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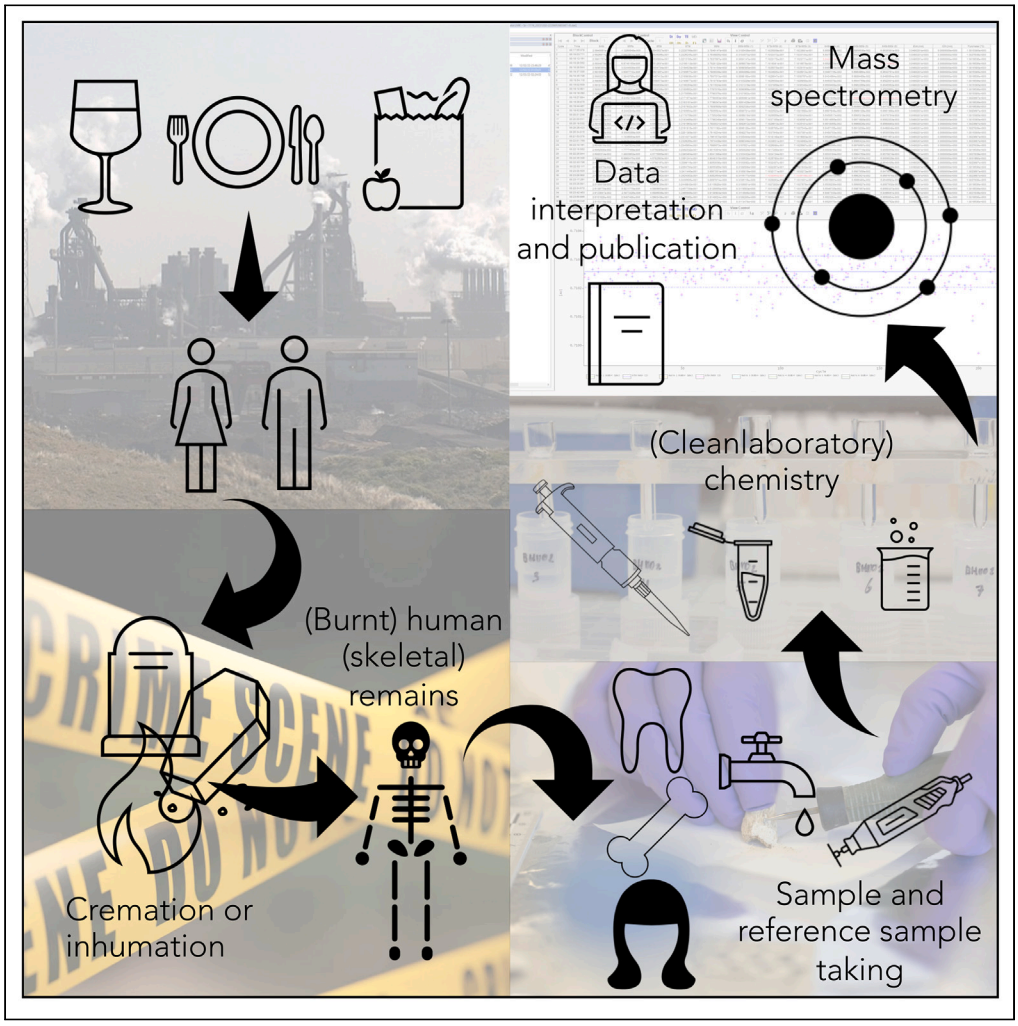

Saskia T.M. Ammer, Nathan Routhledge, Gareth R. Davies, Arian C. van Asten, Suzan J.A. Verdegaal-Warmerdam, Lisette M. Kootker

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Highlights

Sr-O-C-N-H isotope data of modern humans and drinking water from the Netherlands

$\delta^{15}N$ values of human hair cannot be utilized to differentiate from other countries

Variations in hair $\delta^{13}C$ enable the exclusion the Netherlands as a region of origin

Limited availability of reference data makes interpretation of data difficult

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Enhancing the contemporary human and water isotope reference database for the Netherlands: New insights from Sr-O-C-N-H isotope data

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SUMMARY

The determination of an individual's geographic origin is an essential aspect of forensic investigations. When primary identifiers cannot be used to make a positive identification, isotope analysis can be utilized to provide new leads. Modern reference data are essential for accurate interpretation of human isotopic data in terms of diet and origin. This article presents Sr-O-C-N-H isotope data of modern individuals (hair, dental enamel, and dentine collagen) and drinking water from the Netherlands. The δ^{15}N values of human hair fall within the range of values observed worldwide and cannot be utilized to differentiate from other countries. Distinct disparities in the hair δ^{13}C are evident between European countries and other regions, making it possible to exclude the Netherlands as a region of origin. Comparing Dutch dental isotope data to those of other nations has proven difficult due to the limited availability of reference data. The same limitation applies to tap water δ^{2}H data.

INTRODUCTION

Identification of human remains is paramount in forensic investigations. The primary identifiers used for identification are DNA profiling, fingerprint ridge analysis, roentgenography, and comparative analysis of dental records.[1,2] However, these techniques require a known record from the person of interest, or family in the case of DNA, to make a positive identification. In cases where primary identifiers cannot be used or provide no match, other techniques must be utilized to provide new leads that could reveal the identity of the deceased individuals. Isotope analysis has become more prominent in forensic science, since first being used to aid human identification in 1982, where the composition of stable isotopes in human tissue was seen to vary from person to person.[3–12] Nevertheless, its full potential has not been exploited, and several isotope systems are yet to be applied in medicolegal casework.[13,14] Most applications of forensic isotope analysis focus on the reconstructions of the timeline of geographic movements and dietary habits.[6,15,16] The isotope systems of, *inter alia*, strontium (^{87}Sr/^{86}Sr), lead (^{20x}Pb/^{20x}Pb), oxygen (δ^{18}O), and hydrogen (δ^{2}H) can be used to gain valuable insights into the geological or even geographical location the person grew up in or where the deceased has spent the last years to months of life.[17–20] Carbon (δ^{13}C), nitrogen (δ^{15}N), sulfur (δ^{34}S), but also, less commonly used in current practice, calcium ($\delta^{44/42}$Ca), zinc (δ^{66}Zn), and stable strontium (δ^{88}Sr) can provide valuable data on an individual's diet.[21–23]

The elements mentioned previously are incorporated into our tissues through the food and water we consume and the air we breathe, as well as other factors, such as bathing water.[24–31] The isotope ratios measured in various tissues, such as hair keratin, bones (apatite and collagen), and dental elements, provide insights into provenance and diet during various stages of life. Dental enamel of permanent dentition, for instance, mineralizes during childhood, incorporating the isotope ratio of the foods consumed during that specific period.[32] In contrast, due to bone remodeling, the isotope composition of bone apatite and collagen can vary in and between the skeletal elements. For example, depending on the age at death, the isotope composition of trabecular bone represents the average isotope composition of the consumed food during the approximately last five years of life, while cortical bone can represent the last decades prior to death.[5,33–38] Isotopically speaking, the collagen will be representative of the dietary protein intake, with the bioapatite being representative of the whole diet.[39–41] Theoretically, using multiple isotope systems and multiple types of human tissues, very specific insights can be gained about recent and childhood provenance and diet. However, the accuracy of the interpretation of isotope data heavily depends on the presence and quality of relevant reference datasets. Absence of reference data or the presence of small, unrepresentative datasets may often lead to the identification of large potential regions of origin, hampering the application potential of isotope data in medicolegal casework.

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Table 1. Descriptive statistics of the results for hair keratin, dental enamel (bioapatite), dentine (collagen), and tap water samples

	Hair		Enamel			Dental collagen		Tap water		
	$\delta^{15}N$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$^{87}Sr/^{86}Sr$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{2}H$	$^{87}Sr/^{86}Sr$
Sample size (*n*)	28	28	73	73	159	38	38	98	98	143
Minimum	8.4	−21.6	−7.4	−14.8	0.70896	10.2	−22.0	−9.5	−66.9	0.70837
Maximum	10.6	−20.4	−5.0	−12.8	0.70942	11.9	−19.9	−4.4	−30.1	0.71278
Mean	9.2	−20.9	−6.5	−13.9	0.70919	11.0	−20.7	−7.3	−49.7	0.70927
SD	0.4	0.3	0.5	0.5	0.00024	0.4	0.4	0.9	6.5	0.00067

Key: $\delta^{15}N$, $\delta^{13}C$, and $\delta^{18}O$ in per mil (‰) versus AIR, VPDB, and VPDB (enamel) and VSMOW (tap water), respectively. SD, standard deviation (σ).

Isotope research in archaeology is based on the premise that the palaeodiet was dominated by locally grown foods. However, the isotopic disparities in the Dutch geological subsurface (Sr) and nutritional availability, which were discernible in prehistoric and historical eras through the composition of consumed foods and consequently in individuals, are not always observable in the contemporary period.[18,42] Today, globalization of food production and distribution has led to some disconnection of consumers from the location of food production. This "global supermarket-diet" has the potential to override the "local" (i.e., geological and environmental) isotope signatures, leading to isotope ratios that are not indicative of specific geological or geographical origins. Consequently, available strontium isotope landscape maps, *Sr isoscapes* in short, that are frequently used for identification of potential regions of origin in archaeological isotope research, e.g., in the study by West et al., Kootker et al., Willmes et al., Snoeck et al., and Lugli et al.,[43–47] are not necessarily applicable in forensic casework. Relevant reference databases and isoscape maps are specifically required for the use in modern (forensic) contexts.[18,24,48–50]

The application of isotope analysis in Dutch forensic casework increased significantly since its introduction in 2008. The collaboration between the Netherlands Forensic Institute (NFI), the national police force, and the Vrije Universiteit Amsterdam has resulted in a wider acceptance of the method to gather new information about the geographical origin and ultimately the identification of the unknown dead. In 2023, INTERPOL and the Belgian, Dutch, and German police launched "Operation Identify Me", an ultimate attempt to identify 22 unknown females.[51] The information in cases referring to possible regions of (childhood) origin is extracted from isotope analysis. As the significance of isotope analysis for forensic sciences is increasing, it is essential to increase the accuracy of the interpreted isotope data. Consequently, the growing and successful implementation of isotope research in Dutch forensic science calls for the need to expand the available relevant reference datasets and the development of reference maps specifically for modern (forensic) research.

Unfortunately, to date, reference or baseline isotope data from modern human individuals from the Netherlands are scarce. However, a recent paper reported 153 modern human enamel and 143 tap water $^{87}Sr/^{86}Sr$ measurements from the Netherlands, defining a first "Dutch" strontium isotope signature with Sr ratios ranging between 0.7088 and 0.7099.[18] In contrast, there are extremely limited available modern Dutch human C-N-O-H isotope data.[15,17] Therefore, this paper presents newly generated isotope data from tap water (O-H), dentine collagen and hair keratin (C-N), and dental enamel (Sr-C-O), updating (Sr) and expanding (O-C-N-H) the published isotopic reference datasets. This publication will contribute to a better understanding of the Sr-C-N-O-H isotope composition of or accessible to the modern Dutch population and add to the ever-growing modern-day human tissue and tap water database of the Netherlands, while tying the new data into the previously published data.

RESULTS

The Dutch human hair, dental elements, and tap water database now consists of 776 data points for the isotope systems of carbon, nitrogen, oxygen, hydrogen, and strontium. A detailed breakdown of the data added by this study can be found in the STAR Methods section. All $\delta^{15}N$ and $\delta^{13}C$ isotope values for human hair; $\delta^{18}O$, $\delta^{13}C$ values and $^{87}Sr/^{86}Sr$ for dental enamel; $\delta^{13}C$ and $\delta^{15}N$ isotope values for dentine-derived collagen, and $\delta^{18}O$, $\delta^{2}H$ values and $^{87}Sr/^{86}Sr$ for tap water can be found in the Supplementary Data file. An overview of the descriptive statistics of the results can be found in Table 1.

Human hair

A total of 28 hair samples were analyzed for their $\delta^{15}N$ and $\delta^{13}C$ isotope values (Supplementary Data). The metadata from the questionnaires completed by the donors can also be found in the Supplementary Data. No formal analyses were conducted on the metadata; however, they can be used for future research. The $\delta^{15}N$ values recorded range from 8.4‰ to 10.6‰, with a mean of 9.2‰ ± 0.4‰ (1σ). The $\delta^{13}C$ isotope values average −20.9‰ ± 0.3‰ (1σ), with the minimum being −21.6‰ and the maximum being −20.4‰ (Table 1). A visual representation of the distribution of these isotope values can be found in Figure 1.

Human dental elements

Dental enamel

A total of 73 dental enamel samples were analyzed for O and C isotopes. Furthermore, we added six individuals to the previously published $^{87}Sr/^{86}Sr$ dataset,[14] which now consists of 159 data points (Supplementary Data). The $\delta^{18}O$ values recorded range from −7.4‰ to −5.0‰, with

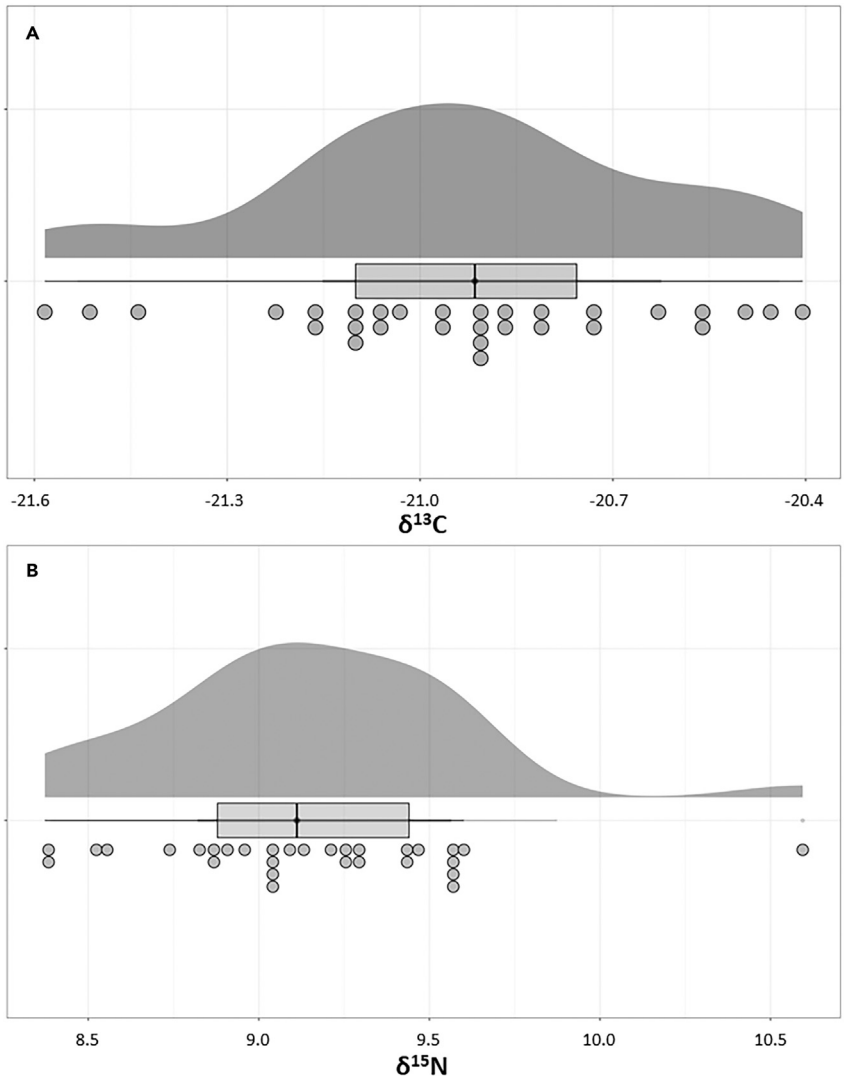


Figure 1. Raincloud plots of the isotope values found in modern human hair from the Netherlands

(A) $\delta^{13}C$ and (B) $\delta^{15}N$ isotope values in per mil (‰). The raincloud (half-density) plot (top) enhances the traditional boxplot (middle) by highlighting multiple modalities (an indicator that groups may exist) and how distributions vary compared to the median and inner-quartile range. The half-dotplot (lowest), which is similar to a histogram, indicates the number of samples (number of dots) in each bin.

the mean being −6.5‰ ± 0.5‰ (1σ). The average $\delta^{13}C$ isotope value is −13.9‰ ± 0.5‰ (1σ), with the minimum being −14.8‰ and the maximum being −12.8‰. The $^{87}Sr/^{86}Sr$ ranges from 0.70896 to 0.70942, averaging 0.70919 ± 0.00024 (1σ, Table 1). A visual representation of the distribution of these isotope values can be found in Figure 2.

Dental collagen

A total of 38 tooth dentine collagen samples were analyzed for their $\delta^{13}C$ and $\delta^{15}N$ isotope values (Supplementary Data). The $\delta^{13}C$ values average −20.7‰ ± 0.4‰ (1σ), with the minimum being −22.0‰ and the maximum being −19.9‰. The $\delta^{15}N$ values recorded range from 10.2‰ to 11.9‰, with the mean being 11.0‰ ± 0.4‰ (1σ, Table 1). A visual representation of the distribution of these isotope data can be found in Figure 3.

Tap water

Of a total of 143 tap water samples, 98 samples were analyzed for their $\delta^{18}O$ and $\delta^{2}H$ isotope values. All 143 samples were previously analyzed for their Sr isotope ratios (Data S1).[52] The $\delta^{18}O$ values range from −9.5‰ to −4.4‰, with the mean being −7.3‰ ± 0.9‰ (1σ). The $\delta^{2}H$ isotope values average −49.7‰ ± 6.5‰ (1σ), with the minimum being −66.9‰ and the maximum −30.1‰. The $^{87}Sr/^{86}Sr$ ranges from 0.70837 to

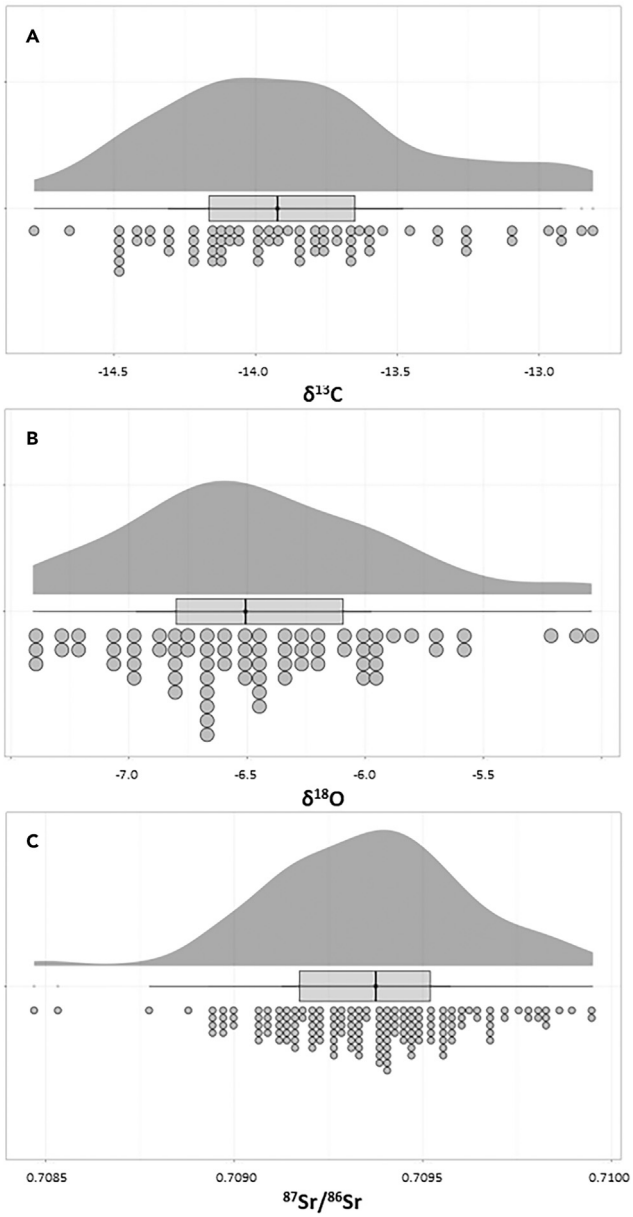


Figure 2. Raincloud plot of the isotope values and ratios found in human dental enamel from the Netherlands

(A) $\delta^{13}C$ and (B) $\delta^{18}O$ isotope values in per mil (‰), and (C) $^{87}Sr/^{86}Sr$. The raincloud (half-density) plot (top) enhances the traditional boxplot (middle) by highlighting multiple modalities (an indicator that groups may exist) and how distributions vary compared to the median and inner-quartile range. The half-dotplot (lowest), which is similar to a histogram, indicates the number of samples (number of dots) in each bin.

0.71278, averaging 0.70927 ± 0.00067 (1σ, Table 1). A visual representation of the distribution of these isotope values can be found in Figure 4.

DISCUSSION

Human hair

The hair keratin isotope data obtained in this study reflect the dietary intake in the months to years leading up to the donation in 2021. When comparing these data to older cold forensic cases, it is important to consider the sample date. Over time, dietary preferences and the availability of raw ingredients may have undergone shifts or alterations. Consequently, the Dutch hair C and N isotope data presented in this paper (collected in 2021) may not accurately represent the hair isotope values in significantly older cases. Furthermore, it is

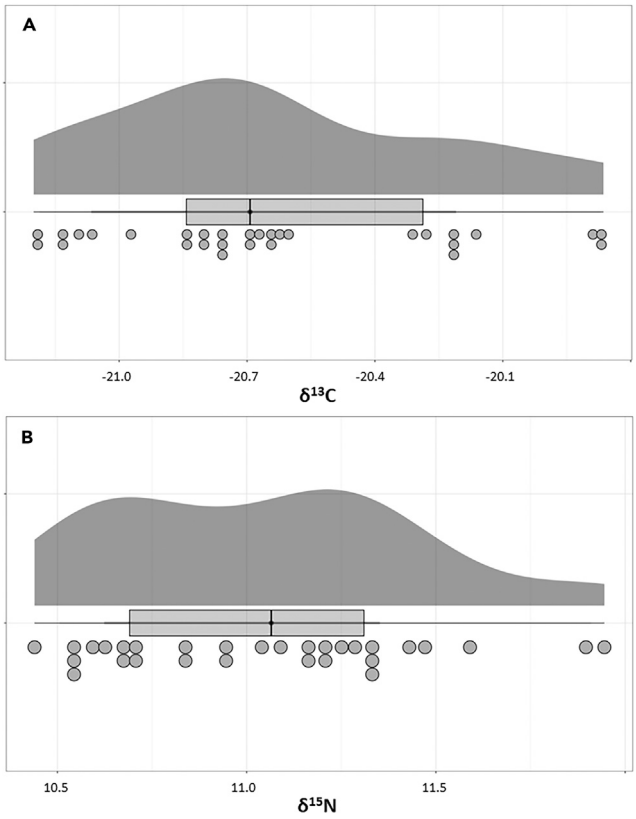


Figure 3. Raincloud plot of the isotope values found in human tooth collagen from the Netherlands

(A) $\delta^{13}C$ and (B) $\delta^{15}N$ isotope values in per mil (‰). The raincloud (half-density) plot (top) enhances the traditional boxplot (middle) by highlighting multiple modalities (an indicator that groups may exist) and how distributions vary compared to the median and inner-quartile range. The half-dotplot (lowest), which is similar to a histogram, indicates the number of samples (number of dots) in each bin.

notable that the samples were analyzed in bulk, as described in the STAR Methods, which means that potential variations within the hair were not observable.[15]

Nitrogen isotope data

The collected hair keratin nitrogen isotope data from the Netherlands show a range of 2.2‰. This outcome may partially be attributed to the limited spatial diversity in the collection of samples, as most donors resided in the coastal provinces of Noord- and Zuid-Holland. Nevertheless, the overall isotopic variation may also be constrained owing to the widespread adoption of a dominant "supermarket-diet" and the substantial geographic expanse of the country. However, the range is comparable with the range observed in other countries (Figure 5). It is therefore likely that that amount of protein consumed is not dependent on food availability or geographical origin but varies greatly due to the personal preferences of the individual.

The Dutch $\delta^{15}N$ values are compared to published data from other countries in Figure 5. The descriptive statistics of all countries can be found in the Supplementary Data in tabular format. An additional six hair samples were incorporated in this discussion sampled in 2004 and 2013 from the Netherlands which were previously published,[56] thereby expanding the Dutch dataset to a total of 34 data points. The $\delta^{15}N$ values of human hair from the Netherlands fall within the range of values observed worldwide and, therefore, cannot be utilized to differentiate from the countries represented in this study.[3,49,54–56] However, based on the current data, if $\delta^{15}N$ values outside the range observed in the Netherlands are encountered in an unidentified individual, the Netherlands can be excluded as a possible source.

Carbon isotope data

The $\delta^{13}C$ variation observed in this study is narrow (1.2‰), akin to the nitrogen isotope data. The underlying factors contributing to this limited variation are likely consistent with those mentioned previously. In Figure 6 and the Supplementary data (tabular format), the Dutch dataset is compared to available data from various countries worldwide. Unlike the nitrogen isotope data, distinct disparities are evident between European countries and other regions globally. The $\delta^{13}C$ values have less variance and are lower than the countries in the Americas and on the Asian and African continents. The available European data are similar, with the Netherlands occupying an intermediate position. Therefore,

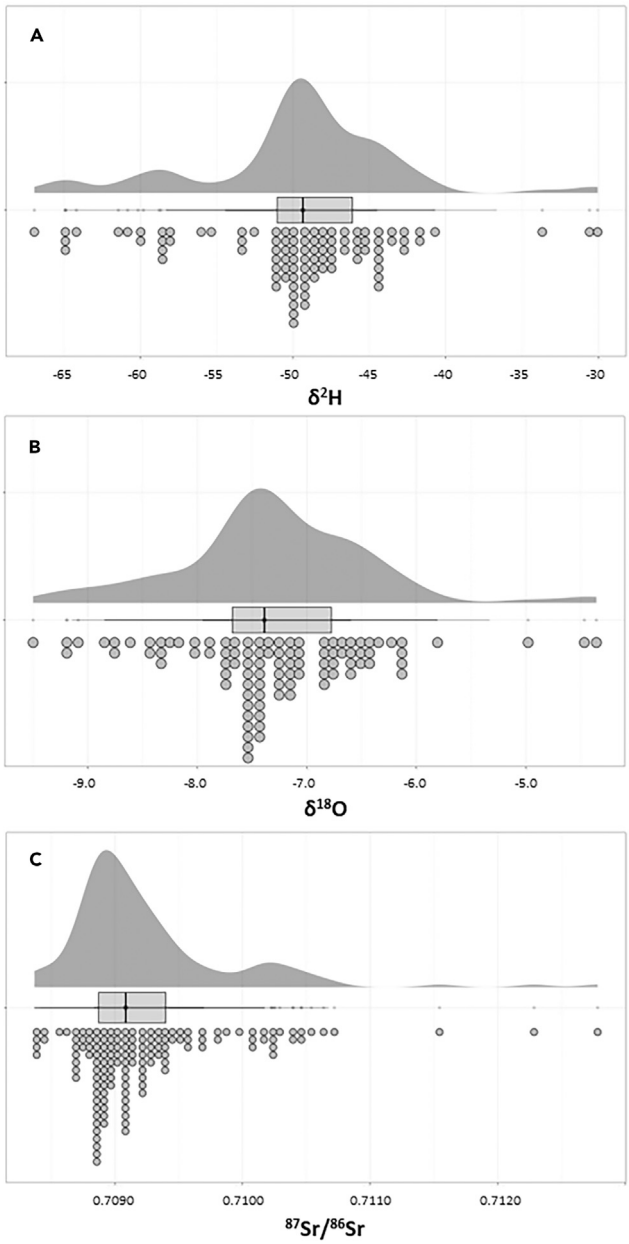


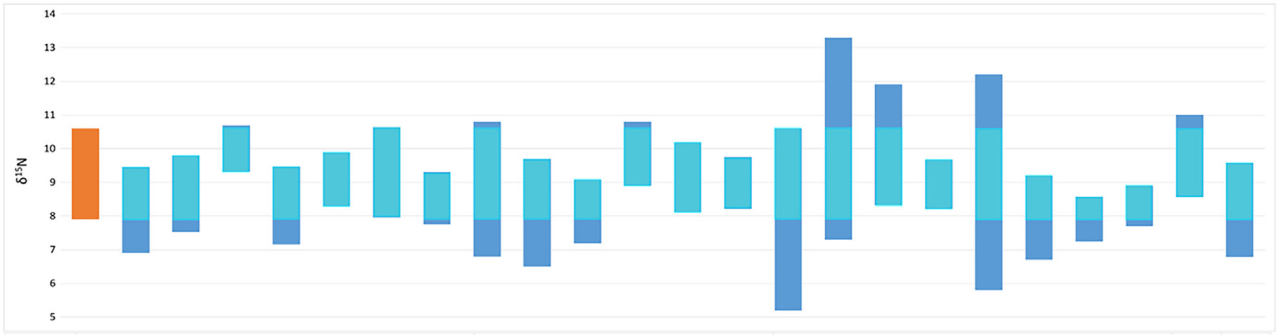
Figure 4. Raincloud plot of the isotope values and ratios found in tap water from the Netherlands

(A) δ^2H and (B) δ^{18}O isotope values in per mil (‰), and (C) ^{87}Sr/^{86}Sr. The raincloud (half-density) plot (top) enhances the traditional box-plot (middle) by highlighting multiple modalities (an indicator that groups may exist) and how distributions vary compared to the median and inner-quartile range. The half-dotplot (lowest), which is similar to a histogram, indicates the number of samples (number of dots) in each bin.

similarly to the δ^{15}N data, it is not feasible to identify an unknown individual as Dutch solely based on δ^{13}C values. However, it is possible to exclude the Netherlands as a plausible region of origin if the data deviate from the recorded values from the Netherlands.

Human dental elements

Dental elements record the dietary intake of an individual during the early years of life until adolescence. Depending on the dental element, enamel of the permanent dentition is formed between birth and ca. the age of 16.[32,58] Formation of the primary root dentine of the third molar starts at an approximate age of 15.5 years and is commonly completed by the age of 25.[32,59] However, the continuous formation of secondary dentine throughout the lifespan of the tooth allows for the recording of an ongoing isotope signature of the food consumed in later stages of life.[32] Because the roots in this study are bulk analyzed, the data presented here are dominated by the primary dentine recorded during youth



	Europe								the Americas						Asia								Oceania	Africa
	the Netherlands	Northern Germany	Southern Germany	Denmark	Italy	UK/Ireland	France	Poland	Mexico	USA	Canada	Colombia	Brasil	Costa Rica	China	Mongolia	Russia	Japan	India	Pakistan	Iran	Lebanon	Australia	Ethiopia
Sample Size	34	55	98	12	33	28	24	19	101	206	15	38	22	17	74	20	40	20	38	11	8	11	9	3
Mean	9.1	8.2	8.5	9.9	8.4	8.8	8.9	8.7	9.2	8.8	8.3	9.9	9.0	8.8	8.1	9.6	9.6	8.8	8.1	8.2	7.9	8.3	9.5	8.2
SD	0.5	0.6	0.5	0.4	0.7	0.4	0.7	0.4	0.6	0.4	0.5	0.6	0.6	0.5	1.1	1.4	0.7	0.5	1.7	0.7	0.5	0.4	0.9	1.4
Min	7.9	6.9	7.5	9.3	7.2	8.3	8.0	7.8	6.8	6.5	7.2	8.9	8.1	8.2	5.2	7.3	8.3	8.2	5.8	6.7	7.3	7.7	8.6	6.8
Max	10.6	9.5	9.8	10.7	9.5	9.9	10.6	9.3	10.8	9.7	9.1	10.8	10.2	9.8	10.6	13.3	11.9	9.7	12.2	9.2	8.6	8.9	11.0	9.6
Range	2.7	2.6	2.3	1.4	2.3	1.6	2.7	1.6	4.0	3.2	1.9	1.9	2.1	1.5	5.4	6.0	3.6	1.5	6.4	2.5	1.3	1.2	2.4	2.8

Figure 5. Comparison of δ^{15}N human hair values from various countries

Range of human hair δ^{15}N values from the Netherlands (this study and previously published data[41]), and selected countries, such as Mexico,[49] United States,[53] Colombia,[54] China,[55] India,[55] Mongolia,[55] Pakistan.[55] The data for all other countries are taken from the study by Lehn et al.[56] All isotope values in per mil (‰). More reference data can be found elsewhere.[9,57]

and adolescence. The contribution of the continuously forming secondary dentine is negligible due to the low quantities formed.[60] Consequently, the generated data may represent a diet consumed several decades ago, depending on the age of the donor. As a result of changing dietary habits over time, the Dutch data presented in this paper may not accurately reflect the expected C and N isotope data in significantly older or more recent cases.

Dental enamel

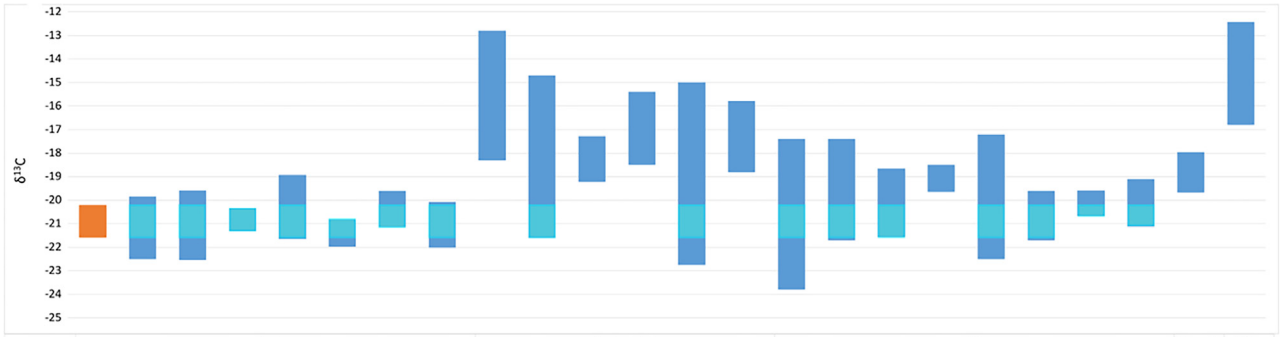
The overall variation in apatite δ^{18}O and δ^{13}C is limited (2.4‰ and 2.0‰, respectively) and barely larger than the observed intra-dental variation in modern Dutch individuals (2.0‰).[17] The variation in ^{87}Sr/^{86}Sr data is also limited (0.00046) and exceeds the observed intra-dental variation of 0.0002 by a factor of 2.[17] Moreover, all data are within the earlier published range that is considered indicative for the modern Dutch population (0.7088–0.7099).[18] Unfortunately, very limited data for European comparison are available. The ^{87}Sr/^{86}Sr recorded in Dutch teeth overlaps with many other countries, although not with Mexico and Brazil (Table 2), underlining the need for a multi-isotope approach. The Dutch oxygen isotope data overlap with the Bulgarian, although modern human dental enamel δ^{18}O reference data are even more scarce than that of strontium. The Dutch δ^{13}C data fall within the range of available reference data, and therefore does not allow for distinction (Table 2). Using established conversion equations,[61–63] the $\delta^{18}O_{VPDB}$ data can be converted to $\delta^{18}O_{VSMOW}$ and $\delta^{18}O_{DW}$ to allow comparisons with tap water or environmental water data.[61–63] The converted $\delta^{18}O_{VSMOW}$ ranges between 23.3‰ and 25.7‰ and the $\delta^{18}O_{DW}$ between −11.6‰ and −7.8‰. The human $\delta^{18}O_{DW}$ data exceed the minimum $\delta^{18}O_{DW}$ observed in the tap water samples (−9.5‰, see "Tap water"), underlining the difficulty to use conversation equations as a manner to compare primary dietary sources (e.g., drinking water) to human tissues data.

Dentine collagen

The δ^{15}N and δ^{13}C values in the dentine-derived collagen samples show limited variability (1.7‰ and 2.1‰, respectively). In accordance with previously published data, which indicated that bone collagen was relatively enriched compared to the same individual's hair keratin (0.86‰–1.50‰ in nitrogen and 0.6‰–1.4‰ in carbon),[56,69] this study found that the tooth dentine collagen was enriched relative to the hair keratin (average 1.8‰ in nitrogen and 0.2‰ in carbon). However, it must be noted that the number of samples in the datasets is different, and the data do not come from the same individual. This may account for the relatively significant difference in δ^{15}N and limited enrichment in δ^{13}C. Additional research is needed to investigate the use of hair keratin isotope data as a proxy for bone or dental collagen.

Tap water

The first oxygen and hydrogen isotope data of Dutch tap water show a greater variation in δ^{18}O compared to the human enamel data (5.1‰ vs. 2.4‰), and a range of 36.8‰ in δ^{2}H (−66.9‰ and −30.1‰). The variation in δ^{18}O can be explained by the rather complex tap water system in the Netherlands, using different environmental and natural resources (precipitation, ground water, river water, etc.).[18] The range in δ^{2}H is large (36.8‰), considering the proximity to the sea and limited elevation range, which are commonly considered the driving forces in the variability



	Europe								the Americas						Asia								Oceania	Africa
	the Netherlands	Northern Germany	Southern Germany	Denmark	Italy	UK/Ireland	France	Poland	Mexico	USA	Canada	Colombia	Brasil	Costa Rica	China	Mongolia	Russia	Japan	India	Pakistan	Iran	Lebanon	Australia	Ethiopia
Sample Size	34	55	98	12	34	28	24	19	101	206	15	38	22	17	74	20	40	20	38	11	8	11	9	3
Mean	-20.9	-20.9	-20.8	-20.9	-20.4	-21.3	-20.3	-21.2	-15.6	-16.8	-18.1	-16.8	-16.6	-17.2	-20.3	-20.0	-20.4	-19.1	-19.8	-20.6	-20.1	-19.7	-18.9	-14.9
SD	0.3	0.5	0.4	0.3	0.6	0.3	0.5	0.5	1.0	0.8	0.5	0.8	1.7	1.0	1.5	1.0	0.6	0.4	1.2	0.7	0.4	0.6	0.7	2.2
Max	-20.2	-19.8	-19.6	-20.4	-18.9	-20.8	-19.6	-20.1	-12.8	-14.7	-17.3	-15.4	-15.0	-15.8	-17.4	-17.4	-18.7	-18.5	-17.2	-19.6	-19.6	-19.1	-18.0	-12.4
Min	-21.6	-22.5	-22.5	-21.3	-21.7	-22.0	-21.1	-22.0	-18.3	-21.6	-19.2	-18.5	-22.8	-18.8	-23.8	-21.7	-21.6	-19.6	-22.6	-21.7	-20.7	-21.1	-19.7	-16.8
Range	1.4	2.7	3.0	1.0	2.7	1.2	1.5	1.9	5.5	6.9	1.9	3.1	7.8	3.0	6.4	4.3	2.9	1.1	5.3	2.1	1.1	2.0	1.7	4.4

Figure 6. Comparison of $\delta^{13}C$ human hair values from various countries

Range of human hair $\delta^{13}C$ values from the Netherlands (this study and previously published data[41]), and selected countries, such as Mexico,[49] United States,[53] Colombia,[54] China,[55] India,[55] Mongolia,[55] Pakistan.[55] The data for all other countries are taken from the study by Lehn et al.[56] All isotope values in per mil (‰).

of stable light isotope values. For comparison, the range in $\delta^{2}H$ in Mexico, a country 47 times the size of the Netherlands with elevation ranging up to 5,675 m, is only twice as large, 82.4‰.[70] The range in the Netherlands is in part controlled by the origin of river water in Germany and the Alps.

Limitations of the study

One notable limitation of this research, as well as many previous studies, is that the donated hair samples used in the analysis are already relatively "clean" compared to hair found in forensic contexts, which may be contaminated by decomposition fluids, rain, soil, or other external influences. However, it is important to acknowledge that even in "clean" hair samples, other exogenous factors such as the use of shampoo, conditioner, relaxing agents, etc., can still influence the biogenic isotope composition and should therefore not be disregarded. Previous research, however, provided evidence that the carbon and nitrogen isotope systems were relatively robust, resulting in limited diagenetic alterations in hair $\delta^{13}C$ and $\delta^{15}N$ over time and providing more reliable data than H-O-Sr isotope ratios.[71] Nevertheless, further (experimental) research is necessary to explore the recovery of endogenous isotope signatures from both "dirty" (soiled) and "clean" hair samples, considering various isotope systems.[71,72] While this research has contributed to the expanding database of isotope values and ratios, albeit with a restricted number of samples, it is important to note that the spatial coverage of all human tissue data is still limited.

The analysis of samples in bulk leaves the intra-individual variation as an unexplored aspect in forensic isotope research. This highlights the need for further investigation in this area. Furthermore, this study handled the data as bulk data, without taking the metadata from the questionnaires into account. To better understand deviant data points with regards to dietary habits, pathological conditions, cultural heritage, etc., and to increase the interpretational accuracy of the data, comparative analyses of metadata and isotope values should be conducted in the future.

Additionally, this study did not incorporate Pb isotope data, which has demonstrated significant potential in forensic isotope research for determining geographic origin in cases of unidentified deaths (unpublished data Vrije Universiteit Amsterdam). To effectively utilize Pb isotope research on a broader scale in the future, it is crucial to expand the reference dataset with relevant human data.

The ability to compare the Dutch dental enamel and dentine collagen isotope data to other country's data is limited due to sheer lack of availability. It is thus of essence to increase the publicly available data of identified modern human tissues, especially dental elements, and bones. Examples of successful sample and data acquisition are IDIS (collaboration of the NFI and the Vrije Universiteit Amsterdam) and the currently ongoing Project FIND-EM in the United States.

Furthermore, this study showed that it remains difficult to use tap water data for human relocation. As previously published by Kootker et al., tap water has a limited impact on isotope intake in Dutch individuals. This is likely due to an increasing globalization of food distribution, making tap water an unsuitable reference material for forensic investigations of isotopes in modern dental enamel within the Netherlands.

Conclusions

The accurate determination of an individual's geographic origin is a fundamental aspect of forensic investigations. Isotope analysis potentially plays a crucial role in this process by providing information about an individual's dietary patterns, environmental exposure, and mobility history. By incorporating the reference isotope values and ratios specific to the Dutch inhabitants and environment, the accuracy and reliability of isotope analysis in forensic investigations within the Netherlands can be significantly improved. Specifically, it enhances our ability to distinguish between

Table 2. Strontium ratios and oxygen and carbon isotope values from selected countries for comparison of dental enamel

	$^{87}Sr/^{86}Sr$	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)
Brasil[64]	0.71015 to 0.71566 (n = 75)	–	−16.66 to −13.40 (n = 75)
Vietnam[65]	0.70805 to 0.71823 (n = 23)	−10.61 to −5.04 (n = 48)	−17.25 to −6.32 (n = 48)
USA[65]	0.70797 to 0.71061 (n = 26)	−12.57 to −3.75 (n = 158)	−12.88 to −7.77 (n = 158)
Mexico[66]	0.7044 to 0.7064 (n = 19)	–	–
Norway[67]	0.70773 to 0.71769 (n = 8)	–	–
Bulgaria[68]	0.70833 to 0.70908 (n = 15)	−7.6 to −5.4 (n = 12)	−12.7 to −10.6 (n = 12)
Germany[a]	0.70867 to 0.71046 (n = 7)	–	–

[a]Unpublished data Vrije Universiteit Amsterdam.

individuals originating from different geographic areas, which is crucial for narrowing down the pool of potential matches in unidentified cases. In this publication, the data of 28 hair (for nitrogen and carbon), 159 enamel (for strontium, of which 73 also for oxygen and carbon), 38 dentine, and 143 tap water (for strontium, of which 98 also for oxygen and hydrogen) samples were presented and analyzed. Furthermore, if possible, they were compared to available data from other countries.

The $\delta^{15}N$ values of human hair from the Netherlands fall within the range of values observed worldwide and, therefore, cannot be utilized to differentiate from the countries represented in this study. Unlike the nitrogen isotope data, distinct disparities in the hair carbon isotope data are evident between European countries and other regions globally. Yet, it is not feasible to identify an unknown individual as Dutch solely based on $\delta^{13}C$ values. However, it is possible to exclude the Netherlands as a plausible region of origin if the data deviate from the recorded values from the Netherlands. Furthermore, the human dental enamel $\delta^{18}O_{DW}$ data exceed the minimum $\delta^{18}O_{DW}$ observed in the tap water samples (−9.5‰, see "Tap water"), underlining the difficulty to use conversation equations as a manner to compare primary dietary sources (e.g., drinking water) to human tissues data.

The task of comparing Dutch Sr-O-C-N isotope data from dental enamel and dentine collagen to that of other (European) nations has encountered significant challenges due to the limited availability of publicly accessible data. The scarcity of European reference data further hampers the possibility of conducting a meaningful comparison specifically involving the Dutch dental enamel $^{87}Sr/^{86}Sr$. The same limitation applies to the tap water $\delta^{2}H$ data. Nevertheless, these data are of utmost significance as they establish a foundation for future comparisons and enable researchers to include or exclude the Netherlands as a potential country of origin.

The findings of this study make a significant contribution to the growing database of isotope values and ratios worldwide. This expanded dataset holds immense value in the context of forensic investigations, particularly in enhancing our understanding of modern human isotopic signatures within the Netherlands. Further, it strengthens our ability to determine the geographic origin of unknown individuals, facilitating victim identification efforts and contributing to the advancement of forensic science in the Netherlands.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Human hair
  - Human dental elements
  - Tap water
- METHOD DETAILS
  - Human hair
  - Human dental elements
  - Tap water
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.109561.

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AUTHOR CONTRIBUTIONS

S.T.M.A.: conceptualization, methodology, supervision, validation, formal analysis, investigation, data curation, writing – original draft, review and editing, and visualization. NR.: data collection, formal analysis, investigation, and writing – review and editing. G.R.D.: supervision, funding acquisition, and writing – review and editing. A.C.v.A.: supervision, funding acquisition, and writing – review and editing. S.J.A.V.-W.: formal analysis and writing – review and editing. L.M.K.: conceptualization, methodology, supervision, validation, formal analysis, investigation, data curation, project administration, writing – original draft, and review and editing.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Human Hair	This study	1
Human Hair	This study	2
Human Hair	This study	3
Human Hair	This study	4
Human Hair	This study	10
Human Hair	This study	11
Human Hair	This study	12
Human Hair	This study	15
Human Hair	This study	18
Human Hair	This study	19
Human Hair	This study	20
Human Hair	This study	21
Human Hair	This study	23
Human Hair	This study	24
Human Hair	This study	25
Human Hair	This study	26
Human Hair	This study	28
Human Hair	This study	29
Human Hair	This study	30
Human Hair	This study	31
Human Hair	This study	32
Human Hair	This study	35
Human Hair	This study	39
Human Hair	This study	41
Human Hair	This study	42
Human Hair	This study	43
Human Hair	This study	44
Human Hair	This study	45G
Human Hair	This study	45B
Human Hair	This study	45M
Human Tooth	Kootker et al.[18]	Breda 13
Human Tooth	Kootker et al.[18]	Breda 1
Human Tooth	Kootker et al.[18]	Helmond 2
Human Tooth	Kootker et al.[18]	Folter 3
Human Tooth	Kootker et al.[18]	Zwartsluis 4
Human Tooth	Kootker et al.[18]	Folter 7
Human Tooth	Kootker et al.[18]	Folter 8
Human Tooth	Kootker et al.[18]	Helmond 11
Human Tooth	Kootker et al.[18]	Strijen 15
Human Tooth	Kootker et al.[18]	Erasmus 1
Human Tooth	Kootker et al.[18]	Helmond 14

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Human Tooth	Kootker et al.[18]	Strijen 14
Human Tooth	Kootker et al.[18]	Z-Heerlen 2
Human Tooth	Kootker et al.[18]	Strijen 17
Human Tooth	Kootker et al.[18]	Helmond 16
Human Tooth	Kootker et al.[18]	Zwartsluis 8
Human Tooth	Kootker et al.[18]	Zwartsluis 3
Human Tooth	Kootker et al.[18]	Folter 4
Human Tooth	Kootker et al.[18]	Folter 5
Human Tooth	Kootker et al.[18]	Strijen 6
Human Tooth	Kootker et al.[18]	Breda 14
Human Tooth	Kootker et al.[18]	Twente 4
Human Tooth	Kootker et al.[18]	Helmond 5
Human Tooth	Kootker et al.[18]	Zwartsluis 2
Human Tooth	Kootker et al.[18]	Veldhoven 8
Human Tooth	Kootker et al.[18]	's-Hertogenbosch 15
Human Tooth	Kootker et al.[18]	Folter 1
Human Tooth	Kootker et al.[18]	Breda 3
Human Tooth	Kootker et al.[18]	Z.Heerlen 8
Human Tooth	Kootker et al.[18]	Erasmus 11
Human Tooth	Kootker et al.[18]	's-Hertogenbosch 17
Human Tooth	Kootker et al.[18]	Maastricht 16
Human Tooth	Kootker et al.[18]	Sneek 12
Human Tooth	Kootker et al.[18]	Breda 4
Human Tooth	Kootker et al.[18]	Erasmus 13
Human Tooth	Kootker et al.[18]	Strijen 16
Human Tooth	Kootker et al.[18]	Folter 2
Human Tooth	Kootker et al.[18]	Zwartsluis 6
Human Tooth	Kootker et al.[18]	Folter 6
Human Tooth	Kootker et al.[18]	Zeeland 8
Human Tooth	Kootker et al.[18]	Helmond 3
Human Tooth	Kootker et al.[18]	Helmond 8
Human Tooth	Kootker et al.[18]	Veldhoven 9
Human Tooth	Kootker et al.[18]	Friesland 14
Human Tooth	Kootker et al.[18]	Helmond 6
Human Tooth	Kootker et al.[18]	Drenthe 16
Human Tooth	Kootker et al.[18]	Geldermalsen 8
Human Tooth	Kootker et al.[18]	Maastricht 15
Human Tooth	Kootker et al.[18]	Geldermalsen 5
Human Tooth	Kootker et al.[18]	Breda 18
Human Tooth	Kootker et al.[18]	Z.Heerlen 7
Human Tooth	Kootker et al.[18]	Steenwijk 7
Human Tooth	Kootker et al.[18]	Breda 2
Human Tooth	Kootker et al.[18]	Helmond 10
Human Tooth	Kootker et al.[18]	's-Hertogenbosch 18
Human Tooth	Kootker et al.[18]	's-Hertogenbosch 7

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Human Tooth	Kootker et al.[18]	Maastricht 3
Human Tooth	Kootker et al.[18]	Maastricht 17
Human Tooth	Kootker et al.[18]	Geldermalsen 1
Human Tooth	Kootker et al.[18]	Erasmus 3
Human Tooth	Kootker et al.[18]	Geldermalsen 4
Human Tooth	Kootker et al.[18]	Steenwijk 1
Human Tooth	Kootker et al.[18]	Sneek 3
Human Tooth	Kootker et al.[18]	Zwartsluis 5
Human Tooth	Kootker et al.[18]	Heerlen 10
Human Tooth	Kootker et al.[18]	Friesland 18
Human Tooth	Kootker et al.[18]	Strijen 10
Human Tooth	Kootker et al.[18]	's-Hertogenbosch 2
Human Tooth	Kootker et al.[18]	Breda 8
Human Tooth	Kootker et al.[18]	Veldhoven 16
Human Tooth	Kootker et al.[18]	Helmond 7
Human Tooth	Kootker et al.[18]	Geldermalsen 7
Human Tooth	Kootker et al.[18]	Maastricht 6
Human Tooth	Kootker et al.[18]	Sneek 1
Human Tooth	Kootker et al.[18]	Friesland 10
Human Tooth	Kootker et al.[18]	Helmond 15
Human Tooth	Kootker et al.[18]	Helmond 13
Human Tooth	Kootker et al.[18]	Erasmus 14
Human Tooth	this publication	VU_DB
Human Tooth	Kootker et al.[18]	Sneek 6
Human Tooth	Kootker et al.[18]	Zwartsluis 1
Human Tooth	Font et al.[3,4]	IDID_013NL
Human Tooth	Font et al.[3,4]	IDID_014NL
Human Tooth	Font et al.[3,4]	IDID_015NL
Human Tooth	Font et al.[3,4]	IDID_016NL
Human Tooth	Font et al.[3,4]	IDIS_001NL
Human Tooth	Font et al.[3,4]	IDIS_002NL
Human Tooth	Font et al.[3,4]	IDIS_003NL
Human Tooth	Font et al.[3,4]	IDIS_004NL
Human Tooth	Font et al.[3,4]	IDIS_005NL
Human Tooth	Font et al.[3,4]	IDIS_006NL
Human Tooth	Font et al.[3,4]	IDIS_007NL
Human Tooth	Font et al.[3,4]	IDIS_008NL
Human Tooth	Font et al.[3,4]	IDIS_009NL
Human Tooth	Font et al.[3,4]	IDIS_010NL
Human Tooth	Font et al.[3,4]	IDIS_011NL
Human Tooth	Font et al.[3,4]	IDIS_012NL
Human Tooth	Font et al.[3,4]	IDIS_017NL
Human Tooth	Font et al.[3,4]	IDIS_018NL
Human Tooth	Font et al.[3,4]	IDIS_019NL
Human Tooth	Font et al.[3,4]	IDIS_020NL

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Human Tooth	Font et al.[3,4]	IDIS_021NL
Human Tooth	Font et al.[3,4]	IDIS_022NL
Human Tooth	Font et al.[3,4]	IDIS_023NL
Human Tooth	Font et al.[3,4]	IDIS_024NL
Human Tooth	Font et al.[3,4]	IDIS_025NL
Human Tooth	Font et al.[3,4]	IDIS_026NL
Human Tooth	Font et al.[3,4]	IDIS_027NL
Human Tooth	Font et al.[3,4]	IDIS_028NL
Human Tooth	Font et al.[3,4]	IDIS_029NL
Human Tooth	Font et al.[3,4]	IDIS_030NL
Human Tooth	Kootker et al.[18]	Almelo 1
Human Tooth	Kootker et al.[18]	Folter 2-C
Human Tooth	Kootker et al.[18]	Heerlen 5
Human Tooth	Kootker et al.[18]	Sneek 11
Human Tooth	Kootker et al.[18]	Sneek 13
Human Tooth	Kootker et al.[18]	Sneek 14
Human Tooth	Kootker et al.[18]	Sneek 4
Human Tooth	Kootker et al.[18]	Steenwijk 4
Human Tooth	Kootker et al.[18]	Steenwijk 6
Human Tooth	Kootker et al.[18]	Strijen 3
Human Tooth	Kootker et al.[18]	Strijen 8
Human Tooth	Kootker et al.[18]	Zeeland 1
Human Tooth	Kootker et al.[18]	Zeeland 3
Human Tooth	Kootker et al.[18]	Zeeland 6
Human Tooth	Plomp et al.[73]	28-R14a
Human Tooth	Plomp et al.[73]	29-R11
Human Tooth	Plomp et al.[73]	30-R13
Human Tooth	Plomp et al.[73]	34-R5
Human Tooth	Plomp et al.[73]	35-R9
Human Tooth	Plomp et al.[73]	36-F1
Human Tooth	Plomp et al.[73]	37-F3
Human Tooth	Plomp et al.[73]	38-F4
Human Tooth	Plomp et al.[73]	39-F8
Human Tooth	Plomp et al.[73]	40-F11
Human Tooth	Plomp et al.[73]	41-F12
Human Tooth	Plomp et al.[73]	42-F13
Human Tooth	Plomp et al.[73]	43-R6
Human Tooth	Plomp et al.[73]	44-M4
Human Tooth	Plomp et al.[73]	45-M5
Human Tooth	Plomp et al.[73]	47-M14
Human Tooth	Plomp et al.[73]	48-ZH1
Human Tooth	Plomp et al.[73]	49-ZH3
Human Tooth	Plomp et al.[73]	50-ZH4
Human Tooth	Plomp et al.[73]	51-ZH9
Human Tooth	Plomp et al.[17]	Drenthe-15

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Human Tooth	Plomp et al.[17]	Drenthe-3
Human Tooth	Plomp et al.[17]	Friesland-6
Human Tooth	Plomp et al.[17]	Friesland-7
Human Tooth	Plomp et al.[17]	Limburg-6
Human Tooth	Plomp et al.[17]	Twente-1
Human Tooth	Plomp et al.[17]	Twente-2
Human Tooth	Plomp et al.[17]	Twente-6
Human Tooth	Plomp et al.[17]	Zuid Holland 13
Human Tooth	this publication	AO
Human Tooth	this publication	JVDS
Human Tooth	this publication	SM
Human Tooth	this publication	VU_DB
Human Tooth	this publication	VU_JK
Tap Water	Kootker et al.[18]	Tiel II
Tap Water	Kootker et al.[18]	Gouda
Tap Water	Kootker et al.[18]	Leiderdorp
Tap Water	Kootker et al.[18]	Alphen aan de Rijn
Tap Water	Kootker et al.[18]	Vianen
Tap Water	Kootker et al.[18]	Enspijk
Tap Water	Kootker et al.[18]	Ridderkerk
Tap Water	Kootker et al.[18]	Schijndel
Tap Water	Kootker et al.[18]	Zwolle
Tap Water	Kootker et al.[18]	Utrecht Zuilen II
Tap Water	Kootker et al.[18]	Serooskerke
Tap Water	Kootker et al.[18]	Amstelveen
Tap Water	Kootker et al.[18]	Heemstede
Tap Water	Kootker et al.[18]	Haarlem
Tap Water	Kootker et al.[18]	Ijsselstein
Tap Water	Kootker et al.[18]	Aalsmeer
Tap Water	Kootker et al.[18]	Vijlen
Tap Water	Kootker et al.[18]	Eindhoven
Tap Water	Kootker et al.[18]	Heiloo
Tap Water	Kootker et al.[18]	Valkenburg (L)
Tap Water	Kootker et al.[18]	Wageningen
Tap Water	Kootker et al.[18]	Velp
Tap Water	Kootker et al.[18]	Heerlen
Tap Water	Kootker et al.[18]	Mechelen
Tap Water	Kootker et al.[18]	Nijmegen II
Tap Water	Kootker et al.[18]	Nijmegen
Tap Water	Kootker et al.[18]	Maastricht
Tap Water	Kootker et al.[18]	Echt
Tap Water	Kootker et al.[18]	Arnhem Centrum
Tap Water	Kootker et al.[18]	Hengelo
Tap Water	Kootker et al.[18]	Ede
Tap Water	Kootker et al.[18]	Ermelo

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Tap Water	Kootker et al.[18]	Kampen II
Tap Water	Kootker et al.[18]	Almere Centrum
Tap Water	Kootker et al.[18]	Borger
Tap Water	Kootker et al.[18]	Susteren
Tap Water	Kootker et al.[18]	Amersfoort
Tap Water	Kootker et al.[18]	Arnhem Zuid
Tap Water	Kootker et al.[18]	Doorn
Tap Water	Kootker et al.[18]	Geleen
Tap Water	Kootker et al.[18]	Almere
Tap Water	Kootker et al.[18]	Nieuwegein
Tap Water	Kootker et al.[18]	Bunnik
Tap Water	Kootker et al.[18]	Apeldoorn
Tap Water	Kootker et al.[18]	Hoenderloo
Tap Water	Kootker et al.[18]	Sittard
Tap Water	Kootker et al.[18]	Kampen
Tap Water	Kootker et al.[18]	Brunssum
Tap Water	Kootker et al.[18]	Utrecht Zuilen
Tap Water	Kootker et al.[18]	Almelo
Tap Water	Kootker et al.[18]	Utrecht
Tap Water	Kootker et al.[18]	Enschede
Tap Water	Kootker et al.[18]	Winterswijk
Tap Water	Kootker et al.[18]	Haaksbergen
Tap Water	Kootker et al.[18]	Veenendaal
Tap Water	Kootker et al.[18]	Venlo
Tap Water	Kootker et al.[18]	Steenwijk
Tap Water	Kootker et al.[18]	Meppel
Tap Water	Kootker et al.[18]	Den Helder
Tap Water	Kootker et al.[18]	Oldenzaal
Tap Water	Kootker et al.[18]	Horst
Tap Water	Kootker et al.[18]	Lottum
Tap Water	Kootker et al.[18]	Diever
Tap Water	Kootker et al.[18]	Hilversum
Tap Water	Kootker et al.[18]	Leiden
Tap Water	Kootker et al.[18]	Gennep
Tap Water	Kootker et al.[18]	Doetinchem
Tap Water	Kootker et al.[18]	Appingedam
Tap Water	Kootker et al.[18]	Dronten
Tap Water	Kootker et al.[18]	Wassenaar
Tap Water	Kootker et al.[18]	Rotterdam
Tap Water	Kootker et al.[18]	Berkel
Tap Water	Kootker et al.[18]	Breda
Tap Water	Kootker et al.[18]	Tilburg
Tap Water	Kootker et al.[18]	Oostvoorne
Tap Water	Kootker et al.[18]	Hardenberg
Tap Water	Kootker et al.[18]	Bergen op Zoom

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Tap Water	Kootker et al.[18]	Groningen II
Tap Water	Kootker et al.[18]	America
Tap Water	Kootker et al.[18]	Groningen
Tap Water	Kootker et al.[18]	Middelburg
Tap Water	Kootker et al.[18]	Hoorn
Tap Water	Kootker et al.[18]	Poeldijk
Tap Water	Kootker et al.[18]	Emmeloord
Tap Water	Kootker et al.[18]	Zwijndrecht
Tap Water	Kootker et al.[18]	Heerenveen
Tap Water	Kootker et al.[18]	Achtmaal
Tap Water	Kootker et al.[18]	Dalfsen
Tap Water	Kootker et al.[18]	Roosendaal
Tap Water	Kootker et al.[18]	Nuis
Tap Water	Kootker et al.[18]	Delft
Tap Water	Kootker et al.[18]	Den Andel
Tap Water	Kootker et al.[18]	Hoogeveen
Tap Water	Kootker et al.[18]	Venray
Tap Water	Kootker et al.[18]	Roermond
Tap Water	Kootker et al.[18]	Leeuwarden
Tap Water	Kootker et al.[18]	Franeker
Tap Water	Kootker et al.[18]	Harlingen
Tap Water	Kootker et al.[18]	's-Gravenhage
Tap Water	Kootker et al.[18]	's-Gravenhage II
Tap Water	Kootker et al.[18]	Den Bosch II
Tap Water	Kootker et al.[18]	Den Bosch
Tap Water	Kootker et al.[18]	Texel
Tap Water	Font et al.[3,4]	LF14
Tap Water	Font et al.[3,4]	LF52
Tap Water	Font et al.[3,4]	LF53
Tap Water	Font et al.[3,4]	LF20
Tap Water	Kootker et al.[18]	Boxmeer
Tap Water	Kootker et al.[18]	Bredevoort
Tap Water	Kootker et al.[18]	Breukelen
Tap Water	Kootker et al.[18]	Delft II
Tap Water	Font et al.[3,4]	LF18
Tap Water	Kootker et al.[18]	Drachten
Tap Water	Font et al.[3,4]	LF51
Tap Water	Kootker et al.[18]	Emmen
Tap Water	Kootker et al.[18]	Enkhuizen
Tap Water	Font et al.[3,4]	LF19
Tap Water	Font et al.[3,4]	LF25
Tap Water	Font et al.[3,4]	LF50
Tap Water	Font et al.[3,4]	LF26
Tap Water	Kootker et al.[18]	Kerkrade
Tap Water	Font et al.[3,4]	LF23

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Tap Water	Kootker et al.[18]	Lelystad
Tap Water	Font et al.[3,4]	LF49
Tap Water	Kootker et al.[18]	Norg II
Tap Water	Kootker et al.[18]	Norg III
Tap Water	Kootker et al.[18]	Ouddorp
Tap Water	Kootker et al.[18]	Reuver
Tap Water	Font et al.[3,4]	LF58
Tap Water	Font et al.[3,4]	LF55
Tap Water	Font et al.[3,4]	LF56
Tap Water	Font et al.[3,4]	LF57
Tap Water	Kootker et al.[18]	Soesterberg
Tap Water	Kootker et al.[18]	Stadskanaal
Tap Water	Kootker et al.[18]	Uden
Tap Water	Font et al.[3,4]	LF84
Tap Water	Font et al.[3,4]	LF86
Tap Water	Font et al.[3,4]	LF85
Tap Water	Kootker et al.[18]	Velp NB
Tap Water	Kootker et al.[18]	Vianen II
Tap Water	Kootker et al.[18]	Wijk en Aalburg
Tap Water	Kootker et al.[18]	Winschoten
Tap Water	Kootker et al.[18]	Zwijndrecht II
Deposited data		
Human Tooth Data	Figshare	Kootker et al.[74]
Human Hair Data	Figshare	Ammer et al.[75]
Tap Water Data	Figshare	Kootker et al.[52]
Software and algorithms		
	R: A Language and Environment for Statistical Computing. http://www.r-project.org/	

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact and corresponding authors Saskia Ammer (s.t.m.ammer@vu.nl).

Materials availability

This study did not generate new unique reagents. There are restrictions to the availability of reagents due to that the human materials in this study is in its entirety few, therefore considered to be a limited resource. The samples are physically stored at the Archaeological and Forensic sample preparation laboratory, Department of Earth Sciences, Vrije Universiteit Amsterdam, the Netherlands.

Data and code availability

- De-identified human hair and tooth isotope data are deposited on Figshare and publicly available as of the date of publication.[74,75]
- Tap water isotope data supporting the findings of this study are deposited on Figshare and publicly available as of the date of publication.[52]
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The current modern-day human reference database of the Netherlands consists of human hair and dental elements. Tap water data is also included in this reference database as tap water is used for hydration, food preparation, as well as hygiene. A questionnaire was used associated with the collection of the hair samples (Questionnaire S1). The hair samples were collected between February and June of 2021. The dental elements were collected between 2010 and 2023. The tooth collection remains ongoing, we are thus able to continue adding to the database on a regular basis. Detailed information about the sampling locations of the tap water samples are published elsewhere.[18]

Human hair

For this study, a total of 28 hair samples were collected from anonymous donors. For the collection of the hair samples, written instructions were given to the donors how to cut and package the hair. The donors were also provided with a questionnaire asking questions about their diet and recent geographic whereabouts (Supplementary Data and Questionnaire S1). The samples were collected between February and June of 2021. The hair samples were wrapped in aluminium foil, placed in sealed envelopes and either collected in person or sent to the Vrije Universiteit Amsterdam. Any samples of individuals who spent time outside of the Netherlands during the respective growth period of the hair sample were excluded from this study. Hair samples were categorized based on colour using the Fischer-Saller scale (Figures S1 and S2).[76] Hair type was determined using the method used by hairdressers established by Andre Walker, based on the morphology of the hair.[77] Each sample was photographed and characterized for hair colour and type (Data S1, Figures S1 and S2). An overview of the answers to the questionnaire of the hair samples, as well as their hair colour and type categorization can be found in the Supplementary Data file. Human scalp hair grows at an average of 1 cm per month, and since the elemental exchange between hair and blood ceases when it dies off, it retains the biogenic and dietary information, preserving a longitudinal record of stable isotope signatures.[15,78] In this study, the average represented timeframe is approximately six months, equalling $\pm$6 cm hair length.

Human dental elements

The use of the human dental elements for scientific research was approved by the Medical Ethics Review Committee of the Amsterdam UMC, location VUmc. The samples were provided by dentist who extracted the elements, accompanied by a questionnaire about the dietary preferences and whereabouts of the donors during childhood (Questionnaire S2). None of the individuals relocated during the formation and mineralization period of the dental enamel and thus remained stationary in their respective place of residence in the Netherlands.

Tap water

For this study, a total of 98 tap water samples were analysed for their $\delta^{18}O$ and $\delta^{2}H$ isotope values to add to the existing database of 143 tap water samples analysed for $^{87}Sr/^{86}Sr$.[3,18] Analytical details for the Sr isotope analysis are provided in detail in Kootker et al.[18]

METHOD DETAILS

Human hair

Approximately 50-100 mg of hair from each sample was placed into 2 ml glass vials. The samples were cleaned by filling the vials with a two parts chloroform one part methanol solution and placed in an ultrasonic bath for 10 minutes. The methanol-chloroform mixture was then replaced with milli-Q water and placed back in the ultrasonic cleaner for 10 minutes. This process was performed three times before further leaching the samples with 0.01M HCL and Milli-Q again. The samples were then dried on a hotplate at 60°C and subsequently cut into circa 3mm pieces using scissors and stored in vials for sub sampling. Approximately 0.45 mg $\pm$ 10% of hair from all the samples were weighed into 5 x 9 mm tin capsules and weighed, along with standards of USGS40, USGS41, USGS42, and USGS43.

The $\delta^{15}N$ and $\delta^{13}C$ isotope values were measured using a Flash NC 1112series Elemental Analyser coupled to a Thermo Finnigan DeltaPlus XP Isotope Ratio Mass Spectrometer (IRMS) at the Earth Sciences Stable Isotope Laboratory, Vrije Universiteit Amsterdam. Instrument precision was better than 0.17‰ (1σ) for C-N isotopes based on replicate analysis of standard reference materials USGS42 ($\delta^{13}C$ = –21.06 $\pm$ 0.13‰ and $\delta^{15}N$ = 8.02 $\pm$ 0.15‰ (1σ, n =24) and USG43 ($\delta^{13}C$ = –21.43 $\pm$ 0.10 ‰ and $\delta^{15}N$ = 8.32 $\pm$ 0.17 ‰ (1σ, n =6)). The samples were calibrated using USGS40 and USGS41. The stable isotope results are expressed as δ (delta) values in per mil (‰) relative to Vienna Peedee Belemnite (VPDB) for $\delta^{13}C$ and atmospheric nitrogen (AIR) for $\delta^{15}N$.

Human dental elements

Enamel powder was collected from the crown of the tooth using a Proxxon diamond tipped burr inside a fume hood, class 100. The burr was cleaned between each sample by being immersed in Milli-Q water, 10% hydrochloric acid (HCl), Milli-Q water again, and then ethanol. After removing the outermost surface of the enamel, circa 1-3 mg of dental enamel powder was collected using a diamond-tipped burr. The samples were taken from the mesial or distal lobe of either the buccal or lingual surface, depending on the physical quality of the molar and the presence of carious lesions.

For the $\delta^{18}O$ analyses, circa 0.3 $\pm$ 10% mg enamel powder was weighed into a glass Exetainer® vial with screw-capped pierceable butyl rubber septa and transferred to the Stable Isotope laboratory at the Vrije Universiteit Amsterdam. The prepared vial was placed in a sample block interspaced with calibrations standards LSVEC, BCT (replacement for NBS19) and NBS18 and the in-house standards VICS for a linearity correction. The standard IAEA-603 is also analysed during each run as a control. After flushing the vials with helium, samples and standards

were acidified with water-free H_3PO_4 (100%) at 45°C and allowed to react for 24 hours. The gas mixture was analysed using a Thermo Finnigan Delta plus IRMS with a GasBench II. The isotopic values are reported as δ (delta) values in ‰ units. Values were normalized to international standard IAEA-603 ($\delta^{18}O$ = 2.34 ± 0.13‰ and $\delta^{13}C$ = 2.52 ± 0.05‰ (1σ, n =16)) and are reported relative to the Vienna Peedee Belemnite (VPDB) standard.

For the $^{87}Sr/^{86}Sr$ analysis, all sample preparation and analyses were conducted at the Vrije Universiteit Amsterdam, the Netherlands. The dental enamel and tap water samples were sealed in acid-cleaned polyethylene Eppendorf centrifuge tubes and transported to the class 100 clean laboratory. The sample residues were dissolved in 500 µl 3M HNO_3 for ion exchange chromatography. A detailed description of the Sr extraction/chromatography and the sample loading protocol are provided elsewhere.[79] The strontium isotope compositions were measured on a ThermoFinnigan Triton *Plus* thermal ionisation mass spectrometer (TIMS). The ratios were determined using a static routine and were corrected for mass-fractionation to $^{86}Sr/^{88}Sr$ of 0.1194. The NBS987 standard gave a mean $^{87}Sr/^{86}Sr$ of 0.710257 ± 0.000006 (n = 113) during the period of analysis (2021). The current certified value of NBS987 is 0.71034 ± 0.00026 (certificate issue date 19 June 2007); event though accepted values vary significantly, between approximately 0.71024 to 0.710263.[80,81] The procedural blanks contained on average 56 pg strontium (n = 9). The dataset was analysed using SPSS 25.0 (IBM SPSS Statistics for Macintosh, Armonk, IBM Corp.).

Thirty-eight of the teeth that have previously been analysed for their Sr isotope composition in dental enamel were now selected to create a first database of $\delta^{13}C$ and $\delta^{15}N$ isotope signatures in human tooth dentin. A Dremel diamond wheel was used to remove a portion of the dental root. The wheel was cleaned between each sample by being immersed in Milli-Q water, 10% HCl, Milli-Q water again, and then ethanol. The fragment was weighted using a digital balance and placed inside a 16 x 100 mm Elkay test tube (Elkay Labs, 2021). Subsequently 10 ml of 0.6M HCl was added to the test tubes to dissolve the bioapatite. The test tubes were stored in a fridge at 4°C for 48 hours. Subsequently, HCl was removed, and the firmness of the dentine was examined. If the dentine showed stiffness, then 10ml of 0.6M HCl was added back into the test tube and placed back in the fridge for another 48 hours. If the root had a sponge-like texture, then 9 ml of 0.01M HCl (pH3) was added using a dispenser and placed in an oven at 80°C for 48 hours. Following the removal of samples from the oven, the collagen was filtered using a 9 ml Ezee filter. The filtered collagen solution was then transferred into a weighed test tube, covered with parafilm, and stored in a freezer at -20°C. The collagen sample was then lyophilised for 48 hours, and reweighed. Approximately 0.50 mg ±10% of collagen was weighted into 5 x 9 mm tin capsules, along with control standards of USGS40, USGS41, USGS42, and USGS43.

The $\delta^{13}C$ and $\delta^{15}N$ values were measured using an elemental analyser (NCA500; ThermoQuest Italia, Rodana, Italy) coupled with an isotope ratio mass spectrometer (Delta Plus; Thermo-Quest Finnigan, Bremen, Germany) at the Earth Sciences Stable Isotope Laboratory, Vrije Universiteit Amsterdam. Instrument precision was better than 0.24 ‰ (1σ) for coupled C-N isotopes based on replicate analysis of standard reference materials USGS42 ($\delta^{13}C$ = –21.13 ± 0.17 ‰ and $\delta^{15}N$ = 8.04 ± 0.20 ‰ (1σ, n =5)) and USGS43 ($\delta^{13}C$ = –21.34 ± 0.14 ‰ and $\delta^{15}N$ = 8.44 ± 0.24 ‰ (1σ, n =9)). The stable isotopes results are expressed as δ (delta) values in per mil (‰) relative to Vienna Peedee Belemnite (VPDB) for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. The integrity of the collagen samples was assessed based on the atomic C:N ratio, the N and C abundances and collagen yield.[82–85] The average C/N (mol/mol) was 3.54 ± 0.13 (1σ, n = 43).

Tap water

Tap water samples and standards were transferred to 2 ml crimp vials using a micro-pipet, and a clean tip for every sample and standard. Vials were then closed with a crimp cap. Samples were measured on a Picarro Inc L2140-i Wavelength-scanning cavity ring-down spectrometer at the Stable Isotope Laboratory, Department of Earth Sciences, Vrije Universiteit Amsterdam, in sets of 10, bracketed by four in-house laboratory standards. At least seven replicate analyses were performed per vial. The average and standard deviation of the last four injections were calculated for all samples and standards.

All standards are in-house water-standards that have been calibrated using the VSMOW and VSLAP (international water-standards from IAEA). Three of these standards are used for calibration of the samples. The fourth standard (KONA) is used as a control standard to determine the accuracy and precision of the measurement. The standard deviations of the isotopic values of KONA are <0.2 ‰ and <2 ‰ for $\delta^{18}O$ and $\delta^{2}H$ respectively. The calibrated values for KONA are –0.045 ‰ for $\delta^{18}O$ and 0.6 ‰ for $\delta^{2}H$.

QUANTIFICATION AND STATISTICAL ANALYSIS

Mean and standard deviation were calculated from Excel for Sr, O, H, C, and N isotope data (see Table 1). Figures 1, 2, 3, and 4 were made using R. Figures 5 and 6 using Excel and Photoshop.