Carbon, nitrogen, and sulfur stable isotopic reconstruction of human diet in a mountainous woodland village in Sendaiji in premodern Japan

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Abstract The reconstruction of everyday diets in villages is important for understanding the diversity of diets and commerce networks of food items in premodern Japan. However, premodern diets in villages have not been well studied compared with those in cities. In this study, stable isotope analyses were performed on 23 adult human skeletons excavated from Sendaiji, a mountainous woodland village of underground Christians in Osaka in premodern Japan. No significant isotopic differences was found between individuals identified as Buddhists and those identified as Christians or between females and males. The total mean carbon, nitrogen, and sulfur isotope ratios were –21.1 ± 0.4‰, 11.6 ± 1.0‰, and 8.9 ± 1.3‰, respectively. The carbon isotope ratios in Sendaiji were the lowest among the studied premodern populations probably because these individuals consumed woodland foods that are affected by the canopy effect. No significant correlation between sulfur and nitrogen isotope ratios was apparent, suggesting that there was little contribution from marine foods or marine fertilizers to the diet of individuals in premodern Sendaiji. The relatively high nitrogen isotope ratios in Sendaiji were possibly because of the denitrification in paddy rice fields, ammonium uptake by paddy rice, use of animal fertilizers, and/or consumption of freshwater fish. To our knowledge, this is the first detailed bioarchaeological study of the premodern diet in a mountainous village in western Japan.

Key words: dietary reconstruction, Edo period, Sendaiji, stable isotope analysis, underground Christian

Introduction

Although the diet of townspeople in the Edo period (AD 1603–1867) in Japan has been reconstructed in detail, the diet of ordinary people living in villages has not been studied in similar depth. Historical (Hanley, 1997; Ehara et al., 2009; Harada, 2009), archaeological (Vaporis, 1998), and bioarchaeological (Kusaka et al., 2011; Tsutaya et al., 2016a; Maruyama et al., 2018) studies have revealed that the diet of ordinary people in the Edo period mainly comprised C3 crops, such as rice and vegetables, and fishes and poultry were sometimes consumed. However, most of this evidence has come from larger cities, such as Edo (present-day Tokyo), Osaka, and Kyoto, and the everyday diet of people living in villages is not well documented. Although there are some historical documents describing the menus of feasts and ceremonies in villages (Masuda and Ehara, 2005; Yoda et al., 2012), these dietary menus usually differ from everyday diets.

The reconstruction of the everyday diet in villages is important for understanding the diversity of diets and commerce networks of food items in the Edo period. Efficient commerce networks had been developed in the Edo period, and people could purchase foods produced in different regions of Japan (Hanley, 1997; Ehara et al., 2009; Harada, 2009). Conversely, people in the Edo period had greater regional diversity in their diet than people in present-day Japan (Yoned et al., 2011; Kusaka et al., 2016). Bioarchaeological investigations can provide direct evidence of food items consumed by ordinary people in the past, an issue that is sometimes difficult to reconstruct through historical studies.

This study investigates diets in Sendaiji, a mountainous woodland village in Osaka in premodern Japan, by analyzing the carbon, nitrogen, and sulfur stable isotope ratios in excavated human skeletons. Sendaiji provides an ideal first case for the detailed bioarchaeological reconstruction of diets in a mountainous village that is located far from the big cities in premodern western Japan. Sendaiji is also known as a village of underground Christians (kakure Christians).
Christianity was imported into Japan in the mid-16th century but was prohibited after the late 16th century, and those of Christian faith were executed by the Toyotomi and Tokugawa Shogunates. The faithful kept their religious beliefs secret to escape execution, and were thus known as 'underground Christians.'

Sendaiji and underground Christians

Sendaiji is located in the Settsu mountain area of Ibaraki city, Osaka, Japan (Figure 1). The village lies at an altitude of approximately 250 m, and the Saho and Ohiwa rivers run nearby.

Sendaiji is known as a village of underground Christians. A famous Christian lord (daimyo), Ukon Takayama, reigned in this area during the late 16th century. The premodern burial stones of Christians, Christian medals, and icons have been discovered in this village, and have been dated to the 1590s–1620s (Osaka Center for Cultural Heritage, 2015). After the prohibition of Christianity in the late 16th century and continuing executions after the Shimabara rebellion in 1637, those of the Christian faith kept their religious beliefs secret and went underground. Underground Christians behaved in public like Buddhists but maintained their religious beliefs and performed ceremonies secretly.

The Sendaiji site cluster was discovered during the construction of the Shin-meishin highway, which connects Nagoya and Kobe, and was excavated during 2012–2013. A total of four sites—Sendaiji West, Sendaiji Kurusu-yama, Sendaiji Shisaka, and Hinato—were discovered and dated as originating from the 10th to the 19th centuries (Osaka Center for Cultural Heritage, 2015). The Sendaiji West site is divided into sectors 3/4, 5, and 6. Among these, the Sendaiji West site sector 6 (SW6) and the Sendaiji Kurusu-yama site (SK) were the targets of interests of this study. The individual descriptions of the other sites in the Sendaiji site cluster can be found in the excavation report (Osaka Center for Cultural Heritage, 2015).

A total of 37 burials were found at the SK site, and some of them appeared to be of Christians. Christian burials were a rectangular pit, and human bodies were buried in a supine position, similar to the other Christian burials excavated at the Shimo-otowa and Takatsuki Castle sites in Osaka; these burials were clearly different from the contemporary Buddhist burials (Osaka Center for Cultural Heritage, 2015). However, no grave goods specific to Christians were found at the SK site. Excavated grave stones and radiocarbon dating of a human skeleton suggest that the SK cemetery was used from the late 16th to early 17th centuries (Osaka Center for Cultural Heritage, 2015). Because the chronological period of the SK site spans before and after the prohibition of Christianity in premodern Japan, the SK skeletons analyzed in this study might not necessarily be those of underground Christians.

Stable isotope analysis

Carbon and nitrogen stable isotope analyses have been used for dietary reconstruction in the past (Lee-Thorp, 2008; Makarewicz and Sealy, 2015). The carbon stable isotope ratios ($\delta^{13}C$ values) of terrestrial higher plants systematically differ according to the type of photosynthesis: C₄ plants typically exhibit >10‰ higher $\delta^{13}C$ values than C₃ plants (O’Leary, 1988). Plants living under closed canopy forests usually have $\delta^{13}C$ values that are several permille lower than those living in drier open habitats, a phenomenon known as the canopy effect (Medina and Minchin, 1980; van der Merwe and Medina, 1991). These differences are passed on to consumers within the same ecosystem, with a slight increase (isotopic enrichment).

Nitrogen stable isotope ratios ($\delta^{15}N$ values) indicate stepwise enrichments within the food chain (Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984). Marine organisms, such as fishes and sea mammals, have higher $\delta^{15}N$ values than terrestrial organisms, and freshwater fishes have intermediate values (Schoeninger et al., 1983; Schoeninger and DeNiro, 1984). Terrestrial plants also tend to have elevated $\delta^{15}N$ values when they are fertilized with organic fertilizers, such as fermented fish and animal excreta (Szpak, 2014).

Sulfur stable isotope ratios ($\delta^{34}S$ values) are fairly homogenous in marine organisms (from +15‰ to +19‰) due to the uniformity of the marine water $\delta^{34}S$ value (Rees et al., 1978), but those of terrestrial and freshwater vary widely (from −20‰ to +20‰) (Nehlich, 2015). Therefore, $\delta^{34}S$ values have been used as a proxy for marine food consumption. Plants grown using marine fertilizers also reflect the marine
signal in their $\delta^{34}S$ values (Szpak et al., 2019).

Stable isotopic differences among food sources are reflected in the tissues of their consumers, which exhibit specific isotopic differences. The carbon, nitrogen, and sulfur stable isotope ratios in consumers’ tissues are typically 5‰, 2–6‰, and 0.5‰ higher than those in tissues of prey (Lee-Thorp, 2008; Nehlich, 2015), although these values vary widely due to the nutritional status of consumers and the macronutrient composition of the prey tissues (Howland et al., 2003; Vanderklift and Ponsard, 2003; Robbins et al., 2005). The stable isotope ratios in bone collagen reflect those of the protein fraction of the diet (Tieszen and Fagre, 1993). By considering these factors, the palaeodiet, especially for dietary protein sources, can be reconstructed by measuring the stable isotope ratios of human skeletal collagen (e.g., Yoneda et al., 2004; Kusaka et al., 2011; Tsutaya et al., 2016a).

Materials and Methods

Bone samples

Bone samples were taken from 18 and 5 adult individuals from the SW6 and SK sites, respectively (Table 1). All the skeletons from the SW6 and SK sites were buried in Jodo and Christian burials, and are therefore regarded as Buddhists and Christians, respectively. The sex of the adults was estimated from the morphology of the pelvis (Phenice, 1969; Bruzek, 2002) and cranium (Walker, 2008).

Stable isotope analysis

Collagen was extracted from bone samples by using a method described by Tsutaya et al. (2017). Bone pieces weighing 100–300 mg and an additional 250–650 mg were sampled for carbon/nitrogen and sulfur stable isotope analyses, respectively. After the surfaces were cleaned with a dental drill and drill bit, the bone samples were soaked in 0.2 M NaOH to remove exogenous organic matter. Bone samples were washed with pure water, freeze-dried, and decalcified with 0.25 N HCl at 4°C for a few days. The decalcified samples were washed with pure water and then heated in weak HCl solution (pH 4.5) at 80°C for >48 h for gelatinization. Gelatinized samples were filtered using a glass fiber filter (Whatman GF/F), and the resultant gelatin samples were freeze-dried. The NaOH treatment for samples for sulfur isotope analysis was done after decalcification following the method of Tsutaya et al. (2018).

Carbon and nitrogen stable isotope ratios were measured from approximately 350 μg of extracted gelatin by elemental analyzer–isotope ratio mass spectrometry (EA-IRMS; Thermo Flash 2000 elemental analyzer, Thermo ConFlo III interface, and Thermo Delta V mass spectrometer) at the University Museum, University of Tokyo, Japan. All samples were measured in duplicate. Stable isotope ratios were calibrated by referring to the proposal by Szpak et al. (2017). The $\delta^{13}C$ and $\delta^{15}N$ values were calibrated against the laboratory working standard (L-alanine: $\delta^{13}C = –19.6 \pm 0.2$‰; $\delta^{15}N = 8.7 \pm 0.2$‰) purchased from SI Science Co., Ltd. (Saitama, Japan), whose values were determined by the NBS 19 and the International Atomic Energy Agency (IAEA) Sucrose ANU (calibrated against Pee Dee Belemnite) and IAEA N1 and IAEA N2 (calibrated against AIR) international standards, respectively. On the basis of repeated measure-

| ID  | Site | Burial | Sex   | Element   | $\delta^{13}C$ (%) | $\delta^{15}N$ (%) | $\delta^{34}S$ (%) | %C | %N | %S   | C/N | C/S | N/S | Yield (%) |
|-----|------|--------|-------|-----------|-------------------|-------------------|-------------------|----|----|------|-----|-----|-----|-----------|
| sj13| SW6  | 13     | Male  | Long bone | –21.5             | 10.9              | –                  | 41.5| 14.4| –    | 3.4 | –   | –   | 2.1       |
| sj15| SW6  | 15     | Male  | Rib       | –20.9             | 12.6              | 7.2                | 44.1| 16.2| 0.144| 3.2 | 816.9| 258.3| 11.0      |
| sj18| SW6  | 18     | Female| Long bone | –21.3             | 10.9              | –                  | 45.8| 16.7| –    | 3.2 | –   | –   | 6.0       |
| sj19| SW6  | 19     | Male  | Rib       | –20.5             | 13.7              | 9.2                | 43.7| 15.9| 0.147| 3.2 | 792.4| 247.6| 7.3       |
| sj20| SW6  | 20     | Female| Phalange  | –20.1             | 11.2              | –                  | 46.4| 17.1| –    | 3.2 | –   | –   | 17.1      |
| sj24| SW6  | 24     | Male  | Rib       | –20.7             | 13.3              | –                  | 44.1| 15.7| –    | 3.3 | –   | –   | 3.5       |
| sj25| SW6  | 25     | Female| Rib       | –20.9             | 11.3              | 9.6                | 45.9| 16.9| 0.147| 3.2 | 833.4| 263.1| 16.0      |
| sj26| SW6  | 26     | Female| Rib       | –20.9             | 12.5              | –                  | 45.0| 16.2| –    | 3.2 | –   | –   | 5.1       |
| sj27| SW6  | 27     | Female| Rib       | –21.1             | 11.4              | –                  | 45.1| 16.6| –    | 3.2 | –   | –   | 5.2       |
| sj30| SW6  | 30     | Male  | Rib       | –21.3             | 11.6              | –                  | 44.2| 15.8| –    | 3.3 | –   | –   | 4.5       |
| sj35| SW6  | 35     | Unknown| Rib     | –22.3             | 11.7              | –                  | 42.8| 13.6| –    | 3.7 | –   | –   | 3.6       |
| sj36| SW6  | 36     | Female| Rib       | –21.2             | 11.1              | –                  | 44.2| 16.1| –    | 3.2 | –   | –   | 3.7       |
| sj40| SW6  | 40     | Unknown| Rib     | –20.6             | 12.4              | –                  | 45.4| 16.6| –    | 3.2 | –   | –   | 9.5       |
| sj91| SW6  | 91     | Male  | Rib       | –21.5             | 10.9              | –                  | 44.1| 15.7| –    | 3.3 | –   | –   | 2.1       |
| sj103| SW6 | 103    | Unknown| Rib     | –21.3             | 10.0              | –                  | 44.5| 15.7| –    | 3.3 | –   | –   | 3.1       |
| sj106| SW6 | 106    | Female| Rib       | –21.4             | 11.7              | 10.4               | 43.5| 15.9| 0.148| 3.2 | 782.8| 245.6| 11.3      |
| sj108| SW6 | 108    | Unknown| Rib + cranium | –20.8             | 10.5              | 8.1                | 45.4| 16.2| 0.165| 3.3 | 733.3| 225.0| 5.7       |
| sj119| SW6 | 119    | Female| Rib       | –21.9             | 11.5              | –                  | 43.4| 14.4| –    | 3.5 | –   | –   | 4.4       |
| sk7 | SK   | 7      | Unknown| Rib      | –20.7             | 10.1              | –                  | 42.2| 15.5| –    | 3.2 | –   | –   | 3.6       |
| sk27| SK   | 27     | Unknown| Rib      | –20.9             | 12.7              | –                  | 39.9| 14.1| –    | 3.3 | –   | –   | 1.9       |
| sk29| SK   | 29     | Male  | Rib       | –21.4             | 10.8              | –                  | 43.4| 14.8| –    | 3.4 | –   | –   | 1.9       |
| sk39| SK   | 39     | Female| Rib       | –21.2             | 11.7              | –                  | 43.4| 14.8| –    | 3.4 | –   | –   | 1.8       |
| sk40| SK   | 40     | Female| Rib       | –20.9             | 12.2              | –                  | 43.2| 14.3| –    | 3.5 | –   | –   | 2.7       |
ments of the calibration standard, for both $\delta^{13}C$ and $\delta^{15}N$, precision was determined to be $\pm 0.1\%$ standard deviation (SD). Accuracy or systematic error was not determined.

Approximately 25 mg of the extracted gelatin was loaded into tin capsules and measured for sulfur isotopes by EA-IRMS (Thermo Flash 2000 elemental analyzer, Thermo ConFlo interface, and Thermo Delta Plus Advantage mass spectrometer) at SI Science Co., Ltd. Elemental concentrations and isotope ratios were calibrated against the international standards (NBS123, IAEA S-1, and IAEA S-2). The analytical precision was determined to be $\pm 0.3\%$ SD.

**Statistics**

All statistical test was done by using R software version 3.5.1 (R Core Team, 2018).

### Results

**Preservation of bones**

The preservation of collagen in the bones varied (Table 1). Overall, one (sj35) of the 23 samples did not meet the criteria for acceptable C/N ratios (2.9–3.6) (DeNiro, 1985). This sample was excluded from further analysis. All samples produced acceptable gelatin yields (≥1%) (van Klinken, 1999).

Most samples for sulfur stable isotope analysis did not meet the criteria for acceptable sulfur atomic concentrations (0.15–0.35%) (Nehlich and Richards, 2009) but did meet that for acceptable atomic C/S and N/S ratios (600 ± 300 and 200 ± 100, respectively) (Nehlich and Richards, 2009). However, because all samples showed acceptable C/S and N/S ratios, none of these samples was excluded from further analysis.

**Carbon and nitrogen stable isotope ratios**

The $\delta^{13}C$ and $\delta^{15}N$ values of adult individuals are presented in Figure 2 and summarized in Table 2. The mean $\delta^{13}C$ and $\delta^{15}N$ values were $-21.1 \pm 0.4\%$ and $11.6 \pm 1.0\%$ at the SW6 site and $-21.0 \pm 0.3\%$ and $11.5 \pm 1.1\%$ at the SK site, respectively. Individual variations in $\delta^{15}N$ values were greater than those in $\delta^{13}C$ values (Figure 2). Mann–Whitney $U$-tests revealed no significant difference in $\delta^{13}C$ and $\delta^{15}N$ values between individuals at the SW6 and SK sites, between total females and total males, and between SW6 females and SW6 males (Table 3).

Figure 3 presents the adult human isotope ratios and the isotopic ranges of past and modern food sources in Japan (Yoneda et al., 2004) with a fixed offset from dietary protein to collagen of 5‰ for $\delta^{13}C$ and 4‰ for $\delta^{15}N$ (Lee Thorp, 2008). Stable isotope ratios from other populations in the Edo period are also presented in Figure 3 for comparison.

![Figure 2. Carbon and nitrogen stable isotope ratios for human bone collagen from Sendaiji. Total mean and 1SD of $\delta^{13}C$ and $\delta^{15}N$ values are also indicated.](image)

![Table 2. Summary of $\delta^{13}C$ and $\delta^{15}N$ values (‰) of Sendaiji skeletons](table)

| Sex     | $\delta^{13}C$ (%) | $\delta^{15}N$ (%) |
|---------|--------------------|--------------------|
| Female  | -21.1 ± 0.4        | 11.5 ± 0.5         |
| Male    | -21.1 ± 0.4        | 12.0 ± 1.2         |
| Unknown | -20.9 ± 0.4        | 11.1 ± 1.3         |

Although the mean $\delta^{15}N$ value in Sendaiji is similar to that in the other populations, the mean $\delta^{13}C$ value in Sendaiji is the lowest. Among the comparative populations, only that at the SKT871 site is located in Osaka and represents townspeople (Tsutaya et al., 2017). Mann–Whitney $U$-tests revealed significantly lower $\delta^{13}C$ ($-1.0\%$, $U = 8$, $P < 0.001$) values in Sendaiji than at the SKT871 site.

**Sulfur stable isotope ratios**

Because a large amount of collagen is required for the analysis (i.e. >25 mg), sulfur stable isotope analysis was
performed on five selected individuals at the SW6 site (Table 1). Sulfur stable isotope analysis was performed to evaluate marine contributions. If the individual variation in δ\(^{15}\)N values in Sendaiji (Figure 2) was a result of different marine contributions, δ\(^{34}\)S values, a proxy for marine diets, should correlate with δ\(^{15}\)N values. Therefore, the targets of sulfur stable isotope analysis were selected from individuals representing a wide range of δ\(^{15}\)N values (Table 1).

The mean δ\(^{34}\)S value in Sendaiji skeletons was 8.9 ± 1.3‰ and was not within the 100% marine range (from +15‰ to +19‰) (Figure 4). A Spearman’s rank correlation test revealed no significant correlation between δ\(^{15}\)N and δ\(^{34}\)S values (\(R^2 = 0.01, P = 0.95\)). This result suggests that the variation in δ\(^{15}\)N values was not caused by the use of marine foods or marine fertilizers.

### Discussion

#### Intrapopulation difference in diet

Although the chronological periods at the SW6 and SK sites differ, no significant intersite difference was detectable in the δ\(^{13}\)C and δ\(^{15}\)N values in Sendaiji (Table 3), suggesting that Buddhists and Christians ate similar diets. Historical and ethnographic studies have suggested that there was little dietary difference between Buddhists and Christians in premodern Japan. Although some food taboos for Christians appear in historical documents written by premodern Christian evangelists (Murakami and Yanagiya, 1969) and in an ethnographic study of modern Japanese human populations that retain premodern underground Christian traditions (kakure-kirishitan) (Miyazaki, 2018), typical prohibited foods comprised meat and eggs, and these taboo foods were usually only applied at specific times of the year. Furthermore, the replacement of Christian ceremonial foods with locally available foods was reported in the ethnographic studies of modern kakure-kirishitan populations; for example, bread was replaced with raw fish and rice, and wine was replaced with sake (Japanese rice wine) (Miyazaki, 2018). This evidence is generally consistent with the results of this study. Regardless of their religious beliefs, premodern villagers in Sendaiji appeared to obtain and consume locally available foods without strong religious preferences in diet.

The isotopic analysis conducted as a part of this study revealed no significant differences between females and males in Sendaiji (Table 3). The physical observations of the Sendaiji skeletons showed that relatively severe antemortem tooth loss was evident in this population, and was more apparent in males than in females (Osaka Center for Cultural Heritage, 2015). Moreover, spondylitis deformans was more evident in males than in females in the Sendaiji skeletal population (Osaka Center for Cultural Heritage, 2015). These sex-specific differences in skeletal health are assumed to be associated with sex-specific differences in subsistence activity (Osaka Center for Cultural Heritage, 2015). It is possible that these sex-specific differences in subsistence activity are associated with dietary differences (Berbesque et al., 2016); however, no sex-specific differences in diet were identified in the Sendaiji skeletal population (Table 3).

#### Diet in Sendaiji

The lowest mean δ\(^{13}\)C values in Sendaiji among the Edo period populations studied so far (Figure 3) might be caused by a diet depending on the forest ecosystem. Sendaiji is located in a remote mountainous woodland area, and food sources from closed canopy forests typically produce lower δ\(^{13}\)C values (Medina and Minchin, 1980; van der Merwe and...
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Analysis of palaeo-pollens at the Sendaiji sites showed that the woodland comprised oaks and red pine distributed around Sendaiji at least during the 11th to the 14th centuries (Osaka Center for Cultural Heritage, 2015). The ethnographic interviews of modern elderly Sendaiji inhabitants revealed that foods from the woodland mountains, such as persimmon fruits, chestnuts, and mushrooms, were gathered in the early 20th century (Osaka Center for Cultural Heritage, 1999). If such woodland foods were consumed in premodern Sendaiji, skeletons would have low δ\(^{13}\)C values. Although there is a general trend toward lower δ\(^{13}\)C values in Edo-period human hair in western compared with eastern Japan (Maruyama et al., 2018), the δ\(^{13}\)C values of the Sendaiji population are still significantly lower than those of the SKT871 population in Osaka, western Japan. Therefore, an additional driver for low δ\(^{13}\)C values, probably foods from the woodland, would affect the carbon isotopic signature of the Sendaiji population.

On the other hand, higher δ\(^{15}\)N values (Figure 3) were reported in premodern human skeletons from mountainous villages in Yamanashi (Anrakuji site: Yoneda, 2000) and Nagano (Kamizawa-one and Hodokubo sites: Yoneda et al., 1996) in eastern Japan. Their higher δ\(^{15}\)N values were probably the result of the consumption of marine fish or \(C_4\) plant foods (Figure 3).

Although it is possible that the relatively high δ\(^{15}\)N values of the Sendaiji skeletons (Figure 3) resulted from the consumption of marine foods or use of marine fertilizers, the result of sulfur isotope analysis indicates that this is probably not the case (Figure 4). The ethnographic interviews revealed that salted fish was purchased in Sendaiji in the early 20th century (Osaka Center for Cultural Heritage, 1999), but its protein contribution seems to be small in premodern Sendaiji. Salted marine fish was transported for sale to mountainous villages in premodern Japan, but it is hypothesized that these products were consumed mostly for salt intake but not for fish meat (Miyamoto, 1985). Crops grown with marine fertilizers show a marine signal in their δ\(^{34}\)S values (Szpak et al., 2019), and a historical document, *Ikegami-ke monjo*, which describes everyday life around Ibaraki, Osaka, during 1595–1943 indicates that marine fish fertilizer for crops was purchased in merchants in the city of Osaka by premodern Sendaiji inhabitants (Osaka Center for Cultural Heritage, 1999). The sulfur stable isotope analysis of premodern rice hulls excavated in Unseiji, Akashi, Hyogo, western Japan, suggests that marine fertilizer was used around Akashi in the Edo period (Tsutaya et al., 2016b). Despite this evidence, the results of this study suggest that the elemental contribution from marine fertilizer to human skeletons was small in Sendaiji.

Alternately, denitrification, ammonium uptake by paddy rice, animal fertilizer, and/or the consumption of freshwater fish are possible causes of the relatively high δ\(^{15}\)N values in the Sendaiji skeletons (Figure 3). Paddy rice fields generate suitable conditions for bacterial denitrification, where nitrate (NO\(_3^-\)) in the soil is reduced to dinitrogen monoxide (N\(_2\)O), and \(^{15}\)N is enriched in the remaining nitrate (Xing et al., 2002). Denitrification increases the δ\(^{15}\)N values of nitrate in soil (Mariotti et al., 1988), and plants take up and assimilate such \(^{15}\)N-enriched nitrate. Also, nitrification of ammonium in the soil results in higher δ\(^{15}\)N values in ammonium, and paddy rice uptakes ammonium as one of the nitrogen sources (Yoneyama et al., 1990). Ethnographic interviews have revealed that cattle were kept, and their excreta was used as fertilizers for crops, in Sendaiji in the early 20th century (Osaka Center for Cultural Heritage, 1999). The use of human excreta as fertilizer was common in premodern Japan, and human excreta was sold as a fertilizer in Osaka as early as early the beginning of the 17th century (Hanley, 1997; Takigawa, 2004). Animal fertilizers usually produce high δ\(^{15}\)N values (Szpak, 2014), and manuring using animal excrement further increases its δ\(^{15}\)N values (Bogaard et al., 2007; Kim et al., 2008). If the δ\(^{15}\)N values of crops were elevated according to the use of animal fertilizers, the consumption of these crops should be reflected in the increased levels of δ\(^{15}\)N in human skeletons. Finally, freshwater fish also show higher δ\(^{15}\)N values (e.g., Yoneda et al., 2004), and ethnographic interviews have revealed that eels were obtained from the nearby Saho river in Sendaiji in the past (Osaka Center for Cultural Heritage, 1999). To investigate these hypotheses further, the analysis of plant/leaf remains and stable isotope analysis of individual amino acids (Naito et al., 2013; Styring et al., 2015) would be beneficial.

**Conclusions**

The carbon, nitrogen, and sulfur stable isotope analyses of human skeletal collagen from premodern Sendaiji, Osaka, Japan, revealed the following evidence for diet in Sendaiji from the 16th to 19th centuries:

- No dietary differences according to religious beliefs (Buddhist or Christian) or sexes existed.
- Contributions from marine food protein or marine fertilizers were not evident.
- The lowest δ\(^{13}\)C value in Sendaiji among the Edo period populations could be caused by the consumption of food sourced from woodland.

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