Paleodiet of Hunter-Gatherers in Japan Estimated by ^{13}C - ^{15}N and Lipid Analyses¹⁾

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Reconstruction of paleodiets for hunter-gatherers in Japan is carried out using the major nutritive elements. (1) Carbohydrate sources are examined using carbon isotope data from charcoal in burnt “cookies” and pottery residues. Measurements of charcoal in burnt cookies indicated no evidence of C4 plant use during the prehistoric age in Japan. (2) Protein sources are identified using carbon and nitrogen isotope ratios measured on collagen extracted from human bones. Among hunting-fishing-gathering people in Japan, marine protein intake was about 70–90 % for people in Hokkaido including the Ainu people, while Jomon people in Honshu and Kyushu had a wider variation of 20–60 % between coastal and inland people. (3) Lipid sources are identified from fatty acids and sterols extracted from pottery residues and coprolites by GC and GC-MS. Coprolites and pottery sherds from shellmidden sites indicated that animal foods were dominant lipid sources in the diet, and marine food resources were not constantly, but occasionally used.

I. Paleodiet analyses

Paleodietary studies of prehistoric hunting-fishing-gathering people in Japan have been carried out using a number of different analytical methods. Prehistoric diet compositions have been determined from data on faunal and floral remains excavated from archaeological sites (SAKAZUME, 1960 ; KANEKO, 1982 ; SUZUKI, 1986 ; AKAZAWA, 1986). Problems with this method result from variable preservation conditions of the remains, particularly floral remains which are found in only a few appropriate situations, such as water-logged sites.

Paleobiomass analysis provided new perspectives for paleodiet studies (KOIKE, 1986a). Paleobiomass analysis for the Murata River area on the east coast of Tokyo Bay (KOIKE, 1986b) provided a detailed estimation of biomass for marine and terrestrial food resources associated with the human population in the study area. Hunting and collecting pressure based on age compositions of faunal remains (KOIKE and OHTAISHI, 1987) indicated the balance between

paleobiomass of food resources and human consumption.

Application of methods, such as GC (gas chromatography) and GC-MS (GC-mass spectrometry), enables direct analysis of chemical residues in coprolites and also from pottery and burnt stones that may have been used for cooking (THORNTON *et al.*, 1970 ; RÖTTLANDER, 1978 ; NAKANO, 1984 ; KOIKE, 1987). The sterol composition of the residues is used to identify animal, plant or bacterial origins of foods (KOIKE, 1991). Fatty acids are used to differentiate aquatic foods, with long-chain polyunsaturated fatty acids, from terrestrial foods with medium-chain fatty acids (KOIKE and TSUCHIYA, 1988).

Human burials provide another sources of information on diet through stable isotope analysis using collagen extracted from human bones (DENIRO and EPSTEIN, 1978). Carbon isotope ratios allow differentiation of marine and terrestrial foods as protein sources (CHISHOLM *et al.*, 1983 ; SHOENINGER *et al.*, 1983). Stable carbon analysis for paleodiet was carried out during

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1987 and 1989 (CHISHOLM *et al.*, 1988) measuring diet species of living animals and plants (KOIKE and CHISHOLM, 1988), and also human bone collagen from Kyushu, Hokkaido and central Japan. Nitrogen isotope ratios indicate trophic level in ecological food chains (MINAGAWA and WADA, 1984); whether plants or animals were the main protein sources in the human diet (MINAGAWA and AKAZAWA, 1988; KOIKE, 1990).

In this paper, analytical methods for paleodiet studies using the major nutritive elements are discussed. Carbohydrate sources are examined using carbon isotope data from charcoal in burnt cookies and pottery residues. Protein sources were identified using carbon and nitrogen isotope ratios measured on extracted human bone collagen. Lipid sources were identified from fatty acids and sterols extracted from pottery residues and coprolites.

II. Carbohydrate sources in paleodiet

Charcoal samples of burnt cookies and pottery residues were used for both $\delta^{13}\text{C}$ measurement and lipid analysis. Therefore, charcoal samples were first treated by chloroform-methanol to extract lipids. About 10 mg of lipid-free charcoal sample were crushed and placed in a Quartz tube with about 1 g of CuO , and heated in an oven at 700°C for 2 hours. Purified CO_2 samples were measured on a Varian CH7 stable isotope mass-spectrometer in the Department of Earth Science at Nagoya University.

To determine the standard carbon isotope ratios for Japanese edible plants, about 50 edible plants were measured (KOIKE and CHISHOLM, 1988). Among the plant foods available in Japan, only 3 species (*Echinochloa crusgalli* -11.6‰ , *Panicum miliaceum* -10.9‰ and *Setaria italica* -11.1‰) were found to be C4 plants. All these are domestic and were probably introduced during historic times. The average values for C3 plants is 25.5‰ . Among measurements of acorns and wild nuts, $\delta^{13}\text{C}$ values for climax forest species of trees such as *Fagus crenata* and *Quercus mongolia* show more negative values, between -28 and -26‰ , while those for secondary forest species of tree such as chestnut were generally less negative around -24‰ .

Measurements of charcoal from burnt cookies

Fig. 1 $\delta^{13}\text{C}$ measurement of charcoal and pottery residues

● : burnt cookies ○ : pottery residues

provide no evidence of C4 plants during prehistoric times in Japan (Fig. 1). This suggests that $\delta^{13}\text{C}$ measurements using human collagen can allow differentiation of marine foods and terrestrial food for protein source in Japanese prehistoric samples, because there will be no overlap of C4 plant and marine food species values.

Measurements of charcoal from pottery residues showed that most of these samples had similar values to those of burnt cookies, suggesting that these came from similar plant resources. The exception was pottery sherds from the Mawaki site which were found with a number of dolphin bones, where $\delta^{13}\text{C}$ values were -20‰ indicating there may have been marine foods in the charcoals.

III. Protein sources in paleodiet

Bone samples are used in our work not only for paleodiet studies, but also for genetic research (HORAI *et al.*, 1989; TAKENAKA and KOIKE, 1989). About 1 g of bone sample is crushed to 1 mm or less and placed in a cellulose tube for decalcification with 1 N HCl at 4°C . The supernatant from well-preserved bone is used for DNA sequence analysis. If the residue contains fine clay or some admixture other than collagen, then it is heated at 60°C , at pH 3 in HCl solution, for at least 10 hours to denature the collagen and

Fig. 2 Location of sites analysed

○ : Jomon ▲ : Ainu

recover it as gelatin. About 10 mg of freeze-dried extracted collagen or gelatin are placed, in vacuo, in a Pyrex or quartz tube with about 1 g of CuO, and combusted in a preheated oven, at 520 °C for Pyrex or 700 °C for quartz, for 2 hours.

The resulting CO₂ samples are measured on the same mass spectrometer at Nagoya University. Purified N₂ samples are measured on a Finnegan MAT 251 mass spectrometer at the Research Center for Agriculture, Tsukuba.

To determine standard values of food resources, carbon and nitrogen isotope ratios for living animals and plants were measured, not only for the major taxa throughout Japan, but also for well defined ecological locations in the Tokyo Bay area. About 50 samples of primary producers and about 80 samples of their consumers, from the tidal flat of the Obitsu River in Tokyo Bay were measured (KOIKE *et al.*, 1989). $\delta^{13}\text{C}$ values for the primary producers fell into three groups : salt marsh plants having normal

Fig. 3 Average dietary $\delta^{13}\text{C}$ values for hunter-gatherers from each site analysed

C3 plant values of -26 to -24 ‰. Benthonic diatoms at -19 to -16 ‰, and seagrasses, including attached diatoms at -11 to -15 ‰. Plotted $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ distributions also show the same 3 groups (WADA, 1980 ; KOIKE, 1990). Intertidal bivalves and other species available at the tidal flats as for human populations were at -14 to -17 ‰ in $\delta^{13}\text{C}$ values and at +12 to +15 ‰ in $\delta^{15}\text{N}$ values.

As an example of a terrestrial ecosystem, the Hakusan Mountain area in Central Japan was selected. In this location black bears and their foods, which also include edible wild plants used by humans, were measured (KOIKE, 1990). Land mammals, such as sika deer, wild boar and black bear collagen had $\delta^{13}\text{C}$ values of -20 to -22 ‰ (-23.5 to -25.5 ‰ estimated for meat) and $\delta^{15}\text{N}$ values of +2 to +4 ‰.

$\delta^{13}\text{C}$ analyses were carried out on about 850 human bone samples from 44 sites in Hokkaido, Honshu and Kyushu (Fig. 2). The prehistoric

Fig. 4 Typical $\delta^{13}\text{C}$ values for average diets of hunter-gatherer in various locations around the world

hunter-gatherers in Japan include both the Jomon Period people and those of later periods in Hokkaido. Rice agriculture started in the Yayoi Period in Honshu and Kyushu. The main purpose of the isotopic analysis was to estimate the relative importance of marine and/or terrestrial protein in the diet of these people.

Hunter-gatherers in Hokkaido, from Jomon to modern Ainu, yielded average diet $\delta^{13}\text{C}$ values between -17 and -21 ‰ (Fig. 3). These coastal people seem to have obtained about 80 to 100 % of their protein from marine foods, similar to the intake of the coastal inhabitants of west coast Canada, Greenland and Scandinavia (Fig. 4).

Shellmound people in Honshu and Kyushu show a wide variation in $\delta^{13}\text{C}$ values, between -19.4 and -24.7 ‰, indicating that about 10 to 50 % of their dietary protein came from marine resources. Consequently, results for hunter-gatherers in central and western Japan indicate a greater reliance on terrestrial food than do results for the northern people. In addition, these results indicate that the marine-terrestrial proportions in diet varied according to the geographic location of the sites, possibly through variations in available food resources. During

the Yayoi Period people appear to have obtained about 50 to 80 % of their protein from terrestrial food. There is little variation in average $\delta^{13}\text{C}$ values between Yayoi sites, suggesting an increase in reliance on feeding strategies that are relatively independent of environmental conditions, e.g., the use of agriculture.

As an example of individual differences within a site, $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ measurements plotted for the Saihiro shellmound site near Tokyo show good correlation with each other, being tightly clustered along a regression line. This must result from their strategies for getting protein, that is, use of terrestrial resources mainly consisting of plants, as indicated by the low $\delta^{15}\text{N}$ values, and of marine resources from lower trophic levels.

Differences in the trophic levels exploited are shown by $\delta^{15}\text{N}$ measurements (Fig. 5). Low values for the Jomon people in Honshu indicated that they depended on both plants and herbivores from lower trophic levels, as well as shellfish and fish for their protein. Higher values for hunter-gatherers in Hokkaido indicate that they relied on higher trophic level foods than people in Honshu and Kyushu, perhaps indicating greater dependency on herbivores and

Fig. 5 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for hunter-gatherers in Japan

larger fish. It is interesting that the Sakhalin Ainu seem to have relied on even higher trophic level foods than the Hokkaido Ainu, probably being more dependent on sea mammals.

These results show some of the observable variations in diet among Japanese hunter-gatherers, compared with other coastal populations in the world. Since these dietary data are taken directly from bone samples of the people we are interested in, individual isotopic variations may be combined with other archaeological evidence, such as burial conditions, to strengthen dietary interpretations. Further, the same collagen/gelatin samples may provide radiocarbon dates. In addition, DNA sequence analysis using the same bone sample may provide genetic information for the person examined (HORAI *et al.*, 1989).

IV. Lipid analysis in paleodiet

Lipid analysis is a non-destructive method which enables us to detect small amounts of lipids in archaeological materials. However application of lipid analysis to paleodiet has some difficulties due to alteration of lipids over long time periods, and contamination by lipids from the soil.

To check for contamination by lipids from the soil, the following conditions should be satisfied; 1) the extraction rate of the total lipids for the archaeological material analysed is considerably higher than that of surrounding soils. 2) Free fatty acid (FFA) composition and sterol composition for the material analysed are different from those for the soil, containing original cooked lipids from plants and animals.

The next step in the lipid analysis is to consider the degeneration of the extracted lipids. After heating to 60 °C, with stirring for 7 days (KOIKE and TSUCHIYA, 1988), unsaturated FFA rapidly degenerated in the air. FFA C20:5 and C22:6 decreased by about 40% from those present before the experiment, and FFA C16:2, C18:2 and C22:2 were also decreased by 80% from those present before the experiment. Besides the chemical degeneration, bacteria in the soil also act to increase saturated FFAs.

Total lipids are extracted 3 times with chloroform-methanol (2:1) under sonication for 30 minutes each. After mixing with 1% BaCl₂ the chloroform layer is evaporated, and the extracted lipids are measured, as total lipids. The total lipids are methylated with 5% HCl-MeOH, and the FFA and sterols collected using

TLC developed with hexane-ether-acetic acid (80 : 30 : 1).

Saturated FFAs from C14 to C28 and unsaturated FFAs Cn : 1, Cn : 2, Cn : 4 and Cn : 5 are detected by gas chromatography, with a 20 % DEGS packed column, using a column temperature of 190 °C rising to 220 °C at 1 °C per minute. Sterols are identified by gas chromatography with 2 % OV-17 packed column at a column temperature of 270 °C. Further identification of FFAs and sterols is carried out using gas chromatography mass spectrometry (JOEL GC-MS DX303) with a megabour column and a scan range of mass number 50 to 500, and a cycle time of 1 second.

122 edible wild plants and 56 species of aquatic animals have been examined to compile standard data on their fatty acid and sterol compositions (KOIKE and TSUCHIYA, 1988; KOIKE, 1991). Sterols are useful to classify lipids as animal, plant or bacterial in origin. Sterols in animals are mainly limited to cholesterol, except for coprostanol in droppings or coprolites. Ergosterol was detected only in *Basidiomycota*, and aquatic plants produced unique sterols, for example fucosterol in *Chromophyta*.

The dominant sterols in most of the terrestrial plants analysed were β -sitosterol, campesterol and stigmasterol. β -sitosterol was especially abundant in the acorns of *Fagales* and roots of *Liliales* and *Araceae*. Buds and young leaves were relatively abundant in campesterol, and green vegetables had relatively high levels of stigmasterol. Other sterols were detected in a limited number plants : Brassicasterol in *Brassica campestris* and *Ficus carica*, 7-stigmasterol in *Thea sinensis*, *Chamaecyparis obtusa* and others, Avenosterol in *Poncirus trifoliata*, *Xanthoxylum piperitum* and *Pteridium aquilinum*. Cholesterol is also detected in a number of plants, especially oily nuts such as walnuts and pine-nuts.

While sterols are useful for classifying as animal, plant or algae, and to get information about plant groups, FFAs are useful to distinguish terrestrial resources from aquatic resources. Aquatic animals produce more highly unsaturated FFAs, while FFAs for the terrestrial animals are limited to C16 and C18 forms. Differences between plants and animals

Fig. 6 GC-MS spectrum of sterols extracted from coprolite at the Soya site

connected by an ecological food chain are usually small ; saturated FFA levels in animals are comparatively higher than those in plants. Cluster analysis using FFA composition for 63 aquatic samples showed that analysed samples could be classified as dolphins (rich in FFAs C14 : 0, C14 : 1, C16 : 1 and C18 : 1), molluscan shells (rich in FFAs C20 : 1 and C22 : 2), tropical fish from Okinawa (rich in the FFAs C18 : 0 and C20 : 4), fresh-water fish (relatively high in C18 : 0, C18 : 2 and C22 : 0), and other oceanic fishes.

About 180 pottery sherds and 30 burnt stones from the Earliest to the Latest Jomon Periods and the Historic Period were analysed. Pottery sherds from the Juno and Miyakubo water-logged sites contained higher amounts of total lipids, at 0.3 to 2 mg/g sample, while those from settlement sites on terraces were between 0.05 to

0.5 mg/g sample. FFA composition showed different preservation conditions : saturated types of FFA usually increased in worn pottery sherds of poor preservation. Sterol compositions for these samples were useful to differentiate animal lipids with cholesterol, from plant lipids with β -sitosterol, campesterol and stigmasterol.

40 coprolites from the Tagara, Enmeiin, Soya and Torihama shellmound sites were analysed for FFA and sterol compositions. An example of a Mass-spectrum for sterols extracted from a coprolite from the Soya site is shown (Fig. 6). The sterol composition was high in cholesterol, accompanied by β -sitosterol and campesterol, suggesting that their diet contained much animal foods, probably fish, but mixed with plant foods.

These methods of analysis add a further tool for paleodiet studies in different time period. Lipid analysis using the coprolite provide dietary informations for a meal in a day, and lipid analysis and carbon isotope measurements using pottery residues indicate foods cooked during a season or more, while isotopic measurements using human collagen shows average dietary values during a few years. Thus, these analytical methods for the paleodiet study will enable to explain exploitation strategies of the hunter-gatherers and their adaptation to the environment.

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