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# Stable Isotope Analysis of Prehistoric Human Bone from the Furuyashiki Site, Kamikita Town, Aomori, Japan: A Pilot Study Report

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A human bone sample from an Early Jomon burial recovered at the Furuyashiki site in Kamikita Town, Aomori Prefecture in the northern Tohoku region of Honshu, Japan, was subjected to stable isotopic analysis in a pilot study of the local prehistoric diet. The bone sample was reasonably well preserved. Although it was somewhat mineralized with carbonates, it was possible to extract sufficient collagen for the analysis. The results of the isotopic analysis suggest that  $49 \pm 29$  % of this individual's protein derived from marine food species. This indicates that the individual's marine food intake in terms of protein is likely to have been lower than the average of Hokkaido Jomon people, but higher than that of Jomon people in central Honshu. The implications of this result are discussed in relation to the interpretation of archaeological data from the Sannai Maruyama site, which is also located in northern Tohoku.

## INTRODUCTION

Over the past two decades, stable isotope analysis of carbon and nitrogen of human bone collagen has provided extremely useful information regarding dietary patterns of prehistoric Jomon people on the Japanese Islands [e.g., CHISHOLM *et al.* 1992; MINAGAWA and AKAZAWA 1992]. Results of these analyses indicate strong similarities between skeletal remains excavated from Jomon sites in Hokkaido (the northernmost island among the four large islands of Japan; Figure 12.1) and those of historic Ainu in Hokkaido and Sakhalin: both groups of people were heavily reliant on marine food in terms of their protein intake. On the other hand, samples from Jomon sites in the central part of Honshu (the largest island of Japan) indicate that the primary food source for central Honshu Jomon was terrestrial food, including both C<sub>3</sub> plant and meat. These include samples from the Kanto, Chubu and southern Tohoku regions. It is particularly interesting to note that bone samples not only from inland sites but also from coastal shellmiddens, such as Sanganji in Fukushima Prefecture and Kosaku in Chiba Prefecture, show patterns of relatively low dependence on marine food [e.g., MINAGAWA and AKAZAWA 1992]. These results led to questions about the traditional interpretation of coastal Jomon people being primarily littoral foragers.

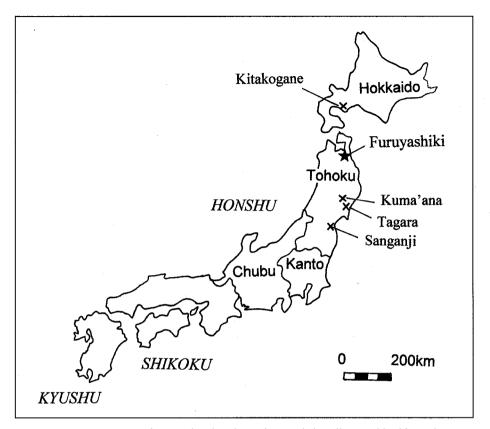


Figure 12.1 Map of Japan showing the regions and sites discussed in this study (★ indicates the approximate location of the Furuyashiki site).

Between Hokkaido and central Honshu, the northern part of the Tohoku region (the northernmost region of Honshu Island; see Figure 12.1) is an area in which virtually no stable isotope analysis of Jomon skeletal remains have been conducted. In this area, many large Jomon settlements have been reported, among which is the Early to Middle Jomon Sannai Maruyama site (for an overview of Jomon archaeology in this region, see the Okada paper in this volume). The common presence of shellmiddens associated with both marine fish and terrestrial mammal bones [e.g., HACHINOHE CITY MUSEUM 1988], as well as ample evidence of nut storage [e.g., WATANABE 1976], suggest that the Jomon people in this region exploited a variety of food resources. However, because very few quantitative studies of faunal and floral remains have been reported (with a few exceptions such as the work by D'ANDREA 1995), and because systematic regional settlement pattern analyses have yet to be conducted, we know relatively little about the subsistence base and resulting settlement systems of the Jomon people in this region. In this regard, stable isotope analysis of samples can shed new light on Jomon diet, subsistence and settlement systems in this region.

As a first step to approach this issue, we took the opportunity to analyze an Early Jomon

skeletal sample from the Furuyashiki site in Kamikita Town, Aomori Prefecture. The following is the report of this pilot study.

## THE FURUYASHIKI SITE AND THE BONE SAMPLE

The Furuyashiki site is located in Kamikita Town, Kamikita County, Aomori Prefecture. Geographically, the site is on a river terrace of the Nakatsu River, a small river that flows into Ane-numa, a small lake located south of the Ogawara Lake. The site is approximately 34-36 meters above sea level, and is associated with Early to Late Jomon features and artifacts, including a Middle Jomon shellmidden. The location of the site is shown in Figure 12.2.

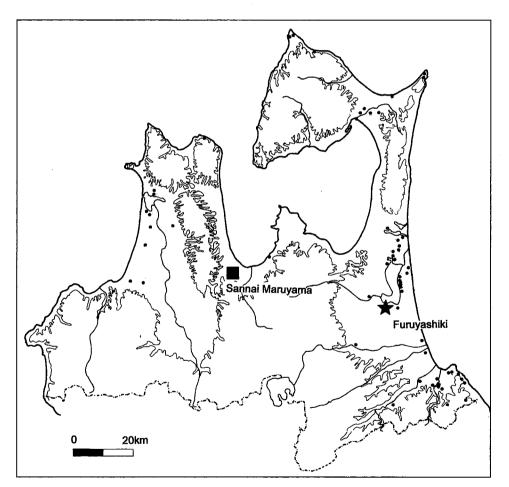


Figure 12.2 Map of Aomori Prefecture showing the location of the Furuyashiki site (solid star), the Sannai Maruyama site (solid square), and other Jomon sites associated with shellmiddens or shell layers (small, solid circles) (modified from HACHINOHE CITY MUSEUM 1988).

The human skeletal remains examined in this study were excavated from a flask-shaped burial pit (Feature No. 2) dated to the Lower-Ento-d phase of the Early Jomon period. The excavation was conducted by the Board of Education of Kamikita Town [1983, 1986]. According to the excavation report [BOARD OF EDUCATION OF KAMIKITA TOWN 1986], the depth of the pit was 1.4 meters, and the floor area measured 1.8 meters in diameter. The skeletal remains were those of an adult female, whose estimated age was approximately 20 years old [MORIMOTO 1986]. The floor of the pit was covered by a layer of shells, on which the body of the deceased was placed. Two deep jars (Lower-Ento-d style) were placed over the body. A stone tablet (ritual object) and a polished stone axe were also recovered within the pit. The top part of the pit was covered by scallop shells [MORIMOTO 1986].

A bone fragment from this individual was provided for stable isotopic analysis by courtesy of the Board of Education of Kamikita Town. The preservation of the bone sample was reasonably good. Although it was somewhat mineralized with carbonates, it contained sufficient collagen for the analysis.

#### ANALYSIS

#### **Dietary Inferences from Stable Isotopic Analysis**

The analysis of the stable isotopes of carbon and nitrogen from human bone collagen has provided useful information about the relative importance of marine versus terrestrial food alternative groups as protein sources in local prehistoric diets [e.g., CHISHOLM *et al.* 1982, 1983, 1992; HAYDEN *et al.* 1987; KOIKE and CHISHOLM 1988; MINAGAWA and AKAZAWA 1992; ROKSANDIC *et al.* 1988; SCHOENINGER and DENIRO 1984; SCHOENINGER *et al.* 1983; SEALY and VAN DER MERWE 1985, 1986; TAUBER 1981; WALKER and DENIRO 1986]. The technique is relatively well known by now, so only a few important details are mentioned here.

Analytical results are expressed, as del values, in parts per mil (‰), as follows:

 $\delta (\%) = \{ [R \text{ sample } / R \text{ standard}] - 1 \} \times 1000$ where

 $R = ({}^{13}C/{}^{12}C) \text{ or } ({}^{15}N/{}^{14}N).$ 

The carbon standard is the internationally used PDB standard, the nitrogen standard is N<sub>2</sub> in air.

Samples are cleaned and contaminant carbon is removed before analysis [CHISHOLM 1989; CHISHOLM *et al.* 1992]. Samples are combusted and the resulting gases are analyzed in an isotope ratio mass spectrometer, in this case in the Department of Earth Sciences at the University of British Columbia. The precision of analyses for this instrument is  $\pm 0.1\%$  or better for CO<sub>2</sub>, and  $\pm 0.2\%$  or better for N<sub>2</sub>. Instrumental error therefore does not contribute noticeably to uncertainties in the results.

#### Carbon

We know, from numerous studies, that different major plant groups (Calvin-Benson or C3

photosynthetic pathway plants, C<sub>4</sub> or Hatch-Slack plants, Crassulacean Acid Metabolism [CAM] plants, and marine plants) follow chemically different pathways, characterized by different isotope ratios [e.g., SMITH and EPSTEIN 1971; VOGEL 1978; O'LEARY 1981; VAN DER MERWE 1982]. The C<sub>3</sub> group includes flowering plants, trees and shrubs, and most temperate zone grasses. The majority of C<sub>4</sub> species are xeric environment grasses, including maize, some millets, some sorghums, cane sugar, some amaranths and some chenopods. Some millets and barnyard grasses occur in Japan and could be a minor factor in isotopic analysis, particularly if local herbivores were eating them in quantity and thus introducing them to the food chain. The Crassulacean Acid Metabolism (CAM) plants are succulents, such as pineapple and various cacti, and have values that usually reflect the environment in which they are growing. Luckily for diet analysis the CAM plants are not very common in the diet of herbivores or humans, nor are they common in northern Japan. In most cases isotopic analyses in Japan will address relative proportions of marine versus C<sub>3</sub> terrestrial contributions to human diet.

We also know that when both terrestrial and marine animals eat, their metabolisms recombine food-derived chemicals, producing differences of about 1‰ in isotope ratios between trophic levels for similar tissues, particularly muscle tissue [BENDER *et al.* 1981; DENIRO and EPSTEIN 1978; MCCONNAUGHEY and MCROY 1979; SCHOENINGER 1985]. Consequently, the values for meat are only slightly displaced from those of the foods that the animals eat, which allows us to combine values for meat and plants from the same food chains to obtain averages for alternative groups of human food.

There also is a difference between the diet and extracted bone protein or collagen of the consumers of approximately  $4.5\pm0.5\%$  for lipid / fat free samples as used here [e.g., CHISHOLM 1986; KOIKE and CHISHOLM 1988] to approximately 5‰ for samples with the lipids left in [VAN DER MERWE 1982; VAN DER MERWE and VOGEL 1978]. This allows us to reconstruct the isotope ratio for their *average* diet. This average diet value may then be compared to, and interpolated between, the average values for the diet alternatives available, for example, of C<sub>3</sub> plants or marine food species, in order to estimate the relative proportion of each alternative, as a *protein* source, in that consumer's diet [AMBROSE and NORR 1993; CHISHOLM 1986, 1989]. This procedure works only if variation in diet species values is small.

## Nitrogen

Nitrogen is also metabolized and recombined by the body, reflecting the food source that provided it. For nitrogen, the increment between diet and consumer collagen is not quite as well understood, but seems to be approximately 4 to 5‰. What is particularly useful about nitrogen values is that the inter-trophic level difference between similar tissues is approximately 3 to 4‰ [AMBROSE 1986; MINAGAWA and WADA 1984; SCHOENINGER and DENIRO 1984; SCHWARCZ 1991], which provides better discrimination of trophic levels than does carbon. As Ambrose [1986] and others have observed, marine plants have  $\delta^{15}$ N values approximately 4‰ higher than terrestrial ones. Species from the equivalent trophic levels in marine and terrestrial food chains maintain this difference. The isotopic values for both carbon and nitrogen isotopes in food species groups are summarized in Figure 12.3.

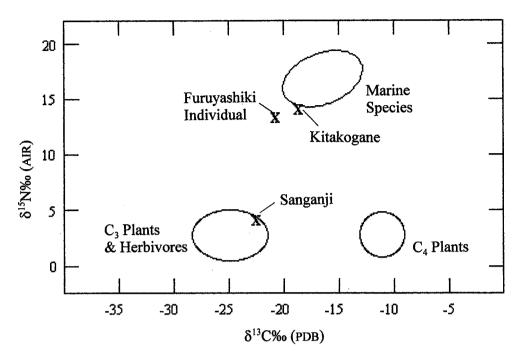


Figure 12.3 Approximate distribution of various potential food groups according to carbon versus nitrogen values, showing the Furuyashiki individual's reconstructed diet results as well as the Kitakogane and Sanganji reconstructed diet averages [after AMBROSE 1986; CHISHOLM *et al.* 1992; KOIKE and CHISHOLM 1988; MINAGAWA and AKAZAWA 1992]. Kuma'ana and Tagara results are not plotted due to lack of nitrogen results.

#### Sources of Variation that must be Considered

There are a number of potential sources of variation inherent in the dietary alternative species that may affect the results of isotopic studies of diet. For example, in the terrestrial environment, geographic and seasonal variations in the proportions of C<sub>3</sub> and C<sub>4</sub> grass species [TEERI and STOWE 1976; TIESZEN *et al.* 1979] could affect grazing herbivores. However, this is not likely a major problem in Japan, since there are few C<sub>4</sub> species present. Marine species, such as anadromous fish and sea mammals, will reflect the average values of the water reservoirs through which they pass. Fontugne and Duplessy [1981], Sackett *et al.* [1965] and Wong and Sackett [1978] have observed a relationship between water temperature and  $\delta^{13}$ C values for marine plankton. Seasonal upwelling of colder water along coastal zones will affect local temperatures and may introduce isotopically different carbon than that of local surface waters. Cold versus warm marine currents would also affect the values for marine species, Further, terrestrial runoff of biogenic carbon, particularly in intertidal and estuarine species, may influence isotopic carbon in marine species, particularly in areas like Tokyo Bay [HAINES and MONTAGUE 1979; RAU *et al.* 1981].

In view of these potential sources of variation, the first step of any complete palaeodiet study must be the determination of *local* values for the archaeologically indicated dietary alternatives. In the vicinity of the Furuyashiki site, there are no extant data that allow determination of local marine or terrestrial diet averages. However, reported values for elsewhere in Japan [CHISHOLM *et al.* 1992; KOIKE and CHISHOLM 1988; MINAGAWA and AKAZAWA 1992] (see Figure 12.3) can still be used as approximate indicators of the food groups and trophic levels exploited by the human consumers examined here. It is important to note that for the limited number of measured Japanese samples, the marine diet species variation is great, thereby severely limiting the precision of estimates of the relative proportions of marine versus terrestrial protein in the local human diet.

#### **Isotope Results and Discussion**

## Food Values

Carbon isotope ratios for Japanese food species have been presented and discussed elsewhere [CHISHOLM *et al.* 1992, KOIKE and CHISHOLM 1988]. Those results indicate that there are only minor variations between Japan and other regions in plant species values. The differences elsewhere are approximately 14‰ between C<sub>3</sub> (average =  $-25.0 \pm 1.38\%$ ) and C<sub>4</sub> plant species (average =  $-11.1 \pm 0.4\%$ ), and about 8 to 9‰ between the C<sub>3</sub> terrestrial and marine (average =  $-16.3 \pm 2.06\%$ ) food alternatives. The differences between major food groups are similar in Japan. This permits the type of analysis we are reporting here. One problem is that the presence of both cold and warm ocean currents off the coast of Japan affects the isotope values for the marine species measured in Japan. This will affect the precision of diet reconstructions, at least until we have a more extensive data base on food species than we do at present.

With a few exceptions, the values for herbivores in Japan are similar to those of the  $C_3$  plant species that they seem to be eating. This means that the herbivores and  $C_3$  plants may be grouped as one diet alternative, which can be compared to the  $C_4$  plants and marine species alternatives (Figure 12.3). One important point that simplifies our analysis is that human  $C_4$  intake is not likely very high. This low  $C_4$  presence has been discussed by Minagawa and Akazawa [1992] and by Chisholm *et al.* [1992]. This means that the comparison that we make here is essentially between the  $C_3$  plant/herbivore group and the marine species. To calculate the relative proportions of each group in a consumer's diet, we determine the value for that consumer's average diet (measured carbon value + increment between diet and consumer's collagen; see above) and then interpolate it between the values for the two food group averages.

Because of the large standard deviations on the Japanese marine food group averages, the maximum uncertainty of proportion estimates is calculated by combining the uncertainties on the marine and terrestrial food averages and on the individual's reconstructed diet, in quadrature, i.e., taking the square root of the sum of the squares of the uncertainties. This gives a result of 2.49, which is  $2.49/8.7 \ge 29\%$  of the interval between the two food alternatives. This uncertainty results from the wide variation in values of the food species used in this preliminary study and is not unusual for an individual or individuals being compared with general food

species data. It will be reduced significantly by obtaining analyses of larger numbers of local samples of food species if and when they become available.

This high overall uncertainty will apply to all diet reconstructions using Japanese samples. However, it will not interfere with comparisons of data sets from different sites and times; it will only restrict our ability to determine the exact proportions in which the different foods were present in individual's diets. To minimize this uncertainty in future studies, we will need more data on local food species for each study area.

#### Human Results

In this pilot study, one sample was prepared and analyzed. As noted above, the sample came from an Early Jomon burial at the Furuyashiki site in Kamikita Town, Aomori Prefecture. The sample gave a low yield of extracted collagen, of about 0.9 percent. However, the appearance was similar to other good collagen extracts obtained from similar samples from Japan. The C:N value was 3.2 (weight) which agrees with the safe range of DeNiro [1985] and Schoeninger *et al.* [1989]. Thus, there is no evidence for contamination in the sample.

The analytical values for this individual are:  $\delta^{13}C = -16.2\%$  and  $\delta^{15}N = 17.8\%$ . Correcting them for the increment between this consumer's collagen and average diet gives values of  $\delta^{13}C = -20.7\%$ ,  $\delta^{15}N = 13.4\%$ . These are plotted in Figure 12.3. The results for the Furuyashiki individual fall near a line connecting the terrestrial C<sub>3</sub> diet groups values with the marine food values. This indicates that there was little or no C<sub>4</sub> plant input in the diet.

For Japan the interval between the carbon values for the marine food [-16.3‰] and C<sub>3</sub> terrestrial food [-25.0‰] alternatives is 8.7‰, and the individual's reconstructed diet value of -20.7‰ is removed from the C<sub>3</sub> alternative by 4.3%.  $4.3 / 8.7 \times 100 = 49\%$ , so the protein in the diet is in the proportions of 49% marine and 51% terrestrial. The result is rounded to approximately 50% marine foods for its dietary protein, with the other 50% obtained from terrestrial sources.

The nitrogen results support the approximately 50:50 marine-terrestrial proportion estimate. However, the nitrogen data for food species are sparse and thus cannot provide exact proportion estimates.

While the results for this individual do fall close to a line connecting the  $C_3$  and marine food species values, they are displaced slightly from the line. This displacement could result from a higher nitrogen value than for people whose results fell on the line. Eating foods from a higher trophic level could cause this difference. In the case of marine food alternatives it would suggest that the consumer(s) ate more fish or marine mammals than shellfish. In the case of terrestrial food alternatives it would suggest that the consumer(s) ate more terrestrial meat than  $C_3$  plants.

### Comparisons with samples from other Jomon sites

The results for, and hence the diet of, this individual differ somewhat from those of individuals from other sites in the southern Tohoku region and Hokkaido, including Tagara (Iwate Prefecture) [Association FOR THE PRESERVATION OF CULTURAL PROPERTIES IN MIYAGI

PREFECTURE 1986] and Kuma'ana (Miyagi Prefecture) [IWATE PREFECTURAL MUSEUM 1979] measured by Chisholm and Koike [1999], and Sanganji (Fukushima Prefecture) and Kitakogane (Hokkaido) measured by Minagawa and Akazawa [1992] (Table 12.1; for locations of these sites, see Figure 12.1). This individual seems to have had a slightly higher marine intake than people from Sanganji, which is located approximately 300 kilometers south. The Furuyashiki results indicate a lower marine intake than for people from Hokkaido and Sakhalin, in fact the carbon value is virtually the same as the results from Tagara, a site not far from Sendai City in southern Tohoku.

 Table 12.1
 Comparison of results for Tohoku and Hokkaido sites (data from this study, CHISHOLM and KOIKE [1999], and MINAGAWA and AKAZAWA [1992]).

Site	δ13C	δ15N	n=
Sanganji	-18.3±0.6	8.6±0.9	13
Kuma'ana	-19.3±0.81		6
Tagara	-16.4±0.69		3
Furuyashiki	-16.2	17.8	1
Kitakogane (M & A)	-14.2±0.4	18.1±0.5	9
Kitakogane (C & K)	-14.5±0.5		8

Note: Kitakogane samples were measured independently by Minagawa and Akazawa and by Chisholm and Koike (carbon only). Differences in results between the two data sets are due to the different number of samples measured and possibly to instrument differences, and are not statistically significant.

There are a few possible explanations for these differences. The Furuyashiki site is located approximately 200-300 kilometers north of Tagara, Kuma'ana and Sanganji, and 200 kilometers south of Kitakogane. Accordingly, the isotope data may suggest a north to south preference for marine resources: residents of the Furuyashiki site ate more marine food than sourthern Tohoku Jomon people, but less than Hokkaido Jomon people. However, some of the difference could be due to geographic variation in isotope ratios found in marine food species [CHISHOLM *et al.* 1992]. In addition, it should be kept in mind that, because only one individual was analyzed in this pilot study, there is always the possibility that the sample represents someone with an atypical diet. Given these possibilities, further research is required to obtain an adequate data base for reliable interpretation of palaeodiet in this region.

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