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ACCURATE AGE ESTIMATION USING ^{14}C CONTENT IN JAPANESE HUMAN TEETH

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ABSTRACT. We investigated age estimation of persons using 45 Japanese human teeth that consisted of mainly the third molar. We measured the radiocarbon content in tooth enamel hydroxyapatite from the crown as well as those in collagen and bioapatite in the root of the same tooth. Age estimations from tooth enamel apatite yielded an absolute age shift of 1.7 ± 1.4 yr from the true age. This value suggests that estimating the age of Japanese people from tooth enamel is as accurate as the previous studies conducted in Europe and the USA. The ^{14}C analysis of roots suggested that bioapatite is formed slightly later than collagen in the same tooth roots. The appropriate formation age ranges suggested by the tooth enamel analysis can be optimized and confirmed by using the additional $F^{14}C$ values of the root samples. We also noticed a characteristic trend of enamel $\delta^{13}C$ values in comparison with the formation ages of the crowns of the teeth. The $\delta^{13}C$ value tends to increase gradually from –13.0‰ to –11.5‰ from the 1960s to the 1970s, probably being caused by changes in the Japanese diet. This trend may support the selection of proper tooth formation age as supplemental data in addition to ^{14}C content analyses.

KEYWORDS: radiocarbon, human tooth, forensic science, date of birth, tooth enamel.

INTRODUCTION

In the late 1950s to early 1960s, the radiocarbon content in the atmosphere increased dramatically as a result of atmospheric nuclear weapons testing. This artificial rapid increase in the ^{14}C content is called the bomb effect. Bomb-produced ^{14}C atoms were oxidized to form carbon dioxide (CO_2), and were incorporated into plants by photosynthesis. Living on plants or animals that have eaten these plants means that the ^{14}C content in the human body reflects the ^{14}C content of atmospheric CO_2 at the time. Hua and Barbetti (2004, 2007) summarized the high ^{14}C content of contemporary materials affected by the bomb effect. The formation ages of contemporary living materials can be commonly estimated by using bomb-produced ^{14}C. An important task in forensic science is identifying victims of accidents or crime. The ^{14}C contained in human tooth enamel provides the most accurate estimates of the birth year, one the most important data points to identify the victims. In fact, recent studies (Spalding et al. 2005; Alkass et al. 2011, 2013) have revealed that bomb-produced ^{14}C atoms can be successfully used to estimate the age of a victim by using ^{14}C variation in the hydroxyapatite fraction of the tooth enamel. Although the applications of ^{14}C technique in odontology have been validated in Europe and the USA, similar systematic studies have not been performed yet in Japan. To realize this project, it was necessary to examine the differences in the duration of tooth formation in Japanese people compared with other nationalities, to determine the zonal effect on the temporal variations of ^{14}C content in the atmosphere, and also to take into account the Japanese diet, which may change the ^{14}C content of the human teeth.

Tooth enamel is the hardest part of the human body and it is most resistant to destruction by natural processes. The most important aspect of tooth enamel is that no turnover of enamel occurs after its formation is completed. Although there are several studies that have estimated the date of birth (DOB) of individuals from the ^{14}C content in tooth enamel, these teeth are mainly from high latitudes in the Northern Hemisphere, from countries such as Sweden, Scotland, and North America (Spalding et al. 2005; Cook et al. 2006; Alkass et al. 2010, 2011, 2013). On the other hand, systematic studies on teeth from the mid-latitudes are quite limited (Wang et al. 2010; Alkass et al. 2011). The precision of age estimation by using tooth enamel

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apatite is determined by the enamel formation time, depending on nationalities as well as temporal and spatial variations of atmospheric ^{14}C contents. As for a genetic factor, for example, the tooth formation age of Japanese (Mongolian) people is different from that of Caucasians. We needed to compile all available data concerning the formation ages for tooth enamel as well as tooth root at the different tooth positions for Japanese.

As an environmental factor, ^{14}C variations in the atmosphere can be divided into five zones from north to south, according to the different ^{14}C peaks from nuclear weapons tests: NH zone 1, NH zone 2, NH zone 3, SH zone 3, and SH zone 1–2 (Hua et al. 2013). Previous works using tooth enamel ^{14}C mainly focused on samples from NH zone 1, but rarely from NH zone 2. Japanese tooth enamel, which could provide a typical example from NH zone 2 (Figure 1), has not been studied sufficiently. In addition, there may be some other effects from diet. Japanese people tend to some extent to eat seafood that is depleted in ^{14}C. The problems of the Japanese diet as well as the Suess effect in CO_2 production by fossil fuel consumption influence the ^{14}C contents in tooth carbonaceous components, which may disturb precise estimation of human DOB, as will be discussed with sample $F^{14}C$ values below. In this study, we aimed to confirm that age estimation from Japanese tooth enamel can determine the precise DOB of individuals and to establish a forensic method for Japanese teeth.

Alkass et al. (2011) reported a possibility that the $\delta^{13}C$ value of tooth enamel might provide information about the geographical origin of individuals. We describe the $\delta^{13}C$ value of Japanese tooth enamel apatite to study a possible trend peculiar to the diet of Japanese people.

SAMPLE MATERIAL

Forty-five tooth samples were selected for ^{14}C analysis from various human teeth supplied by the Faculty of Dentistry, Aichi Gakuin University, Nagoya, Aichi Prefecture, Japan. The tooth samples were collected from patients who lived in and around Nagoya. The year of birth and

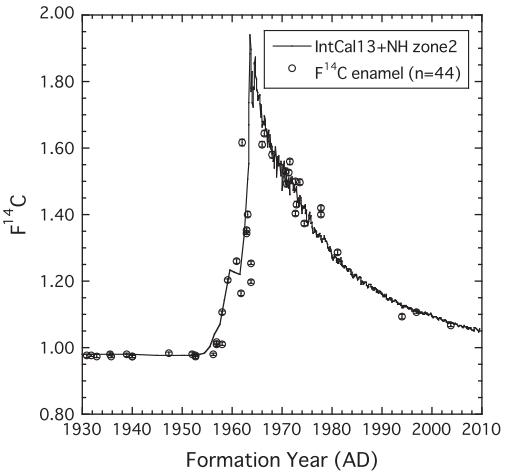


Figure 1 $F^{14}C$ value vs. formation year of crown enamel apatite. The formation year of enamel apatite fraction was estimated from the true date of birth (DOB) of the tooth owner and average enamel formation age estimated in the previous studies (see Table 4). Solid line indicates $F^{14}C$ values of IntCal13 (<1950) and NH zone 2 (>1950) calibration data sets.

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the types of the selected teeth are shown in Tables 1–3. The year of birth ranges from 1919 to 1991 for 13 males, 18 females, and 14 unknowns.

As seen in Tables 1–3, we analyzed mainly the third molar. The third molar is often extracted even from young patients at the dental clinic, in some cases, even though it is not carious. The third molar is considered useless in daily life, but it is prone to become carious, so it is extracted more often. As a result, we have collected third molar samples easily from participating dentists. We also intended to investigate one type of tooth systematically.

Precise estimation of tooth formation age is based on systematic variation of ^{14}C content in atmospheric CO_2. The atmospheric ^{14}C variation mainly resulted from atmospheric nuclear-bomb testing in the late 1950s to early 1960s and is formed by two leading trends as shown in Figure 1: a rising trend from 1955 to 1963 and a falling trend from 1963 and afterwards. Therefore, ^{14}C content of the single carbonaceous fraction from a human tooth offers two possibilities about formation age of the tooth, one belonging to the period from 1955 to 1963 and the other from 1963 and onward. Our major study concentrated on verifying the effectiveness for estimation of human DOB by tooth enamel bioapatite fraction, but also to collect secondary additive information for the more precise and convincing age estimation by using other carbonaceous fractions from the same tooth, which possess information of different formation ages. As for the candidates for the secondary information, we decided to use bioapatite and gelatin-collagen fractions separated from the root part of the same tooth. It has been confirmed by dentists that the crown part is formed first and the root part is completed in a few years after crown formation. Alternatively, another tooth at a different position in the same individual that was formed at a period different from the first tooth can be analyzed. In this study, ^{14}C analyses of bioapatite and collagen extracted from the root portion of the same tooth were performed.

METHOD

Carbon Isotope Ratio Analysis

Physical Separation of Tooth

A tooth was divided into the crown and the root portions with a dental cutting tool. The separated samples were cleaned in distilled water (dH_2O) in an ultrasonic bath for several hours and dried.

Chemical Treatment

CO_2 Extraction from Enamel Apatite of Tooth Crown

The crown enamel fraction was separated chemically according to Spalding et al. (2005) as described briefly in the following. The crown fragments were weighed and samples (400–700 mg) were placed in a vial with 10N NaOH solution for 2–3 hr, and then treated ultrasonically for about 5–6 hr. The NaOH solution was removed and the samples were rinsed with dH_2O three times. Next, the samples were immersed in 10N NaOH again and treated at 70°C on a block heater overnight. This process was repeated every 24 hr for 2–3 weeks until the sample color suggested that all dentin fractions were dissolved and removed. After the pure enamel fraction was obtained, it was washed 10 times with dH_2O until the solution reached pH 7, and then it was stored in a clean vial.

Tooth enamel is composed of approximately 96% hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and 4% other inorganic materials. Its carbon content is about 0.4–0.7% w/w. To collect CO_2, the enamel

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Table 1 List of tooth enamel samples whose $F^{14}C$ values were greater than 1.00, including details of tooth samples, $\delta^{13}C$ and $F^{14}C$ values, estimated formation ages of teeth, and estimated date of birth (DOB) of person.

Sample #	Sex	Tooth #	Enamel formation age (yr)	$\delta^{13}C$	$F^{14}C$	Calibrated date of formation range (2σ)	Calibrated date of DOB range (2σ)	Estimated DOB	Estimated DOB (average)	True DOB of person	True DOB of person − estimated DOB	Absolute difference
306	M	Lower Left 8	12.3	–13.9	1.20250 ± 0.00534	1958.65–1959.37	1946.4–1947.1	1946.7 ± 0.4	1947.4	1946.9	–0.5	0.5
						1960.44–1960.67	1948.1–1948.4	1948.3 ± 0.1				
308	—	Upper Left 8	11.5	–13.2	1.00883 ± 0.00478	1955.00–1956.10	1943.5–1944.6	1944.1 ± 0.5	1944.1	1945.5	1.5	1.5
309	—	Upper Right 8	11.5	–13.3	1.01619 ± 0.00475	1955.41–1956.20	1943.9–1944.7	1944.3 ± 0.4	1944.3	1945.5	1.2	1.2
310	—	Lower Left 8	12.3		1.16414 ± 0.00582	1958.28–1959.03	1946.0–1946.7	1946.4 ± 0.4	1946.4	1949.5	3.1	3.1
312	—	Upper Left 8	11.5	–12.7	1.25958 ± 0.00652	1961.67–1961.98	1950.2–1950.5	1950.3 ± 0.2	1950.3	1949.5	–0.8	0.8
313	—	Lower Left 8	12.3	–12.8	1.25511 ± 0.00580	1961.63–1961.97	1949.3–1949.7	1949.5 ± 0.2	1949.5	1951.5	2.0	2.0
314	—	Upper Right 8	11.5	–13.1	1.35432 ± 0.00724	1962.33–1962.67	1950.8–1951.2	1951.0 ± 0.2	1951.0	1951.5	0.5	0.5
316	—	Upper Left 8	11.5	–13.3	1.34514 ± 0.00639	1962.28–1962.62	1950.7–1951.0	1950.9 ± 0.2	1950.9	1951.5	0.7	0.7
317	—	Lower Right 8	12.3	–14.2	1.19774 ± 0.00532	1958.66–1959.32	1946.4–1947.0	1946.7 ± 0.3	1946.7	1951.5	4.8	4.8
327	—	Lower Left 8	12.3	–12.8	1.01175 ± 0.00474	1955.25–1956.07	1943.0–1943.8	1943.4 ± 0.4	1943.4	1945.6	2.2	2.2
328	F	Lower Right 8	12.3	–12.2	1.61724 ± 0.00992	1963.29–1963.37	1951.0–1951.1	1951.0 ± 0.0	1951.0	1949.8	–1.2	1.2
337	F	Upper Left 7	6.5	–11.7	1.61150 ± 0.00885	1966.36–1966.39	1959.8–1959.8	1959.8 ± 0.0	1960.9	1959.4	–1.5	1.5
						1966.77–1968.22	1960.2–1961.6	1960.9 ± 0.7				
						1968.55–1968.73	1962.0–1962.1	1962.0 ± 0.1				
338	M	Lower Left 8	12.3	–11.5	1.06802 ± 0.00517	2002.23–2007.86	1989.9–1995.6	1992.7 ± 2.8	1993.6	1991.5	–2.1	2.1
						2008.48–2008.85	1996.2–1996.6	1996.4 ± 0.2				
						2009.50–2009.52	1997.2–1997.2	1997.2 ± 0.0				
339	F	Lower Right 8	12.3	–11.9	1.10748 ± 0.00537	1995.23–1995.33	1982.9–1983.0	1983.0 ± 0.0	1985.4	1984.5	–0.9	0.9
						1995.56–2000.09	1983.3–1987.8	1985.5 ± 2.3				
340	—	Upper Right 8	11.5		1.09346 ± 0.00513	1997.25–1997.31	1985.8–1985.8	1985.8 ± 0.0	1988.6	1982.5	–6.1	6.1
						1997.83–2002.88	1986.3–1991.4	1988.9 ± 2.5				
2(743)	M	Lower Left 8	12.3	–12.1	1.58024 ± 0.00926	1966.99–1967.08	1954.7–1954.8	1954.7 ± 3.8	1956.4	1955.6	–0.8	0.8
						1967.34–1969.53	1955.0–1957.2	1956.1 ± 6.2				
						1969.77–1969.89	1957.5–1957.6	1957.5 ± 6.6				
						1970.29–1970.39	1958.0–1958.1	1958.0 ± 7.1				
54(1518)	F	Upper Left 8	11.4		1.10521 ± 0.00566	1957.70–1958.29	1946.3–1946.9	1946.6 ± 0.3	1946.6	1946.7	0.1	0.1
7(2308)	M	Upper Right 8	11.7	–11.7	1.40403 ± 0.00761	1973.29–1973.58	1961.6–1961.9	1961.7 ± 0.1	1962.9	1961.0	–1.9	1.9
						1973.90–1975.84	1962.2–1964.1	1963.2 ± 1.0				
8(2382)	F	Lower Left 8	12.3	–12.3	1.55893 ± 0.00893	1967.02–1967.05	1954.7–1954.8	1954.7 ± 0.0	1956.6	1959.2	2.6	2.6
						1968.00–1969.98	1955.7–1957.7	1956.7 ± 1.0				
						1970.24–1970.43	1957.9–1958.1	1958.0 ± 0.1				
						1970.78–1970.79	1958.5–1958.5	1958.5 ± 0.0				
9(1485)	F	Lower Left 8	12.3	–11.8	1.39963 ± 0.00747	1973.30–1973.57	1961.0–1961.3	1961.1 ± 0.1	1962.3	1965.4	3.1	3.1
						1973.91–1975.85	1961.6–1963.6	1962.6 ± 1.0				

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13(1537)	F	Lower Left 8	12.3	–11.4	1.49752 ± 0.00854	1968.90–1968.98	1956.6–1956.7	1956.6 ± 0.0	1958.6	1961.2	2.6	2.6
						1969.68–1969.71	1957.4–1957.4	1957.4 ± 0.0				
						1970.01–1970.23	1957.7–1957.9	1957.8 ± 0.1				
						1970.44–1972.95	1958.1–1960.7	1959.4 ± 1.3				
67(3711)	F	Lower Right 8	12.3	–10.5	1.28715 ± 0.00654	1979.08–1980.83	1966.8–1968.5	1967.7 ± 0.9	1968.2	1968.8	0.6	0.6
						1981.10–1981.12	1968.8–1968.8	1968.8 ± 0.0				
						1981.43–1981.56	1969.1–1969.3	1969.2 ± 0.1				
						1981.94–1981.95	1969.6–1969.7	1969.6 ± 0.0				
62(2303)	F	Lower Left 8	12.3	–11.7	1.49255 ± 0.00827	1968.92–1968.97	1956.6–1956.7	1956.6 ± 0.0	1959.0	1958.7	–0.3	0.3
						1969.69–1969.70	1957.4–1957.4	1957.4 ± 0.0				
						1970.02–1970.21	1957.7–1957.9	1957.8 ± 0.1				
						1970.54–1972.96	1958.2–1960.7	1959.5 ± 1.2				
						1973.61–1973.64	1961.3–1961.3	1961.3 ± 0.0				
16(4333)	M	Lower Right 8	12.3	–10.5	1.43107 ± 0.00777	1972.99–1974.88	1960.7–1962.6	1961.6 ± 0.9	1962.0	1960.7	–1.3	1.3
						1975.52–1975.53	1963.2–1963.2	1963.2 ± 0.0				
19(4366)	F	Lower Right 8	12.3	–12.9	1.52556 ± 0.00861	1968.42–1968.49	1956.1–1956.2	1956.2 ± 0.0	1958.0	1959.0	1.0	1.0
						1968.82–1969.03	1956.5–1956.7	1956.6 ± 0.1				
						1969.25–1971.09	1957.0–1958.8	1957.9 ± 0.9				
						1971.31–1972.21	1959.0–1959.9	1959.5 ± 0.5				
31(2312)	M	Upper Left 8	11.7		1.37333 ± 0.00733	1974.68–1976.71	1963.0–1965.0	1964.0 ± 1.0	1964.0	1962.8	–1.2	1.2
36(2294)	F	Lower Left 8	12.3	–12.0	1.41964 ± 0.00765	1973.01–1974.93	1960.7–1962.6	1961.7 ± 1.0	1962.1	1965.4	3.3	3.3
						1975.40–1975.80	1963.1–1963.5	1963.3 ± 0.2				
37(2290)	F	Lower Left 8	12.3	–12.5	1.49958 ± 0.00833	1968.90–1968.98	1956.6–1956.7	1956.6 ± 0.0	1958.6	1960.4	1.8	1.8
						1969.67–1969.72	1957.4–1957.4	1957.4 ± 0.0				
						1970.00–1970.23	1957.7–1957.9	1957.8 ± 0.1				
						1970.44–1972.49	1958.1–1960.2	1959.2 ± 1.0				
						1972.70–1972.92	1960.4–1960.6	1960.5 ± 0.1				
50(1524)	F	Upper Left 8	11.4	–13.2	1.53154 ± 0.00869	1967.03–1967.04	1955.6–1955.6	1955.6 ± 0.0	1958.2	1959.3	1.1	1.1
						1968.27–1968.52	1956.9–1957.1	1957.0 ± 0.1				
						1968.79–1971.01	1957.4–1959.6	1958.5 ± 1.1				
						1971.33–1972.10	1959.9–1960.7	1960.3 ± 0.4				
39(2375)	M	Upper Left 8	11.7	–13.0	1.40133 ± 0.00752	1962.60–1962.86	1950.9–1951.2	1951.0 ± 0.1	1951.0	1951.4	0.4	0.4
64(1546)	F	Lower Right 8	11.4	–12.2	1.64491 ± 0.00970	1965.68–1967.88	1954.3–1956.5	1955.4 ± 1.1	1955.4	1955.1	–0.3	0.3
										Average ± 1σ	0.4 ± 2.1	1.7 ± 1.4

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Table 2 List of tooth enamel samples whose $F^{14}C$ values were less than 1.00, including details of tooth samples, $\delta^{13}C$ and $F^{14}C$ values, estimated formation ages of teeth, and estimated date of birth (DOB) of person.

Sample #	Sex	Tooth #	Enamel completion age (yr)	$\delta^{13}C$	$F^{14}C \pm$ err	Calibrated date of formation range (2σ)	Calibrated date of DOB range (2σ)	Estimated DOB	True DOB of person	True DOB of person − estimated DOB	Absolute difference
299	—	Lower Right 8	12.3	–13.3	0.97663 ± 0.00462	1916–1955	1904–1943	1923 ± 20	1919.5	–4	4
300	—	Upper Right 8	11.5	–13.4	0.97568 ± 0.00454	1917–1955	1906–1944	1925 ± 19	1919.5	–5	5
319	—	Upper Right 7	6.5	–13.1	0.97379 ± 0.00448	1918–1955	1911–1948	1930 ± 19	1933.5	4	4
324	F	Upper Right 8	11.4	–13.5	0.98098 ± 0.00449	1909–1955	1898–1944	1921 ± 23	1940.5	20	20
325	M	Upper Right 8	11.7	–13.7	0.97213 ± 0.00464	1923–1955	1911–1943	1927 ± 16	1941.0	14	14
330	M	Lower Left 7	6.7	–13.6	0.97470 ± 0.00465	1917–1955	1910–1948	1929 ± 19	1926.2	–3	3
331	F	Upper Left 7	6.5	–13.4	0.97490 ± 0.00454	1918–1955	1911–1948	1930 ± 19	1929.1	–1	1
332	F	Lower Right 7	6.4	–13.3	0.98152 ± 0.00449	1909–1955	1903–1949	1926 ± 23	1929.1	4	4
335	M	Lower Left 7	6.7	–14.1	0.97875 ± 0.00451	1914–1955	1907–1948	1928 ± 21	1932.2	4	4
336	M	Upper Right 7	6.6	–12.7	0.97313 ± 0.00401	1927–1955	1920–1948	1934 ± 14	1933.3	–1	1
341	—	Upper Left 6	2.7	–9.8	0.98023 ± 0.00444	1911–1955	1908–1952	1930 ± 22	1953.5	23	23
58(3296)	M	Lower Right 8	12.3	–13.1	0.98263 ± 0.00438	1907–1947	1895–1935	1915 ± 20	1935.1	20	20
41(2366)	M	Upper Right 8	11.7	–13.1	0.97690 ± 0.00437	1917–1955	1905–1943	1924 ± 19	1941.0	17	17
									Average ±1σ	7 ± 10	9 ± 8

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Table 3 List of enamel (enl) separated from tooth crowns, gelatin collagen (GC), and apatite (apt) separated from tooth root samples, including details of tooth samples, $\delta^{13}C$ and $F^{14}C$ values, estimated formation ages of teeth, and estimated date of birth (DOB) of person.

Sample #	True DOB of person	Sex	Tooth #	Sample name	Enamel/root formation age	$\delta^{13}C$	$F^{14}C$	Calibrated date of formation range (2σ)	Calibrated date of DOB range (2σ)	Estimated DOB	Estimated DOB (average)	True DOB − estimated DOB	True DOB − estimated DOB (selected)
299	1919.5	—	Lower Right 8	299–enl	12.3	–13.3	0.97663 ± 0.00462	1646–1697	1634–1685	1659 ± 26	1659	260	
								1724–1815	1712–1803	1757 ± 46	1757	162	
								1834–1878	1822–1866	1844 ± 22	1844	76	
								1916–1955	1904–1943	1923 ± 20	1923	–4	–4
				299–GC	18.0	–18.8	0.99327 ± 0.00451	1690–1729	1672–1711	1692 ± 20	1692	228	
								1810–1925	1792–1907	1850 ± 58	1850	70	
								1955–1956	1937–1938	1938 ± 1	1938	–18	–18
				299–apt	18.0	–14.5	1.01655 ± 0.00483	1955.43–1956.22	1937.4–1938.2	1937.8 ± 0.4	1937.8	–18.3	–18.3
58	1935.1	M	Lower Right 8	58–enl	12.3	–13.1	0.98263 ± 0.00438	1668–1781	1656–1769	1712 ± 57	1712	223	
								1797–1892	1785–1880	1832 ± 48	1832	103	
								1907–1947	1895–1935	1915 ± 20	1915	20	
								1950–1955	1938–1943	1940 ± 3	1940	–5	–5
				58–GC	18.0	–19.2	1.00534 ± 0.00458	1707.48–1718.42	1689.5–1700.4	1695.0 ± 5.5	1695.0	240.1	
								1825.84–1831.91	1807.8–1813.9	1810.9 ± 3.0	1810.9	124.1	
								1885.44–1912.64	1867.4–1894.6	1881.0 ± 13.6	1881.0	54.1	
								1954.66–1956.20	1936.7–1938.2	1937.4 ± 0.8	1937.4	–2.3	–2.3
				58–apt	18.0	–14.0	1.05709 ± 0.00486	1956.68–1957.47	1938.7–1939.5	1939.1 ± 0.4	1939.1	–4.0	–4.0
								2005.20–2005.52	1987.2–1987.5	1987.4 ± 0.2	1988.5	–53.4	
								2005.81–2009.52	1987.8–1991.5	1989.7 ± 1.9			
325	1941.1	M	Upper Right 8	325–enl	11.7	–13.7	0.97213 ± 0.00464	1524–1559	1512–1547	1530 ± 18	1530	411	
								1564–1570	1552–1558	1555 ± 3	1555	386	
								1631–1691	1619–1679	1649 ± 30	1649	292	
								1729–1810	1717–1798	1758 ± 41	1758	183	
								1923–1955	1911–1943	1927 ± 16	1927	14	14
				325–GC	17.8	–19.3	1.08257 ± 0.00500	1957.39–1957.99	1939.6–1940.2	1939.9 ± 0.3	1939.9	1.2	1.2
								2000.16–2004.90	1982.4–1987.1	1984.7 ± 2.4	1984.7	–43.6	
				325–apt	17.8	–14.4	1.10198 ± 0.00520	1957.68–1958.23	1939.9–1940.4	1940.2 ± 0.3	1940.2	0.9	0.9
								1996.04–2000.98	1978.2–1983.2	1980.7 ± 2.5	1980.7	–39.6	
308	1945.5	—	Upper Left 8	308–enl	11.5	–13.2	1.00883 ± 0.00478	1955.00–1956.10	1943.5–1944.6	1944.1 ± 0.5	1944.1	1.5	1.5
				308–GC	17.6	–18.8	1.27823 ± 0.00662	1961.80–1962.17	1944.2–1944.6	1944.4 ± 0.2	1944.4	1.1	1.1
								1979.25–1981.16	1961.7–1963.6	1962.6 ± 1.0	1963.3	–17.8	
								1981.37–1981.98	1963.8–1964.4	1964.1 ± 0.3			
				308–apt	17.6	–14.0	1.29660 ± 0.00768	1961.90–1962.40	1944.3–1944.8	1944.6 ± 0.3	1944.6	0.9	0.9
								1977.98–1978.05	1960.4–1960.5	1960.4 ± 0.0	1961.2	–15.7	
								1978.56–1980.79	1961.0–1963.2	1962.1 ± 1.1			

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Table 3 (*Continued*)

Sample #	True DOB of person	Sex	Tooth #	Sample name	Enamel/root formation age	$\delta^{13}C$	$F^{14}C$	Calibrated date of formation range (2σ)	Calibrated date of DOB range (2σ)	Estimated DOB	Estimated DOB (average)	True DOB − estimated DOB	True DOB − estimated DOB (selected)
54	1946.7	F	Upper Left 8	54–enl	11.4	—	1.10521 ± 0.00566	1957.70–1958.29	1946.3–1946.9	1946.6 ± 0.3	1946.6	0.1	0.1
								1995.26–1995.29	1983.9–1983.9	1983.9 ± 0.0	1986.5	–39.8	
								1995.60–2000.18	1984.2–1988.8	1986.5 ± 2.3			
								2000.42–2000.68	1989.0–1989.3	1989.2 ± 0.1			
				54–GC	17.5	–18.5	1.51844 ± 0.00845	1963.13–1963.28	1945.6–1945.8	1945.7 ± 0.1	1945.7	1.0	1.0
								1968.43–1968.47	1950.9–1951.0	1951.0 ± 0.0	1951.8	–5.1	
								1968.86–1969.02	1951.4–1951.5	1951.4 ± 0.1			
								1969.27–1969.28	1951.8–1951.8	1951.8 ± 0.0			
								1969.62–1969.76	1952.1–1952.3	1952.2 ± 0.1			
								1969.96–1972.24	1952.5–1954.7	1953.6 ± 1.1			
				54–apt	17.5	—	1.49946 ± 0.00865	1963.06–1963.23	1945.6–1945.7	1945.6 ± 0.1	1945.6	1.1	1.1
								1968.90–1968.98	1951.4–1951.5	1951.4 ± 0.0	1952.6	–5.9	
								1969.67–1969.72	1952.2–1952.2	1952.2 ± 0.0			
								1970.00–1970.23	1952.5–1952.7	1952.6 ± 0.1			
								1970.44–1972.50	1952.9–1955.0	1954.0 ± 1.0			
								1972.70–1972.93	1955.2–1955.4	1955.3 ± 0.1			
315	1951.5	—	Upper Left 8	315–GC	17.6	–18.6	1.57424 ± 0.00904	1963.23–1963.32	1945.6–1945.7	1945.7 ± 0.0	1945.7	5.8	
								1967.00–1967.07	1949.4–1949.5	1949.4 ± 0.0	1950.5	1.0	1.0
								1967.35–1967.43	1949.8–1949.8	1949.8 ± 0.0			
								1967.68–1969.94	1950.1–1952.3	1951.2 ± 1.1			
								1970.27–1970.40	1952.7–1952.8	1952.7 ± 0.1			
				315–apt	17.6	–14.2	1.52490 ± 0.00855	1963.16–1963.29	1945.6–1945.7	1945.6 ± 0.1	1945.6	5.9	
								1968.42–1968.49	1950.8–1950.9	1950.9 ± 0.0	1951.9	–0.4	–0.4
								1968.83–1969.03	1951.2–1951.4	1951.3 ± 0.1			
								1969.23–1971.10	1951.6–1953.5	1952.6 ± 0.9			
								1971.31–1972.72	1953.7–1955.1	1954.4 ± 0.7			
317	1951.5	—	Lower Right 8	317–enl	12.3	–14.2	1.19774 ± 0.00532	1958.66–1959.32	1946.4–1947.0	1946.7 ± 0.3	1946.7	4.8	4.8
								1984.06–1984.37	1971.8–1972.1	1971.9 ± 0.2	1973.0	–21.5	
								1984.85–1987.99	1972.6–1975.7	1974.1 ± 1.6			
				317–GC	18.0	–18.4	1.54494 ± 0.00871	1963.21–1963.30	1945.2–1945.3	1945.3 ± 0.0	1945.3	6.2	
								1967.03–1967.04	1949.0–1949.0	1949.0 ± 0.0	1951.4	0.1	0.1
								1968.03–1970.83	1950.0–1952.8	1951.4 ± 1.4			
								1971.99–1972.06	1954.0–1954.1	1954.0 ± 0.0			
				317–apt	18.0	–14.5	1.50727 ± 0.00849	1963.09–1963.25	1945.1–1945.3	1945.2 ± 0.1	1945.2	6.3	
								1968.89–1969.00	1950.9–1951.0	1950.9 ± 0.1	1952.5	–1.0	–1.0
								1969.65–1969.73	1951.7–1951.7	1951.7 ± 0.0			
								1969.99–1972.47	1952.0–1954.5	1953.2 ± 1.2			
								1972.76–1972.85	1954.8–1954.9	1954.8 ± 0.0			

samples were hydrolyzed with 105% orthophosphoric acid (H_3PO_4) pretreated in advance. The samples were immersed in 0.25N HCl for about 1 hr to remove any carbonaceous materials on their surfaces. The samples were rinsed three times with dH_2O and dried on a heating block at 95°C overnight. A portion of each enamel sample (220–250 mg) was crushed and acidified with 105% H_3PO_4 at 90°C in an evacuated forked test tube with a block heater to extract CO_2 from the enamel apatite fraction.

CO_2 Extraction from Bioapatite Portion of Tooth Root

Seven root samples given in Table 3 were halved vertically. One half was used to extract CO_2 from bioapatite component and the other half for collagen separation. Following the process used by Alkass et al. (2013), CO_2 was extracted by decomposing the apatite fraction. Root samples were immersed in 2% NaClO in a vial for 24 hr, and then they were rinsed 10 times with dH_2O. The samples were pretreated in 0.1N HCl for about 30 min, washed 10 times with dH_2O, and dried on a heating block at 95°C. Following a procedure similar to that used for enamel apatite, the samples with bioapatite component were treated with 105% H_3PO_4 in a forked test tube, and CO_2 was collected from a pulverized portion of the sample.

Collagen Extraction from Tooth Root

Collagen extraction from root samples was performed according to Longin (1971) and Minami et al. (2004). Root samples were powdered, placed in cellulose tubes, and treated with 0.4–0.6N HCl in beakers. The HCl solution was stirred magnetically, and the samples were decalcified in a refrigerator at 4°C for 24 hr. The HCl solution was removed, and the beakers were filled with dH_2O. The dH_2O was changed once an hour more than five times until the solution pH was around 7. Next, the acid-treated samples were centrifuged and divided into the solution and solid content fractions. The solution was freeze-dried to obtain the acid-soluble collagen fraction. The solid content was rinsed with dH_2O, and then treated with 0.1N NaOH (2–3 mL) for 2 hr. The solid residue was rinsed with 0.1N HCl, and then treated with a weak HCl solution of pH 3 in a block heater at 90°C overnight to solubilize the solid collagen. The liquid fraction was collected and freeze-dried to obtain solid gelatin collagen. The collagen (5–7 mg) was put in evacuated quartz tubes with CuO (500 mg) and reduced Cu (400 mg), and heated at 850°C for 2 hr to generate CO_2.

Separation of Carbon Dioxide, Graphitization, and Carbon Isotope Analysis

The CO_2 generated was purified with a glass vacuum system. The separated CO_2 was divided into two streams: one for $^{13}C/^{12}C$ ratio analysis with an isotope ratio mass spectrometer (IRMS) and the other for ^{14}C analysis by accelerator mass spectrometry (AMS). Part of the CO_2 was reduced to graphite on an iron catalyst under a hydrogen atmosphere (Nakamura et al. 2004). All three carbon isotopes in the graphite material prepared from the tooth samples, a NIST HOxII standard, and ^{14}C blank material (limestone No. 57952, Kishida Chemical Co. Ltd., Japan) were measured by AMS (Tandetron 4130, HVE, the Netherlands) at Nagoya University, Japan (Nakamura et al. 2004, 2007). The resulting $^{14}C/^{12}C$ and $^{13}C/^{12}C$ ratios were then used to calculate the ^{14}C contents of the samples as follows: $F^{14}C = (1.00/0.7459) \times [(^{14}C/^{12}C)_{spl}/(^{14}C/^{12}C)_{HOxII}]$, with correction for carbon isotopic fractionation (Tables 1–3) (Stuiver and Polach 1977; Mook and van der Plicht 1999; Reimer et al. 2004).

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Data of Tooth Formation Age

It is necessary to obtain both enamel and tooth root formation ages for precise estimation of human DOB, as discussed previously. The process of tooth formation has been examined in previous odontology studies. For example, Nolla (1960) checked 3402 X-ray photos of North American young persons aged between 25 and 279 months. Both the age and duration of tooth formation were determined by the sex of the person and the types of teeth. Smith (1991) reported that the tooth formation age of North Americans is different from that of people from other countries. Therefore, it is important to know the accurate completion age and maturing duration of tooth for Japanese people to estimate his/her birth year from tooth information. Many studies on tooth formation in Japanese people have been published, and these data were used in this study. Tanaka (1988), Nishihara and Daito (1989), Tanaka and Daito (1992), and Daito et al. (1997) published data on the enamel formation age in Japanese people. They discussed molar, central, wisdom, and lower molar teeth. We compiled the data for the enamel formation age from these four studies (Table 4). However, since the tooth root completion ages for the Japanese have not published yet, we had to use the data for North Americans summarized by Nolla (1960).

Table 4 Enamel formation age of each type of tooth, upper or lower, male or female, or averaged, for Japanese teeth.

		Enamel formation age of the Japanese						
		Boys		Girls		Average		
Type of tooth*		Left	Right	Left	Right	Left	Right	Reference
Upper tooth	1	3.9	3.9	3.8	3.8	3.8	3.8	Nishihara and Daito (1989)
	2	4.8	4.8	4.4	4.4	4.6	4.6	
	3	4.8	4.8	4.2	4.2	4.5	4.5	
	4	4.8	4.9	4.6	4.8	4.7	4.8	Tanaka (1988)
	5	6.0	6.1	5.9		6.0	6.0	
	6	2.7	2.6	2.7	2.8	2.7	2.7	
	7	6.6		6.5		6.5	6.5	
	8	11.7		11.4		11.5		Tanaka and Daito (1992)
Lower tooth	1	3.7	3.7	3.6	3.6	3.6	3.6	Nishihara and Daito (1989)
	2	4.1	4.1	3.9	3.9	4.0	4.0	
	3	4.7	4.7	4.2	4.2	4.4	4.4	
	4	5.3		5.0		5.2		Daito et al. (1997)
	5	6.1	6.2	5.9	6.0	6.0	6.1	
	6	2.6		2.6		2.6		
	7	6.7		6.4		6.5		
	8	12.3		12.3		12.3		Tanaka and Daito (1992)

*Type of tooth: 1–2: incisor, 3: canine, 4–5: premolar, 6–8: molar.

Determination of the DOB Using ^{14}C Data

The ^{14}C content in each carbonaceous fraction of the tooth reflects that of the atmospheric CO_2 at the time of tooth formation. The following procedure was used to estimate the DOB of individuals from the $F^{14}C$ value in each fraction:

(1) The $F^{14}C$ values of carbonaceous fractions were calibrated to calendar dates with the CALIBomb program developed by the Center for Climate, the Environment, and Chronology

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at Queen's University, Belfast, UK (http://www.calib.qub.ac.uk/CALIBomb/), using the IntCal13 data set in the period before 1950 and the bomb ^{14}C data set (NH zone 2) after 1950. Since we used original ^{14}C data sets, instead of smoothed ones, calibration results offered us candidates of multiple nearby ranges as the possible ages for most of the tooth samples. We thus averaged them as given in Table 1, with errors covering full possible age ranges. Also, the calibration program inevitably produced two possible age ranges, one in the rising part and the other in the falling part of the bomb curve. We selected the possible age ranges in one part of the bomb curve that is consistent with the real DOB of the tooth owner, as given in Tables 1–3.

(2) The formation age of relevant carbonaceous fraction separated from the tooth that was compiled from the previous studies is subtracted from the formation year of each carbonaceous fraction evaluated above to estimate the DOB.

RESULTS

Carbon Stable Isotope Ratios

Figure 2 shows the $\delta^{13}C$ values of the tooth enamel apatite, the root collagen, and the root apatite. The enamel $\delta^{13}C$ value ranged from –14.2‰ to –9.8‰, with an average value of –12.6 ± 1.0‰ for 40 teeth. The root $\delta^{13}C$ values were from –19.3‰ to –18.4‰ (average: –18.8 ± 0.3‰) for gelatin collagen from seven tooth roots, and from –14.5‰ to –14.0‰ (average: –14.3 ± 0.2‰) for root bioapatite from six tooth roots. The exact values are given in Tables 1–3.

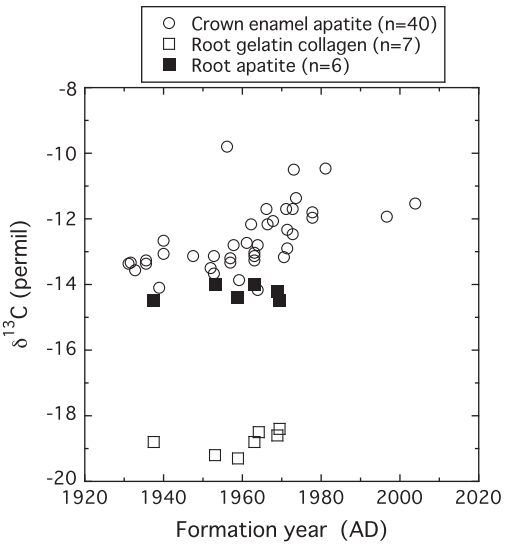


Figure 2 $\delta^{13}C$ values of crown enamel apatite, gelatin collage, and apatite of tooth root vs. formation year of each tooth fraction. The formation year was calculated from the true date of birth (DOB) of the tooth owner and average formation age for the relevant tooth fraction evaluated from data compiled from the previous studies (see text in detail).

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$F^{14}C$ Values of Tooth Enamel Apatite

Tables 1 and 2 list the experimental results and detailed information about the samples. Figure 1 shows $F^{14}C$ values obtained for enamel apatite components. For the samples whose enamel fractions were formed after 1955 with $F^{14}C$ values greater than 1.00, precise enamel formation ages were obtained. The average systematic deviation of the estimated age from the real age was -0.4 ± 2.1 yr, and the average of the absolute deviation between them was 1.7 ± 1.4 yr. The correlation coefficient between the true DOB and the estimated DOB was excellent ($R^2 = 0.976$; Figure 3). However, enamel fractions of 13 tooth samples were formed before the bomb-testing period (~1955), and they all showed the pre-bomb $F^{14}C$ values of less than 1.00 (Figure 1). The formation age estimation by ^{14}C analysis of tooth enamel for the pre-bomb period showed large errors, and the estimation of DOB did as well. The correlation was low between the true DOB and the estimated DOB for the 13 samples ($R^2 = 0.024$, Figure 3).

$F^{14}C$ values of the teeth from Japanese people may be lower than the accepted calibration data sets of IntCal13 and NH zone 2, since Japanese people eat seafood more abundantly than other nationalities. $F^{14}C$ values of seafood normally lower by about 5% than $F^{14}C$ values of atmospheric CO_2. However, no signal of such offset can be seen in Figure 1 that shows the relation of $F^{14}C$ value and formation year of tooth enamel, including 13 tooth enamel samples whose $F^{14}C$ values less than 1.00. $F^{14}C$ values (<1.00) of the 13 tooth enamel samples were quite consistent with IntCal13 data sets with 1σ errors of about ±0.0040 to ±0.0047. Thus, we can conclude that the seafood intake by Japanese people does not show any evident offsets in the $F^{14}C$ values of tooth carbonaceous fractions. In addition, our experimental results also suggest that the Suess effect, which refers to the ^{14}C depletion in atmospheric CO_2 as the result of dead CO_2 release in

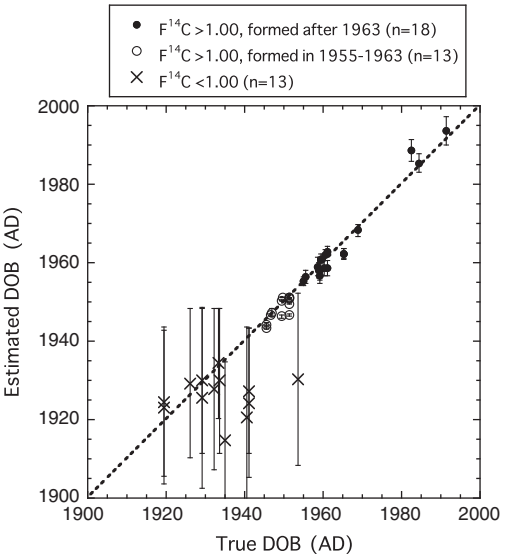


Figure 3 Estimated DOB vs. true DOB for crown enamel apatite. DOB of the tooth owner was estimated from enamel formation year deduced from its $F^{14}C$ value by comparison with IntCal13 and NH zone 2 atmospheric ^{14}C data sets covering post-bomb period, and average tooth enamel formation age evaluated in the previous studies (Table 3, see text in detail). The dotted line indicates expected DOB = true DOB.

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the atmosphere by fossil fuel combustion, is negligible in the evaluation of DOB values from relevant $F^{14}C$ values of tooth samples. In fact, the calibration data set NH zone 2 includes the Suess effect, since the NH zone 2 data is composed of atmospheric CO_2 from the 20th and 21st centuries (Hua et al. 2013).

$F^{14}C$ Values of Tooth Roots

Figure 4 shows the results of $F^{14}C$ values obtained from seven pairs of collagen and bioapatite components in the same tooth roots. Figure 4 also includes $F^{14}C$ value for the tooth enamel fraction from the relevant tooth. In Figure 4, the $F^{14}C$ values were plotted against the formation year of tooth roots. This figure clearly suggests the difference in timing for completing tooth crown formation and tooth root formation of the same tooth. The $F^{14}C$ values of gelatin collagen and those of bioapatite were almost consistent with each other and showed a similar temporal trend, with the correlation coefficient as high as $R^2 = 0.837$. However, a detailed comparison suggested systematic differences between the two carbon components, as discussed in the next section.

DISCUSSION

$\delta^{13}C$ Value of Tooth Enamel Apatite

The $\delta^{13}C$ values of –14.2‰ to –9.8‰ were obtained for 40 enamel apatite samples from Japanese teeth as shown in Figure 2. Alkass et al. (2011) reported that the $\delta^{13}C$ values of tooth enamel apatite samples were –15.4‰ to –13.9‰ for Swedish teeth, –11.8‰ to –10.1‰ for Uruguayan teeth, and –13.4‰ to –12.0‰ for Middle Eastern teeth. Alkass et al. (2013) also reported that the values were –10.2‰ to –8.4‰ for North American (Texas) teeth, and –7.8‰ to +0.1‰ for Mexican teeth. Thus, the $\delta^{13}C$ values for Japanese teeth are slightly higher than those for Swedish teeth, and lower than those for North American teeth. The variations in $\delta^{13}C$ values can be caused by the diet. For example, rather low $\delta^{13}C$ values of –15.4‰ to –13.9‰ for

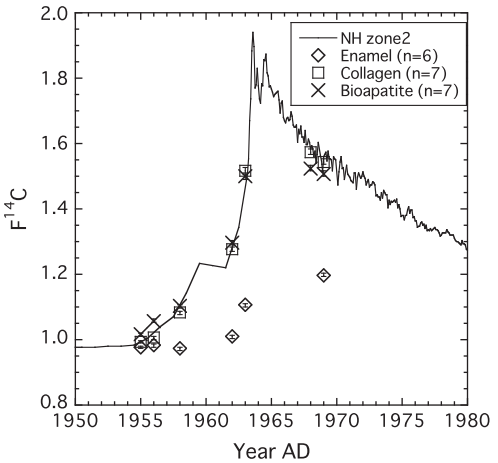


Figure 4 Annual variations of $F^{14}C$ values for three kinds of carbonaceous fractions separated from the same tooth, and $F^{14}C$ values of atmospheric CO_2 (NH zone 2) vs. the year of tooth root completion. Seven teeth with different formation years were analyzed. The three types of fractions were gelatin collagen, bioapatite separated from tooth roots, and tooth crown enamel apatite.

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Swedish teeth can be explained by the Swedish diet containing large amounts of potato with $\delta^{13}C$ value of around –26‰. On the other hand, the values of –10.2‰ to –8.4‰ for North American (Texas) teeth can be by the American diet containing corn with its $\delta^{13}C$ value of about –10‰. The $\delta^{13}C$ values of –14.2‰ to –9.8‰ for Japanese tooth enamel may be affected by the Japanese diet of seafood with $\delta^{13}C$ values of –16‰ to –13‰. Thus, it is acceptable that the $\delta^{13}C$ result for Japanese teeth is intermediate between the $\delta^{13}C$ values for Swedish and North American teeth.

Figure 2 suggests the possibility that the Japanese diet has changed over time. The $\delta^{13}C$ values ranged from –14‰ to –13‰, except for tooth 341 (–9.8‰), for the samples that were formed before around 1960, whereas the samples formed from 1960 to 1975 showed the more variable $\delta^{13}C$ values from –14‰ to –11‰. Finally, the samples formed after 1975 showed the $\delta^{13}C$ values higher than –12.5‰. Warinner and Tuross (2009) and Froehle et al. (2010) report a strong relationship between animal bone collagen and diet $\delta^{13}C$ values. van der Sluis et al. (2015) addressed the effects of diet on $\delta^{13}C$ values of human teeth. Due to fossil fuel combustion, CO_2 concentration is increasing by 0.3–0.4% per year in the later half of 20th century, as observed at Mauna Loa Observatory, at Hawaii Island (http://www.esri.noaa.gov/gmd/obop/mlo/). The $\delta^{13}C$ value of the emitted CO_2 is about –26‰, lowering the $\delta^{13}C$ value of atmospheric CO_2 (–7‰ to –9‰). The $\delta^{13}C$ value of teeth showed an opposite temporal trend (increase in $\delta^{13}C$ value with time), so the diet effect for the Japanese tooth could be stronger. However, we need to analyze more teeth to confirm this tendency in the $\delta^{13}C$ values. If the tendency is confirmed, the $\delta^{13}C$ of tooth samples will provide additional information to select the periods of tooth formation precisely.

Sample 341, the first molar, showed a higher $\delta^{13}C$ value (–9.8‰). Formation of the first molar starts at the age of zero or before birth and is completed at the age of around 2–3 yr old (Table 4). Fuller et al. (2006) studied $\delta^{13}C$ of hairs and nails of infants, and argued that there was an isotopic enrichment in the ^{13}C level during the breastfeeding age. Eerkens et al. (2011) also reported a similar result by using tooth collagen samples. Therefore, the high $\delta^{13}C$ value can be explained by the enrichment of nutrition in early childhood. Only one first molar was analyzed in this research, and we should analyze more first molar samples to confirm this speculation.

As shown in Figure 2, the average $\delta^{13}C$ values of apatite and gelatin collagen fractions separated from the same Japanese tooth roots were -14.3 ± 0.2‰ and -18.8 ± 0.3‰, respectively. This tendency in $\delta^{13}C$ value between the apatite and gelatin collagen fractions is similar to that reported by Sullivan and Kruger (1981). The difference in the two average values was 4.6 ± 0.5‰ for six tooth root samples. The $\delta^{13}C$ values of root apatite were about 1% lower than those of enamel apatite for the tooth formation years older than 1960.

$F^{14}C$ Values for Collagen and Bioapatite from Tooth Roots

Figure 4 shows the variation in atmospheric $F^{14}C$ values (NH zone 2 data set) and the $F^{14}C$ results of root analysis. The $F^{14}C$ values of the root collagen were consistently lower than those of the root bioapatite when the roots were formed before the bomb peak (1963) except for the youngest one (sample #54), and the opposite was observed when the roots were formed after 1963. This means that the formation age of root apatite is later than that of root collagen.

Generally, the formation age of tooth is possibly determined by using $F^{14}C$ values of root apatite and root collagen without knowing the $F^{14}C$ value of tooth enamel. The time difference

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of the formation ages of root apatite and root collagen discussed above can be used to assign the $F^{14}C$ value belonging to rising part or falling part of the ^{14}C variation curve, without temporal information by tooth enamel analysis. However, sample #54 (real DOB: 1946.7, Tables 1 and 3) has offered an example that it is difficult to determine correctly the formation age of tooth by using only the pair of $F^{14}C$ values for root apatite and root collagen. For sample #54, the DOB estimated from root collagen was 1945.6–1946.8 or 1950.9–1954.7, and that from the root apatite was 1945.6–1945.7 or 1951.4–1955.4 (Table 3). Because the root collagen was formed earlier than the root apatite, it is mistakenly concluded that the DOB was 1952 ± 2, whereas the real DOB was 1946.7. The time gap between the formation of root collagen and root apatite is considerably shorter than that between the enamel and the root, and it is difficult to estimate the age using only two carbonaceous fractions from the root. Thus, the analysis of the tooth formation age including both tooth enamel and tooth root portions is preferable. In fact, Kawanishi (1959) showed that there is a large variation in root formation age among individuals. In addition, Cook et al. (2006) reported that there is possible secondary dentition in the tooth root. Therefore, age estimation using only tooth roots may lead to incorrect conclusions. The $F^{14}C$ values for tooth root components should be used to support the accurate age estimation by means of tooth enamel analyses.

Age Estimation by Combination of Tooth Enamel and Root Bioapatite

Age estimation using the $F^{14}C$ values measured for both tooth enamel apatite and root bioapatite was examined for six tooth samples, as summarized in Table 3. For sample #299 (real DOB: 1919.5), the $F^{14}C$ value of tooth enamel was 0.9766 ± 0.0046, and the estimated DOB values were 1659 ± 26, 1757 ± 46, 1844 ± 22, and 1923 ± 20. Considering the $F^{14}C$ value of bioapatite (1.0166 ± 0.0048, and the estimated DOB is 1937.8 ± 0.4), the DOB expected from tooth enamel was in the period 1923 ± 20 and was consistent with the true DOB of 1919, though the estimation error is large.

For sample #58 (real DOB: 1935.1), the tooth enamel $F^{14}C$ value was 0.9826 ± 0.0044, and the estimated DOB values were 1712 ± 57, 1832 ± 48, 1915 ± 20, and 1940 ± 3. The $F^{14}C$ value of root apatite was 1.0571 ± 0.0049, and the estimated DOB values were 1939.1 ± 0.4, 1987.4 ± 0.2, and 1989.7 ± 1.9. Thus, it is more likely that the DOB was 1940 ± 3 estimated from enamel and 1939.1 ± 0.4 from the tooth root.

For sample #325 (real DOB: 1941.1), the tooth enamel $F^{14}C$ value was 0.9721 ± 0.0046, and the estimated DOB values were 1530 ± 18, 1555 ± 3, 1649 ± 30, 1758 ± 41, and 1927 ± 16. However, the $F^{14}C$ value of root bioapatite was 1.1020 ± 0.0052 and the estimated DOB values were 1940.2 ± 0.3 and 1980.7 ± 2.5. Hence, the DOB of 1927 ± 16 from tooth enamel and that of 1940.2 ± 0.3 from the root were likely.

For samples #308 (real DOB: 1945.5), #54 (real DOB: 1946.7), and #317 (real DOB: 1951.5), $F^{14}C$ values for both tooth enamel and root bioapatite showed a clear bomb effect ($F^{14}C > 1.00$) and the expected DOB values and corresponding real DOB values were almost consistent with each other, as summarized in Table 3.

The estimated DOB ranges may not always include the real DOB of individuals. In particular, the root bioapatite may not show the actual DOB because of the variations in root formation periods and possible secondary dentine. However, in many cases, the estimated DOB of tooth enamel can be narrowed to a single range by the assistance of the $F^{14}C$ values from tooth root data.

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Age Estimation by Tooth Enamel Apatite

Teeth with Enamel Apatite Fractions Whose $F^{14}C$ Values Were Less Than 1.00

The $F^{14}C$ values between atmospheric and 13 pre-bomb tooth enamel values did not show good agreement (Figure 3). This may be mainly because of the temporally flat pattern of atmospheric ^{14}C variation, compared with the bomb-pulse ^{14}C pattern. There were only slight temporal changes in the atmospheric ^{14}C; thus, the estimated DOB values of samples had wide age ranges possible. For these 13 samples, the errors for age estimations were so large that it was impossible to determine a short DOB period. It is expected that among the teeth with $F^{14}C$ values less than 1.00 in the tooth enamel, a fair estimate of DOB would be given if the tooth root showed levels exceeding 1.00. However, for samples #299 (real DOB: 1919.5) and #58 (1935.1), the estimated DOB values from the $F^{14}C$ value of the tooth root did not show acceptable agreement with the real DOB (Table 3). Therefore, for pre-bomb samples, it is normally necessary to combine ^{14}C data and information obtained from other forensic investigation methods.

For tooth #341 (real DOB: 1953.5, first molar), the $F^{14}C$ value of tooth enamel was measured as 0.9802 ± 0.0044. Since the first molar normally completes enamel formation at the age of 2.7 yr old, enamel formation should have ended in 1956.2. Thus, tooth #341 should have some effects from bomb-produced ^{14}C. However, its $F^{14}C$ value denied the influence from the bomb ^{14}C. The expected enamel completion year of 1956.2 is on the border for the presence of bomb influence, and we should analyze more first molar samples to confirm this trend.

Teeth with Enamel Apatite Fractions Whose $F^{14}C$ Values Were Greater Than 1.00

The average systematic deviation of the estimated DOB value from the correct value was -0.4 ± 2.1 yr for 31 tooth enamel samples with $F^{14}C$ values >1.00 (Figure 3), and the average of the deviations (i.e. the absolute distance of the estimated DOB value and the correct value) was 1.7 ± 1.4 yr. This accuracy of the DOB estimation for Japanese samples is similar to the previous studies in foreign countries. The absolute deviation for Swedish teeth (Spalding et al. 2005) was 1.6 ± 1.3 yr, and that of North American teeth (Alkass et al. 2013) was 1.8 ± 1.3 yr. There were no significant differences between the results from our tooth samples and those from previous studies. Consequently, there were no specific local or regional effects that influenced the ^{14}C content in Japanese teeth, and age estimation by Japanese teeth is as accurate as that in previous studies in other countries if we use the appropriate tooth formation age for Japanese teeth.

To discuss the age estimation results by teeth in more detail, post-bomb samples were divided into pre-bomb-peak teeth, formed between 1955 and 1963, and post-bomb-peak teeth, formed after 1963.

Teeth with Enamel Formation Ages between 1955 and 1963

A variation of $F^{14}C$ values was relatively large for 13 tooth enamel samples formed during 1955–1963. In this period, the correlation coefficient, R^2, was 0.667 ($n = 13$, Figure 3), though the absolute deviation between the estimated DOB and the real DOB was not so large as 1.5 ± 1.3 yr on average. The low correlation coefficient may be caused by the quick increase in atmospheric ^{14}C. Similar changes in $F^{14}C$ values have been reported previously. The rapid changes in $F^{14}C$ values of atmospheric CO_2 caused large fluctuations in the $F^{14}C$ values in plants and animals (Alkass et al. 2011). Their results showed that the average deviation was 1.9 ± 1.2 yr with a R^2 value of 0.751 ($n = 10$) for tooth enamel samples formed during the rising part of the bomb ^{14}C curve.

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Teeth with Enamel Formation Ages after 1963

During the declining part of the bomb ^{14}C curve, $F^{14}C$ values of 18 tooth enamel samples were similar to those of atmospheric CO_2, and the average absolute deviation in the estimation was 1.8 ± 1.4 yr. The estimated DOB values for all post-bomb-peak teeth showed excellent agreement with the true DOB with R^2 of 0.969 ($n = 18$). The true DOB and the estimated DOB are compared in Figure 3. No local effect was observed because there was no specific change in the variation of the $F^{14}C$ values of tooth enamels compared with atmospheric ^{14}C change.

CONCLUSION

$F^{14}C$ values greater than 1.0 (the sample is obviously influenced by bomb-produced ^{14}C) of tooth enamel apatite can be used to obtain the DOB of individuals accurately. To select and confirm one correct DOB value among several possible age ranges suggested by the $F^{14}C$ value of tooth enamel, the carbon fractions in the root portion of the same tooth should be utilized. Gelatin collagen and bioapatite from the tooth root are suitable because they both were formed 6–8 yr after the enamel completion, although the root bioapatite seems to be formed a bit later than the root collagen. It is preferable to use tooth enamel apatite and root bioapatite because these tissues are resistant to natural weathering.

The average absolute deviation between the estimated DOB and the real DOB in age estimation using Japanese tooth enamel is 1.7 ± 1.4 yr, which is similar to those obtained in previous studies conducted in other countries. In the case of Japanese teeth, samples formed after the bomb peak (1963) may be affected by the change in diet, as suggested by the small but evident increase of $\delta^{13}C$ values, although this effect on ^{14}C content of the tooth is small and negligible in the DOB estimation when looking at the ^{14}C variations in the tooth samples. On the contrary, for age estimation from Japanese teeth formed in the post-bomb period, the $\delta^{13}C$ value of the tooth enamel fraction may be at least applicable as a guide to discriminate between tooth formation during the rising and the falling part of the bomb-effect curve. In conclusion, age estimation using Japanese teeth is accurate, and the results reported herein will assist with criminal investigations and forensic work to identify cadavers.

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