Original

Estimating Living Age Using Stable Isotopes in Japanese Radicular Dentin

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(Accepted for publication, December 17, 2019)

Abstract: We investigated the feasibility of estimating the living age of Japanese individuals and distinguishing sex on the basis of results from stable isotope analysis of stable carbon (13C) and nitrogen (15N) isotopes in radicular dentin. In this study, collagen fibers were extracted from the radicular dentin of mandibular second molars in Japanese individuals born between 1891 and 1964, and the carbon and nitrogen isotope ratios (δ13C and δ15N values, respectively) were calculated. The results showed that regression formulae for δ13C and δ15N values were highly reliable for estimating the living age of Japanese individuals, but no sex difference was evident. Use of radicular dentin thus has potential as a technique for estimating the living age of Japanese individuals, whose diet has changed greatly over the years.

Key words: Stable isotope analysis, Japanese, Carbon isotope ratio (δ13C), Nitrogen isotope ratio (δ15N), Estimation of living age

Introduction

Age estimation is an essential part of identifying unidentified bodies. Even after the soft tissues have decomposed, the teeth, bones, and other hard tissues retain their morphology and are thus often used for age estimation. Observations of the degree of ossification of the epiphyseal cartilages, the number of carpal bones that have appeared, and the level of calcification and eruptive state of teeth enable estimation of the stage of growth and development with a high degree of accuracy.1-4. Observations of the degree of fusion of the cranial suture and of the levels of tooth abrasion and pulp cavity stenosis also enable estimation of adult age.5-7. However, the wide individual variations between humans mean that age estimates made using these morphological characteristics entail a substantial margin of error.8,9. The establishment of other methods of estimating age based on evidence from qualitative analytical data in addition to these morphological techniques is therefore highly desirable to enable more accurate estimations of age.

Stable isotope analysis is a technique used in environmental science and ecology to investigate environmental changes and food chains.10-13. Stable isotopes are substances with identical chemical properties but slightly different mass due to differences in the number of neutrons in the atomic nucleus, and these isotopes occur in nature in specific proportions.13,14. The relative proportions of these stable isotopes are known as stable isotope ratios, and are known to reflect differences in factors such as climate, soil, and diet.14,15. Regan et al. carried out stable isotope analysis using stable carbon (13C) and oxygen (18O) isotopes, and reported that this approach was extremely effective in distinguishing between the remains of American soldiers and Vietnamese individuals.14. Stable isotope analysis has also been used to distinguish soldiers who died on European battlefields in World War II, with one study reporting that the stable isotope ratios of strontium and oxygen in teeth differed significantly between British soldiers and soldiers of other European nationalities.16. In addition, stable isotope analyses of carbon (13C) and nitrogen (15N) isotopes have been used to analyze a variety of bones found in Hokkaido, contributing to our understanding of social structures in the prehistoric Okhotsk culture.17. The presence of 13C is strongly associated with diet and living environment, while 15N is more highly concentrated further up the food chain, accumulating in the proteins that represent the main constituent of collagen fibers.18,19,20. The collagen fibers in human bony tissue are formed and reformed throughout the lifetime of the individual as a process known as remodeling, in which bone is broken down by osteoclasts and regenerated by osteoblasts.21-24. Teeth, however, do not undergo such remodeling, meaning that the composition of the collagen fibers created at the time of tooth formation remains almost unchanged from the time of tooth eruption.25-28. Dentin in particular contains a large amount of collagen fibers, which are an organic substance, and only a small amount of new secondary dentin is added over the course of a lifetime.29,30. The carbon and nitrogen isotope ratios (δ13C and δ15N values, respectively) in tooth dentin are thus highly likely to provide an important clue for estimating living age.

Our objective in this study was to investigate the feasibility of estimating living age and distinguishing sex on the basis of the results of stable isotope analyses for 13C and 15N isotopes in radicular dentin from the mandibular molar radicular dentin of Japanese individuals.

Materials and Methods

Specimens

The study sample comprised 32 mandibular second molars from Japanese individuals born between 1891 and 1964. These teeth were ob-
Stable isotope analysis of $^{13}$C and $^{15}$N

All specimens were prepared for carbon-nitrogen stable isotope analysis using the modified gelatinization method $^{28,29}$. First, approximately 0.5–1.0 g of radicular dentin was extracted from each specimen, using a drill fitted with a diamond disk. This dentin was cleaned ultrasonically with ultrapure water, then immersed in 0.1 N NaOH to remove exogenous organic matter. Cleaning with ultrapure water was again performed, then the tissue was freeze-dried and fragmented into small chunks. These fragmented specimens were then decalcified in 1.2 N HCl solution at 4°C. The remaining portions were extracted and gelatinized for 12 h at 90ºC, after which they were filtered with a glass fiber filter and freeze-dried.

The stable isotopes in approximately 0.4 mg of gelatinized specimens were measured by isotope-ratio mass spectrometry (IRMS) using a mass spectrometer (Delta 5 advantage, Thermo Fisher Scientific Inc, Waltham, MA, USA) connected to an elemental analyzer (Flash 2000, Thermo Fisher Scientific Inc, Waltham, MA, USA) with an interface (ConFlo 3, Thermo Fisher Scientific Inc, Waltham, MA, USA) at the Laboratory of Radiocarbon Dating of the University Museum, the University of Tokyo. The analytical standard deviation (SD) was approximately 0.1‰ for both carbon and nitrogen. Elemental concentrations and isotope ratios were calibrated against laboratory alanine and histidine standards.

The association between $\delta^{13}$C and $\delta^{15}$N and living age was evaluated by regression analysis using SPSS statistical software (IBM Co, New York, NY, USA).

Results

Table 2 shows the $\delta^{13}$C and $\delta^{15}$N values and the atomic C/N ratio for each specimen. According to Van Klinken et al., a specimen is sufficiently pure if the C/N ratio is 2.9–3.6 $^{25}$, and all the specimens in this study were of sufficient purity for analysis according to this standard. Fig. 1 shows the $\delta^{13}$C and $\delta^{15}$N values in tooth samples from Japanese individuals. Wide variations were seen in the distribution of $\delta^{15}$N values for Japanese individuals born between 1891 and 1964. Fig. 2 shows the $\delta^{13}$C and $\delta^{15}$N values by year of birth. The $\delta^{13}$C value exhibited a linear association with birth year using the regression line equation $y = 13.568x + 2189.8$ ($R^2 = 0.4232$), and that for $\delta^{15}$N value also exhibited a linear association with birth year with the regression line equation $y = -13.865x + 2106.3$ ($R^2 = 0.507$). Comparisons by sex found no gender differences for the $\delta^{13}$C and $\delta^{15}$N values at any age (Fig. 3).
Figure 1. Stable isotope ratios of $^{13}\text{C}$ and $^{15}\text{N}$ in tooth dentin from Japanese individuals.
○: Japanese individuals born between 1891 and 1964. ×: Contemporary Japanese individuals (Kusaka et al.)

Figure 2. Associations between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the tooth dentin of Japanese individuals and age
As the human race spread around the world, it developed dietary patterns in line with the characteristics of the local environments. In modern times, however, the globalization of food production has led to the rapid homogenization of these formerly diverse dietary habits. With the advance of global urbanization and the Westernization of lifestyles, the dietary patterns of industrialized countries appear to be increasingly converging toward a Western diet. This trend is very obvious in Japan, and has undergone a dramatic transformation in which the rice-based diet prevalent for over 2000 years is being replaced by a wheat-based diet centered around bread and pasta. Meat consumption also rose from approximately 300 g/person/year in the 1910s to approximately 5.2 kg/person/year in the 1960s, and this amount has since only continued to increase. We found wide variations in the distribution of δ¹³C and δ¹⁵N values of Japanese individuals born between 1891 and 1964. This contrasts with a report from Kusaka et al. that the distribution of δ¹³C and δ¹⁵N values in the contemporary population is concentrated at a single point. This suggested that changes in the distribution of stable isotope ratios seen in modern Japanese since the 1890s may reflect the huge changes that have occurred in the Japanese diet. The regression equations for δ¹³C and δ¹⁵N values may thus be sufficiently reliable for use in estimating the living age of Japanese individuals. However, no gender differences were evident for stable isotope ratios at any age. Tsutaya et al. used stable isotope analysis for research into the Okhotsk culture, and suggested that dietary differences between men and women reflecting differences in social roles may allow the sex of individuals to be distinguished. However, differences in the social roles of Japanese women were only directly connected to dietary differences up until the 17th Century. The absence of a significant gender difference in stable isotope ratios in modern Japan may be due to the absence of any great differences in diets between the sexes.

Stable isotope ratios in hair and nails enable the analysis of differences in the living environment, diet, and climate and soil of the region of origin, and various studies have already pointed out the great value of this approach in the process of narrowing down the identity of unidentified bodies to specific individuals. However, the climate in most of Japan is warm and wet, and unidentified bodies discovered after earthquakes and other mass disasters often consist only of bones and teeth. Dentin is surrounded by enamel and cementum, and the collagen fibers contained undergo very little denaturation. This represents one major advantage of our method using dentin in living age estimation. However, this method of evaluation has some limitations. Font et al. reported that although soldiers from the island nation of Great Britain could be distinguished from those from the European mainland, separating the countries of origin of those European soldiers was impossible. Japan is an island nation that was formerly closed off from the rest of the world, but the major dietary changes that have occurred particularly since the 1900s mean that using the stable isotope ratios of ¹³C or ¹⁵N for living age estimation is highly likely to prove feasible even for members of the same Japanese population. Our results suggested that this may offer a promising method.

Acknowledgments

This study was supported by a research grant from the Japan Society for the Promotion of Science; contract grant number: 18K09643, 17K11808 and was partly supported by a grant of Multidisciplinary Research Center for Jaw Disease (MRCJD): Achieving Longevity and Sustainability by Comprehensive Reconstruction of Oral and Maxillofacial Functions.
Conflict of Interest

The authors have no COI exists.

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