Geographical Patterns of Human Diet Derived from Stable-Isotope Analysis of Fingernails

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KEY WORDS  carbon; food; human nail; isotope ratios; nitrogen

ABSTRACT  Carbon and nitrogen isotope ratios of human fingernails were measured in 490 individuals in the western US and 273 individuals in southeastern Brazil living in urban areas, and 53 individuals living in a moderately isolated area in the central Amazon region of Brazil and consuming mostly locally grown foods. In addition, we measured the carbon and nitrogen isotope ratios of common food items to assess the extent to which these isotopic signatures remain distinct for people eating both omnivorous and vegetarian diets and living in different parts of the world, and the extent to which dietary information can be interpreted from these analyses. Fingernail $^{13}$C values (mean ± standard deviation) were $-15.4 ± 1.0$ and $-18.8 ± 0.8%$ and $^{15}$N values were $10.4 ± 0.7$ and $9.4 ± 0.6%$ for southeastern Brazil and western US populations, respectively. Despite opportunities for a “global supermarket” effect to swamp out carbon and nitrogen isotope ratios in these two urbanized regions of the world, differences in the fingernail isotope ratios between southeastern Brazil and western US populations persisted, and appeared to be more associated with regional agricultural and animal production practices. Omnivores and vegetarians from Brazil and the US were isotopically distinct, both within and between regions. In a comparison of fingernails of individuals from an urban city and isolated communities in the Amazonian region, the urban region was similar to southeastern Brazil, whereas individuals from isolated nonurban communities showed distinctive isotopic values consistent with their diets and with the isotopic values of local foods. Although there is a tendency for a “global supermarket” diet, carbon and nitrogen isotopes of human fingernails hold dietary information directly related to both food sources and dietary practices in a region. Am J Phys Anthropol 131:137–146, 2006.

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Food supply and consumption have shaped the contemporary world (Diamond, 1999). While in the past the energy intake through food consumption was basically related to the process of food acquisition (O’Keefe and Cordain, 2004), today people living in large urban centers have access to a wide range of food products derived from a very broad geographic range (“global supermarket diets”). However, in rural and isolated areas, particularly in developing countries, a significant portion of food in the diet may still be derived from locally produced food items. Stable-isotope analyses can help us evaluate these patterns.

Differing amounts of both heavy and light naturally occurring isotopes of carbon ($^{13}$C/$^{12}$C) and nitrogen ($^{15}$N/$^{14}$N) are present in food items, and consumers incorporate an integrated isotopic signal into their tissues based on all the food they assimilate. As a result, carbon ($^{13}$C) and nitrogen ($^{15}$N) isotope ratios of consumers have been used to infer diet in controlled and semiconrolled studies of animals (DeNiro and Epstein, 1978, 1981; Tieszen et al., 1983; Minagawa and Wada, 1984; Ambrose and Norr, 1993; Sutoh et al., 1993; Tieszen and Fagre, 1993; Sponheimer et al., 2003a,b). Variations in $^{13}$C values of about $20\%$ are found among different foods, occurring largely because of differences in the $C_3$ and $C_4$ photosynthetic pathways (Farquhar et al., 1989). The range of $^{15}$N values found in both plants and animals is also about $20\%$, but is based largely on trophic-level differences (Peterson and Fry, 1987). Trophic-level, muscle-tissue enrichments can vary by $0.5–4.8\%$ for $^{13}$C and 1.0–6.0% for $^{15}$N (DeNiro and Epstein, 1978, 1981; Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984; Ambrose and DeNiro, 1986; Sutoh et al., 1987; Hare et al., 1991; Ambrose and Norr, 1993; Ambrose, 2000; Sponheimer et al., 2003a,b). The stable isotopes of animals, including humans, represent a balance between dietary intake and loss. In general, when animals are close to a steady-state nitrogen balance and their food source has a constant $^{15}$N value, their $^{15}$N values do not exhibit significant fluctuations.

Carbon and nitrogen isotope ratio analyses have been commonly used to reconstruct historical and paleodiets, using both humans and other animal systems (Ambrose and DeNiro, 1986; Boutton et al., 1991; Macko et al., 1999; O’Connell and Hedges, 1999a; Privat et al., 2002) and 1.0–6.0% for $^{15}$N (DeNiro and Epstein, 1978, 1981; Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984; Ambrose and DeNiro, 1986; Sutoh et al., 1987; Hare et al., 1991; Ambrose and Norr, 1993; Ambrose, 2000; Sponheimer et al., 2003a,b). The stable isotopes of animals, including humans, represent a balance between dietary intake and loss. In general, when animals are close to a steady-state nitrogen balance and their food source has a constant $^{15}$N value, their $^{15}$N values do not exhibit significant fluctuations.

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as well as contemporary diets (Webb et al., 1980; Nakamura et al., 1982; Minagawa et al., 1986; Schoeller et al., 1986; Katzenberg and Krouse, 1989; Minagawa, 1992; Yoshinaga et al., 1996; O’Connell and Hedges, 1999a; Tokui et al., 2000; O’Connell et al., 2001; Bol and Pflieger, 2002). Hair and collagen are two of the most commonly analyzed tissue types. However, there are some limitations to interpreting these data, as 1) they infer fractionation factors between an assumed diet and hair or collagen in humans based on uncontrolled studies, or 2) they assume that fractionation factors from controlled animal studies are directly applicable to humans. Given these assumptions, appropriate caution is warranted when quantitative interpretations are made of the data. Nevertheless, results from studies of the modern human diet have strengthened our interpretations of historical diets (Yoshinaga et al., 1996; Bol and Pflieger, 2002).

Earlier studies of contemporary human tissues reached several important conclusions. First, there can be distinct carbon and/or nitrogen isotope ratio differences among inhabitants of different countries or among individuals with different diets (e.g., among omnivores, vegetarians, and vegans) (Nakamura et al., 1982; Minagawa et al., 1986; O’Connell and Hedges, 1999b; Tokui et al., 2000; Bol and Pflieger, 2002). Second, there are significant correlations between the consumption of major food items (such as cereal, meat, dairy, fruits, and vegetables) and the isotopic composition of human hair (Nakamura et al., 1982; Minagawa et al., 1986; Minagawa, 1992; O’Connell and Hedges, 1999b). Third, the δ^{13}C values of hair keratin correlate well with those of the diet, being enriched relative to the diet (DeNiro and Epstein, 1978; Minagawa et al., 1986; Sponheimer et al., 2003b). Fourth, the δ^{15}N values of proteins, including hair keratin, also correlate well with diet, but are enriched relative to the diet (Nakamura et al., 1982; Minagawa et al., 1986; Tokui et al., 2000; Sponheimer et al., 2003a).

The apparent variation in this diet-hair fractionation factor may be the consequence of differential contributions of dietary inputs into the isotope ratios of nonessential amino acids that contribute to the keratin in hair (Schwarz and White, 2004). Approximately two thirds of the C and N in keratin are derived from nonessential amino acids. When only one ingredient containing protein is present in diet (as shown in controlled-feeding experiments), it seems to be effective in determining the degree of routing of protein, as demonstrated by Ambrose and Norr (1993). However, for natural diets, especially human diets, it means that there is an opportunity for mixing of the δ^{13}C of carbohydrate and oil sources to impact the δ^{15}N values of hair keratin. In synthetic tissues, the δ^{13}C of carbohydrate and oil sources to impact the δ^{15}N values of human tissues, as reflected in fingernail keratin.

MATERIALS AND METHODS

Sampling

All samples for this study were collected between September 2000–September 2004. Sampling efforts were concentrated in Brazil and the United States (US), but we also obtained samples from countries in Europe. The δ^{13}C and δ^{15}N values of contemporary human fingernails were analyzed for 273 individuals from Brazil, 490 individuals from the US, and 35 individuals from five countries in Western Europe (referred to hereafter as W-EUROPE). This survey included adults only, spanning a wide range of ages. Volunteered samples were provided to us as clippings from the outer edge of the fingernail. Fingernails were chosen because it is a noninvasive sampling, and nail keratin could integrate diet over the last 6 months (O’Connell et al., 2001). The Brazilian population represented people living in urban centers in the southeastern region of Brazil (referred to hereafter as SE-BRAZIL). The US population represented people living in urban centers in the western region of the US (referred to hereafter as W-USA). Sample sizes from Brazil and the US were sufficient to make statistical comparisons between the two regions.

It is important to recognize from the outset that we are not sure that every individual sampled had lived in the region for a period of time long enough for the fingernail to have fully acquired the isotopic signal of that particular region. However, as suggested by O’Connell et al. (2001), we assumed that it would take at least 5–6 months for the isotopic signal of the diet to register in the fingernail keratin at the end of the nail. In addition, it is important to emphasize that most of our samples were obtained from middle-class individuals living in urban centers, where they had access to a large variety of food items from different geographical origins.

Individuals from the SE-BRAZIL and W-USA populations answered a brief questionnaire providing general details of their diet, i.e., the types of food consumed (cereals, fruits, vegetables, legumes, tubers, beef, dairy, poultry, eggs, pork, processed meats, and seafood) and the frequency of animal protein consumption (daily, weekly, monthly, or never). The SE-BRAZIL and W-USA populations were separated into two groups according to their answers in the questionnaires: omnivores or ovolacto-vegetarians (referred to hereafter as vegetarians).

Additionally, 22 individuals from Santarém, Pará, Brazil (hereafter referred to as CITY), provided fingernail samples. Santarém is an isolated city situated in the central Amazon at the confluence of the Amazon and Tapajós Rivers (2.26 South (latitude), 54.42 West (longitude)). Its population of 100,000 inhabitants is linked to the rest of Brazil only by limited air flights to the rest of the country, by commercial boat traffic that imports food into the city, or by a single road (BR 163) linking Santarém to the east and west regions of the Amazon. This region is well-recognized as having many small communities along the rivers or within the forest. Most people living in these places are caboclo, a term used for descendents of Indians and Portuguese dependent on small-scale agriculture and fishing or hunting for subsistence (Murrieta and Dufour,
Diet patterns from fingernail isotope ratios

Stable-isotope ratio analysis

A dried subsample of 1–2 mg was loaded into tin capsules for \( \delta^{13}C \) and \( \delta^{15}N \) analyses. Stable-isotope ratio analyses were conducted using a Finnigan isotope ratio mass spectrometer interfaced with an elemental analyzer. All samples were analyzed in a continuous-flow mode. For all samples originating from Brazil and for a portion of the US samples, we used a Delta Plus isotope ratio mass spectrometer (Finnigan-MAT) interfaced with an Elemental Analyzer (model 1110, Carlo Erba, Milan, Italy). Brazilian samples were analyzed at the Laboratório de Ecologia Isotópica, Centro de Energia Nuclear na Agricultura—Universidade de São Paulo, Brazil, while US food samples were analyzed at the Stable Isotope Ratio Facility for Environmental Research (SIRFER) at the University of Utah (Salt Lake City, UT). For W-USA fingernails, we used a GV Instruments mass spectrometer (Optima and Isoprime) interfaced to a Carlo Erba NA 1500 or 2500 elemental analyzer at the US Geological Survey facilities (Menlo Park, CA).

Stable-isotope ratios were measured relative to internationally recognized standards. We intercalibrated the laboratories running internal standards, that had been previously calibrated to the international standards. Stable-isotope values are reported in “delta” notation as \( \delta \) values in parts per thousand (‰), where \( \delta \)‰ = \( (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1,000 \), where \( R \) is the molar ratio of the rare to the abundant isotope \( ^{13}C/^{12}C \) or \( ^{15}N/^{14}N \) in the sample and the standard. The standard used for carbon is Pee Dee Belemnite (PDB), and the standard for nitrogen is atmospheric air. The precision of isotope ratio measurements was ±0.15‰ and ±0.3‰ for \( \delta^{13}C \) and \( \delta^{15}N \), respectively.

Statistical analyses

Data distribution was evaluated by the Kolmogorov-Smirnoff test. When the data were normally distributed, parametric tests were applied. Student’s \( t \)-test for independent samples was used to test for differences between omnivores and vegetarians in terms of \( \delta^{13}C \) and \( \delta^{15}N \) values. Differences among geographical regions, the populations in Santarém, and/or among food items were tested using one-way analysis of variance (ANOVA), followed by the post hoc Tukey test. Cluster analysis (an exploratory technique) was applied to the questionnaire data both for SE-Brazil and W-USA populations. All statistical analyses were performed using the software STATISTICA, version 6.1 for Windows (StatSoft, Inc., 2004). Differences at the ≤0.05 level are reported as significant.

RESULTS

Geographical patterns of contemporary omnivores reflected in \( \delta^{13}C \) and \( \delta^{15}N \) values of fingernails

We observed no statistical differences in \( \delta^{13}C \) and \( \delta^{15}N \) values of fingernails between females and males \( (P > 0.05) \). Nor was there a significant isotope ratio dependence on the age of individuals \( (P < 0.05) \). Therefore, we pooled the \( \delta^{13}C \) and \( \delta^{15}N \) data within each geographical region (SE-BRAZIL, W-USA, and W-EUROPE) to test for differences among them.

Fingernails sampled in SE-BRAZIL had higher \( \delta^{13}C \) and \( \delta^{15}N \) values (mean ± standard deviation) \( (−19.4 ± 1.0\%e \) and \( 10.4 ± 0.7\%e \), respectively) than fingernails from

<table>
<thead>
<tr>
<th>TABLE 1. Average of C and N isotopic compositions for all C₃ and C₄ plants, animals and products, and seafood sampled independently</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food</td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>Plants C₃</td>
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<td>Plants C₄</td>
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<tr>
<td>Animal and products</td>
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<td>Seafood</td>
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⁹ Values (%e) are mean ± standard deviation; numbers in parentheses indicate sample size. Different letters in column indicate differences among food types, tested using one-way ANOVA and then post hoc test of Tukey \( (P < 0.05) \).
W-EUROPE (Plants C3) the cereals from omnivores in W-USA, while the category more closely related to fingernail vores SE-Brazil population. On the other hand, the category derived products were separated into two groups: individuals who consumed any kind of meat (omnivores), independent of the frequency of meat consumption, and those who did not consume meat (vegetarians). These positive correlations between δ15C and δ15N values are likely not fortuitous, and are discussed further below.

**Case study: Santarém, Amazon region**

From the questionnaires, we recognized that the subsistence activities of the LAKE community are based on cassava cultivation and production of cassava flour and fishing, while in the FOREST community, the diet is based on the cultivation of cassava (C3), rice (C4), beans (C3), and maize (C4) (Murrieta and Dufour, 2004). They also collect a variety of fruits in the forest, and wildlife hunting is more important for subsistence than fishing. To some extent, the diet in the RIVER community was intermediate between those of the LAKE and FOREST communities. And as expected, people from the CITY of Santarém have a more diverse diet than that of the LAKE, RIVER, and FOREST communities, but curiously enough they consume chicken more frequently in their meals than fish.

The δ13C and δ15N values of fingernails collected in the CITY of Santarém were not significantly different from the isotopic composition of fingernails of omnivores from SE-BRAZIL. However, the fingernails from individuals living in the RIVER, LAKE, and FOREST communities in the Santarém region were depleted in 13C compared to individuals from the CITY of Santarém and from SE-BRAZIL (P < 0.05) (Fig. 2). No significant differences were found among the δ13C values for fingernails from RIVER, LAKE, and FOREST residents (Fig. 2). On the other hand, the δ15N values of fingernails from these rural communities were significantly higher than those found in the CITY of Santarém and in SE-BRAZIL. However, fingernails from the RIVER community were more enriched in 15N than the other isolated communities (P < 0.05) (Fig. 2).

**Evaluating the isotopic contributions of food components to fingernails**

We analyzed staple food items consumed in SE-BRAZIL (represented by Piracicaba) and in W-USA (represented...
by Salt Lake City) with the purpose of evaluating the extent to which isotopic differences among food items might be correlated with isotopic differences that occurred in fingernails between these two geographical regions.

The food items were first classified into four broad categories: animal products, seafood, C3 plants, and C4 plants. In order to help us understand differences between omnivores and vegetarians, we first pooled all samples of those four broad categories, independent of the region from which the food item was sampled (Table 1).

The δ15N signature of C3 and C4 plants, animal products, and seafood differed among them (P < 0.05) (Table 1), with C4 plants having the lowest average δ15N value, and seafood by far the highest δ15N values (Table 1). And as expected, the δ13C values of C3 plants were lower than those found for C4 plants, animal products, and seafood (P < 0.05) (Table 1).

We then partitioned food items into finer categories that allowed a more appropriate comparison of the potential isotope ratio differences in “global supermarket” food items between regions. The C3 plants were divided into FAO categories (cereal C3, tuber, vegetable, and fruit). Among the FAO plant categories, none of the six classifications exhibited any significant differences in δ13C values between the two regions (Table 2). All of the δ13C values were indistinguishable. However, 3 of 6 plant categories had significantly different δ15N values (cereal C3, tuber, and fruit). In each of these three cases, the food items from Brazil were heavier in δ15N value by 0.9–6.2‰ (Table 2). Overall, it appeared that the δ15N values of US plant food items (excluding the N2-fixing legume category) exhibited limited variation among groups. Thus, changes in the δ15N values of cereal C3, tubers, and fruit in Brazil led to the significant δ15N differences in category comparisons between the two countries.

When pooled together into the broad category of animals and products, there were significant differences in the δ15N values of food items from Piracicaba and Salt Lake City (P < 0.05) (Table 2). When separated into the six FAO animal and meat product categories, 4 of the 6

![Fig. 1. Distribution of δ13C and δ15N values of fingernails (grey symbols) and mean ± standard deviation (in black) for omnivores (solid symbols) and vegetarians (open symbols) from contemporary SE-Brazil and W-USA populations. Grey symbols correspond to 109 omnivores of SE-BRAZIL, 114 vegetarians of SE-BRAZIL, 263 omnivores of W-USA, and 51 vegetarians of W-USA who answered questionnaire.](image1)

![Fig. 2. Distribution of δ13C and δ15N values of fingernails values (grey symbols) and mean ± standard deviation (in black) for urban SE-Brazil and residents in CITY of Santarem (solid symbols) and more isolated communities in Brazilian Amazon region (open symbols). Grey symbols correspond to 109 SE-BRAZIL omnivores, 22 CITY, 6 RIVER, 15 FOREST, and 10 LAKE residents, respectively, who answered questionnaire.](image2)
categories again exhibited significantly different $\delta^{15}N$ values. In three of these cases (beef, pork, and dairy), $\delta^{15}N$ values were higher in Piracicaba than in Salt Lake City ($P < 0.05$). The opposite was observed for poultry ($P < 0.05$), where there was a small (0.8%) difference between the two regions. There were no significant differences in $\delta^{15}N$ values for the processed meat and egg categories between Piracicaba and Salt Lake City. In addition, there were no significant differences in $\delta^{15}N$ values for the broad category seafood.

When $\delta^{13}C$ values of food items were pooled together by country into the broad category of animal products, there were significant differences in all categories of food items from Piracicaba and Salt Lake City, with the exception of processed meats ($P < 0.05$) (Table 2). Six of seven animal categories differed in their $\delta^{13}C$ values between regions, with differences ranging from 1.1–6.7%. In each case, animal food items from Brazil were more $^{13}C$-enriched than in the US.

Calculating the potential impact of $C_4$ plants in animal food items

One of the most evident differences in the isotope ratios of animal food items between Brazil and the US appeared to be differences in their average $\delta^{13}C$ values (Table 2). We recognize that the two major plant sources for animals, $C_3$ and $C_4$ plants, have contrasting $\delta^{13}C$ values, although marine-derived feed can complicate interpretations. If we make the initial assumption that all feed for animals was derived from a terrestrial source, then we can estimate the relative proportion of $C_3$ and $C_4$ plants to the animal diets using an isotopic mass balance:

$$\%C_4 = (\delta^{13}C_{\text{animal}} - \delta^{13}C_{C_3}) / (\delta^{13}C_{C_4} - \delta^{13}C_{C_3})$$

where $\%C_4$ is the relative proportion of $C_4$ protein sources in the diet, $\delta^{13}C_{\text{animal}}$ is the average isotopic composition of animal products (−16.8‰) or any of the subcategories, and $\delta^{13}C_{C_3}$ and $\delta^{13}C_{C_4}$ are the average isotopic composition of $C_3$ (−26.1‰, n = 151) and $C_4$ (−11.2‰, n = 16) plants shown in Table 1, respectively. Using this approach, we calculate that in today’s “global supermarket” society, that more than 60% of the diet of the broad category of animal products was derived from $C_4$ plants. This is an especially large percentage, showing the disproportional impact of $C_4$ plants on human food supplies, since $C_4$ taxa account for less than 1% of the Earth’s plant species (Sage and Monson, 1999), and yet represent 25% of terrestrial primary productivity (Still et al., 2003). Even considering a high-estimate fractionation factor of 1% for $^{13}C$ between plants and herbivores, $C_4$ carbon is a very large component of dietary input.

Using Equation (1), we estimated that 75% and 58% of the animal diets were derived from $C_4$ plants in Piracicaba (Brazil) and Salt Lake City (US), respectively. This difference was especially large in the FAO category beef, where a difference of approximately 7% in $\delta^{13}C$ values was observed between Piracicaba and Salt Lake City. Poultry, egg, and dairy were more $^{13}C$-enriched in Piracicaba ($P < 0.05$).

DISCUSSION

Keratin as an isotopic tool

Naturally occurring stable isotopes of C and N have often been measured in both humans (Nakamura et al., 1982; Minagawa et al., 1986; Schoeller et al., 1986; Katzenberg and Krouse, 1989; Minagawa, 1992; Yoshinaga et al., 1996; Macko et al., 1999; O’Connell and Hedges, 1999b; Tokui et al., 2000; O’Connell et al., 2001; Schwarcz and White, 2004; Prowse et al., 2005) and animals (DeNiro and Epstein, 1978, 1981; Tieszen et al., 1983; Schoeninger and DeNiro, 1984; Sponheimer et al., 2003a,b).

Given that approximately two thirds of the carbon and nitrogen atoms in keratin are derived from nonessential amino acids, a mixing or scrambling of the dietary component isotopic signals is possible. Such a scrambling of isotopes from different food sources will make it difficult to provide precise quantitative assessment of the contribution of each component; nevertheless, a semiquantitative assessment is possible. Dietary protein may not be the only carbon source contributing to the protein of hair or fingernails (Minagawa, 1992). There is evidence from animals that hair $\delta^{13}C$ values reflect the carbon isotopic composition of dietary protein rather than the composition of the whole diet (Tieszen and Fagre, 1993). However, the contribution of C from carbohydrates and lipids to the protein component (keratin formation) is still unknown. Despite these complications, tissues such as hair or nail offer an isotopic signal that is systematically related to bulk dietary inputs.

There has been a tendency to meld results from animal and human studies to estimate the fractionation factor between diet and keratin (such as in hair and fingernails), which may or may not lead to some constraints when fully interpreting isotopic data. While most studies focused on hair, O’Connell et al. (2001) showed that the isotopes in hair and fingernail were highly correlated with each other, as might be expected since both tissues are composed primarily of keratin.

Thus, given the existing literature (already cited in the text), we interpret our fingernail data in a semiquantitative manner, based on the consensus of previous studies that $\delta^{13}C$ and $\delta^{15}N$ values are enriched by 1‰ and 3‰ relative to diet, respectively.

Regional isotopic signals in a “global supermarket” environment

Regional isotope ratio signals persist despite a “global supermarket” that would tend to homogenize the potential isotopic differences among geographical regions. As in previous studies of the isotope ratios of human hair, one of the most striking results that we found was the significant difference among populations living in different parts of the world. Table 3 summarizes the literature, showing that regional differences in carbon and nitrogen isotope ratios can appear, despite the advent of a globalization resulting in what may be referred to as a “global supermarket.” Although there can be extensive isotope overlap, carbon and nitrogen isotope ratio differences are apparent for populations living in each of the different continents. Although individuals living in urban centers have access to a large variety of foodstuffs from diverse geographical areas, the data suggest a continued coupling between diet and the regional landscape. In that respect, it is useful to further investigate the causes that led the SE-BRAZIL population to have such high values of $\delta^{13}C$ and $\delta^{15}N$, which to some extent were also observed by Minagawa et al. (1986), Katzenberg and Krouse (1989), and Tokui et al. (2000), but with no clear explanation for this pattern.
Why are diets from urban regions of SE-BRAZIL and W-USA isotopically distinct?

There are several means by which human keratin can result in a high $\delta^{15}N$ value. First, a high $\delta^{15}N$ value could indicate a diet with a higher proportion based on a marine diet instead of plants, since marine food sources have the very highest $\delta^{15}N$ values (e.g., Table 2). Second, a high $\delta^{15}N$ value could indicate a diet with a higher proportion based on terrestrial animals that received feed grown with animal-based fertilizers instead of a diet based on plants. Lastly, a high $\delta^{15}N$ value could indicate a diet with a higher proportion based on terrestrial animals that received feed grown in arid zones instead of a diet based on plants, since it is known that plants from arid and semiarid regions tend to have higher $\delta^{15}N$ values (Heaton, 1987; Robinson, 2001). However, most major agricultural operations today in advanced agriculture use synthetic fertilizers, which have relatively low $\delta^{15}N$ values near 0‰. Therefore, the lower $\delta^{15}N$ values between two regions based on terrestrial diets might simply reflect animals fed plants grown on these artificial fertilizers vs. the absence of significant fertilizer inputs.

There is no evidence from our surveys to indicate that marine food sources played a significant role in the diets of individuals from SE-BRAZIL or W-USA. Thus, let us explore the possibility that individuals from SE-BRAZIL had higher $\delta^{15}N$ values because they were deriving more of their energy intake from animal products. The $\delta^{13}C$ values from SE-BRAZIL are also higher than in other regions (W-USA and W-EUROPE), although a striking difference between the $\delta^{13}C$ of Europeans and North Americans was reported, with North American values being approximately 4% higher (Schoeller et al., 1980, 1986). This was attributed to the widespread use of maize and cane sugar in the US vs. the high use of beet sugar and lesser use of maize in Europe.

As discussed earlier, two likely explanations for the relatively high $\delta^{13}C$ values are trophic enrichment and consumption of $C_4$ plants, either directly or through the consumption of animals fed on a $C_4$-rich diet. Judging from the significant differences in $\delta^{13}C$ between animals and animal products in SE-BRAZIL and W-USA (Table 2), particularly beef, $C_4$ plants appeared to be a much more important component of animal diet in SE-BRAZIL.

We could not extract quantitative animal-consumption data from our surveys, but we could examine general animal-consumption patterns for each region provided by the FAO (FAOSTAT, 2004). These data indicate that all meat consumption in the US (126.5 kg per capita year$^{-1}$) is nearly twice that of Brazil (73 kg per capita year$^{-1}$). We also looked for differences in meat consumption between these two geographical regions by comparing surveys of food consumption available for the city of Campinas in the State of São Paulo (Galeazzi and Falco, 1998), located approximately 80 km from Piracicaba, and for the western region of the US (USDA Continuing Survey of Food Intakes by Individuals, 1994–1996). The inhabitants of Campinas derived 24% of their

**TABLE 3. Carbon and nitrogen isotope ratios for contemporary humans living in different regions of world**

<table>
<thead>
<tr>
<th>Region</th>
<th>Local</th>
<th>Tissue</th>
<th>Diet</th>
<th>$\delta^{15}C$</th>
<th>$\delta^{15}N$</th>
<th>$N$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>Chicago, IL</td>
<td>Hair</td>
<td>O</td>
<td>−16.4 ± 0.9</td>
<td>9.6 ± 0.6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>Chicago, IL</td>
<td>Hair</td>
<td>O</td>
<td>−16.6 ± 0.6</td>
<td>11.8 ± 0.4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>São Paulo</td>
<td>Hair</td>
<td>O</td>
<td>−14.5 ± 1.0</td>
<td>7.3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>Munich</td>
<td>Hair</td>
<td>O</td>
<td>−20.4 ± 0.5</td>
<td>10.1 ± 0.3</td>
<td>1</td>
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<tr>
<td>Netherland</td>
<td>Hair</td>
<td>O</td>
<td>−20.4 ± 0.7</td>
<td>10.4 ± 0.4</td>
<td>5</td>
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<td></td>
</tr>
<tr>
<td>Japan</td>
<td>Hair</td>
<td>O</td>
<td>−18.1 ± 0.5</td>
<td>9.2 ± 0.4</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>Tokyo</td>
<td>Hair</td>
<td>O</td>
<td>−18.0 ± 0.8</td>
<td>10.3 ± 0.5</td>
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</tr>
<tr>
<td>Japan</td>
<td>Tokyo, Arita</td>
<td>Hair</td>
<td>O</td>
<td>−18.2 ± 0.4</td>
<td>9.2 ± 0.4</td>
<td>42</td>
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</tr>
<tr>
<td>China</td>
<td>Hair</td>
<td>O</td>
<td>−19.7 ± 0.9</td>
<td>9.7 ± 0.4</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Korea</td>
<td>Hair</td>
<td>O</td>
<td>−19.1 ± 0.7</td>
<td>8.5 ± 0.6</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>Oxford</td>
<td>Hair</td>
<td>O</td>
<td>−20.2 ± 0.7</td>
<td>8.7 ± 0.5</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>Oxford</td>
<td>Hair</td>
<td>OLV</td>
<td>−21.9 ± 0.3</td>
<td>6.9 ± 0.5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>Oxford</td>
<td>Hair</td>
<td>V</td>
<td>−20.9 ± 0.8</td>
<td>9.8 ± 0.6</td>
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<tr>
<td>UK</td>
<td>Southwest England</td>
<td>Hair</td>
<td>O</td>
<td>−20.8 ± 0.4</td>
<td>8.5 ± 0.6</td>
<td>32</td>
<td></td>
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<tr>
<td>UK</td>
<td>Southwest England</td>
<td>Hair</td>
<td>OLV</td>
<td>-21.2 ± 0.3</td>
<td>6.7 ± 0.7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>Southwest England</td>
<td>Hair</td>
<td>V</td>
<td>−21.7 ± 0.1</td>
<td>9.2</td>
<td>6</td>
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<tr>
<td>Canada</td>
<td>Hair</td>
<td>O</td>
<td>−18.3</td>
<td>9.1</td>
<td>1</td>
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<td></td>
</tr>
<tr>
<td>USA</td>
<td>Hair</td>
<td>O</td>
<td>−18.2</td>
<td>8.4</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>Hair</td>
<td>O</td>
<td>−20.4</td>
<td>8.4</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chile</td>
<td>Hair</td>
<td>O</td>
<td>−20.3</td>
<td>8.6 ± 0.8</td>
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<td></td>
</tr>
<tr>
<td>UK</td>
<td>Oxford</td>
<td>Hair</td>
<td>O</td>
<td>−21.2 ± 0.5</td>
<td>9.3 ± 0.7</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>Oxford</td>
<td>Nail</td>
<td>O</td>
<td>−21.4 ± 0.4</td>
<td>9.4 ± 0.6</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>W-USA</td>
<td>Nail</td>
<td>O</td>
<td>−18.8 ± 0.8</td>
<td>8.5 ± 0.6</td>
<td>455</td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>W-USA</td>
<td>Nail</td>
<td>OLV</td>
<td>−19.8 ± 0.9</td>
<td>10.4 ± 0.7</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>SE-BRAZIL</td>
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<td>O</td>
<td>−15.4 ± 1.0</td>
<td>10.0 ± 0.7</td>
<td>155</td>
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</tr>
<tr>
<td>Brazil</td>
<td>SE-BRAZIL</td>
<td>Nail</td>
<td>OLV</td>
<td>−16.9 ± 1.4</td>
<td>10.0 ± 0.8</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>CITY of Santarém</td>
<td>Nail</td>
<td>O</td>
<td>−16.3 ± 0.9</td>
<td>11.8 ± 1.0</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>FOREST</td>
<td>Nail</td>
<td>O</td>
<td>−19.1 ± 1.0</td>
<td>11.7 ± 0.3</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>LAKE</td>
<td>Nail</td>
<td>O</td>
<td>−19.4 ± 0.9</td>
<td>12.8 ± 0.6</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>RIVER</td>
<td>Nail</td>
<td>O</td>
<td>−20.0 ± 0.9</td>
<td>9.6 ± 0.8</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>W-EUROPE</td>
<td>Nail</td>
<td>O</td>
<td>−20.2 ± 1.2</td>
<td>9.2 ± 0.4</td>
<td>35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Isotopic values (‰) are mean ± standard deviation.
2 Type of diet: O, omnivore; OLV, ovo-lacto-vegetarian; V, vegan.
3 Number of living individuals sampled.
4 References: 1, Nakamura et al., 1982; 2, Schoeller et al., 1986; 3, Minagawa et al., 1986; 4, Minagawa, 1992; 5, O’Connell and Hedges, 1999b; 6, Bol and Pfieger, 2002; 7, O’Connell et al., 2001; 8, this study.

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**References:**
daily energy from animal products; in the western region of the US, this percentage increased to 31%. Together these data argue against a higher per capita meat consumption as the contributing factor for the differences in fingernail isotope ratios between the two regions.

An alternative explanation for the higher $\delta^{15}\text{N}$ values found in SE-BRASIL is the higher $\delta^{13}\text{C}$ values of plants utilized for human and animal consumption in this region. For instance, the average $\delta^{13}\text{N}$ for the broad category plants $C_3$ in SE-BRASIL was almost 2% higher than in W-USA (Table 2). This difference can be due either to higher $\delta^{13}\text{N}$ values in Brazilian crops, or to a higher use of ammonium nitrate fertilizers in the US (FAOSTAT, 2004). The use of synthetic fertilizers in the US in 2000 was much less application of N fertilizer (Palm et al., 2004). On the other hand, fingernails of urban inhabitants of Santarém, located a few kilometers away from the isolated rural communities, had a distinctly different isotopic composition, much closer to the urban centers of SE-BRASIL than to the rural areas of Amazonia.

The nutritional study conducted by Murrieta and Dufour (2004) in two villages of Ituqui island, close to the LAKE community, pointed out that cassava flour (farinha) was responsible for 25–34% of the total energy consumption in those villages. Fish was the second most important item, representing 18–26% of total energy consumption. The isotope-food mass balance using food consumption from Murrieta and Dufour (2004) produced, as expected, lower $\delta^{13}\text{C}$ values than in SE-BRASIL and the CITY of Santarém (Table 4). Considering a fractionation of 3% between diet and nail, the mass balance cal-

### TABLE 4. Isotopic diet mass balance for Santarém isolated communities

<table>
<thead>
<tr>
<th>Food</th>
<th>Aracampa</th>
<th>São Benedito</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Energy (%)</td>
<td>$\delta^{13}\text{C}_\text{food}$ (%)</td>
</tr>
<tr>
<td>Root</td>
<td>34.0</td>
<td>-26.5</td>
</tr>
<tr>
<td>Fish</td>
<td>17.5</td>
<td>-27.9</td>
</tr>
<tr>
<td>Sugar</td>
<td>11.5</td>
<td>-11.0</td>
</tr>
<tr>
<td>Dairy</td>
<td>7.3</td>
<td>-15.2</td>
</tr>
<tr>
<td>Cereal C_3</td>
<td>6.8</td>
<td>-25.9</td>
</tr>
<tr>
<td>Oil</td>
<td>4.6</td>
<td>-26.0</td>
</tr>
<tr>
<td>Poultry</td>
<td>3.2</td>
<td>-15.5</td>
</tr>
<tr>
<td>Fruit</td>
<td>2.5</td>
<td>-26.9</td>
</tr>
<tr>
<td>Legume</td>
<td>1.3</td>
<td>-25.9</td>
</tr>
<tr>
<td>Cereal C_4</td>
<td>1.0</td>
<td>-10.7</td>
</tr>
<tr>
<td>Beef</td>
<td>0.4</td>
<td>-10.4</td>
</tr>
<tr>
<td>Pork</td>
<td>0.4</td>
<td>-17.6</td>
</tr>
<tr>
<td>Vegetable</td>
<td>0.0</td>
<td>-27.8</td>
</tr>
<tr>
<td>Egg</td>
<td>0.0</td>
<td>-15.5</td>
</tr>
<tr>
<td>Animal fat</td>
<td>0.0</td>
<td>-12.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>90.5</td>
<td>-23.0</td>
</tr>
</tbody>
</table>

1 Food energy data from Murrieta and Dufour (2004). Averages of $\delta^{13}\text{C}_\text{food}$ were taken from Table 2. No fractionation was considered between diet and fingernail.
2 $\delta^{13}\text{C}$ of this item is one found for cassava flour that is main type of food used in rural areas of Amazon (Murrieta and Dufour, 2004).
3 We used average values provided by Oliveira (2003).
4 We assumed that all sugar and sweeteners had C_4 origin (sugar cane).
5 According to Murrieta and Dufour (2004), main oil used is produced from soybean.
6 Amount of energy provided by C_4 cereal (maize) was estimated by food energy provided by cereals (908.4 calories per capita day$^{-1}$) and energy provided by maize (167.8 calories per capita day$^{-1}$) according to FAO Statistical Database (2004) for Brazil. We assumed that 18% (167.8/908.4) of total food energy provided by cereals came from maize. In addition, Murrieta and Dufour (2004) attested use of maize as food on several occasions.

### The Santarém case study

So far, we have discussed differences in fingernails of individuals living in urban centers, where they have access to a great variety of food items, from local foods to those originating thousands of kilometers away. Although we saw significant differences among geographical regions, the global food market tends to increase variability among urban individuals living in the same region, and perhaps to decrease differences among urban individuals living in different regions.

The Santarém case study provides an unusual opportunity to more closely examine the role of external food sources in the differentiation of stable-isotope ratios of fingernails. The results observed in rural isolated communities in the Santarém region confirmed the results of nutritional surveys that emphasized the importance of local products such as cassava and a high consumption of fish or game as a source of protein and energy (Peres, 2000; Peres and Lake, 2003; Murrieta and Dufour, 2004). On the other hand, fingernails of urban inhabitants of Santarém, located a few kilometers away from the isolated rural communities, had a distinctly different isotopic composition, much closer to the urban centers of SE-BRASIL than to the rural areas of Amazonia.
Diet patterns from fingernail isotope ratios

Table 5. Carbon and nitrogen isotope ratios (‰) of animals (nail) and tambaqui fish (muscle) tissues from Brazilian Amazon region

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>δ¹³C</th>
<th>δ¹⁵N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dasyprocta sp.</td>
<td>Agouti</td>
<td>−23.2</td>
<td>6.8</td>
</tr>
<tr>
<td>Tayassu sp.</td>
<td>Collared peccary</td>
<td>−23.4</td>
<td>5.6</td>
</tr>
<tr>
<td>Bradypus tridactylus</td>
<td>Sloth</td>
<td>−23.2</td>
<td>10.4</td>
</tr>
<tr>
<td>Colossoma macropomum¹</td>
<td>Tambaqui</td>
<td>−27.9</td>
<td>9.8</td>
</tr>
</tbody>
</table>

¹ From Oliveira (2003).

Calculations for Aracampina and São Benedito (isolated communities) provided fairly good agreement in the δ¹³C values observed for our three isolated communities studied (Table 4). It is also important to remember that sugar is the third most important food item in the diet of those villages. In Brazil, sugar is made exclusively from sugar cane, which is a C₄ plant. Therefore, a small increase in sugar consumption would increase the estimated δ¹⁵N values of fingernails (Yoshinaga et al., 1996).

The δ¹⁵N values of fingernails from isolated communities (RIVER, LAKE, and FOREST) in the Santarém region were most enriched. The higher δ¹⁵N values for the RIVER and LAKE communities were likely due to the high consumption of fish. One of the region’s most popular fish for consumption is Colossoma macropomum, with a δ¹⁵N value of about 9.0‰ (Table 5). People living in RIVER and LAKE communities not only consume fish, but also cassava and cereals (Murrieta and Dufour, 2004). As already discussed, the δ¹⁵N of these food items was also high in Brazil. In the Santarém region, δ¹⁵N values were especially high, as can be seen for locally produced rice (6.6‰) and cassava (6.9‰). Therefore, it seems that the high δ¹⁵N of Amazon caboclos living in the LAKE and especially the RIVER community was associated with a combination of high fish consumption and plants with enriched δ¹⁵N values.

Consistent with both the questionnaire and literature (e.g., Peres, 2000), people from the forest community consumed a fair amount of local wild game and ate much less fish. According to Peres and Lake (2003), for instance, collared peccary (Tayassu sp.) and agouti (Dasyprocta agouti) were regularly consumed by people living in the forest. The isotopic composition of fingernails from these two herbivorous animals, and also from the sloth (Bradypus tridactylus), which is herbivorous but not regularly consumed by the local people (Peres, 2000) (Table 5), could give us an idea of the natural range of δ¹⁵N in this region of the Amazon.

Although Santarém is located at the confluence of the Amazon and Tapajós Rivers, two major rivers of the Amazon basin, apparently people living in this city switched their diet preferences (for city dwellers). As a consequence, the average δ¹³C of their fingernails was 3‰ more enriched than those of rural inhabitants in the region, and δ¹⁵N was lower by ~2‰. Migration from isolated communities to urban centers in the Amazon basin is a common pattern today (Instituto Brasileiro de Geografia e Estatística, 2004). Consequently, there may be a progressive change from a diet based on locally produced food to a diet where people have easy access to a large range of food products (“supermarket diets”).

Conclusions

Based on the food items consumed, the carbon and nitrogen isotope ratios of fingernails from modern human populations in Brazil and the US recorded dietary information. Although the current global economic structure allows people to have access to a greater range of food products (“supermarket diets”), distinct differences in carbon and nitrogen isotope ratios can still be detected in comparisons between urban populations in SE-Brazil and W-USA because of regional differences in food resources. Therefore, despite global trends toward greater dietary homogenization, the carbon and nitrogen isotope ratio data of human fingernails indicate that regional dietary information is recorded in fingernails. In a study of the Santarém region (Brazilian Amazon basin), urbanization effects can be detected. Fingernail data confirmed that fingernails from people in more isolated areas of the forest and river regions away from Santarém recorded distinctive isotopic values, which were consistent with their diets and with the isotopic values of very local foods.

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Literature Cited


1 From Oliveira (2003).


