll}$ values in tooth dentine from modern-day humans. From the results of former studies on the apatite oxygen ratios in human bone or enamel, we expect that the $\delta^{18}O$ values in the dentine will be associated with the isotope ratios of the person's drinking water or the local precipitation. Our main concern was to examine whether the dentine collagen $\delta^{2}H$ values can also be used as a climate proxy for the isotopic composition of local water at the individuals' whereabouts. Furthermore, we will test if there are any direct relationships between the $\delta^{13}C_{carb}$, $\delta^{13}C_{coll}$, $\delta^{15}N_{coll}$, and $\delta^{34}S_{coll}$ values in modern human dentine. As the teeth were extracted within a few days after the death of the persons, we can assume that diagenetic changes of dentine by environmental factors such as soil pH, soil hydrology, ambient temperature, or microbial degradation had not occurred.[31]

2 | SAMPLING AND METHODS

2.1 | Materials

With permission of the ethic commission at the LMU Munich, 29 permanent teeth were collected anonymously from corpses for whom autopsies were ordered by prosecution authorities. After cleaning and removing adhesions mechanically, each tooth was divided in two parts, the crown and the root section, by a small saw. The whole root section, which is mainly composed of dentine, was ground by a ball mill, and tweezers removed the dental nerve. The pulverized dentine was degreased with petroleum ether (40–60°C) for 3 hours using Soxhlet extraction.

2.2 | Preparation of collagen

Collagen was extracted from dentine following previous reports.[32,33] About 400 mg of the defatted dentine material was demineralized with 1M hydrochloric acid for 40 min at room

temperature. After centrifugation and neutralization with demineralized water, the residue was gelatinized with 1mM hydrochloric acid (pH 3) for 16–18 hours at 90°C. The gelatin solution was pressure-filtered through a glass-microfiber disc and a membrane filter (cellulose nitrate, 5 μm). Afterward, the filtered solution was frozen and freeze-dried. The dry collagen was used for stable isotope measurements of hydrogen, carbon, nitrogen, and sulfur, with an isotope ratio-mass spectrometer.

2.3 | Bulk isotope analysis of collagen

Collagen δ^2H values were measured using the comparative equilibration method.[34] Collagen samples and laboratory reference materials were stored under identical conditions for at least 3 days before analysis to enable hydrogen exchange with hydrogen from ambient air moisture. After equilibration, 150 μg of the samples, international and in-house casein reference materials,[35] and in-house collagen reference materials were weighed into tin capsules in triplicate and dried under vacuum for at least 24 hours to remove all adhering humidity. The δ^2H standards comprised two samples of in-house collagen standards from pig and beef with measured δ^2H values of −53.1‰ (±3.0) and −70.5‰ (±2.4). Samples and the standards were loaded into a helium-flushed autosampler. Hydrogen gas produced by high temperature conversion at 1450°C was analyzed isotopically. The international reference material for the calibration of the hydrogen reference gas was NBS 22 with the value of −120‰ versus V-SMOW (certified value according to the IAEA [Vienna, Austria] document).[36] In addition, a common reference material (benzoin, 2-hydroxy-1,2-diphenylethan-1-one) from AG Stabilisotopenanalytik of GDCh (Gesellschaft Deutscher Chemiker, Frankfurt am Main, Germany)[37] with a value of +150‰ versus V-SMOW was applied for scale calibration.

Until now, no international collagen reference materials with certified isotope values have been available. From earlier investigations, it is known that collagen contains about 20% of exchangeable hydrogen.[38,39] This hydrogen, mainly from OH groups, is able to exchange with hydrogen atoms from ambient air humidity and does not originate from the metabolism of the organism that formed collagen. Several attempts have been made to account for the δ^2H values of the exchangeable hydrogen positions and to calculate "true" values for the nonexchangeable hydrogen in collagen. The main problem, even if we know the δ^2H values of the air humidity quite well, is that we cannot simply say that the δ^2H values of the exchangeable hydrogen positions are the same as those for the air humidity. First, we must take into account that when samples are dried, material water vapor is removed, causing isotopic fractionation. Second, the exchange of hydrogen between hydroxyl groups and the remaining air humidity may also proceed with isotopic fractionation. It has been assumed that these effects result in an 80‰ enrichment of ^{2}H in the exchangeable positions over ambient air δ^2H values.[40] Based on that, from the known δ^2H values of air humidity and about 20% of the total hydrogen in collagen being exchangeable, the δ^2H values for the nonexchangeable hydrogen of collagen samples analyzed can be calculated from the measured bulk δ^2H values.[38,41,42] We also considered the observation that vapor δ^2H values at 20°C (room temperature) are less variable.[43] Several authors have based the nonexchangeable δ^2H values in collagen samples on different assumptions, leading to inconsistent results in relevant publications. Thus, to obtain comparable δ^2H values, there is an urgent need for international collagen reference materials. As already mentioned, these do not yet exist. Consequently, we present the measured bulk δ^2H_{coll} values based on the δ^2H values of our internal collagen standards. It should be stressed here that for our purpose, testing the correlation of δ^2H values of collagen and δ^{18}O values of apatite from dentine, the effect of applying either measured bulk δ^2H (δ^2H_{coll}) or calculated nonexchangeable δ^2H ($\delta^2H_{coll\ nex}$) values of collagen on the resulting correlations is not substantial.

For multielement measurement of C-, N-, and S-isotopes, 3.0 mg of collagen was weighed into tin capsules in quadruplicate. The internal standards used for calibration were casein and two different in-house collagen standards, from pig (δ^{13}C: −13.18‰ [±0.09], δ^{15}N: 4.55‰ [±0.09], δ^{34}S: 5.41‰ [±0.21]) and from beef (δ^{13}C: −18.05‰ [±0.07], δ^{15}N: 5.99‰ [±0.05], δ^{34}S: 5.54‰ [±0.35]). For δ^{15}N and δ^{34}S values, scale calibrations with inorganic reference materials were performed (IAEA-NO-3 and USGS25 for δ^{15}N; IAEA-S-1, IAEA-SO-5, and IAEA-SO-6 for δ^{34}S). Scale calibration for δ^{13}C values was performed with organic materials (NBS 22 [oil] and IRMM-BCR 657 [glucose]).[44] Bulk stable isotope ratio measurements of the bioelements (δ^{13}C, δ^{15}N, and δ^{34}S values) in collagen samples were performed according to Sieper et al.[44]

Measurement of the light elements was carried out using elemental analyzer–isotope ratio mass spectrometry (EA–IRMS); for C–N–S simultaneous analysis we used an Elementar Vario Cube EL elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) connected with an Isoprime mass spectrometer (Isoprime Ltd, Cheadle Hulme, UK) or an Elementar Pyrocube connected with an Isoprime VisIon mass spectrometer. For H isotope analysis, a Thermo high temperature conversion unit connected with a Thermo XP plus isotope ratio mass spectrometer (Thermo Fisher, Bremen, Germany) was used. The high temperature conversion unit was equipped with a helium-flushed autosampler, the reaction column was filled with glassy carbon chips, and the temperature of the furnace was set to 1450°C. The reaction gases were passed through an absorption tube containing sodium hydroxide on support and another one containing phosphorus pentoxide. All δ values were indicated in per mil (‰) relative to international reference standards (δ^2H_{VSMOW}, $\delta^{13}C_{VPDB}$, $\delta^{15}N_{AIR}$, and $\delta^{34}S_{VCDT}$) as follows: $\delta = [(R_{sample}/R_{standard}) - 1]$, where R is the ^{2}H/^{1}H, ^{13}C/^{12}C, ^{15}N/^{14}N, or ^{34}S/^{32}S ratio. The analytical precisions using at least triplicate measurements were ±2‰ for δ^2H values, ±0.1‰ for δ^{13}C values, ±0.2‰ for δ^{15}N values, and ±0.3‰ for δ^{34}S values.

2.4 | Apatite preparation

To remove organic compounds, 5 mL 4% NaOCl solution was added to 100 mg pulverized dentine and thoroughly mixed. After 2 days, the NaOCl solution was replaced. On the third day, the supernatant was removed, and the pellet was rinsed with distilled water several times. The pellet was stirred with 5 mL 1M calcium acetate–acetic acid buffer overnight. After several rinses with distilled water, the apatite was dried at 40°C.

2.5 | $\delta^{18}O$ and $\delta^{13}C$ analyses on structural carbonate of dentine

For carbonate isotope analyses 2.0 ± 0.1 mg of the dry dental apatite (section 2.4) was weighed into borosilicate glass autosampler vials. Then, these vials were closed with a septum cap and flushed with helium for 700 seconds. CO_2 gas was produced by adding 7–9 droplets of 103% H_3PO_4 using a syringe. The samples were equilibrated for at least 3 hours at a constant temperature (70°C) in a GasBench II on-line gas preparation and introduction system for isotope ratio mass spectrometry (Thermo Fisher). The equilibrated CO_2 was measured using a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher). The results were calibrated using international standards (NBS 18, NBS 19), using the certified values from the IAEA document.[36,45,46] An in-house laboratory standard (Solnhofen limestone, $\delta^{18}O$: −5.12; $\delta^{13}C$: −0.98) was used for additional quality control. The average precision was 0.1‰ for both $\delta^{13}C$ and $\delta^{18}O$ values. All $\delta^{18}O$ values of the teeth carbonates were measured in per mil (‰) relative to the international carbonate reference standard, Vienna Pee Dee Belemnite (VPDB). The $\delta^{18}O$ values were converted from the VPDB scale to the Vienna Standard Mean of Ocean Water (VSMOW) scale using the equation of Kim et al[47]: $\delta^{18}O$ (VSMOW) = 1.03092 $\delta^{18}O$ (VPDB) + 0.03092.

2.6 | Dentine phosphate preparation

For the precipitation of dental phosphate as silver phosphate, the method of Joachimski et al[48] was applied. Three milligrams of the pretreated apatite sample was dissolved in 115 μL of 2M HF in an Eppendorf® tube for 20 hours. Thereafter, 115 μL of 2N KOH was added for neutralization. The solution was then centrifuged, and the supernatant was carefully removed using a pipette. After adding 1500 μL of a freshly prepared $AgNO_3$ solution, the (open) tubes were heated overnight in a water bath at 60°C. The precipitated silver phosphate crystals were rinsed several times with distilled water and removed from the tube surface using an ultrasonic bath. The crystals were dried at 60°C and crushed using a spatula.

2.7 | $\delta^{18}O$ analysis in dentine phosphate

A quantity of 0.92–0.94 mg of dry silver phosphate was weighed into silver capsules (4 × 6 mm) in triplicate. Prior to analysis, the samples were stored in a vacuum-drying cabinet at 60°C for 48 hours to remove water vapor. After drying, the samples were immediately filled in an autosampler flushed with helium. The samples were pyrolyzed at 1490°C in a HT Oxygen Analyzer (HEKAtech GmbH, Wegberg, Germany) coupled to a Delta V Advantage isotope ratio mass spectrometer. The measured gas (CO) was purified and isolated by passing the pyrolysis products through traps filled with Carbosorb and $MgClO_4$ and through a GC column (70°C). The $\delta^{18}O$ values were calibrated using the international benzoic acid standards IAEA 601 ($\delta^{18}O$ = 23.3‰) and IAEA 602 ($\delta^{18}O$ = 71.4‰) and an additional laboratory α-cellulose standard ($\delta^{18}O$ = 31.4‰ [±0.2‰]; Sigma-Aldrich, St. Louis, MO, USA). Further details are reported in Mayr et al.[49]

3 | RESULTS

In our study we collected 29 teeth from 28 modern-day adults: for 22 of them the place of birth of the person is known, while 7 are from unknown corpses. The locations from which the human individuals originate cover a latitudinal range between 41.4° S and 56.8° N and a longitudinal range between 12.9° E and 147.1° E (Table 1). The mean annual isotope ratios of precipitation at the places of birth[50] span a range of 111‰ for $\delta^{2}H$ values and 13.8‰ for $\delta^{18}O$ values. The period of dentine formation of the permanent teeth used in this study (Table 1) is from the third to the 16th year.[4] We are operating on the assumption that for each individual the residential region during the period of tooth growth is the same as the place of their birth.

Table 2 presents the intradentine stable isotope ratios for carbon ($\delta^{13}C_{coll}$), nitrogen ($\delta^{15}N_{coll}$), sulfur ($\delta^{34}S_{coll}$), and hydrogen ($\delta^{2}H_{coll}$) in collagen; $\delta^{18}O$ values in phosphate ($\delta^{18}O_{phos}$); and $\delta^{18}O$ and $\delta^{13}C$ values in the structural carbonate ($\delta^{18}O_{carb}$, $\delta^{13}C_{carb}$).

Before $\delta^{18}O_{phos}$, $\delta^{18}O_{carb}$, and $\delta^{13}C_{carb}$ analyses on bioapatite, we treated the dentine material with NaOCl and acetic acid buffer to remove organic matter and secondary carbonate. The dentine $\delta^{18}O_{phos}$ values of the pretreated samples range from +12.6‰ to +22.7‰. In a further step, we measured the $\delta^{18}O$ and $\delta^{13}C$ values of the structural carbonate in the raw dentine material. By processing the samples in this way, we wanted to test any possible influences of the pretreatment procedure on the $\delta^{18}O_{carb}$ and $\delta^{13}C_{carb}$ values. The pretreatment procedure decreased the quantity of raw dentine material on average by 47% (Table 1). For pretreated apatite samples, the $\delta^{18}O_{carb}$ values were from +20.0‰ to +29.5‰ and the $\delta^{13}C_{carb}$ values from −15.9‰ to −6.3‰; for untreated samples the $\delta^{18}O_{carb}$ values were from +21.2‰ to +28.4‰ and the $\delta^{13}C_{carb\ untreated}$ values from −15.9‰ to −8.9‰. The mean offsets between pretreated and untreated samples are −0.7 ± 0.7‰ for $\delta^{18}O$ values and 0.5 ± 0.6‰ for $\delta^{13}C$ values (Table 2).

TABLE 1 Dentine samples: origin of the donors, local mean annual precipitation values,[50] percentage of prepared collagen or apatite, and calculated precipitation values by the use of $\delta^{18}O$ dentine values[12,15]

Person	Tooth number	Country of birth	Latitude (decimal deg; North positive)	Longitude (decimal deg; East positive)	Elevation (m)	δH^2 (annual precipitation)[50]	$\delta^{18}O$ (annual precipitation)[50]	% collagen	% dentine after sample pretreatment
15	M1	China	31.23	121.47	16	−50	−7.9	10.6	44
16	M1	Austria	47.81	13.05	431	−63	−8.9	9.5	39
17	M1	Germany	47.73	12.88	467	−63	−9.0	10.3	42
18	I2	Eritrea	15.32	38.93	2331	4	0.7	11.8	48
19	I2	Somalia	3.84	45.30	229	23	1.4	7.1	42
20	I2	Greece	37.26	23.13	239	−38	−6.5	11.0	44
21	I1	Syria	33.52	36.39	650	−32	−6.6	7.2	42
22	I2	Afghanistan	33.55	68.42	2178	−37	−7.0	11.9	44
23	I2	Australia	−41.40	147.10	17	−23	−4.1	11.9	44
24	M1	Russia	56.77	43.24	111	−88	−12.2	13.2	43
25	I2	Romania	44.30	23.80	100	−50	−7.1	9.0	41
26	C	Eritrea	15.32	38.93	2331	4	−0.7	9.3	45
27	C	Egypt	29.31	30.84	31	14	1.4	11.4	40
28	C	Kazakhstan	43.22	76.85	853	−75	−10.2	10.1	40
29	PM2	Kazakhstan	42.90	71.40	623	−48	−7.6	14.7	50
30	PM1	Turkey	38.42	27.14	11	−29	−5.2	10.2	57
31	I1	Ukraine	46.77	36.80	24	−60	−8.3	6.8	60
32	I2	N/K						13.2	61
33	PM1	N/K						11.9	60
34	PM1	N/K						12.4	62
35	C	N/K						14.9	64
36	M1	Kazakhstan	49.58	82.60	288	−87	−12.4	7.8	59
37	M1	N/K						12.3	64
38	M2	N/K						11.8	62
39	M2	N/K						13.7	67
40	M1	Germany	52.52	13.41	36	−58	−8.1	13.8	67
41	M2	Germany	52.52	13.41	36	−58	−8.1	13.3	68
42	M1	Bulgaria	42.51	27.46	19	−59	−8.8	16.8	67
43	M1	Poland	52.73	15.24	26	−60	−8.4	14.9	62

All δ values are presented as ‰ values.
N/K: country of birth unknown.

The relationships between apatite and collagen isotope ratios were investigated using linear regression. Table 3 shows the linear correlation equations between the measured δ values for oxygen in apatite phosphate or in the structural carbonate and hydrogen in collagen. The dentine collagen δ^2H values ranging from −70‰ to 0‰, and the δ^2H_{coll} values in the modern dentine samples are significantly correlated to the $\delta^{18}O_{phos}$ values as well as to the $\delta^{18}O_{carb}$ values. All sample values are within the 95% confidence interval.

Figure 1 illustrates the relationship between the $\delta^{18}O_{phos}$ and $\delta^{18}O_{carb\ pretreated}$ values of each dentine sample. The data in Table 2 show that the mean $\Delta_{carb\text{-}phos}$ spacing is 6.6 ± 0.7‰ for pretreated and 7.2 ± 0.7‰ for untreated samples (Table 2). With respect to different climatic settings in the regions of origin, $\Delta_{carb\text{-}phos}$ in the modern dentine samples remains nearly constant. Figure 2 shows the correlation between the $\delta^{18}O_{phos}$ or $\delta^{18}O_{carb\ pretreated}$ value and the δ^2H value of each dentine sample.

The dentine collagen $\delta^{13}C$ values range from −20.7‰ to −15.4‰. The $\delta^{13}C_{coll}$ and $\delta^{13}C_{carb}$ values are highly correlated (Table 3), and the mean difference between the values for the two compounds is 5.0 ± 1.0‰ (pretreated apatite) or 4.6 ± 0.7‰ (untreated apatite).

The collagen $\delta^{15}N$ values range from 6.7‰ to 12.8‰ and the collagen $\delta^{34}S$ values from 3.6‰ to 10.7‰. Table 4 shows the correlations between all measured isotope ratios in apatite and in collagen. Pearson correlation coefficients indicate no clear relationship between the $\delta^{15}N$ values and the isotope ratios of any

TABLE 2 Dentine samples: stable isotope values

Person	$\delta^{13}C_{coll}$	$\delta^{15}N_{coll}$	$\delta^{34}S_{coll}$	$\delta^{2}H_{coll}$	$\delta^{18}O_{phos}$	$\delta^{18}O_{carb}$ pretreated	$\delta^{13}C_{carb}$ pretreated	$\delta^{18}O_{carb}$ untreated	$\delta^{13}C_{carb}$ untreated	$\Delta^{18}O_{pretreated-}$ untreated	$\Delta^{13}C_{pretreated-}$ untreated	$\Delta^{18}O_{carb\text{-}phos}$	$\Delta^{13}C_{carb\text{-}coll}$
15	−20.54	12.77	8.63	−47	17.82	23.96	−15.49	24.77	−15.48	−0.8	0.0	6.1	5.1
16	−19.89	11.00	5.66	−42	15.12	22.98	−14.76	22.86	−15.68	0.1	0.9	7.9	5.1
17	−19.27	11.29	4.93	−46	16.13	23.06	−15.25	23.46	−15.53	−0.4	0.3	6.9	4.0
18	−15.43	8.56	5.71	−10	21.92	29.51	−6.30	28.29	−8.90	1.2	2.6	7.6	9.1
19	−17.90	11.76	8.87	−14	22.67	28.82	−13.22	28.45	−13.45	0.4	0.2	6.2	4.7
20	−18.67	10.91	6.43	−32	16.93	22.28	−15.32	23.72	−15.14	−1.4	−0.2	5.4	3.4
21	−19.80	8.96	8.60	−34	17.83	24.58	−15.36	24.85	−15.51	−0.3	0.2	6.7	4.4
22	−19.92	6.67	7.17	−36	16.80	23.27	−14.49	24.17	−15.30	−0.9	0.8	6.5	5.4
23	−17.71	10.37	10.66	−26	19.04	24.71	−12.50	25.87	−12.25	−1.2	−0.3	5.7	5.2
24	−17.65	11.42	5.64	−46	15.23	21.73	−13.49	23.33	−13.74	−1.6	0.3	6.5	4.2
25	−18.40	9.67	4.62	−45	15.67	23.42	−13.29	n.d.	n.d.			7.8	5.1
26	−16.67	9.10	6.83	0	21.75	27.92	−9.83	27.36	−11.62	0.6	1.8	6.2	6.8
27	−18.88	12.16	4.93	−5	22.72	28.38	−13.72	n.d.	n.d.			5.7	5.2
28	−17.97	10.51	5.78	−47	14.89	20.91	−14.42	n.d.	n.d.			6.0	3.6
29	−18.00	12.60	6.71	−56	14.70	21.91	−12.88	22.09	−12.81	−0.2	−0.1	7.2	5.1
30	−18.96	9.40	6.24	−31	17.10	24.05	−14.07	24.16	−14.53	−0.1	0.5	6.6	4.9
31	−20.40	9.10	6.30	−59	16.15	22.49	−13.64	23.47	−14.26	−1.0	0.6	6.3	6.8
32	−19.44	11.03	3.57	−43	17.50	23.58	−14.62	23.94	−15.65	−0.4	1.0	6.1	4.8
33	−19.12	9.72	5.74	−54	14.65	21.88	−14.32	n.d.	n.d.			7.2	4.8
34	−19.48	8.95	6.48	−55	16.16	22.52	−14.78	23.53	−15.17	−1.0	0.4	6.4	4.7
35	−18.49	11.86	5.28	−66	13.10	20.31	−13.40	21.20	−13.83	−0.9	0.4	7.2	5.1
36	−18.53	10.53	4.18	−70	12.58	20.03	−13.62	21.33	−14.20	−1.3	0.6	7.5	4.9
37	−19.65	10.44	3.88	−43	15.29	22.61	−14.90	22.84	−15.20	−0.2	0.3	7.3	4.7
38	−19.70	10.77	4.36	−44	15.04	21.70	−14.63	22.64	−14.68	−1.0	0.1	6.7	5.1
39	−19.47	10.78	3.67	−51	17.14	22.90	−14.88	24.13	−15.02	−1.2	0.1	5.8	4.6
40	−20.03	11.36	3.55	−48	16.77	23.24	−15.03	24.94	−15.02	−1.7	0.0	6.5	5.0
41	−20.13	11.54	4.02	−50	17.33	23.15	−15.55	24.74	−15.83	−1.6	0.3	5.8	4.6
42	−19.40	9.39	4.68	−44	17.48	23.08	−14.32	24.24	−15.30	−1.2	1.0	5.6	5.1
43	−20.67	11.07	4.51	−41	15.70	22.22	−15.93	22.80	−15.92	−0.6	0.0	6.5	4.7
MV	−19.0	10.5	5.8	−41	16.9	23.5	−13.9	24.1	−14.4	−0.7	0.5	6.5	5.0
SD	1.2	1.3	1.7	17	2.5	2.3	1.9	1.8	1.6	0.7	0.6	0.7	1.0
Min	−20.7	6.7	3.6	−70	12.6	20.0	−15.9	21.2	−15.9	−1.7	−0.3	5.4	3.4
Max	−15.4	12.8	10.7	0	22.7	29.5	−6.3	28.4	−8.9	1.2	2.6	7.9	9.1

All δ values are presented as ‰ values.

MV: mean value; SD: standard deviation; Min: minimum value; Max: maximum value; n.d.: not determined.

TABLE 3 Linear correlation equations between the measured δ values

Y	*X*	Regression coefficient	Standard error (CI 95%)	Intercept	Standard error (CI 95%)	*R* (Pearson)	*N*
$\delta^{18}O_{phos}$	$\delta^{18}O_{carb\ pretreated}$	1.047	0.055	−7.653	1.307	0.964**	29
$\delta^{18}O_{carb\ pretreated}$	$\delta^{18}O_{phos}$	0.888	0.047	8.446	0.804		
$\delta^{18}O_{phos}$	$\delta^{18}O_{carb\ untreated}$	1.300	0.051	−14.442	1.232	0.983**	25
$\delta^{18}O_{carb\ untreated}$	$\delta^{18}O_{phos}$	0.743	0.029	11.550	0.497		
$\delta^{18}O_{phos}$	$\delta^{2}H_{coll}$	0.138	0.013	22.573	0.574	0.898**	29
$\delta^{2}H_{coll}$	$\delta^{18}O_{phos}$	5.846	0.552	−139.883	9.450		
$\delta^{18}O_{carb\ pretreated}$	$\delta^{2}H_{coll}$	0.127	0.012	28.685	0.526	0.899**	29
$\delta^{2}H_{coll}$	$\delta^{18}O_{carb\ pretreated}$	6.353	0.597	−190.092	14.083		
$\delta^{18}O_{carb\ untreated}$	$\delta^{2}H_{coll}$	0.096	0.012	28.081	0.552	0.848**	25
$\delta^{2}H_{coll}$	$\delta^{18}O_{carb\ untreated}$	7.524	0.980	−222.893	23.713		
$\delta^{13}C_{carb\ pretreated}$	$\delta^{13}C_{coll}$	1.382	0.155	12.279	2.95	0.864**	29
$\delta^{13}C_{coll}$	$\delta^{13}C_{carb\ pretreated}$	0.540	0.061	−11.451	0.852		
$\delta^{13}C_{carb\ untreated}$	$\delta^{13}C_{coll}$	1.166	0.111	7.785	2.117	0.910**	25
$\delta^{13}C_{coll}$	$\delta^{13}C_{carb\ untreated}$	0.710	0.068	−8.810	0.979		

**Correlation is significant at the level of 0.01.

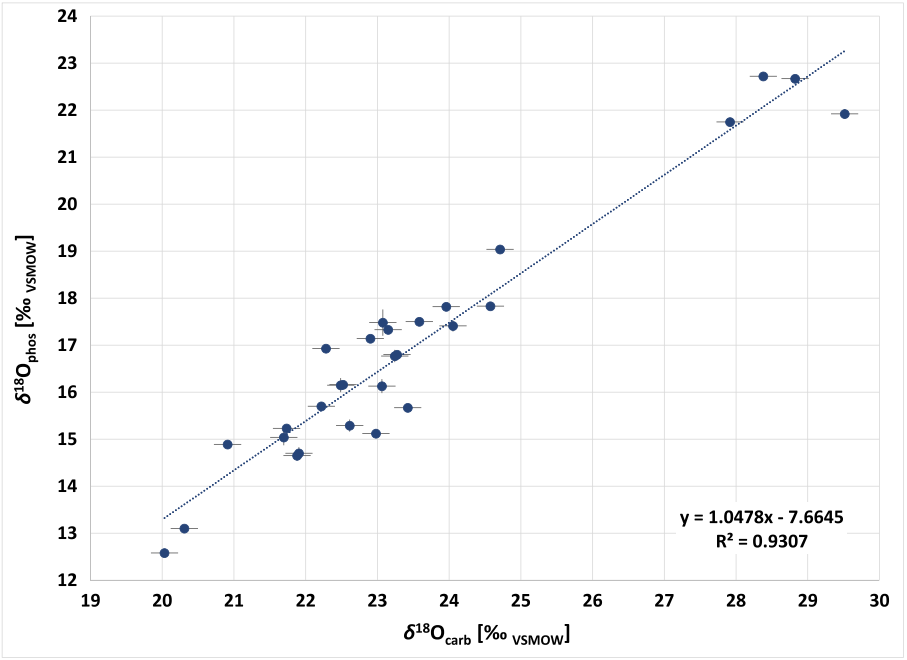

FIGURE 1 Relationship between pretreated apatite $\delta^{18}O_{carb}$ and $\delta^{18}O_{phos}$ values. Error bars: analytical uncertainty for $\delta^{18}O_{carb}$ values and standard deviations for $\delta^{18}O_{phos}$ values [Color figure can be viewed at wileyonlinelibrary.com]

other element. Weak relationships exist between collagen $\delta^{34}S$ values and $\delta^{18}O_{carb}$ or $\delta^{18}O_{phos}$ values.

4 | DISCUSSION

4.1 | Relationship between $\delta^{18}O_{carb}$ and $\delta^{18}O_{phos}$ values

The results of our study show that the $\delta^{18}O_{phos}$ and $\delta^{18}O_{carb}$ values in the bioapatite of the modern human dentine are highly correlated, for $\delta^{18}O_{carb}$ values of both pretreated and untreated apatite samples (R^2 = 0.93, or R^2 = 0.97). Regarding the correlations and slopes, the reported regression formulae between phosphate and carbonate in bone or tooth enamel of modern mammal species,[26,27,51,52] or of archaeological human dental enamel[15] were similar to our regression formula based on the $\delta^{18}O_{phos}$ and $\delta^{18}O_{carb\ pretreated}$ values in the dentine samples. In contrast, the $\delta^{18}O_{phos}$ and $\delta^{18}O_{carb}$ values in North American human dentine of the 18th and 19th centuries[29] show substantially lower correlations. It is obvious that the range of the $\delta^{18}O_{phos}$ values of the archaeological dentine samples was less (only 4.4‰) than that of the modern dentine samples (10.1‰).

The $\Delta_{carb\text{-}phos}$ offsets in the modern dentine of 6.6 ± 0.7‰ or 7.2 ± 0.7‰ are much lower than the usually observed differences of

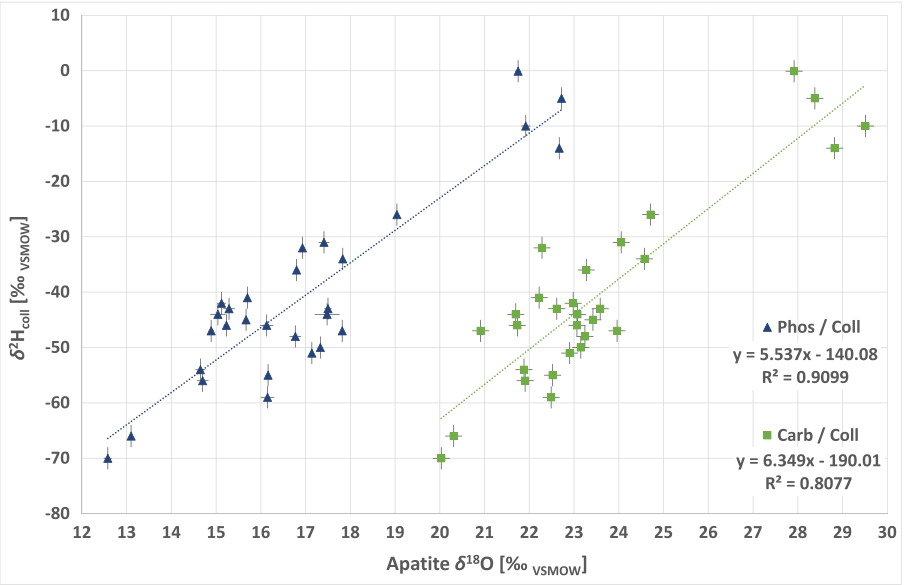

FIGURE 2 Relationships between apatite $\delta^{18}O_{carb\ pretreated}$ or $\delta^{18}O_{phos}$ values and collagen $\delta^{2}H$ values. Error bars: analytical uncertainty for $\delta^{18}O_{carb}$ values and $\delta^{2}H_{coll}$ values and standard deviation for $\delta^{18}O_{phos}$ values [Color figure can be viewed at wileyonlinelibrary.com]

~8‰–10‰ for enamel, dentine, and bone samples.[15,26,27,29,53-55] Particularly with regard to archaeological material, the offset between the $\delta^{18}O_{phos}$ and $\delta^{18}O_{carb}$ values is suggested to be a criterion for the quality control of the analyzed material; the generally accepted $\Delta^{18}O_{carb\text{-}phos}$ value for well-preserved enamel is ~9‰.[26,27,51] With regard to our modern human dentine samples, we can expect that no diagenetic processes have occurred. However, $\Delta_{carb\text{-}phos}$ offsets vary among species but are also dependent on various methodologies used to measure the $\delta^{18}O_{phos}$ and $\delta^{18}O_{carb}$ values, different pretreatment procedures, different precipitation methods for bioapatite phosphate or carbonate extraction methods, and different instruments employed.[28,56,57] The lack of suitable international bioapatite materials makes it difficult to identify the reasons for the deviations (see Fischer et al[58] for discussion). All these factors together complicate the comparison of the results of different studies. Several authors found large $\Delta_{carb\text{-}phos}$ offsets in mammal bones and tooth enamel that were related to species-specific diet, physiology, interspecies, intraspecies, and intratissue variability.[28,54,55] Following an intensive discussion of that issue, Göhring et al[28] finally stated that calculating $\delta^{18}O_{phos}$ values from $\delta^{18}O_{carb}$ values is highly error-prone.[28]

The oxygen in the structural carbonate of the bioapatite derives from blood bicarbonate, which isotopically equilibrates with body water, while the oxygen in the phosphate mainly originates from body water itself. This leads to the conclusion that the $\delta^{18}O_{phos}$ value is more sensitive to the isotopic composition of drinking water than the $\delta^{18}O_{carb}$ value, which is more influenced by solid food components. The highest $\Delta_{carb\text{-}phos}$ offsets appeared in those dentine samples with the lowest $\delta^{18}O$ values. These tooth samples are from continental, mountainous regions (e.g., Kazakhstan), where lower $\delta^{18}O$ values in precipitation and drinking water can be expected. Whereas most of the drinking water is usually of local origin, most of the food might have its origin in more temperate regions and, compared with the local food or drinking water, the aqueous parts of the foreign food are enriched in ^{18}O. While food affects $\delta^{18}O_{carb}$ values more than $\delta^{18}O_{phos}$ values, the $\Delta_{carb\text{-}phos}$ offset in the dentine samples increases with lower drinking water oxygen isotope ratios. A feeding study on small mammals[59] showed similar results. The highest $\Delta^{18}O_{carb\text{-}phos}$ offset in mouse enamel occurred with the most ^{18}O-depleted drinking water, suggesting that food ^{18}O content preferentially contributes to the $\delta^{18}O_{carb}$ values. Major differences between drinking water and food $\delta^{18}O$ values may therefore affect dentine as well as enamel $\delta^{18}O_{carb}$ values.

Furthermore, the results of several studies indicated that bioapatite $\delta^{18}O_{carb}$ values in enamel are higher than those of bone,[60-62] whereas neither the enamel nor the bone $\delta^{18}O_{phos}$ values were consistently higher. Webb et al[62] suggested that different fractionations of blood bicarbonate during bioapatite formation and maturation cause ^{18}O-(and ^{13}C-)enriched structural carbonate in enamel versus bone apatite, and Gehler et al[60] explained this gap by different time intervals in the mineralization of the two tissues. Nevertheless, because the structure of dentine is more similar to that of bone than to enamel, we also expect lower $\delta^{18}O_{carb}$ values in dentine than in enamel. Thus, this may also explain the low $\Delta_{carb\text{-}phos}$ offsets in the modern dentine samples.

4.2 | Relationships between apatite $\delta^{18}O$, collagen $\delta^{2}H$, and local $\delta^{18}O/\delta^{2}H$ precipitation values

One of the main concerns of our study was to examine the relationship between apatite $\delta^{18}O$ and collagen $\delta^{2}H$ values in human dentine. From the strong linear correlation between $\delta^{18}O_{phos}$ and $\delta^{2}H_{coll}$ values ($R^2 = 0.81$), as well as between $\delta^{18}O_{carb}$ and $\delta^{2}H_{coll}$ values ($R^2 = 0.81$ for pretreated or $R^2 = 0.72$ for untreated apatite), we conclude that the calculation of $\delta^{2}H_{coll}$ values from $\delta^{18}O_{phos}$ or $\delta^{18}O_{carb}$ values, and vice versa should be possible for each individual dentine sample. The linear correlation

TABLE 4 Correlations between measured δ values in pretreated apatite and in collagen

		$\delta^{18}O_{carb}$ pretreated[d]	$\delta^{18}O_{carb}$ untreated	$\delta^{18}O_{phos}$	$\delta^{13}C_{coll}$	$\delta^{13}C_{carb}$ pretreated	$\delta^{13}C_{carb}$ untreated	$\delta^{15}N_{coll}$	$\delta^{34}S_{coll}$	$\delta^{2}H_{coll}$
$\delta^{18}O_{carb}$ pretreated	Pearson	1	0.960**	0.964**	0.453*	0.588**	0.593**	−0.125	0.341	0.899**
	Significance		0.000	0.000	0.014	0.001	0.002	0.518	0.071	0.000
	N	29	25	29	29	29	25	29	29	29
$\delta^{18}O_{carb}$ untreated	Pearson		1	0.983**	0.483*	0.549**	0.526**	−0.255	0.440*	0.848**
	Significance			0.000	0.014	0.004	0.007	0.219	0.028	0.000
	N		25	25	25	25	25	25	25	25
$\delta^{18}O_{phos}$	Pearson			1	0.380*	0.475**	0.497*	−0.082	0.363	0.898**
	Significance				0.042	0.009	0.011	0.672	0.053	0.000
	N			29	29	29	25	29	29	29
$\delta^{13}C_{coll}$	Pearson				1	0.864**	0.910**	−0.106	0.231	0.480**
	Significance					0.000	0.000	0.582	0.228	0.008
	N				29	29	25	29	29	29
$\delta^{13}C_{carb}$ pretreated	Pearson					1	0.966**	−0.287	0.186	0.507**
	Significance						0.000	0.131	0.333	.005
	N					29	25	29	29	29
$\delta^{13}C_{carb}$ untreated	Pearson						1	−0.178	0.307	0.519**
	Significance							0.394	0.136	0.008
	N						25	25	25	25
$\delta^{15}N_{coll}$	Pearson							1	−0.130	−0.170
	Significance								0.500	0.378
	N							29	29	29
$\delta^{34}S_{coll}$	Pearson								1	0.352
	Significance									0.061
	N								29	29
$\delta^{2}H_{coll}$	Pearson									1
	Significance									
	N									29

Pearson's correlation.
*Correlation is significant at the level of 0.05.
**Correlation is significant at the level of 0.01.

between $\delta^{2}H_{coll}$ and $\delta^{18}O_{phos}$ values in the modern dentine samples is much better than for human bone samples from North American archaeological sites (R^2 = 0.16 for individual points, or R^2 = 0.49 for site averages).[29] To our knowledge, currently this is the only study that has investigated the relationship between apatite $\delta^{18}O$ and collagen $\delta^{2}H$ values in human tissues.

The $\delta^{2}H_{coll}$ and $\delta^{18}O_{phos}$ values of dentine samples should be helpful for provenancing unknown individuals, in particular, for the reconstruction of the places of residence during childhood and youth. Several authors[12-14,63] have established correlation equations between human enamel $\delta^{18}O_{phos}$ and drinking water $\delta^{18}O$ values, which enable us to narrow down the geographical origin of people. Thus, by the given apatite $\delta^{18}O$ or collagen $\delta^{2}H$ values in dentine we may also predict the local climate at the individual's whereabouts. We checked the accuracy of prediction based on the apatite $\delta^{18}O$ or collagen $\delta^{2}H$ values of the tooth samples; we know the birthplaces of the donors for only 22 of the 29 collected tooth samples. For the evaluation of our results, we have assumed that these persons grew up and stayed at the same approximate locations until the development of dentine had been completed (16th year of life at the latest). To test if there is a direct isotopic connection between the dentine and the climate, we compared the dentine $\delta^{18}O_{phos}$, $\delta^{18}O_{carb}$, and $\delta^{2}H_{coll}$ values of these 22 individuals with the mean annual $\delta^{18}O$ or $\delta^{2}H$ precipitation values at their places of birth ($\delta^{18}O_{precip}$ or $\delta^{2}H_{precip}$, Table 2). As expected, tooth samples originating from hot and arid climatic regions (Eastern Africa) had the highest apatite $\delta^{18}O$ and $\delta^{2}H_{coll}$ values, whereas the lowest values occurred in tooth samples from continental climates (Kazakhstan). However, we cannot claim that meteoric precipitation maps will provide accurate representations of the isotopic composition of human drinking water

obtained from groundwater and surface water. We can only speculate that the $\delta^{18}O$ and $\delta^{2}H$ values of the predicted local precipitation closely match the regional tap or drinking water values.[11] Accurate maps of the isotopic composition of precipitation water are readily available for most areas of the globe, but those of surface and groundwater are relatively limited.[64]

From the regression statistics, apparently there are highly significant linear relationships between the dentine $\delta^{18}O_{phos}$, $\delta^{18}O_{carb}$, and $\delta^{2}H_{coll}$ values (dependent variables) and the mean annual $\delta^{18}O$ or $\delta^{2}H$ precipitation values (independent variables):

$$\delta^{18}O_{phos} = 0.64\,(\pm 0.05)\,\delta^{18}O_{precip} + 21.59\,(\pm 0.39) \quad (1)$$
$$R\,(\text{Pearson}) = 0.94, R^2 = 0.89,$$

$$\delta^{18}O_{carb\ pretreated} = 0.61\,(\pm 0.04)\,\delta^{18}O_{precip} + 27.89\,(\pm 0.33) \quad (2)$$
$$R\,(\text{Pearson}) = 0.95, R^2 = 0.91,$$

$$\delta^{18}O_{carb\ untreated} = 0.46\,(\pm 0.05)\,\delta^{18}O_{precip} + 27.55\,(\pm 0.42) \quad (3)$$
$$R\,(\text{Pearson}) = 0.90, R^2 = 0.81,$$

$$\delta^{2}H_{coll} = 4.03\,(\pm 0.43)\,\delta^{18}O_{precip} - 11.41\,(\pm 3.23) \quad (4)$$
$$R\,(\text{Pearson}) = 0.90, R^2 = 0.82,$$

$$\delta^{2}H_{coll} = 0.52\,(\pm 0.06)\,\delta^{2}H_{precip} - 15.79\,(\pm 2.90) \quad (5)$$
$$R\,(\text{Pearson}) = 0.90, R^2 = 0.81.$$

Pearson correlations indicate that human dentine $\delta^{18}O_{phos}$ values as well as $\delta^{18}O_{carb}$ values are clearly proportional to $\delta^{18}O_{water}$ values, and $\delta^{2}H_{coll}$ values are highly correlated to $\delta^{2}H_{water}$ values as well as to $\delta^{18}O_{water}$ values. This means that the isotope ratios of hydrogen in collagen and oxygen in apatite match very well with the local precipitation $\delta^{18}O$ and $\delta^{2}H$ values. From the slopes of the regression formulae, we conclude that precipitation is on average responsible for 64% of the $\delta^{18}O_{phos}$ values, or 61% of the $\delta^{18}O_{carb}$ values and for 52% of the $\delta^{2}H_{coll}$ values in dentine. However, in addition to that aspect, while local tap water might be the main source of the ingested water, the consumption of variable amounts of bottled water, imported fruits, vegetables, and meats may affect the isotope ratios of modern humans' body water. Referring to the results of Reynard et al[65] on modern hair samples from the East Coast cities of the USA, a range of >±10‰ of $\delta^{2}H$ values was evident at each location; a similar range should reasonably be assumed for $\delta^{2}H_{coll}$ values. Similar variabilities may also apply for modern apatite $\delta^{18}O$ values. The results of a feeding study on rats suggest that variations in the isotopic composition of local water of less than 2.5‰ in $\delta^{18}O$ values and less than 10‰–20‰ in $\delta^{2}H$ values are invisible in the tissues, unless isotopically distinct food resources contribute to regional isotopic separations.[59]

As already mentioned, the results of different studies on human tissues have led to several linear regression equations that allow predictions about the local climate at the residence of an individual from the $\delta^{18}O_{phos}$ or $\delta^{18}O_{carb}$ values. The regression equation calculated for fossil human bones and local meteoric water by Longinelli[14] is quite similar to our regression formula for the calculation of $\delta^{18}O_{water}$ values from modern dental $\delta^{18}O_{phos}$ values (Equation 1). It is also very close to the inverse regression formula for the $\delta^{18}O_{tapwater}/\delta^{18}O_{phos}$ linear relationship of the "superset" reported by Daux et al,[12] while the inverse formula is mentioned by Meier-Augenstein[66] (Equation 6b).

Daux et al[12]

$$\delta^{18}O_{tap\ water} = 1.54\,(\pm 0.09)\,\delta^{18}O_{phos} - 33.72\,(\pm 1.51), \quad (6a)$$

Inverse[66]

$$\delta^{18}O_{phos} = 0.65\,\delta^{18}O_{water} + 21.89. \quad (6b)$$

By using the regression equation of Daux et al[12] (Equation 6a) to calculate $\delta^{18}O_{water}$ values from the modern dentine $\delta^{18}O_{phos}$ values, we obtain a linear relationship correlating well with the predicted $\delta^{18}O$ precipitation values (Figure 3). Chenery et al[15] established a direct relationship between $\delta^{18}O_{phos}$ and $\delta^{18}O_{carb}$ values for human tooth enamel (Equation 7a) and combined this equation with the tap water equation of Daux et al[12] (Equation 6a) to allow a direct calculation of drinking water $\delta^{18}O$ values from the $\delta^{18}O_{carb}$ values in human bioapatite (Equation 7b).

Chenery et al[15]

$$\delta^{18}O_{phos} = 1.0322\,\delta^{18}O_{carb} - 9.6849 \quad (7a)$$

and[15]

$$\delta^{18}O_{drinking\ water} = 1.59\,\delta^{18}O_{carb} - 48.63. \quad (7b)$$

Using Equation 7b for the calculation of $\delta^{18}O_{water}$ values from our modern dentine $\delta^{18}O_{carb\ pretreated}$ values, we obtain a linear relationship, but the calculated $\delta^{18}O_{water}$ values are around 4‰ lower than the local precipitation $\delta^{18}O$ values (Figure 3). We assume that the lower $\Delta_{carb\text{-}phos}$ offsets in our dentine samples than in the archaeological enamel samples are mainly responsible for the consistent shift in the $\delta^{18}O_{water}$ values calculated from the $\delta^{18}O_{carb}$ values.

For the calculation of the precipitation values with respect to the climatic condition at the person's whereabouts from the oxygen isotope ratios in the dentine, we suggest that our established linear regression equations should be applied. This enables direct conversion from the $\delta^{18}O_{phos}$ value (Equation 1) or the $\delta^{18}O_{carb}$ value (Equations 2 and 3, respectively) to the local $\delta^{18}O_{precip}$ value. The same is true for hydrogen or oxygen isotope ratios of the local precipitation, which can be calculated from dentine $\delta^{2}H_{coll}$ values using Equation 4 or Equation 5. We can reasonably assume that the oxygen or hydrogen isotope ratios of tap or drinking water are not very different from those of the mean annual precipitation.[15]

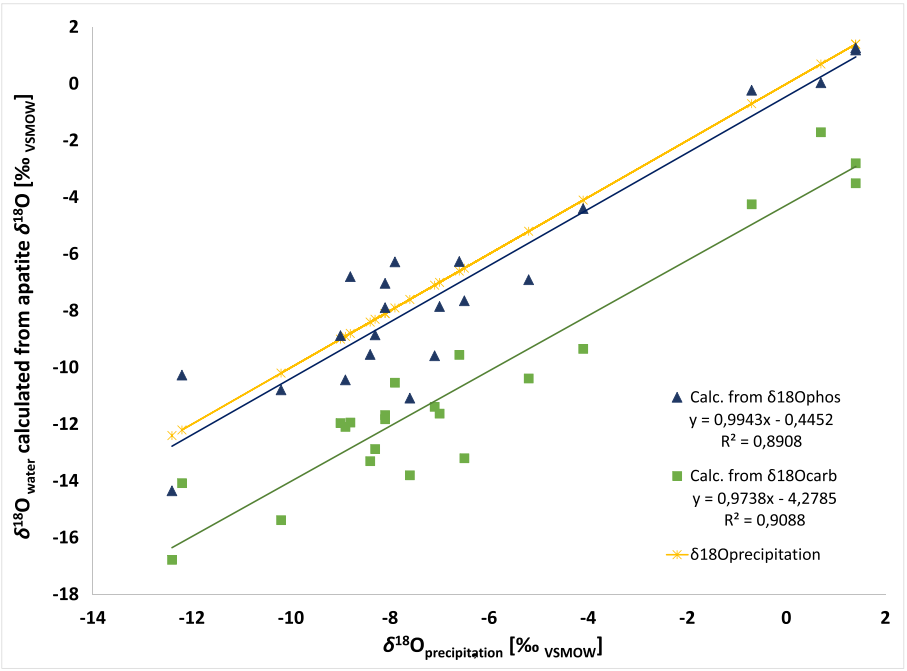

FIGURE 3 Correlation between $\delta^{18}O_{water}$ values calculated from the dentine $\delta^{18}O_{phos}$ values using the formula established by Daux et al[12] (Equation 6a) or from the dentine $\delta^{18}O_{carb\ pretreated}$ using the formula established by Chenery et al[15] (Equation 7b), and the local mean annual $\delta^{18}O_{precip}$ values[50] (yellow line) [12,15,50] [Color figure can be viewed at wileyonlinelibrary.com]

4.3 | Intratissue differences in collagen $\delta^{13}C$ and structural carbonate $\delta^{13}C$ compositions

Carbon in collagen mainly derives from proteins, but structural apatite additionally contains carbon from lipids and carbohydrates. The $\delta^{13}C$ values of both compounds are highly correlated in the dentine. The correlation between the $\delta^{13}C_{coll}$ and the $\delta^{13}C_{carb}$ values in the modern dentine is robust ($R^2 = 0.74$ for pretreated, and $R^2 = 0.83$ for untreated apatite), although the $\Delta^{13}C_{carb\text{-}coll}$ values vary from 3.4‰ to 9.1‰. We suggest that the large isotope range is associated with the highly variable dietary habits in the different countries, ranging from an almost vegan diet up to the consumption of high amounts of animal protein (meat, fish, eggs, dairy products). In addition, the amount of C4-plant (e.g., corn, millet, sorghum) versus C3-plant (e.g., wheat, rye, rice) based food could explain differences in the consumer's carbon isotope ratios.[67]

4.4 | Pretreatment of samples

Before $\delta^{13}C$ and $\delta^{18}O$ analyses on bioapatite in enamel or dentine, the material is commonly treated to remove organic matter and secondary carbonate. Koch et al[68] and Garvie-Lok et al[69] recommended pretreatment of bone and fossil enamel to remove organic compounds and secondary carbonates prior to $\delta^{18}O_{carb}$ analyses. As dentine contains about 30% organic compounds, we decided to pretreat the tooth material before $\delta^{18}O_{phos}$, $\delta^{18}O_{carb}$, and $\delta^{13}C_{carb}$ analyses on the apatite. However, some studies have shown that there is no clear need for any pretreatment of the apatite because only high amounts of organic matter influence carbonate $\delta^{13}C$ and $\delta^{18}O$ values.[70,71] Numerous recent studies have shown that pretreatment can have unintended and inconsistent consequences for the isotopic composition of structural carbonate or phosphate.[56,57,68,69,72-76] Our results indicate no significant mean offsets between pretreated and untreated dentine. However, there was relatively high variability in the offsets among the samples (2.9‰ for $\delta^{18}O$ values and 2.9‰ for $\delta^{13}C$ values).[74] In addition, the great loss of about 50% of the raw dentine material can be considered as being a further drawback of the pretreatment procedure. Pellegrini and Snoeck[75] compared commonly used methods for bioapatite carbonate pretreatments for isotopic measurements on archaeological and modern bone, dentine, or enamel. The results clearly showed that any chemical treatment had rather unpredictable, often significant effects on the original isotopic composition, and this corroborates the need for consistency in the pretreatment of bone and teeth specimens. The authors questioned the necessity of removing organic matter at all from most skeletal samples prior to isotopic analysis.[75] Particularly with regard to modern dentine, it should be proved in further studies whether it is in fact necessary to remove organic content prior to apatite carbonate or phosphate stable isotope analyses.

4.5 | Relationship among isotope ratios of nitrogen and sulfur in collagen and other elements

There was no relationship between the $\delta^{15}N_{coll}$ and $\delta^{2}H_{coll}$ values for the modern dentine samples (Table 3). Reynard and Tuross[65] could also not recognize any correlation between the $\delta^{15}N$ and $\delta^{2}H$ values in hair samples of contemporary humans. By contrast, for archaeological human bone samples, it has been stated that both $\delta^{15}N$ and $\delta^{2}H$ values increase within the food chain and depend on the amount of consumed protein.[30,77] Topalov et al[41] supported this conclusion, finding that hydrogen from both food and water

contributed to δ^2H_{coll} values, albeit using a mouse model and enriched food and water.[65] We reasonably assume that in modern human samples, a trophic effect based on the $\delta^{15}N$ values might be difficult to recognize. For one thing, low $\delta^{15}N$ values of human tissues may be caused by an almost vegan diet. However, lowered $\delta^{15}N$ values may have additional causes. For example, the use of synthetic nitrogen fertilizer in agriculture decreases $\delta^{15}N$ values in modern food products. Most synthetic nitrogen fertilizers have significantly lower $\delta^{15}N$ values than organic fertilizers.[78] Furthermore, a high consumption of legumes leads to decreased $\delta^{15}N$ values. A shift toward lower diet $\delta^{15}N$ values would result if legumes (or animals that fed on them) made up an increasingly larger part of the diet.[79] Contrary to cereals or corn products, pulses bind atmospheric nitrogen ($\delta^{15}N$ value = 0‰). A comparably large proportion of legumes in the diet may have decreased the collagen $\delta^{15}N$ values of some individuals, especially of those from African or Asian regions. Consequently, $\delta^{15}N$ values in modern human tissues may depend not only on the amount of protein from animal sources but also on additional factors. Beyond different individual dietary habits, the high diversity in food production together with worldwide trading of animal feed components could potentially mitigate the relationship between $\delta^{15}N$ and δ^2H values in modern human tissues.

Our results indicate weakly correlated dentine $\delta^{34}S_{coll}$ and $\delta^{18}O_{carb}$ values. A similar pattern was recognized in mammal bones from coastal regions, where the relationship was explained as being the result of the sea spray effect.[80] However, the very weak correlation between isotope ratios from two different precursor pools in two different matrices, one organic and one inorganic, should be treated with extreme caution until such time that more data become available.

5 | CONCLUSIONS

This study has reported the first comparison of stable isotope results in apatite and collagen on modern-day human dentine samples from different climate zones. This enabled the calculation of isotope relationships over a broad range of values.

$\delta^{18}O_{phos}$, $\delta^{18}O_{carb}$, and δ^2H_{coll} values are highly correlated in modern human dentine. From this it follows that there is a direct relationship between oxygen in the apatite phosphate or carbonate and hydrogen in the collagen fraction. As there is almost no turnover of the dentine during an individual's lifetime, the different fractions developed at the same time and received comparable oxygen and hydrogen isotope signals from the ingested water.

The highly significant correlation of $\delta^{18}O_{phos}$, $\delta^{18}O_{carb}$, and δ^2H_{coll} values in the modern dentine samples implies that we may measure the $\delta^{18}O$ values on the apatite fractions or the δ^2H value on the collagen fraction to obtain reliable information about the climatic setting at a person's whereabout.

Consequently, similar to the apatite $\delta^{18}O$ values, the collagen δ^2H value in the dentine can be used as a proxy for the climatic condition at the residence of an individual during childhood and adolescence. With regard to this issue, from the C–N–S–H stable isotope ratios in dentine collagen, sufficient information about the isotopic composition of the food and of the drinking water can be obtained.

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