Forensic Science International 209 (2011) 34–41

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Analysis of ^{14}C and ^{13}C in teeth provides precise birth dating and clues to geographical origin

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ARTICLE INFO

Article history:
Received 1 August 2010
Received in revised form 29 November 2010
Accepted 1 December 2010
Available online 3 January 2011

Keywords:
Age estimation
Forensic odontology
Identification
Radiocarbon
Stable isotope

ABSTRACT

The identification of human bodies in situations when there are no clues as to the person's identity from circumstantial data, poses a difficult problem to the investigators. The determination of age and sex of the body can be crucial in order to limit the search to individuals that are a possible match. We analyzed the proportion of bomb pulse derived carbon-14 (^{14}C) incorporated in the enamel of teeth from individuals from different geographical locations. The 'bomb pulse' refers to a significant increase in ^{14}C levels in the atmosphere caused by above ground test detonations of nuclear weapons during the cold war (1955–1963). By comparing ^{14}C levels in enamel with ^{14}C atmospheric levels systematically recorded over time, high precision birth dating of modern biological material is possible. Above ground nuclear bomb testing was largely restricted to a couple of locations in the northern hemisphere, producing differences in atmospheric ^{14}C levels at various geographical regions, particularly in the early phase. Therefore, we examined the precision of ^{14}C birth dating of enamel as a function of time of formation and geographical location. We also investigated the use of the stable isotope ^{13}C as an indicator of geographical origin of an individual. Dental enamel was isolated from 95 teeth extracted from 84 individuals to study the precision of the ^{14}C method along the bomb spike. For teeth formed before 1955 ($N = 17$), all but one tooth showed negative $\Delta^{14}C$ values. Analysis of enamel from teeth formed during the rising part of the bomb-spike (1955–1963, $N = 12$) and after the peak (>1963, $N = 66$) resulted in an average absolute date of birth estimation error of 1.9 ± 1.4 and 1.3 ± 1.0 years, respectively. Geographical location of an individual had no adverse effect on the precision of year of birth estimation using radiocarbon dating. In 46 teeth, measurement of ^{13}C was also performed. Scandinavian teeth showed a substantially greater depression in average $\delta^{13}C$ (−14.8) than teeth from subjects raised in Japan (−13.5), Middle East and North Africa (−12.7) and South America (−10.9). In summary, isotopic analysis of carbon in enamel from a single tooth can give a good estimate of the year of birth of an individual and also provide information about the geographical origin of the individual. This strategy can assist police and forensic authorities when attempting to solve unidentified homicide cases and may facilitate the identification work associated with mass disasters.

1. Introduction

Estimation of the age at death of deceased individuals, whose decomposed, mutilated or burned remains make identification impossible, represents an important task for forensic pathologists, anthropologists, and forensic odontologists. If no clues are at hand regarding the deceased's identity, the age and the sex of the victim constitute important information that can limit the search for possible matches amongst a large number of alternatives. Whereas the gender usually can be determined by morphological methods or by DNA analysis of the remains, most methods for age determination suffer from poor estimate precision. A number of different methods for estimating the age of deceased persons have been published, most of them based on examination of teeth and bones. Analyses based on morphological methods typically provide age estimation errors in adults of ±10 years (for review see [1,2]).

Since teeth are highly resistant to decomposition, chemical degradation and heat, they constitute a particularly valuable material for forensic analysis. The observation of a gradual conversion of L-aspartic acid to its D-form in teeth led to the development of aspartic acid racemization analysis as a tool for age

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doi:10.1016/j.forsciint.2010.12.002

estimation, first described by Helfman and Bada [3]. Today, this method has become widely used and has been reported to provide a precision of age at death of less than ±5.0 years in adults, depending on which kind of tooth is analyzed (for review, see [2,4]). However, since this method is temperature dependent, it cannot be used in cases exposed to extreme temperatures, such as the analysis of teeth from fire victims. In addition at least 4 teeth of the same type, from the same geographical location should be run in parallel with the test tooth, to ensure accurate age estimation [5]. Lastly, one should also keep in mind that teeth from persons residing near or at the surface of a hot climate such as a dessert can experience accelerated conversion and appear erroneously aged.

Recently, a method to predict year of birth by accelerator mass spectrometry (AMS) analysis of radiocarbon (^{14}C) in dental enamel was developed, showing an average absolute error of 1.6 ± 1.3 years [6]. Similar results were found in a subsequent study by different authors [7]. When applied on teeth collected from subjects raised in Sweden, the average absolute error of the method could be reduced to 1.3 ± 1.0 years [8]. This strategy is based on the incorporation of bomb pulse derived ^{14}C into enamel during tooth formation. The term 'bomb pulse' refers to the drastic increase in atmospheric and biospheric levels of ^{14}C caused by extensive above ground test detonations of nuclear weapons during the period of the cold war (1955–1963) [9–12]. After the Limited Test Ban Treaty was signed in 1963, atmospheric ^{14}C levels began to decrease exponentially. By comparing ^{14}C levels in tooth enamel with reference data on atmospheric ^{14}C levels, an estimation of the year of birth of a particular tooth can be obtained. Using reference information about tooth formation times [13,14], the year of birth of the individual can be calculated.

Newly produced atmospheric ^{14}C reacts with oxygen to form $^{14}CO_2$, which is incorporated into plants by photosynthesis. By eating plants, and animals that live off plants, the ^{14}C concentration in the human body closely parallels that in the atmosphere at any given point in time [6,15]. Radiocarbon levels in the atmosphere have remained almost constant for several thousands of years before the bomb pulse [16]. Above-ground nuclear bomb testing resulted in an increase of ^{14}C into the troposphere, which subsequently entered the atmosphere and dispersed equally around the globe. Nuclear bomb testing was largely restricted to a couple of locations in the northern hemisphere and as such ^{14}C levels in the atmosphere differ somewhat as a function of geographical latitude and time since bomb-testing [10]. As a result, a delay in the distribution to the southern hemisphere was observed [10]. This raises a concern regarding the feasibility of the radiocarbon method to estimate year of birth when teeth from other parts of the world other than Northern Europe (where the method was developed and tested, [6,8]) are concerned. It was therefore the aim of this study to analyze teeth collected from individuals born and raised in different geographical regions to explore the precision of the method across different geographies.

In addition to radiocarbon dating of enamel, we also investigated the use of measuring ^{13}C levels in tooth enamel as a predictor of the geographical origin of an individual. ^{13}C is a stable isotope that constitutes about 1.1% of all carbon. Plants, to a variable degree, can discriminate between ^{12}C and ^{13}C, resulting in differences in the levels of this isotope between different types of plants. Differences in the fixation of CO_2 during photosynthesis distinguish the more common C3 plants from C4 plants. C4-plants have a double fixation step for CO_2 and their photosynthetic pathway is located deeper in the leaves. Isotope fractionation in C4 plants is primarily limited by diffusion [17,18]. This is in contrast to C3 plants which can better discriminate between these isotopes and reduce the binding of ^{13}C and more readily make ^{13}C diffuse out of through the stomatal pores to the ambient air [18]. As a result, C4 plants (which include corn and sugar cane) contain higher amounts of ^{13}C than C3 plants (which include potato, sugar beet, and wheat) [19]. In general, C4 plants tend to grow in hotter or drier climates than C3 plants whose open stomata lose too much water to thrive. This in turn means that animals, including humans, having a diet based mainly of C4 plants, or animals feeding on such plants, will incorporate more ^{13}C than those that have C3 plant based diets. Recently, analysis of ^{13}C (along with other stable isotopes) was applied on hair samples from subjects of different geographical origin and large differences between certain populations were observed [20]. Thus, the second aim of this study was to see if ^{13}C levels in tooth enamel can provide additional information about the geographical origin of an individual.

2. Materials and methods

In total 95 teeth from 84 individuals of known date of birth were analyzed. Teeth were collected by dentists with the following patient information recorded: date of birth (year and month), sex, tooth extraction date, type of tooth and, when possible, country of birth and where the first 15 years of life were spent. For determination of the methodological error of ^{14}C measurements as a function of time, teeth with enamel formation occurring before the bomb pulse, during the rising, and during the falling part of the bomb pulse were analyzed. To examine the possible influence of geographical variation on the incorporation of ^{14}C into dental enamel during tooth formation, teeth collected from different geographical regions were selected. As such, teeth from individuals raised in different continents were studied. Subjects were raised in eight countries and represented four continents including both northern and southern hemispheres. Most of the teeth were pre-molars and molars. A few teeth showed carious lesions and some teeth had plastic or mercury alloy fillings. These teeth were also used, since the fillings detached and/or were dissolved during the alkaline incubation and hence did not interfere with the macroscopic isolation of the enamel. In addition, 35 teeth from 35 individuals treated by Swedish dentists, but where the geographical origin was not recorded, were also included for comparison. Finally, to study the impact of the ^{13}C levels on the ^{14}C determinations, and to evaluate the possibility of predicting the origin of subjects, 46 teeth from 39 individuals where both isotopes could be determined simultaneously, were analyzed.

All teeth were extracted by dentists, typically for orthodontic purpose, or in some cases because of a periodontal condition. Patients were asked for consent to save and use these teeth for research analysis instead of discarding them. Teeth were collected, labelled with the tooth number, year and month of birth, sex, and year and month of tooth extraction. No patient information was passed on from dentist to researcher. Ethical permission was obtained by the Regional Ethical Committee, Stockholm (Dnr 2010/314-31/3). Teeth showing $\Delta^{14}C$ values below −15 did not allow for birth dating, other than they were considered to have been formed before the bomb pulse.

For our calculations, we used reference information about enamel lay-down provided by Nolla [14] and validated in [8]. Nine maturation stages are described by Nolla [14] and we used the middle stage to approximate the time of lay-down for each type of tooth. In all cases studied, except one, the sex of the person was known and the different enamel lay-down times for males and females used accordingly. For the single case lacking information about sex, the average value for male and female was used.

2.1. Preparation for radiocarbon analysis

2.1.1. Enamel preparation

The tooth crown was cut away from the root at the level of the cervical line and incubated in 10 N NaOH at room temperature in a water-bath sonicator (Branson 150). Every 24 h NaOH was replaced and the non-enamel structures removed mechanically using an odontologic electric drill. Purified enamel was then washed three times with DDH_2O, re-submersed in 10 N NaOH and placed again into the sonicator water bath. This procedure was repeated every day for 3–5 days (until all dentin and soft structures were stripped from the enamel). The enamel was then rinsed several times in DDH_2O and dried at room temperature overnight. Finally, the enamel was kept in a glass tube until pre-treated for AMS analysis.

2.1.2. AMS pre-treatment

Aliquots of the enamel samples were placed in culture tubes for pre-treatment to remove the surface carbon that may have coated the enamel between formation and analysis. Since the carbon content of enamel is 0.4–0.6%, 80–150 mg aliquots were typically used to get full sized samples containing 0.4–0.9 mg C for ^{14}C analysis. Enamel samples were immersed in 1.0 N HCl at room temperature for 1.5 h, rinsed 3 times with DDH_2O and placed on a heating block at 95 °C under a lose aluminum foil tent to dry overnight. Powdered samples react vigorously in 1.0 N HCl and were immersed for only a minute or two, rinsed 5 times with DDH_2O and placed on a heating block at 95 °C under a lose aluminum foil tent to dry overnight. The acid pre-treatment was designed to remove the outer surface of the enamel that was exposed to the harsh alkali environment during dentin removal without dissolving too much of the enamel. Base always contains some carbonate that can

potentially exchange with the enamel during the preparation step. Furthermore, alkali solutions remove CO_2 from the atmosphere and produce carbonate and bicarbonate in solution that can precipitate or exchange with that in the enamel. Each dried enamel sample was broken into 5–10 pieces and placed in an individual single-use reactor and again weighed to the nearest 0.1 mg. The HCl etching procedure dissolves a couple milligrams of exterior enamel surface in a 100 mg enamel sample. The enamel samples placed in individual reaction chambers were evacuated, heated and acidified with concentrated orthophosphoric acid at 90 °C. The evolved CO_2 was purified, trapped, and reduced to graphite in the presence of iron catalyst in individual reactors [21,22]. With the enamel aliquots used, nearly all CO_2 samples were >500 μg C. The CO_2 was then split and $\delta^{13}C$ measured by stable isotope ratio mass spectrometry (Supplemental Table 1). Background values were controlled by consistently following procedures, frequently baking sample tubes, periodically cleaning rigs, and maintaining a clean lab [23].

2.2. Accelerator mass spectrometry analysis

Graphite targets were measured using the 10-MV HVEE FN-class tandem electrostatic AMS system at the Center for Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory (LLNL). The operation is similar to that when performing high-precision measurements of 18,000-year-old turbidities used as secondary standards [24]. The system employs a LLNL designed high-output negative ion solid graphite Cs-sputter source [25] which emits 250–350 μg of ^{12}C from a full-sized sample, corresponding to approx. 900 ^{14}C counts per second from a contemporary sample. The FN AMS system routinely achieves 15% total system efficiency for C analyzing $^{14}C^{4+}$ in the detector [26]. Details on the design of the LLNL AMS system and its operation can be found in the literature [24–27]. Enamel samples are usually full sized and contemporary, so analysis times are relatively rapid, generally less than 5 min. The enamel samples are measured for 30,000 ^{14}C counts per cycle for 4–7 cycle repetitions and achieve standard deviations of 0.3–0.8%.

Corrections for background contamination introduced during AMS sample preparation are made by establishing the contributions from contemporary and fossil carbon, following the procedures of Brown and Southon [28]. All data are normalized using six identically prepared NIST SRM 4990B (Oxalic Acid I) primary standards. NIST SRM 4990C (Oxalic Acid II), IAEA-C6 [29], and TIRI [30] wood are used as secondary standards and quality controls to monitor spectrometer performance. The ratio of NIST SRM 4990C to NIST SRM 4990B (Oxalic Acid II/Oxalic Acid I) measured between February 2005 and July 2009 on 22 different sample wheels containing enamel samples had an average value of 1.291 ± 0.002 (1 SD), in agreement with the certified value of 1.293 ± 0.001. ^{14}C-free calcite serves as background material for processing the enamel samples. The enamel samples are organized in groups of 10–14 unknowns bracketed by primary standards with one primary standard in the middle of the group. The secondary standards, primary standards and group of unknowns are measured consecutively as a cycle. Upon completion of a cycle the set of primary standards, secondary standards and unknown samples are measured again until desired precision is achieved. A typical group of 14 enamel samples is measured completely in 2–3 h. The measurement error is determined for each sample and generally ranges between ±0.2 and 0.8% (1 SD). All ^{14}C data are reported using the $F^{14}C$ fraction modern nomenclature developed for post-bomb data [31]. $F^{14}C$ is a concentration unit ($^{14}C/C$) denoting enrichment or depletion of ^{14}C relative to oxalic acid standard normalized for isotope fractionation. Data are also reported as decay corrected $\Delta^{14}C$ following the nomenclature of [32]. $\Delta^{14}C$ was calculated using the equation:

$$\Delta^{14}C = 1000 \times \{F^{14}C \times \exp[\lambda \times (1950 - y)] - 1\}$$

where $\lambda = 1/8267\ yr^{-1}$ and y = year of measurement after 1950 A.D.

2.3. Determining year of birth from ^{14}C data

The average age at which the enamel of each specific tooth is formed has been determined previously and is dependent on the tooth number and gender of the person [13,14]. In one case, the sex had not been recorded by the dentist, so the average time for enamel formation for males and females for this type of tooth was used. The ^{14}C concentration measured in the tooth enamel is plotted onto a curve of atmospheric ^{14}C against time to determine the year of enamel synthesis and date of birth of the individual. The time (in years) taken for the enamel to form is subtracted from the year obtained to give an estimated date of birth (Fig. 1 and Supplemental Table 1). Calibrated ages were obtained by using the CALIbomb Levin dataset [33], where the smoothing in years was set at 1.0 and the 2 Sigma error used. The Levin dataset constitutes a compilation of measurements of atmospheric ^{14}C levels at different geographical locations. To assess the universal feasibility of the Levin dataset, estimates were also calculated using zone-specific CALIbomb reference data. However, for ^{14}C tooth enamel values that fell between 1955 and 1960, the CALIbomb program was not used. In CALIbomb these values are predicted using a straight line from pre-bomb values to 1960. For these age ranges values based on a compilation of multiple data sets were used instead [10].

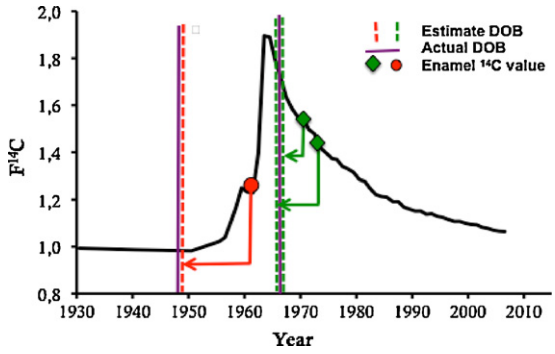

Fig. 1. Schematic illustration of the principle for the birthdating of teeth using analysis of bomb pulse derived ^{14}C. The red dot represents a tooth with a radiocarbon value matching the rising part of the curve at 1961. The angled arrow represents the time of enamel lay down for this particular tooth and ends at the broken vertical line (^{14}C calculated DOB) adjacent to the actual DOB of the person. The green diamonds represent two different teeth (with different laydown times) from the same person, giving different ^{14}C values, allowing for identification of which part of the bomb-curve a person was born. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

If it is not obvious whether an individual is born before or after the peak of the bomb tests, then two teeth with different enamel lay-down times can be analyzed – this distinguishes whether the ^{14}C measurement relates to the rising or falling part of the curve [6]. Additional studies by Cook et al. [7], also show that radiocarbon analysis on the collagen component of the tooth root (combined dentin and cementum) also allows one to determine whether an individual is born on the rising or falling part of the bomb-curve. Since determining which side of the bomb-peak a person's age falls on has been demonstrated previously, we do not include the analysis of two teeth for this purpose here, but have rather chosen the age range appropriate to the known date of birth of the individual.

2.4. Statistical analysis

Regression analysis was used to explore the correlation between estimated and true date of birth in different settings. The difference between different geographical groups regarding ^{14}C and ^{13}C, respectively, was tested with Mann–Whitney U-test. A p-value < 0.05 was considered statistically significant. For all statistical analyses, Statistica 9.1 (Stat Soft Inc., Tulsa, OK) was used.

3. Results

Supplemental Table 1 shows the details of all cases. The same subjects were used for different assessments. Tables 1–3 display the actual and estimated DOB of the individual teeth for three groups, group 1: cases where the tooth enamel was laid down before the bomb pulse, group 2: tooth enamel laid down between 1955 and 1963 (the rising part of the bomb-spike) and group 3: tooth enamel laid down after 1963. In cases where the enamel was formed before the bomb pulse (Table 1, $n = 17$), negative $\Delta^{14}C$ values were found in all cases, except one (J10). Contamination may have caused this elevated ^{14}C concentration (see Section 4). Teeth formed between 1955 and 1963 (Table 2, $n = 12$) showed an absolute error from the true formation time of 1.9 ± 1.4 years (mean; SD). A good correlation was found between the estimated and the actual time of formation ($R^2 = 0.751$; Fig. 2a). Three teeth did not show positive $\Delta^{14}C$ values (OC33, OC53 and OC56); however the calculated time of formation for these teeth was close to the onset of the bomb-pulse (1955). Fig. 2a includes one of these cases (OC56), demonstrating that even a negative $\Delta^{14}C$ value (down to −15) can be plotted. Table 3 contains the data for the teeth formed after 1963 ($n = 66$) where the absolute error from the true formation time is 1.3 ± 1.0 years. All of these teeth contained bomb pulse derived radiocarbon and showed an excellent correlation with the actual time of enamel formation ($R^2 = 0.989$; Fig. 2b). A comparison between the actual and estimated DOB for different $F^{14}C$ values is shown in Fig. 3.

Table 4 and Supplemental Tables 2a–d display the precision in the radiocarbon dating of teeth formed at different geographical

Table 1
^{14}C analysis of teeth formed before 1955.

Person no.	Case no.	Sex	Tooth no.	Enamel formation time (yrs)	Actual DOB	Estimated age
1	FM89	M	24	5.6	1947.3	PB
	FM89		25	6.6		PB
2	OC50	F	34	4.4	1912.8	PB
3	OC54	F	34	4.4	1949.0	PB
4	OC79	F	25	5.6	1937.0	PB
5	J3	F	24	4.9	1950.3	PB
6	J4	M	11	3.2	1932.3	PB
7	J5	M	16	3.3	1928.2	PB
8	J6	M	47	6.5	1912.7	PB
9	J7	M	12	4.0	1917.3	PB
10	J8	F	15	5.6	1911.3	PB
11	J9	M	11	3.2	1917.4	PB
	J10	M	23	4.7	1917.4	Error[a]
12	U1-42	M	42	3.0	1931.4	PB
	U1-43		43	4.3		PB
13	U4-27	M	27	6.5	1946.5	PB
14	U5-12	M	12	4.0	1946.5	PB

PB—pre-bomb value.
[a] An elevated ^{14}C value was recorded, please see text.

Table 2
^{14}C analysis of teeth formed during the rising part of the bomb-curve.

Person no.	Case no.	Sex	Tooth no.	Enamel formation time (yrs)	Actual DOB	Estimated age	Error	Absolute error
1	OC31[a]	M	17	6.5	1952.3	1949.7	−2.6	2.6
2	OC33	M	32	3.0	1953.8	PB	PB	PB
3	OC34	?	33	4.2	1957.3	1957.3	0.0	0.0
4	OC44[a]	M	27	6.5	1952.7	1950.6	−2.1	2.1
5	OC45	M	31	2.5	1958.2	1957.9	0.3	0.3
6	OC53	M	12	4.0	1952.1	PB	PB	PB
7	OC56[a]	M	36	2.4	1955.8	1952.7	−3.1	3.1
8	OC58[a]	M	26	3.3	1956.5	1953.9	−2.6	2.6
9	OC60	F	17	5.8	1955.8	1954.1	−1.6	1.6
10	OC66	M	28	12.6	1949.4	1949.8	0.4	0.4
11	FM19	M	31	2.5	1958.3	1954.0	−4.3	4.3
	FM19	M	32	3.0		1954.1	−4.2	
							Mean	1.9
							SD	1.4

PB—pre-bomb value.
[a] Calculated according Hua and Barbetti [8].

regions. In total 95 teeth from 84 subjects were used for comparison and grouped according to geographical regions: Sweden; Japan + Middle East; and South America. Teeth from Japan and the Middle East were grouped together, since their numbers were limited and according to Calibomb Classification [33], both regions belong to Northern hemisphere zone 2. Table 4 summarizes the precision for these groups and details, including a comparison between results obtained using zone-specific reference values and Levin's dataset, are given in Supplemental Tables 2a–d. No significant difference in either the average factual deviation of the estimation, or the average absolute error was seen between the different geographical groups. The geographically different ^{13}C values did not introduce any error.

In 40 of the teeth from subjects raised in different geographical regions, the amount of enamel also allowed for determination of ^{13}C levels. There was an obvious difference in these levels between subjects of different origins. The lowest depression of ^{13}C was seen in teeth from Sweden (-14.7 ± 0.4), followed by teeth from Japan (-13.5 ± 1.2), Middle East (-13.7 ± 0.6) and South America (-10.9 ± 0.6). The differences between these groups were significant ($p < 0.01$), except between teeth obtained from Japan and Middle East. Table 5 provides details of these results and from this table it can be appreciated that there was no overlap at all between teeth from Sweden and South America regarding the ^{13}C concentration.

4. Discussion

The increasing accessibility to various personal registers in many countries makes the date of birth of an individual even more important than the actual age at death, when it comes to identifying unknown victims. As opposed to the radiocarbon method to detect bomb pulse derived ^{14}C, other methods of age determination of deceased subjects provide information about the age at death rather than the year of birth. By combining the radiocarbon methodology with other methods, such as the aspartic acid racemization analysis, we have recently shown that both the year of birth and year of death can be estimated in obscure cases [8].

Our previous studies have been based on teeth collected by Swedish dentists, where in most cases it was known that the subjects were born and raised in Sweden. Since the initial distribution of bomb pulse derived radiocarbon around the world was somewhat uneven, we wanted to examine the global applicability of this birthdating method by analysis of teeth collected from different geographical locations. We show here that the geographical influence on ^{14}C incorporation during enamel formation does not adversely affect age-estimation precision. Swedish teeth did show a better precision than teeth from other geographical locations; however this population was younger and as shown by the ranging study (Tables 2 and 3 and Fig. 2), it can be concluded that teeth formed during the falling

Table 3
^{14}C analysis of teeth formed during the falling part of the bomb-curve.

Person no.	Case no.	Sex	Tooth no.	Enamel formation time (yrs)	Actual DOB	Estimated age	Error	Absolute error
1	FM01	M	44	5.1	1991.3	1989.9	−1.4	1.4
2	FM02	M	34	5.2	1991.8	1990.8	−1.0	1.0
3	FM03	M	44	4.2	1993.2	1993.5	0.3	0.4
4	FM05	F	44	4.2	1988.8	1988.0	−0.8	0.8
5	FM06	F	14	4.9	1988.8	1985.7	−3.1	3.1
6	FM07	M	34	5.1	1988.6	1987.6	−1.0	1.0
7	FM08	F	44	4.4	1992.5	1991.4	−1.1	1.1
8	FM09	F	24	4.9	1990.5	1990.0	−0.5	0.5
9	FM10	M	21	3.2	1982.0	1981.2	−1.3	1.3
10	FM11	F	35	5.7	1989.2	1989.2	0.0	0.0
11	FM12	F	14	4.9	1991.3	1992.3	1.0	1.0
12	FM25	F	34	4.4	1993.6	1994.0	0.4	0.4
13	FM27	M	14	5.6	1990.8	1989.7	−1.1	1.1
14	FM29	F	24	4.9	1991.8	1991.3	−0.5	0.5
15	FM30	F	14	4.9	1989.1	1987.3	−1.8	1.8
16	FM31	F	17	5.8	1993.4	1992.3	−1.1	1.1
17	FM32	F	14	4.9	1991.1	1990.3	−0.8	0.8
18	FM33	F	14	4.9	1991.5	1990.6	−0.9	0.9
19	FM34	F	34	4.4	1991.4	1990.8	−0.6	0.6
20	FM39	M	34	5.1	1990.8	1989.9	−0.9	0.9
21	FM42	M	14	5.6	1993.2	1992.5	−0.7	0.7
22	FM50	F	14	4.9	1988.3	1987.1	−1.2	1.2
23	FM53	M	14	5.6	1990.9	1989.3	−1.6	1.6
24	FM85	F	47	5.6	1963.7	1963.4	−0.3	0.3
	FM85		17	5.8		1963.4	−0.3	0.3
25	OC29	M	38	13.0	1950.6	1944.8	−5.8	5.8
26	OC41	F	45	5.7	1973.7	1972.7	−1.0	1.0
27	OC42	F	18	11.2	1965.3	1968.0	2.7	2.7
28	OC46	M	48	13.0	1957.4	1956.3	−1.1	1.1
29	OC47	F	28	11.2	1956.8	1955.2	−1.6	1.6
30	OC48	F	38	11.8	1971.2	1970.6	−0.6	0.6
31	OC51	F	28	11.2	1969.3	1968.0	−1.3	1.3
32	OC52	M	28	12.6	1985.7	1983.5	−2.2	2.2
33	OC59	M	18	12.6	1962.6	1961.1	−1.5	1.5
34	OC61	F	28	11.2	1968.8	1968.4	−0.4	0.4
35	OC62	M	28	12.6	1956.7	1956.0	0.7	0.7
36	OC65	M	48	13.0	1964.9	1965.3	0.4	0.4
37	OC67	M	18	12.6	1977.1	1981.0	3.9	3.9
38	OC69	M	37	6.5	1979.4	1977.4	−2.0	2.0
39	OC71	M	27	6.5	1989.6	1990.5	0.9	0.9
40	OC72	F	28	11.2	1977.2	1976.8	−0.4	0.4
41	OC73	F	15	5.6	1988.6	1988.2	0.4	0.4
42	OC80	F	48	11.8	1961.7	1959.1	−2.6	2.6
43	J1	F	13	3.8	1967.3	1966.3	−1.0	1.0
44	J2	M	14	5.6	1964.2	1962.8	−1.4	1.4
45	FM41	M	28	12.6	1967.8	1965.6	−2.0	1.2
	FM41		36	2.4		1968.2	0.4	
	FM41		26	3.3		1967.6	−0.2	
	FM41		14	5.6		1966.5	−1.3	
	FM41		15	6.6		1965.5	−2.3	
46	FM57	F	38	11.8	1955.9	1957.8	1.9	1.9
47	U2-35	M	35	6.5	1966.8	1965.8	−1.0	1.0
48	U3-25	M	25	6.6	1977.4	1976.8	−0.6	0.6
49	U6-28	F	28	11.2	1985.6	1983.9	−1.7	1.7
50	U7-26	F	26	3.0	1995.2	1993.1	−2.1	2.1
51	U8-36	F	36	2.3	1992.3	1993.4	1.1	1.1
52	FM20	M	27	6.5	1975.8	1974.4	−1.4	1.4
53	FM18	M	48	13.0	1970.4	1968.0	−2.4	2.4
54	FM19	M	28	12.6	1958.3	1960.8	2.5	3.1
	FM19		38	13.0		1957.0	−1.3	
55	FM21	F	28	11.2	1985.1	1986.0	0.9	0.9
56	FM58	M	21	3.2	1962.6	1959.8	−2.8	2.8
57	FM04	F	14	4.9	1991.5	1991.5	−2.1	2.1
58	FM13	M	24	5.6	1992.1	1992.1	−2.4	2.4
59	FM14	F	17	5.8	1972.2	1972.2	0.0	0.0
60	FM38	F	15	5.6	1989.1	1987.8	−1.3	1.3
							Mean	1.3
							SD	1.0

part of the curve had a better DOB prediction precision than older teeth.

From 1959 and onwards, multiple atmospheric ^{14}C reference data are available, grouped according to geographical latitude [10,11,33]. While it seems justified to use the reference values provided for the appropriate zone, we show that the Levin data set can give as accurate results as the zone-specific data (Table 4 and Supplemental Tables 2a–d). In particular, this is evident in Supplemental Table 2d, where the birth date is certified, whereas the origin is unknown (although the majority of subjects were

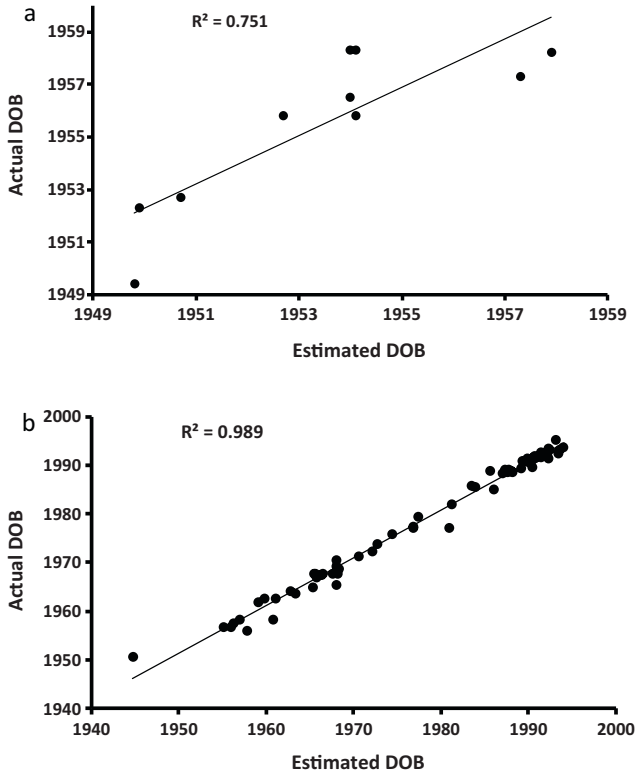

Fig. 2. (a) Radiocarbon analysis of teeth formed during the rising part of the bomb curve show a good correlation with actual formation time (R^2 = 0.751). Note that the data represent the DOB of the person and not the date of enamel formation, with several subjects born before the bomb-spike. Raw data can be found in Table 2 and Supplemental Table 1. The two cases showing Δ^{14}C values below −15 are excluded (see Section 4). (b) An excellent correlation was found between estimated and actual DOB regarding teeth formed after 1963 (R^2 = 0.989). The data match Table 3 and raw data can be found in Supplemental Table 1.

most likely raised in Scandinavia). This is particularly encouraging, since for many unidentified dead bodies, the exact geographical origin is unknown. Having stated that, it should be noted that we have only examined a limited number of teeth from the Southern Hemisphere and since ^{14}C changes lagged there until 1968, it is wise to use Southern hemisphere reference data for subjects raised there prior to 1968.

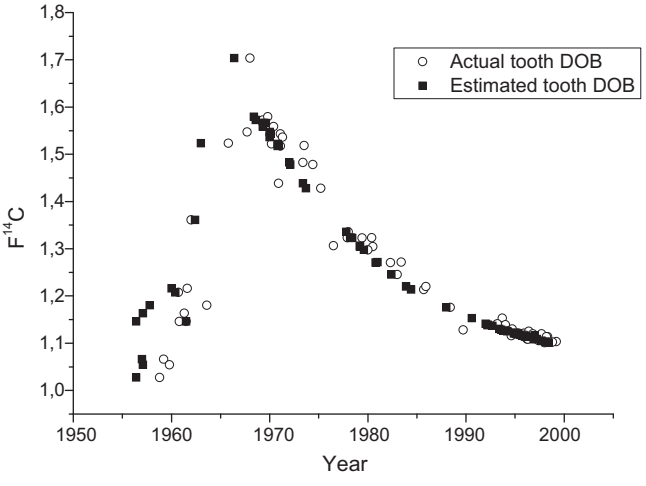

Fig. 3. Distribution of F^{14}C values across the time curve. For teeth formed before 1959, the Hua and Barbetti dataset [10] was used as a reference. These results are given as Δ^{14}C values, however values are shown here as F^{14}C values for purposes of consistency. Please note that the squares and circles represent estimated and actual DOB of the *teeth*, not the individual.

Table 4
Precision of DOB estimates using Levin's data set vs. zone-specific data as reference.[a]

Zone	Levin	Zone data	*N*	Countries
SH	1.4 ± 0.5	1.3 ± 0.8	15	Chile and Uruguay
NH Zone 2	1.9 ± 0.9	1.6 ± 1.0	18	Japan, Middle East and Morocco
NH Zone 1	1.0 ± 0.6	1.2 ± 0.8	27	Sweden
NH Zone 1	1.5 ± 1.3	1.5 ± 1.2	35	Scandinavian?

[a] Details of the calculations are given in Supplemental Tables 2a–d.

The precision of ^{14}C analysis *per se* is dependent on an accurate handling and careful procedures from procurement to instrumentation, since contamination at any step in the protocol may cause dramatically different radiocarbon readings and hence severely compromise correct interpretation of the results. When applying the expedited procedure for analysis using cryo-crushing of teeth, we showed that this theoretical problem is typically not relevant in practice [8], although we did obtain a divergent result in one case that might have been due to contamination of recent carbon remnants in an insufficiently cleaned crushing tube. In the present study, we also obtained a higher radiocarbon value than expected in one case (Table 1, tooth J10). Another tooth (J9) from the same subject showed a pre-bomb concentration and we have not been able to identify the source of contamination of this tooth, which was collected in Japan. The subject was born in 1917 and as such, possible exposure to locally increased ^{14}C levels due to the two Second World War detonations in Japan is unlikely, since these detonations occurred long after the teeth were formed. Rather, the recorded ^{14}C value (Δ^{14}C 47.7; F^{14}C 1.0549) indicates that contamination comes from a contemporary source, most likely introduced during sample processing and handling.

From Fig. 3 it is evident that F^{14}C values below 1.1000 show a lower precision than higher values. If such values are considered to match formation on the rising part of the bomb-curve, caution is warranted. In our series, we obtained negative Δ^{14}C for three teeth that were supposed to have been formed after 1955, two of them even lower than −15 (cases OC33 and OC53). However, their actual formation time was close to the onset of the bomb pulse and during this initial phase, the atmospheric radiocarbon was unevenly distributed. In addition, these two individuals belonged to the group "Scandinavians", where the origin was not recorded, so there is the possibility that they might have been raised in the Southern Hemisphere, where the bomb pulse started later. On the other hand, no pitfalls were encountered with F^{14}C values above 1.1000. Indeed, such values (N = 72, including cases on the rising part of the curve and regardless of geographical origin) showed an absolute average error of 1.4 ± 1.1 years.

^{13}C analysis of tooth enamel was performed to predict the origin of the subjects. The differences were obvious between geographical locations, e.g. Swedish and South American subjects (Table 5). Thus enamel ^{13}C concentration can provide clues as to the geographical region where the subject was raised, and this information may prove valuable for identification work. Recently, analysis of ^{13}C (along with other stable isotopes) was applied on hair samples from subjects from different geographical origins and indeed substantial differences in ^{13}C concentrations between various populations were observed [20]. It is likely that a number of subjects from different geographical regions will adapt to a variable extent to the food culture in the country they reside. This means that e.g. a European citizen settling in the United States may build up ^{13}C levels in the body more typical of other Americans rather than Europeans and that hair analysis for ^{13}C will therefore reflect the food culture where the subject has been staying most recently. Even though a more widespread intake of "fast foods" may, over time, decrease the differences between various

Table 5
$\delta^{13}C$ results in teeth from individuals raised in different geographical regions.

Person no.	Case no.	Sex	Country	Tooth no.	Enamel formation time (yrs)	Actual DOB	$\delta^{13}C$			
							1	2	3	4
1	FM02	M	Sweden	34	5.2	1991.8	−14.60			
2	FM03	M	Sweden	44	4.2	1993.2	−14.90			
3	FM05	F	Sweden	44	4.2	1988.8	−14.38			
4	FM06	F	Sweden	14	4.9	1988.8	−14.87			
5	FM10	M	Sweden	21	3.2	1982.0	−14.49			
6	FM11	F	Sweden	35	5.7	1991.3	−14.63			
7	FM25	F	Sweden	34	4.4	1993.6	−15.04			
8	FM27	M	Sweden	14	5.6	1990.8	−14.74			
9	FM29	F	Sweden	24	4.9	1991.8	−15.04			
10	FM30	F	Sweden	14	4.9	1989.1	−14.76			
11	FM31	F	Sweden	17	5.8	1993.4	−15.02			
12	FM32	F	Sweden	14	4.9	1991.1	−14.69			
13	FM33	F	Sweden	14	4.9	1991.5	−15.15			
14	FM34	F	Sweden	34	4.4	1991.4	−14.79			
15	FM50	F	Sweden	14	4.9	1988.3	−15.10			
16	FM67	F	Sweden	13	3.8	1936.5	−15.35			
17	FM85	F	Sweden	47	5.6	1963.7	−13.89			
	FM85			17	5.8		−14.06			
18	FM89	M	Sweden	24	5.6	1947.3	−14.45			
	FM89			25	6.6		−15.00			
19	J1	F	Japan	13	3.8	1967.3		−11.22		
20	J2	M	Japan	14	5.6	1964.2		−12.06		
21	J3	F	Japan	24	4.9	1950.3		−13.81		
22	J4	M	Japan	11	3.2	1932.3		−12.68		
23	J5	M	Japan	16	3.3	1928.2		−12.94		
24	J6	M	Japan	47	6.5	1912.7		−14.87		
25	J7	M	Japan	12	4.0	1917.3		−14.48		
26	J8	F	Japan	15	5.6	1911.3		−14.87		
27	J9	M	Japan	11	3.2	1917.4		−13.30		
	J10			23	4.7	1917.4		−14.43		
28	FM20	M	Iraq	27	6.5	1975.8			−12.31	
29	FM18	M	Kuwait	48	13.0	1970.4			−12.47	
30	FM19	M	Morocco	31	2.5	1958.3			−13.30	
	FM19			28	12.6	1958.3			−12.74	
	FM19			32	3.0	1958.3			−11.98	
	FM19			38	13.0	1958.3			−13.43	
31	FM57	F	Chile	38	11.8	1955.9				−11.47
32	U1-42	M	Uruguay	42	3.0	1931.4				−10.36
	U1-43			43	4.3	1931.4				−10.50
33	U2-35	M	Uruguay	35	6.5	1966.8				−11.24
34	U3-25	M	Uruguay	25	6.6	1977.4				−11.00
35	U4-27	M	Uruguay	27	6.5	1946.5				−10.05
36	U5-12	M	Uruguay	12	4.0	1946.5				−11.00
37	U6-28	F	Uruguay	28	11.2	1985.6				−11.30
38	U7-26	F	Uruguay	26	3.0	1995.2				−11.84

populations, the fact that recently performed hair analysis still shows geographical differences [20] implies that analysis of tooth enamel will be useful for significant time to come. It can also be concluded that the golden days of ^{14}C analysis are yet to come, since a large part of missing subjects in the near future will be expected to have teeth exposed to the bomb curve and hence, that birthdating using this methodology will be valuable during the coming decades. Approximately 100 AMS facilities are scattered throughout the world, the majority of them capable of measuring ^{14}C. Analysis costs vary with facility and degree of sample clean-up required.

In conclusion, combined analysis of ^{13}C and ^{14}C of tooth enamel can provide useful information for police and forensic identification work, whether it concerns mass disasters, suspected homicide cases, or any case where the identification of the deceased subject is critical.

Acknowledgements

This work was supported by grants from the Human Frontiers Science Program and by NIH/NCRR (RR13461) and was performed in part under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. KA was supported in part by the Swedish National Board of Forensic Medicine. Special thanks are given to the dentists for providing extracted teeth.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.forsciint.2010.12.002.

References

[1] S. Ritz-Timme, C. Cattaneo, M.J. Collins, E.R. Waite, H.W. Schutz, H.J. Kaatsch, H.I. Borrman, Age estimation: the state of the art in relation to the specific demands of forensic practise, Int. J. Legal. Med. 113 (2000) 129–136.
[2] E.R. Waite, M.J. Collins, S. Ritz-Timme, H.W. Schutz, C. Cattaneo, H.I. Borrman, A review of the methodological aspects of aspartic acid racemization analysis for use in forensic science, Forensic Sci. Int. 103 (1999) 113–124.
[3] P.M. Helfman, J.L. Bada, Aspartic acid racemization in tooth enamel from living humans, Proc. Natl. Acad. Sci. U.S.A. 72 (1975) 2891–2894.
[4] S. Ohtani, T. Yamamoto, Strategy for the estimation of chronological age using the aspartic acid racemization method with special reference to coefficient of correlation between D/L ratios and ages, J. Forensic Sci. 50 (2005) 1020–1027.

[5] S. Ohtani, T. Yamamoto, Age estimation by amino acid racemization in human teeth, J. Forensic Sci. 55 (2010) 1630–1633.
[6] K.L. Spalding, B.A. Buchholz, L.E. Bergman, H. Druid, J. Frisen, Forensics: age written in teeth by nuclear tests, Nature 437 (2005) 333–334.
[7] G.T. Cook, E. Dunbar, S.M.X.S. Black, A preliminary assessment of age at death determination using the nuclear weapons testing ^{14}C activity of dentine and enamel, Radiocarbon 48 (2006) 305–310.
[8] K. Alkass, B.A. Buchholz, S. Ohtani, T. Yamamoto, H. Druid, K.L. Spalding, Age estimation in forensic sciences: application of combined aspartic acid racemization and radiocarbon analysis, Mol. Cell Proteomics 9 (2010) 1022–1030.
[9] H. De Vries, Atomic bomb effect: variation of radiocarbon in plants, shells, and snails in the past 4 years, Science 128 (1958) 250–251.
[10] Q. Hua, M. Barbetti, Review of tropospheric bomb ^{14}C data for carbon cycle modeling and age calibration purposes, Radiocarbon 46 (2004) 1273–1298.
[11] I. Levin, B. Kromer, The tropospheric $^{14}CO_2$ level in mid-latitudes of the northern hemisphere (1959–2003), Radiocarbon 46 (2004) 1261–1272.
[12] R. Nydal, K. Lovseth, Distribution of radiocarbon from nuclear tests, Nature 206 (1965) 1029–1031.
[13] M.V. Bolanos, M.C. Manrique, M.J. Bolanos, M.T. Briones, Approaches to chronological age assessment based on dental calcification, Forensic Sci. Int. 110 (2000) 97–106.
[14] C.M. Nolla, The development of the permanent teeth, J. Dental Child. 27 (1960) 254–263.
[15] W.F. Libby, R. Berger, J.F. Mead, G.V. Alexander, J.F. Ross, Replacement rates for human tissue from atmospheric radiocarbon, Science 146 (1964) 1170–1172.
[16] M. Stuiver, P.J. Reimer, E. Bard, J.W. Beck, G.S. Burr, K.A. Hughen, B. Kromer, G. McCormac, J. van der Plicht, M. Spurk, INTCAL98 radiocarbon age calibration, 24000-0 cal BP, Radiocarbon 40 (1998) 1041–1083.
[17] G.D. Farquhar, J.R. Ehleringer, K.T. Hubick, Carbon isotope discimination and photosynthesis, Annu. Rev. Plant Mol. Biol. 40 (1989) 503–537.
[18] M.H. ÓLeary, Carbon isotopes in photosynthesis, BioScience 38 (1988) 328–336.
[19] D.A. Schoeller, M. Minigawa, R. Slater, I.R. Kaplan, Stable isotopes of carbon, nitrogen and hydrogen in the contemporary North American human food web, Ecol. Food Nutr. 18 (1986) 159–170.
[20] E. Mutzel Rauch, C. Lehn, O. Peschel, S. Holzl, A. Rossmann, Assignment of unknown persons to their geographical origin by determination of stable isotopes in hair samples, Int. J. Legal Med. 123 (2009) 35–40.
[21] G.M. Santos, J.R. Southon, K. Druffel-Rodrigez, S. Griffin, M. Mazon, Magnesium perchlorate as an alternative water trap in AMS graphite sample preparation: a report on sample preparation at KCCAMS at the University of California, Irvine, Radiocarbon 46 (2004) 165–173.
[22] J.S. Vogel, J.R. Southon, D.E. Nelson, Catalyst and binder effects in the use of filamentous graphite for AMS, Nucl. Instrum. Methods Phys. Res. Sect. B 29 (1987) 50–56.
[23] P. Zermeño, D.K. Kurdyla, B.A. Buchholz, S.J. Heller, M. Kashgarian, B.R. Frantz, Prevention and removal of elevated radiocarbon contamination in the LLNL/CAMS natural radiocarbon sample preparation laboratory, Nucl. Instrum. Methods Phys. Res. Sect. B 223 (2004) 293–297.
[24] T.P. Guilderson, J.R. Southon, T.A. Brown, High-precision AMS C-14 results on TIRI/FIRI turbidite, Radiocarbon 45 (2003) 75–81.
[25] J.C. Davis, I.D. Proctor, J.R. Southon, M.W. Caffee, D.W. Heikkinen, M.L. Roberts, T.L. Moore, K.W. Turteltaub, D.E. Nelson, D.H. Lloyd, J.S. Vogel, LLNL/UC AMS facility and research program, Nucl. Instrum. Methods Phys. Res. Sect. B 52 (1990) 269–274.
[26] S.J. Fallon, T.P. Guilderson, T.A. Brown, CAMS/LLNL ion source efficiency revisited, Nucl. Instrum. Methods Phys. Res. Sect. B 259 (2007) 106–110.
[27] J.R. Southon, M.L. Roberts, Ten years of sourcery at CAMS/LLNL – evolution of a Cs ion source, Nucl. Instrum. Methods Phys. Res. Sect. B 172 (2000) 257–261.
[28] T.A. Brown, J.R. Southon, Corrections for contamination background in AMS 14C measurements, Nucl. Instrum. Methods Phys. Res. Sect. B 123 (1997) 208–213.
[29] K. Rozanski, W. Stichler, R. Gonfiantini, E.M. Scott, R.P. Beukens, B. Kromer, J. van der Plicht, The IAEA C-14 intercomparison exercise 1990, Radiocarbon 34 (1992) 506–519.
[30] E.M. Scott, The third international radiocarbon intercomparison (TIRI) and the fourth international radiocarbon (FIR) – 1999–2002 – results, analysis and conclusions, Radiocarbon 45 (2003) 293–408.
[31] P.J. Reimer, T.A. Brown, R.W. Reimer, Discussion: reporting and calibration of post-bomb C-14 data, Radiocarbon 46 (2004) 1299–1304.
[32] M. Stuiver, H.A. Pollach, Discussion. reporting of ^{14}C data, Radiocarbon 19 (1977) 355–363.
[33] http://intcal.qub.ac.uk/CALIBomb/frameset.html.