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Carbon Isotope Ratios of Human Tooth Enamel Record the Evidence of Terrestrial Resource Consumption During the Jomon Period, Japan

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ABSTRACT *Objective:* Archaeological remains strongly suggest that the Holocene Japanese hunter-gatherers, the Jomon people, utilized terrestrial plants as their primary food source. However, carbon and nitrogen isotope analysis of bone collagen indicates that they primarily exploited marine resources. We hypothesize that this inconsistency stems from the route of protein synthesis and the different proportions of protein-derived carbon in tooth enamel versus bone collagen. Carbon isotope ratios from bone collagen reflect that of dietary protein and may provide a biased signal of diet, whereas isotope ratios from tooth enamel reflect the integrated diet from all macronutrients (carbohydrates, lipids, and proteins).
Methods: In order to evaluate the differences in inferred diet between the archaeological evidence and bone collagen isotope data, this study investigated carbon isotopes in Jomon tooth enamel from four coastal sites of the Middle to Late–Final Jomon period (5,000–2,300 years BP).
Results: Carbon isotope ratios of human teeth are as depleted as coeval terrestrial mammals, suggesting that C_3 plants and terrestrial mammals were major dietary resources for the Jomon people. Dietary dependence on marine resources calculated from enamel was significantly lower than that calculated from bone collagen. The discrepancy in isotopic ratios between enamel and collagen and the nitrogen isotope ratio in collagen shows a negative correlation on individual and population levels, suggesting diets with variable proportions of terrestrial and marine resources.
Conclusion: This study highlights the usefulness of coupling tooth enamel and bone collagen in carbon isotopic studies to reconstruct prehistoric human diet. Am J Phys Anthropol 158:300–311, 2015.

The Jomon people were hunter-gatherers who inhabited in the Japanese Archipelago from 16,000–2,500 cal BP and, culturally, are characterized by pottery marked with a particular cord pattern (Mizoguchi, 2002; Kobayashi, 2004). The diet of the Jomon people has been studied extensively from abundant excavated remains at archaeological sites. Both terrestrial and marine resources, including nuts, deer, boar, marine fish, and shellfish, were essential resources for the Jomon hunter-gatherers (Tomioka, 2010). Terrestrial plants were considered to be the major food source based on the excavation of plant remains (Yamanouchi, 1964; Watanabe, 1975; Nishida, 1980). Procurement of seasonal food resources and preservation of nuts in underground pits was also important.

The regional variation of their diet and subsistence has been investigated through the analyses of lithic and bone tools (artifacts). Discriminant function analysis of these tool-kits revealed the presence of four geographic domains in response to different environments (Akazawa, 1986, 1999). The coastal Jomon societies of eastern Japan had a procurement system in forested and estuarine/marine littoral ecosystems year round, while the western Jomon people, who lived in forested and freshwater ecosystems, faced a seasonal decline of marine resources in the spring and summer (Akazawa, 1999).

Climate in the Jomon period was considered relatively warmer during the Early and Middle Jomon and then cooler during Late–Final Jomon periods; vegetation change would have paralleled this climatic change (Tsuji et al., 1983; Tsukada, 1986; Yasuda, 1990). One interesting question is whether or not the diet of the Jomon people also changed during this time. The diet of the Jomon has also been investigated through the analysis of the frequency of tooth caries (Turner, 1979; Fujita, 1995; Temple, 2007). Fujita (1995) stated that caries tooth frequency increased from the Early Jomon to the Middle and Late–Final Jomon periods. Temple (2007) found that tooth caries frequencies in teeth of the Middle–Late Jomon period were lower than the Late–final Jomon

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period, which suggests a shift to greater reliance on plant foods following the climatic and environmental change (Temple, 2007). Temple (2011) found different frequencies of tooth caries between males and females, where the caries frequency of females was higher than that of males. This implies greater levels of carbohydrate consumption by females related to gender-based behavioral and dietary differences.

Stable isotope analysis of prehistoric human skeletons is a powerful tool for reconstructing dietary behavior and environment. Carbon and nitrogen isotopes in bone collagen reveal the proportion of terrestrial versus marine resource in diet (Schoeninger et al., 1983; Walker and DeNiro, 1986). Applications of this method to the Jomon skeletal remains in Japan have shown regional variability in the proportion of marine versus terrestrial resources in their diet (Chisholm et al., 1992; Minagawa and Akazawa, 1992; Yoneda et al., 2004; Kusaka et al., 2010). Coastal Jomon people in the mainland of Japan were dependent on marine and terrestrial resources, whereas inland Jomon primarily depended on terrestrial resources (Minagawa, 2001). The possibility of freshwater fish consumption for the inland Jomon was suggested (Yoneda et al., 2004). The diet of the Hokkaido Jomon was characterized by the procurement of large marine mammals (Minagawa, 2001; Naito et al., 2010). Kusaka et al. (2008) investigated intrasite variation of carbon and nitrogen isotope ratios in bone and found dietary dependence on marine resources differed based on tooth ablation types: individuals with type 2C ablation (extraction of maxillary and mandibular canines) in the Inariyama site showed more dependence on marine resources than those of type 4I ablation (extraction of maxillary canines and mandibular incisors;). These studies extensively illuminated the variability of diet based on region, age, and cultural factors.

Although extensive, the previous isotopic studies of Jomon dietary reconstruction have exclusively used bone collagen; tooth enamel, which is comprised almost entirely of hydroxyapatite, has never been examined. Stable isotopes in collagen and hydroxyapatite are derived from different dietary components. Controlled diet experiments of rats and pigs revealed that carbon in bone collagen is primarily derived from protein, while the carbon in bone and enamel hydroxyapatite comes from whole diet, rather than just protein (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Howland et al., 2003; Jim et al., 2004; Warinner and Tuross, 2009). Thus, carbon isotope ratios of bone collagen can be higher than the bulk diet if the dietary contribution of marine animals (high protein content, enriched in ^{13}C) is greater than that of terrestrial animals (high protein content, depleted in^{13}C). Thus, bone collagen carbon isotope values are biased toward the dominant protein source and can lead to an underestimate of the consumption of C_3 plants due to their low protein content.

STABLE ISOTOPES

Carbon isotope analysis of tooth enamel hydroxyapatite has been used on archaeological remains (van der Merwe et al., 2003; Knudson et al., 2009; Loftus and Sealy, 2012; Pfeiffer et al., 2014), although analysis of bone hydroxyapatite is more common (e.g., Lee-Thorp et al., 1989; Ambrose et al., 1997; Harrison and Katzenberg, 2003; Yesner et al., 2003).

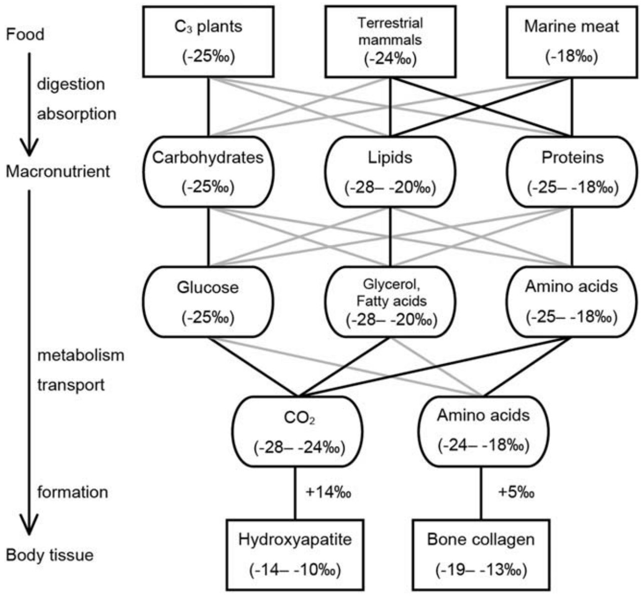

Fig. 1. Schematic diagram of the metabolic pathways of carbohydrates, lipids, and proteins. Carbon isotope ratios in parentheses are reference values for food sources for the Jomon (Krueger and Sullivan, 1984; Schwarcz, 1991; Ambrose and Norr, 1993; Cerling and Harris, 1999; Yoneda et al., 2004; Murray et al., 2009).

The carbon isotope ratio of plants differs depending on photosynthetic pathway, where C_3 and C_4 plants have distinct carbon isotope ratios. C_3 plants, which include most dicotyledons, exhibit the mean value of $-27.5‰$, while C_4 plants, which include many monocotyledons, especially grasses and sedges in warm and arid zones, exhibit a mean value of $-12.5‰$ (Smith and Epstein, 1971). In environments where C_4 plants are not present or are not part of the diet (i.e., for the Jomon people), carbon isotope ratios can be used to distinguish C_3-based diets from marine resources. This is because marine algae generally have higher carbon isotope ratios than terrestrial C_3 plants (Smith and Epstein, 1971; Laws et al., 1995; Fry, 2006).

Carbonate in hydroxyapatite of tooth enamel and bone is synthesized from dissolved CO_2 in blood, and ultimately CO_2 produced by cellular metabolism (Fig. 1; Krueger and Sullivan, 1984; Schwarcz, 1991). Carbon isotope ratios of carbonate in hydroxyapatite reflect those of all macronutrients in the diet (i.e., carbohydrate, lipid, and protein) (Ambrose and Norr, 1993; Tieszen and Fagre, 1993). On the other hand, bone collagen is mainly synthesized from amino acids derived from dietary protein. Thus, carbon isotope ratios of bone collagen reflect those of dietary protein (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Howland et al., 2003; Jim et al., 2004; Warinner and Tuross, 2009). Roughly, 60% of carbon in collagen is derived from dietary protein (Froehle et al., 2010).

To evaluate the diet of the Jomon based on carbon isotope ratios, it is important to note that C_3 plants are dominant in the coastal regions of the eastern and western Japanese main island of Honshu. Therefore, the terrestrial dietary components are primarily C_3-based (depleted in ^{13}C), whereas marine resources are enriched in ^{13}C compared to terrestrial C_3 plants. In the nitrogen system, the greater number of the trophic levels in the marine environment causes higher $\delta^{15}N$ values for marine organisms compared to terrestrial ones.

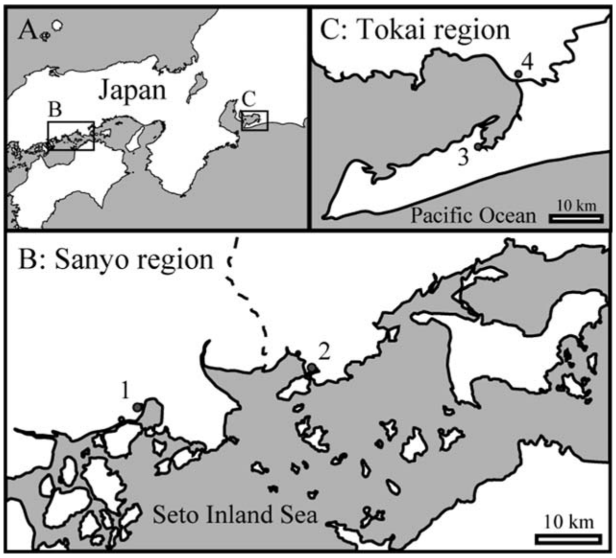

Fig. 2. Map of the Jomon shell mounds included in this study from the Sanyo and Tokai regions: 1, Ota; 2, Tsukumo; 3, Yoshigo; 4, Inariyama.

The purpose of this study is to assess the dietary dependence on terrestrial plants in the diet of the Jomon by measuring carbon isotope ratios of tooth enamel. We compare the calculated dietary dependence on marine resource relative to terrestrial resources using carbon isotope data from tooth enamel and bone collagen from the same individuals. We also assess inter- and intrapopulation diet variability. The enamel of permanent molars forms from childhood through early adolescence whereas bone collagen integrates diet over longer timescales. Therefore, differences in the isotopic signatures of tooth enamel and bone collagen may also reflect dietary changes through life.

MATERIALS AND METHODS

We sampled 116 Jomon individuals from Ota, Tsukumo, Yoshigo, and Inariyama sites, which are located in two coastal regions (Sanyo and Tokai regions) in Japan (Fig. 2, Table 1). These sites were excavated by K. Kiyono and reported in Japanese publications (e.g., Kiyono, 1969). Detailed information of the excavations is available in a recent study, and carbon and nitrogen isotope ratios of bone collagen for 93 of the 116 individuals studied here were reported by Kusaka et al. (2010). The Ota shell mound belongs to the Middle Jomon period (ca. 5,000–4,000 years BP), which is located in Hiroshima Prefecture of the Sanyo region in western Japan. Twenty-three individuals from Ota were used for the analysis. The Tsukumo shell mound of the Late–Final Jomon period (ca. 4,000–2,300 years BP) is located in Okayama Prefecture of the Sanyo region in western Japan. Thirty-eight individuals from Tsukumo were used. The Yoshigo shell mound of the later part of the Late to the Final Jomon period (ca. 3,200–2,800 years BP) is located in Aichi Prefecture of the Tokai region in eastern Japan. Thirty-eight individuals from Yoshigo were used for the analysis. The Inariyama shell mound of the middle part of the Final Jomon period (ca. 2,800–2,500 years BP) is also located in Aichi Prefecture. Seventeen individuals from Inariyama were used for the study. Sex and age at death of human skeletal remains were determined based on the standard method (Buikstra and Ubelaker, 1994). The age at death was categorized into the following classes: adolescent (12–20 years), young adult (20–34 years), middle adult (35–49 years), and old adult (50+ years). Adult individuals that were poorly preserved and could not be classified into these categories were left as adult with indeterminable age.

We also obtained enamel and bone samples from five deer (*Cervus nippon*), three bone samples of boar (*Sus scrofa*), and six fish bone samples from the archaeological collection excavated at Yoshigo shell mound (Table 2). The fish bones are from sea bream (Sparidae) that commonly inhabited in the Pacific coast and the Seto inland sea.

For teeth, we analyzed third molars (M3s), which form from 9 to 13 years in age in humans (Hillson, 1996). Because tooth enamel does not remodel after mineralization (Hillson, 1996), our isotopic analysis of tooth enamel reveals diet from late childhood into early adolescence. Because we drilled the side of each tooth evenly along the crown from the occlusal surface to the enamel–dentine junction, the isotopic signal represents the average diet over the period of tooth mineralization. Because the time of mineralization of each individual can be variable, we did not set narrower age intervals. Tooth enamel comprises larger apatite crystals, is denser, and has a lower organic content than dentin or bone apatite, making it less susceptible diagenesis (Ayliffe et al., 1994; Budd et al., 2000).

Tooth and bone samples for carbon isotope analysis were ultrasonically cleaned in ultrapure water and then dried. A dental drill equipped with a tungsten carbide burr was used to abrade the tooth enamel and bone samples. After abrading the surfaces to remove soil-derived substances, we collected 3 mg samples of enamel and of compact bone. Bone samples were washed with acetic acid (1.0*M*) for 24 h to remove any diagenetic carbonates, rinsed with distilled water, and then dried.

We measured carbon isotope ratios of samples from Ota, Tsukumo, and Yoshigo skeletal remains with an isotope ratio mass spectrometer equipped with GasBench II preparation device (Thermo Fisher Scientific, Inc.) at the Research Institute for Humanity and Nature. Carbon isotope ratios of the Inariyama and deer teeth were measured using an isotope ratio mass spectrometer coupled to a Carboflo device (Thermo Fisher Scientific, Inc.) at the University of Utah. Results are reported in the standard permil (‰) notation where:

$$\delta^{13}\mathrm{C} = (R_{\mathrm{sample}}/R_{\mathrm{standard}} - 1) \times 1000$$

and R_{sample} and R_{standard} are the ratios of $^{13}\mathrm{C}/^{12}\mathrm{C}$ in the sample and standard, respectively. An analogous equation is used for $\delta^{15}\mathrm{N}$. The standards are VPDB and AIR for $\delta^{13}\mathrm{C}$ and $\delta^{15}\mathrm{N}$, respectively. The difference in $\delta^{13}\mathrm{C}$ values between instruments is negligible based on the comparison of the samples from the same teeth. External precision was smaller than ±0.2‰ in both the instruments.

We use the notation $\varepsilon^{13}\mathrm{C}_{\mathrm{enamel\text{-}collagen}}$, to describe isotope enrichment (Cerling and Harris, 1999), which is given as $\varepsilon^{13}\mathrm{C}$ where:

$$\varepsilon^{13}\mathrm{C}_{\mathrm{enamel\text{-}collagen}} = (1000 + \delta^{13}\mathrm{C}_{\mathrm{enamel}})/$$
$$(1000 + \delta^{13}\mathrm{C}_{\mathrm{collagen})} - 1) \times 1000$$

The $\varepsilon^{13}\mathrm{C}$ value gives the true isotopic enrichment (or difference) between two $\delta^{13}\mathrm{C}$ values rather than the

TABLE 1. Carbon isotope ratios of tooth enamel (M3) and carbon and nitrogen isotope ratios of bone

Site	Kiyono collection No.	Sex	Age at death	Tooth ablation	$\delta^{13}C_{enamel}$	$\delta^{13}C_{collagen}$[a]	$\delta^{15}N_{collagen}$[a]	$\varepsilon^{13}C_{enamel\text{-}collagen}$
Ota	664	Female	Middle adult	No ablation	−12.0	−17.1	12.9	5.2
Ota	665	Female	Middle adult	No ablation	−12.2	−17.2	12.1	5.1
Ota	668A	Male	Adult	No ablation	−11.7	–	–	–
Ota	668B	Male	Adult	No ablation	−12.5	–	–	–
Ota	670	Male	Young adult	No ablation	−11.6	–	–	–
Ota	674	Male	Young adult	No ablation	−11.6	−15.4	15.3	3.9
Ota	684	Male	Young adult	No ablation	−11.5	−15.9	13.4	4.5
Ota	687	Female	Young adult	No ablation	−12.4	–	–	–
Ota	688	Female	Young adult	No ablation	−11.8	−16.4	13.2	4.7
Ota	693	Male	Young adult	No ablation	−12.2	−16.3	14.1	4.1
Ota	694	Male	Adult	No ablation	−12.4	−15.6	13.4	3.3
Ota	695	Male	Adult	No ablation	−13.0	–	–	–
Ota	697	Female	Adult	No ablation	−12.2	–	–	–
Ota	702	Male	Young adult	No ablation	−12.4	−16.2	14.1	3.8
Ota	709	Male	Young adult	No ablation	−11.8	–	–	–
Ota	710	Female	Middle adult	No ablation	−12.8	−17.0	13.2	4.3
Ota	711	Female	Young adult	No ablation	−11.9	−17.0	13.0	5.1
Ota	713	Male	Adult	No ablation	−12.0	–	–	–
Ota	717	Female	Young adult	No ablation	−11.9	−16.3	12.9	4.5
Ota	718	Male	Young adult	No ablation	−12.0	–	–	–
Ota	719	Male	Young adult	No ablation	−11.8	–	–	–
Ota	722	Female	Young adult	No ablation	−11.9	−16.4	14.0	4.6
Ota	904	Female	Adult	No ablation	−12.9	−19.6	7.7	6.8
Tsukumo	1	Female	Middle adult	4I	−11.2	−15.3	13.9	4.2
Tsukumo	2	Male	Young adult	2C	−10.7	−16.4	11.9	5.7
Tsukumo	3	Male	Young adult	4I	−11.7	−16.4	12.3	4.8
Tsukumo	4	Female	Young adult	4I	−11.1	−15.4	13.6	4.4
Tsukumo	5	Male	Young adult	2C	−11.2	−15.7	13.0	4.6
Tsukumo	6	Female	Young adult	4I	−10.9	−16.3	12.3	5.5
Tsukumo	7	Female	Young adult	4I	−11.1	−16.3	12.5	5.3
Tsukumo	8	Male	Adolescent	0	−10.8	−14.5	15.0	3.8
Tsukumo	11	Female	Young adult	4I	−12.3	−15.4	13.9	3.2
Tsukumo	12	Female	Adolescent	4I	−11.6	−16.1	12.5	4.5
Tsukumo	13	Male	Middle adult	2C	−11.5	−17.9	10.2	6.4
Tsukumo	14	Female	Young adult	4I	−13.2	−19.5	7.6	6.5
Tsukumo	16	Female	Young adult	4I	−11.7	−16.2	12.5	4.6
Tsukumo	19	Male	Young adult	2C	−10.5	−16.0	12.8	5.6
Tsukumo	23	Female	Young adult	4I	−11.8	−15.6	13.3	3.8
Tsukumo	24	Male	Young adult	2C	−11.3	−16.2	12.6	4.9
Tsukumo	27	Male	Middle adult	0	−9.4	−15.5	13.3	6.3
Tsukumo	30	Male	Young adult	0	−11.5	−15.2	11.7	3.7
Tsukumo	32	Male	Young adult	2C	−10.8	−16.2	12.4	5.5
Tsukumo	33	Male	Middle adult	2C	−11.4	−15.7	13.4	4.3
Tsukumo	34	Female	Young adult	2C	−11.6	−16.1	13.0	4.6
Tsukumo	37	Female	Middle adult	4I	−11.1	−15.7	12.9	4.7
Tsukumo	39	Male	Young adult	2C	−10.9	−19.6	5.4	9.0
Tsukumo	40	Female	Old adult	4I	−12.8	−19.1	8.1	6.4
Tsukumo	41	Female	Old adult	2C	−11.5	−16.0	12.8	4.6
Tsukumo	42	Female	Young adult	2C	−11.5	−16.3	13.0	4.9
Tsukumo	44	Female	Young adult	4I	−12.8	−19.1	9.1	6.4
Tsukumo	46	Male	Middle adult	No ablation	−12.2	−17.4	12.5	5.3
Tsukumo	55	Male	Young adult	2C	−12.1	−15.6	12.3	3.6
Tsukumo	58	Male	Middle adult	0	−12.1	−15.3	14.9	3.2
Tsukumo	63	Unknown	Unknown	4I	−12.5	−17.4	10.7	5.1
Tsukumo	65	Male	Young adult	0	−12.4	−17.8	10.0	5.5
Tsukumo	66	Male	Young adult	2C	−11.3	−17.1	11.0	5.9
Tsukumo	67	Female	Middle adult	4I	−12.0	−17.4	11.0	5.5
Tsukumo	68	Female	Young adult	4I	−11.9	−17.3	10.9	5.5
Tsukumo	151	Male	Middle adult	No ablation	−11.5	−16.0	14.0	4.5
Tsukumo	162A	Female	Middle adult	2C	−11.6	−16.4	12.4	4.9
Tsukumo	164	Female	Young adult	2C	−12.1	−17.1	12.1	5.1
Yoshigo	273	Male	Young adult	4I	−10.3	−17.9	8.7	7.8
Yoshigo	280	Male	Adolescent	4I	−9.6	−14.6	12.7	5.0
Yoshigo	287	Female	Middle adult	2C	−10.6	−15.1	12.4	4.6
Yoshigo	292	Male	Young adult	Unknown	−9.9	−16.0	11.2	6.2
Yoshigo	302	Female	Young adult	2C	−10.6	−16.6	10.7	6.1
Yoshigo	310	Female	Young adult	2C	−11.1	−16.3	10.6	5.3
Yoshigo	316	Male	Young adult	2C	−9.8	–	–	–
Yoshigo	322	Female	Young adult	4I	−11.4	−14.8	12.7	3.5

TABLE 1. Continued

Site	Kiyono collection No.	Sex	Age at death	Tooth ablation	$\delta^{13}C_{enamel}$	$\delta^{13}C_{collagen}$[a]	$\delta^{15}N_{collagen}$[a]	$\varepsilon^{13}C_{enamel\text{-}collagen}$
Yoshigo	333	Male	Young adult	2C	−9.7	–	–	–
Yoshigo	335	Female	Adolescent	2C	−10.1	−16.4	11.4	6.4
Yoshigo	341	Male	Adolescent	0	−10.7	−13.5	14.0	2.9
Yoshigo	342	Female	Middle adult	4I	−11.7	−13.6	14.2	1.9
Yoshigo	345	Male	Middle adult	4I	−12.0	−15.7	11.5	3.7
Yoshigo	349	Male	Young adult	4I	−11.1	–	–	–
Yoshigo	352	Female	Adolescent	4I	−11.5	−15.6	11.0	4.1
Yoshigo	357	Female	Middle adult	2C	−11.8	–	–	–
Yoshigo	363	Male	Middle adult	2C	−10.9	−15.1	12.5	4.2
Yoshigo	366	Male	Middle adult	4I	−13.9	−19.3	6.9	5.6
Yoshigo	375	Male	Young adult	4I	−10.2	−14.6	12.7	4.5
Yoshigo	383	Male	Middle adult	2C	−13.3	−19.3	7.2	6.1
Yoshigo	386	Male	Young adult	4I	−11.9	–	–	–
Yoshigo	388	Male	Young adult	4I	−9.8	−15.0	12.3	5.4
Yoshigo	396	Male	Young adult	4I	−12.0	–	–	–
Yoshigo	404	Female	Adolescent	2C	−10.0	–	–	–
Yoshigo	408	Female	Adult	4I	−13.3	−18.2	8.9	5.1
Yoshigo	419	Male	Young adult	2C	−9.6	−15.5	12.0	6.1
Yoshigo	435	Male	Young adult	2C	−13.2	−19.6	7.1	6.5
Yoshigo	436	Male	Young adult	2C	−11.2	−19.4	7.3	8.3
Yoshigo	460	Female	Young adult	2C	−11.9	−18.1	9.1	6.3
Yoshigo	461	Male	Middle adult	0	−10.2	–	–	–
Yoshigo	481	Male	Middle adult	4I	−12.0	–	–	–
Yoshigo	488	Female	Middle adult	4I	−11.9	−16.1	11.0	4.3
Yoshigo	500	Female	Middle adult	2C	−10.9	−15.5	11.8	4.7
Yoshigo	509	Male	Middle adult	4I	−10.0	–	–	–
Yoshigo	522	Female	Young adult	4I	−9.8	–	–	–
Yoshigo	534	Male	Middle adult	4I	−10.0	–	–	–
Yoshigo	540	Female	Middle adult	4I	−11.3	−17.0	10.4	5.8
Yoshigo	541	Female	Young adult	2C	−10.0	–	–	–
Inariyama	210	Unknown	Adolescent	2C	−12.5	−17.6	8.8	5.2
Inariyama	211	Female	Middle adult	4I	−9.6	−17.6	9.4	8.2
Inariyama	212	Male	Young adult	4I	−12.1	−17.6	9.9	5.5
Inariyama	217	Female	Young adult	4I	−10.6	−17.0	9.7	6.5
Inariyama	218	Male	Young adult	4I	−13.2	−17.6	10.1	4.5
Inariyama	224	Female	Middle adult	4I	−10.4	−16.2	9.7	5.9
Inariyama	228	Female	Adolescent	4I	−12.5	−18.2	8.5	5.8
Inariyama	229	Female	Middle adult	4I	−9.6	−15.4	9.9	5.9
Inariyama	231	Male	Young adult	2C	−11.7	−15.0	11.2	3.3
Inariyama	232	Male	Young adult	2C	−10.0	−15.1	10.4	5.2
Inariyama	233	Male	Middle adult	2C	−10.2	−14.3	11.3	4.2
Inariyama	236	Male	Adolescent	2C	−10.0	−15.1	11.6	5.2
Inariyama	238	Male	Young adult	2C	−9.9	−14.5	10.4	4.7
Inariyama	241	Male	Middle adult	2C	−10.5	−17.7	7.3	7.3
Inariyama	249	Male	Middle adult	4I	−12.8	−18.0	9.3	5.3
Inariyama	251	Male	Young adult	2C	−10.4	−15.1	10.2	4.8
Inariyama	253	Female	Adolescent	4I	−10.6	−16.8	9.3	6.3

[a] Data from Kusaka et al. (2010).

approximate value given by the numerical difference between two $\delta^{13}C$ values (Cerling and Harris, 1999). The $\varepsilon^{13}C_{enamel\text{-}collagen}$ can be an indicator of resource use pattern from marine and terrestrial ecosystems (Clementz et al., 2009). We evaluate the isotopic enrichment between the two tissue types and discuss the implications for the Jomon paleodiet.

RESULTS

Carbon isotope ratios of tooth enamel ranged from −13.9‰ to −9.4‰ among the Jomon individuals (Fig. 3). Values of Ota tooth enamel exhibited a mean value of −12.1 ± 0.4‰ and ranged from −13.0‰ to −11.5‰. Tsukumo tooth enamel showed a mean value of −11.6 ± 0.7‰ and ranged from −13.2‰ to −9.4‰. Yoshigo tooth enamel had a mean value of −11.0 ± 1.2‰ and ranged from −13.9‰ to −9.6‰. Inariyama tooth enamel had a mean value of −11.0 ± 1.2‰ and ranged from −13.2‰ to −9.6‰.

Carbon isotope ratios of tooth enamel differed significantly between four sites (Kruskal-Wallis test, $\chi^2 = 22.53$, $P < 0.01$). The $\delta^{13}C$ values of Ota enamel samples were lower than those of Yoshigo and Inariyama samples, and the Tsukumo enamel samples were not different from other sites (Wilcoxon test, $P < 0.05$).

The mean $\delta^{13}C$ values of deer enamel and bone samples were −12.2 ± 1.0‰ and −12.2 ± 0.9‰, respectively. The mean $\delta^{13}C$ value of suid (wild boar) bone samples was −11.9 ± 0.8‰. These values are typical for terrestrial mammals that eat C_3 plants. In contrast, fish bone samples have a mean $\delta^{13}C$ value of −3.8 ± 1.8‰, much higher than terrestrial mammals, as expected from their marine-based ecology.

TABLE 2. Tooth enamel and bone carbon isotope ratios of Jomon fauna

Experiment No.	Species	Part	δ^{13}C
YC2E	Deer (*Cervus nippon*)	Enamel	−13.1
YC4E	Deer (*Cervus nippon*)	Enamel	−11.1
YC5E	Deer (*Cervus nippon*)	Enamel	−11.3
YC6E	Deer (*Cervus nippon*)	Enamel	−13.3
YC8E	Deer (*Cervus nippon*)	Enamel	−12.3
YC271B	Deer (*Cervus nippon*)	Bone	−12.4
YC301B	Deer (*Cervus nippon*)	Bone	−12.5
YC407B	Deer (*Cervus nippon*)	Bone	−12.8
YC410B	Deer (*Cervus nippon*)	Bone	−12.5
YC494B	Deer (*Cervus nippon*)	Bone	−10.7
YB277B	Boar (*Sus scrofa*)	Bone	−11.0
YB416B	Boar (*Sus scrofa*)	Bone	−12.5
YB440B	Boar (*Sus scrofa*)	Bone	−12.2
YF261B	Fish (Sparidae)	Bone	−5.8
YF301B	Fish (Sparidae)	Bone	−2.4
YF356B	Fish (Sparidae)	Bone	−4.8
YF416B	Fish (Sparidae)	Bone	−0.9
YF459B	Fish (Sparidae)	Bone	−5.0
YF494B	Fish (Sparidae)	Bone	−3.8

Carbon isotope ratios of tooth enamel were compared with carbon and nitrogen isotope ratios of bone collagen previously published (Kusaka et al., 2010). Carbon isotope ratios of tooth enamel and bone collagen were significantly correlated in each site (Fig. 4). Carbon isotope ratios of tooth enamel and nitrogen isotope ratios of bone collagen were significantly correlated ($P < 0.05$) in Ota, Tsukumo, and Yoshigo but not in Inariyama (Fig. 4). A comparison of δ^{13}C values from tooth enamel and bone collagen between the four age classes (adolescent, young adult, middle adult, and old adult) was not significant (Kruskal-Wallis test, $\chi^2 = 3.09$, $P = 0.38$ for tooth enamel; $\chi^2 = 1.97$, $P = 0.58$ for bone collagen).

Carbon isotope ratios of Tsukumo enamel samples differed significantly by tooth ablation type. Those of type 2C individuals were higher than those of type 4I individuals (Wilcoxon test, $\chi^2 = 4.56$, $P < 0.05$; Table 3). In Tsukumo, most of males have type 2C and females have type 4I tooth ablation. Type 2C males exhibited higher isotope values than type 4I females (Wilcoxon test, $\chi^2 = 4.45$, $P < 0.05$, Fig. 5). Dietary difference in relation to sex and tooth ablation types was also found in Inariyama, where type 4I males had higher carbon isotope ratios than type 2C males and type 4I females (Kruskal-Wallis test, $\chi^2 = 6.37$, $P < 0.05$).

DISCUSSION

Carbon isotope ratios of Jomon tooth enamel were as low as those of deer enamel and bone and boar bone samples (Fig. 3). The low δ^{13}C values suggest that the Jomon from the four sites relied mainly on C_3 plants and terrestrial mammals rather than marine resources. To estimate the relative contribution of marine resources to the whole diet, we hypothesized that exclusive reliance on C_3 plants and terrestrial mammals yields an enamel δ^{13}C value of −12.2‰ and −3.8‰ for diets relying exclusively on marine resources. These end member values are derived from taking the average values of deer enamel and fish bone, respectively. According to this linear mixing model, the mean dietary dependence on marine resource of Ota was 1%, that of Tsukumo was 7%, and those of Yoshigo and Inariyama were 14.3% (Table 4). These results suggest that the marine resource contribution to diet was relatively low and that most of dietary resources for the Jomon people who lived in the coastal environment in the Sanyo and Tokai regions of mainland Japan were C_3 plants and terrestrial mammals.

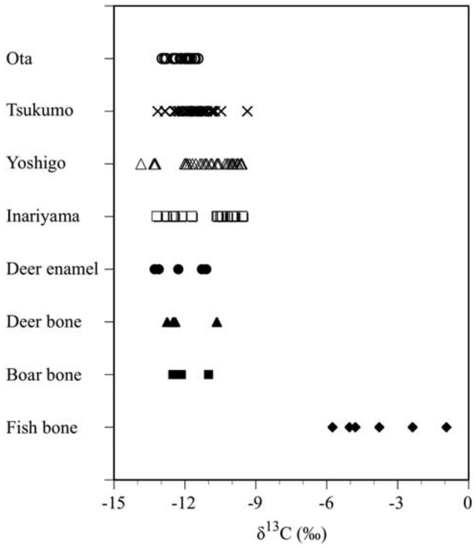

Fig. 3. Carbon isotope ratios of humans and other faunal skeletal remains.

Obviously, this estimation is affected by the isotope ratios assigned to the two end members for terrestrial and marine resource consumers. We assumed the same isotopic enrichment value between diet and bioapatite for human, deer, and fish. When the isotopic enrichment of 14.1‰ is applied to the calculation (Cerling and Harris, 1999), δ^{13}C values of the diet of deer and fish are −26.3‰ and −17.9‰, respectively. These values are within the estimated ranges of C_3 plant values in the Jomon period (−25.4 ± 1.6‰) and marine fish protein (−18.2 ± 1.0‰) (Yoneda et al., 2004), underscoring the abovementioned assumption on the carbon isotope ratios of two end members.

The incorporation of lipids might confound the interpretation of human diet from tooth enamel carbon isotopic ratios. Carnivores show relatively low isotope ratios due to a relatively high lipid diet, where lipids have relatively lower isotope ratios than whole diet (by ca. 2–4‰; Lee-Thorp et al., 1989; Passey et al., 2005; Clementz et al., 2009). Therefore, the lipids in terrestrial and marine meat might cause lower isotope values in human tooth enamel. Naito et al. (2010) reported that the Jomon population of the Kitakogane shell mound in Hokkaido consumed 74% of marine resources based on the nitrogen isotope ratios in amino acids of bone collagen. Since the Jomon population in Hokkaido exploited large marine mammals (e.g., Fur seal) based on archaeological remains, their diet probably included a significant fraction of lipids that would affect the carbon isotope in tooth enamel. Naito et al. (2013) also reported that the inland Jomon relied heavily on terrestrial meat. However, there is no archaeological evidence from the coastal area of the mainland that suggests heavy exploitation of either marine large mammals or terrestrial meat. We assume that their diet did not include a significant amount of lipids.

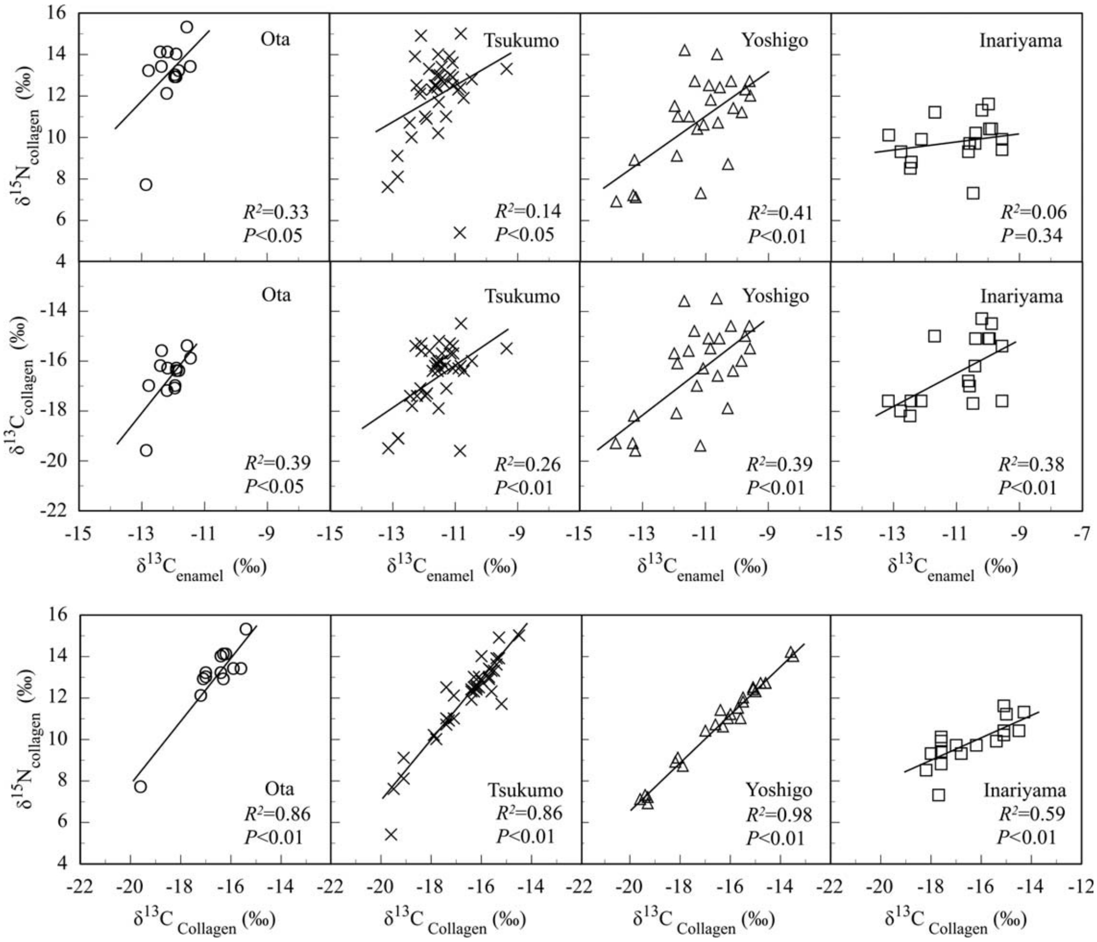

Fig. 4. Comparison of carbon isotope ratios of tooth enamel and carbon and nitrogen isotope ratios of bone collagen in each of the four sites.

TABLE 3. Statistical summary of carbon isotope ratios of tooth enamel

	Ota			Tsukumo			Yoshigo			Inariyama		
	N	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD
All individual	23	−12.1	0.4	38	−11.6	0.7	38	−11.0	1.2	17	−11.0	1.2
Sex												
Male	13	−12.0	0.1	18	−11.3	0.7	22	−11.1	0.3	10	−11.1	0.4
Female	10	−12.2	0.1	19	−11.7	0.6	16	−11.0	0.2	6	−10.5	0.5
Tooth ablation type												
0	–	–	–	5	−11.2	1.2	2	−10.4	0.3	–	–	–
4I	–	–	–	16	**−11.8**[a]	0.7	19	−11.2	1.2	9	−11.3	0.4
2C	–	–	–	15	**−11.3**[a]	0.5	16	−10.9	1.2	8	−10.6	0.4
No ablation	–	–	–	2	−11.9	0.5	–	–	–	–	–	–
Sex and tooth ablation type												
Male, 0	–	–	–	5	−11.2	1.2	2	−10.4	0.3	–	–	–
Male, 4I	–	–	–	1	−11.7	–	12	−11.1	1.3	3	**−12.7**[b]	0.5
Male, 2C	–	–	–	10	**−11.2**[a]	0.5	7	−11.1	1.6	7	**−10.4**[b]	0.6
Female, 4I	–	–	–	14	**−11.8**[a]	0.7	7	−11.5	1.0	6	**−10.5**[b]	1.1
Female, 2C	–	–	–	5	−11.6	0.3	9	−10.8	0.7	–	–	–

[a] Statistically different by Wilcoxon test.
[b] Statistically different by Kruskal-Wallis test.

We also estimated the percentage of marine protein consumption in the diet of the Jomon populations by using carbon isotope ratios of bone collagen, and we compared this result with that derived from tooth enamel hydroxyapatite. We presumed δ^{13}C values of bone collagen of −21.3 and −11.7‰ for the cases of 100% dependence on terrestrial protein and 100% dependence on marine protein, respectively, as inferred from the δ^{13}C values of bone collagen in deer and omnivorous marine fish (Kusaka et al., 2010). This model gives the mean protein procurement from marine resources of 48.6–51.0% with a considerably wide range of estimates among the entire sample (Table 4). This result contrasts to the small contribution of marine resources to total caloric consumption based on tooth enamel carbon isotopes (mean of 1.2–14.3%). The difference in δ^{13}C values of bone collagen between the deer and marine fish is 9.6‰, whereas that of tooth enamel is 8.4‰. This difference only slightly changes the results of the calculation and does not significantly affect this contrasting result.

The carbon isotope ratio of bone collagen reflects that of dietary protein, while the carbon isotope ratio of apatite reflects the whole diet (Ambrose and Norr, 1993; Tieszen and Fagre, 1993). Marine fish and shellfish contain large amounts of protein and small amounts of carbohydrate. In contrast, C_3 plants have high carbohydrate and low protein concentrations compared to marine resources. Therefore, consumption of protein-rich marine resources with higher δ^{13}C values, even if the total caloric contribution to diet was relatively low, results in high δ^{13}C values in bone collagen, but not in tooth enamel.

The abovementioned discussion relies on the assumption that dietary pattern did not change between childhood (9–13 years in age, when the enamel of the third molars is formed) and adulthood. The δ^{13}C value of tooth enamel does not change after mineralization, whereas bone collagen reflects carbon isotopic ratios of diet for about 10 years before death (Stenhouse and Baxter, 1979). The possibility that they switched from a plant-based diet in childhood to a marine resource rich diet in adulthood is unlikely because adolescents, young adults, middle adults, and old adults do not exhibit different isotope signatures in tooth enamel and bone collagen (Table 1). In addition, carbon isotope ratios of tooth enamel and bone collagen were positively correlated in Ota, Tsukumo, and Yoshigo individually. This indicates that the diets between adulthood and childhood were similar throughout an individual's life. In other words, an individual who was dependent on marine resources during childhood continued this dependence in adulthood.

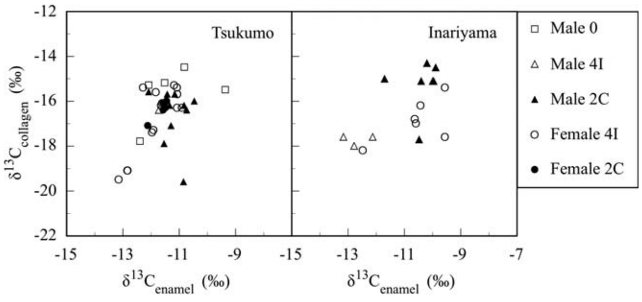

Fig. 5. Carbon isotope ratios of tooth enamel and bone collagen of Tsukumo and Inariyama marked by sex and tooth ablation types.

The difference in marine resource dependence calculated from the δ^{13}C values in tooth enamel and in bone collagen in Table 4 gives insight into the proportion of terrestrial plants versus meat in the terrestrial fraction of diet. If the proportion of terrestrial meat were significantly higher than plants, then protein from meat would be utilized as the main energy source and would also determine the δ^{13}C value of bone collagen. If this were the case, the δ^{13}C values of tooth enamel and bone collagen would be similar. However, this study revealed that this is not the case for the studied Jomon populations. The enamel δ^{13}C values indicate that terrestrial resources (plants or mammals) were a significant part of the Jomon diet. The δ^{13}C values of bone collagen show a small contribution of terrestrial meat, and we, therefore, conclude that terrestrial plants comprised a significant part of the Jomon diet.

From excavated archaeological remains, it has been claimed that the major food source for the Jomon populations was plants based on the intensive excavation of Jomon sites (Yamanouchi, 1964; Watanabe, 1975; Nishida, 1980). Plant remains were particularly common in low wetland sites. Tooth caries frequencies in Jomon populations are within the range of mixed-agricultural and agricultural economies (Turner, 1979) and modern hunter-gatherers (Fujita, 1995). The high rates of tooth caries indicate carbohydrate-rich diets. However, carbon and nitrogen isotope analysis of bone collagen on coastal Jomon skeletons indicated that marine proteins contributed to a significant part of their diet (Minagawa, 2001; Kusaka et al., 2010). Our tooth enamel carbon isotope data support the consumption of terrestrial plants and mammals in whole diet. Even in the inhabitants who left huge shell mounds in the coastal area, most of the energy source was C_3 plants and terrestrial mammals. However, by combining the tooth enamel and bone collagen isotopic data, it becomes evident that the primary source of protein in the Jomon diet was derived from marine resources.

The enrichment between carbon isotope ratios of tooth enamel and bone collagen ($\varepsilon^{13}C_{enamel\text{-}collagen}$) can be used to infer the trophic relationship and dietary variations (Clementz et al., 2009). The average $\varepsilon^{13}C_{enamel\text{-}collagen}$ value from all Jomon individuals is 5.1 ± 1.2‰ with a range from 1.9‰ to 8.9‰. Comparison of $\varepsilon^{13}C_{enamel\text{-}collagen}$ values between the four sites were not significant (Kruskal-Wallis test, $\chi^2 = 6.21$, $P = 0.10$); however, the mean $\varepsilon^{13}C_{enamel\text{-}collagen}$ value from Ota (4.6 ± 0.9‰) was 0.5–1‰ lower than other sites (5.1 ± 1.1‰ for Tsukumo, 5.2 ± 1.4‰ for Yoshigo, 5.5 ± 1.2‰ for Inariyama). The $\varepsilon^{13}C_{enamel\text{-}collagen}$ values and the δ^{15}N values were significantly negatively correlated (Fig. 6A, $r = 0.75$, $P < 0.01$). The means of $\varepsilon^{13}C_{enamel\text{-}collagen}$ values and δ^{15}N values in each site were also significantly correlated (Fig. 6B, $r = 0.98$, $P < 0.01$).

The strong correlation between $\varepsilon^{13}C_{enamel\text{-}collagen}$ and δ^{15}N values illuminates the dietary resource use from the terrestrial and marine ecosystems (Fig. 6). The δ^{15}N

TABLE 4. Comparison of the percent marine food in diet based on carbon isotope ratios in tooth enamel and bone collagen

	% Marine based on enamel				% Marine based on collagen			
Site	Mean	SD	Min	Max	Mean	SD	Min	Max
Ota	1.2	4.8	0 (−9.5)	8.3	48.6	10.9	18.1	61.8
Tsukumo	7.1	8.3	0 (−11.9)	33.3	49.6	12.9	17.4	70.5
Yoshigo	14.3	14.3	0 (−20.2)	31.0	50.7	18.2	17.3	81.0
Inariyama	14.3	14.3	0 (−11.9)	31.0	51.0	14.2	32.2	72.7

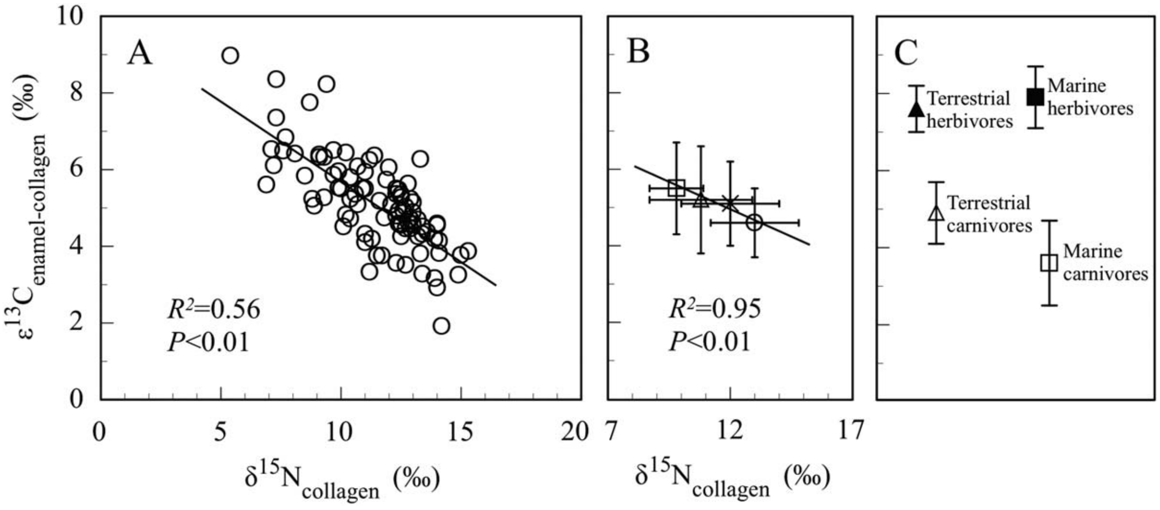

Fig. 6. (**A**) The $\varepsilon^{13}C_{enamel\text{-}collagen}$ values and the $\delta^{15}N$ values of the Jomon skeletal remains; (**B**) the mean $\varepsilon^{13}C_{enamel\text{-}collagen}$ values and the mean $\delta^{15}N$ values of each site with standard deviations: Ota (○), Tsukumo (×), Yoshigo (△), Inariyama (□); (**C**) the $\varepsilon^{13}C_{bioapatite\text{-}collagen}$ values of terrestrial herbivores and carnivores and carnivorous marine cetaceans recalculated from Clementz et al. (2009).

values of bone collagen indicate dietary dependence on marine versus terrestrial meat (Schoeninger et al., 1983; Walker and DeNiro, 1986). The $\varepsilon^{13}C_{enamel\text{-}collagen}$ can be used to address trophic level of animals (Krueger and Sullivan, 1984; Lee-Thorp et al., 1989; Clementz et al., 2009). In the C_3 and C_4 terrestrial ecosystems, terrestrial herbivore mammals show higher $\varepsilon^{13}C_{enamel\text{-}collagen}$ values (7.6‰) than terrestrial carnivores (4.8‰, Clementz et al., 2009). The same value can be assumed for humans in a terrestrial ecosystem. In marine ecosystems, sea cows that eat sea grass (7.8‰) show a higher value than carnivorous marine cetaceans (3.6‰). We can also assume that the incorporation of marine protein and lipid will decrease the $\varepsilon^{13}C_{enamel\text{-}collagen}$ values in humans. The $\varepsilon^{13}C_{enamel\text{-}collagen}$ values for the Jomon populations spans the range of $\varepsilon^{13}C_{enamel\text{-}collagen}$ values of terrestrial and marine herbivores to those of terrestrial and marine carnivores, indicating that their diet varied from terrestrial plant consumption to marine resource consumption as well as terrestrial animal consumption (Fig. 6). The correlation between the $\varepsilon^{13}C_{enamel\text{-}collagen}$ values and the $\delta^{15}N$ values also illustrates the influence of both C_3 plants and terrestrial and marine meat in Jomon diets.

The Ota individuals of the Middle Jomon period exhibited a lower mean carbon isotope ratio of the tooth enamel than Tsukumo, Yoshigo, and Inariyama individuals of the Late–Final Jomon period (Fig. 3). On average, the dietary contribution from the marine resources in Ota was about 6–13%. The shells excavated from Ota include *Lunella coreensis* (Sugai in Japanese) and Oyster (Magaki) that live in shore reefs, and *Batillaria multiformis* (Uminina) that lives in sandy areas, whereas the blood cockle (Haigai), Japanese littleneck (Asari), and common orient clam (Hamaguri), which are common in other shell mounds, are scarce (Inaba, 1971). These species are also found in the excavation of Tsukumo shell mound (Shimada et al., 1920) and in the archaeological sites of the Tokai region (Toizumi, 2000). These shells would be incorporated by Ota people and contributed to the isotopic signal in Ota enamel but do not explain the depleted isotope ratios in Ota enamel. The carbon and nitrogen isotope analysis on bone collagen revealed that the Ota had higher nitrogen isotope ratios than other sites, implying greater incorporation of higher trophic level foods from both marine and terrestrial ecosystems (Kusaka et al., 2010). The prevalence of tooth caries of Ota (3.8%) was lower than those of Tsukumo (9.6%), Yoshigo (11.4%), and Inariyama (5.4%) (Temple, 2007). The low tooth caries frequency of Ota suggests lower plant consumption compared to other sites. The diet of the Ota Jomon people may have included a relatively large amount of lipid-rich terrestrial meat based on the slightly lower mean $\delta^{13}C$ values and may have also been relatively protein-rich based on $\varepsilon^{13}C_{enamel\text{-}collagen}$ and relatively high $\delta^{15}N$ values (Fig. 6B). This dietary difference between sites can be interpreted as a temporal change in diet. The prevalence of tooth caries in the Jomon people documents an increase from the Middle to the Late–Final Jomon period (Temple, 2007). Carbon isotope data indicate that dietary change from a lipid-rich terrestrial diet to a carbohydrate-rich diet, with a relatively protein-rich diet coming from marine resources. These dietary and behavioral changes may have occurred in accordance with cooling climatic conditions and attendant environmental change during the Middle to the Late–Final Jomon periods.

While carbon isotope ratios of tooth enamel and carbon and nitrogen isotope ratios of bone collagen were correlated in Ota, Tsukumo, and Yoshigo, it is not the case for Inariyama (Fig. 4). The nitrogen isotope ratios of Inariyama (at most 11.6‰) are lower than those of other sites, suggesting that Inariyama population might have relied more on marine resources from lower trophic levels (Kusaka et al., 2010). Archaeological evidence suggests that Jomon populations in the region of the Atsumi Peninsula and Mikawa Bay differed locally in terms of marine resource exploitation (Toizumi, 2000, 2008) and we can assume that Inariyama engaged in shellfish gathering without active large game (tuna, sharks) fishing compared to Yoshigo. The unique relationship between carbon isotope ratios of enamel and carbon/nitrogen isotopes of bone collagen in Inariyama might be caused by the incorporation of marine resources that have high carbon isotope and low nitrogen isotope ratios (shellfish rather than large fish). This is also supported by the $\varepsilon^{13}C_{\text{enamel-collagen}}$ and $\delta^{15}N$ values of Inariyama, suggesting a significant contribution of lower trophic level marine foods (Fig. 6B).

Carbon isotope ratios in enamel from Ota did not differ between males and females, but carbon isotope in bone collagen suggests a sex-based dietary difference (Kusaka et al., 2010). This can be interpreted as a result of sexual division of labor during adulthood where males were more engaged in fishing activities while females were more engaged in the collection of plants and small animals. Such sex-based differences in isotope ratios were not found in enamel and collagen of the Yoshigo individuals, suggesting that Yoshigo males and females had similar diets, even if sexual division of labor existed. Temple (2011) found that greater frequencies of molar caries in females compared to males and interpreted that dietary difference existed in relation to sexual division of labor. At present, some sites show evidence for differences in carbohydrate intake between males and females, while others do not (Temple, 2011). Tsukumo females showed lower carbon isotope ratios of tooth enamel compared to males, indicating that more terrestrial resources in the diet of females (Table 3). Since tooth caries frequency increases with age, the carbon isotope ratio in bone hydroxyapatite is suitable to be compared with tooth caries frequency.

Carbon isotope ratios of tooth enamel in Tsukumo and Inariyama individuals were related to sex and/or tooth ablation types. At Tsukumo, type 2C males, who extract maxillary and mandibular canines, have higher isotope ratios than type 4I females, who extract maxillary canines and mandibular incisors. Interestingly, this difference was not found in the values of bone collagen, suggesting Tsukumo people had dietary differences related to gender and/or tooth ablation types during childhood. In Inariyama, carbon isotope ratios of type 2C males and type 4I females were higher than those of type 4I males. This difference was also found in the values of bone collagen (Kusaka et al., 2008). Type 2C males were more dependent on marine resources than type 4I males during adulthood and childhood in Inariyama. Locally different relationships between dietary habit, sex and tooth ablation types indicate the complexity of the Jomon society.

CONCLUSIONS

Carbon isotope ratios of tooth enamel and bone collagen lead to very different dietary reconstructions in prehistoric human populations. In the case of the four Jomon populations studied here, carbon isotope ratios of tooth enamel indicate a high dependence on plant foods for carbohydrates, while bone collagen suggests a high dependence on marine resources for protein, reflecting different synthetic pathways of tooth enamel and bone collagen from diets. Carbon in tooth enamel originates from CO_2 produced through cellular respiration, whereas carbon in bone collagen is mainly derived from dietary proteins. Thus, tooth enamel and bone collagen reflect different aspects of diet. Comparison of carbon isotope ratios of both tissues allows us to obtain a better reconstruction of prehistoric human diets when very different isotope ratios are predicted for energy and protein sources (e.g., terrestrial and marine resources). The discrepancy of isotopic ratios between these tissues also provides evidence of terrestrial-based diets with an important marine component for protein in the Jomon diet.

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