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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

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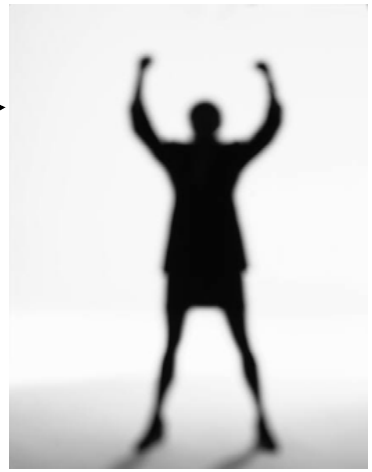
Abstract

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 $\delta^{13}C$ values (mean ± standard deviation) were -15.4 ± 1.0‰ and -18.8 ± 0.8‰ and $\delta^{15}N$ 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, 2006. © 2006 Wiley-Liss, Inc.

Background (I)

Enrichment factor or
Fractionation factor = (body d15N) / (dietary d15N) = 3-4‰

Dietary d15N



d15N in body protein pool

Fractionation factor is stable for sex and age

Background (I)

$\delta^{15}N =$

$$d15N = \{(15N/14N_{(sample)} - 15N/14N_{(\underline{standard})}) / 15N/14N_{(standard)}\} \times 1000$$

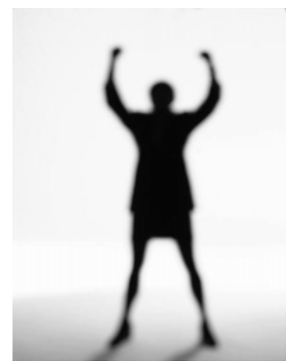
Atomospheric N

$\delta^{13}C =$

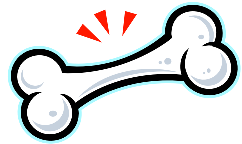
$$d13C = \{(13C/12C_{(sample)} - 13C/12C_{(\underline{standard})}) / 13C/12C_{(standard)}\} \times 1000$$

Fossil remain of shell

d13C: higher in C3 plants (e.g., rice, wheat, potato)
lower in C4 plants (e.g., maize)



Background (I)

In archaeology,  d15N d13C → Diet in the past

In ecology,  d15N d13C → Analysis of food web in the regional ecosystem

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Preconditions: (1) “You are what you eat”
(2) Fractionation factor is stable

The principle is valid for a steady metabolic state.

Recent study: Negative nitrogen balance (nutritional stress, diseases) increase d15N. Positive nitrogen balance??

“Global supermarket diets”

“Diet derived from locally produced foods”

d13C and d15N values of human fingernails from distinct regions to address:

1) Are there recognizable dietary isotope differences among regions?
2) How variable are d13C and d15N values of human fingernails in this ‘‘supermarket diet’’ era?
3) Are there recognizable isotope differences between humans living in urban centers in comparison to more isolated rural populations?

MATERIALS AND METHODS: Sampling

In 2000-2004,

(A) n=273 Brazil (<u>SE</u>. urban); n=490 USA (<u>W</u>, urban);
n=35 <u>W-Europe</u>,
middle-class, adults,
Brief Q: type of foods consumed, Freq Animal protein
→ (a) omnivores or (b) vegetarian

(B) n=22 Brazil (Santare´m, an isolated <u>CITY</u> in Amazon)

(C) n=15 Brazil (<u>Forest</u>), n=6 (<u>River</u>), n=10 (<u>lake</u>),

(D) n=2 agoutis, n=1 peccary, n=2 sloths

(A) urban centers, (B) an Isolated city
(C) Isolated rural communities, (D) wild animals

MATERIALS AND METHODS: Sampling

Fingernails: rinsed twice in distilled water for 20 min (OK?)

Food items in each region:

<u>FAO classification for food commodities (FAO Statistical Database, 2004):</u>

(A) Animal products: beef, poultry, pork, processed meat, dairy, and egg.
(B) Poultry: chicken and turkey.
(C) Processed meat: bacon, ham, pepperoni, salami, and sausage.
(D) Dairy products: milk and cheese.
(E) Seafood: salmon, tuna, crab, shrimp, octopus, and squid.
(F) Vegetable: broccoli, celery, collards, cucumber, lettuce, radicchio, Swiss chard, tomato, and zucchini legume, tuber, fruit.
(G) Cereal C3: barley, oat, rice, and wheat
(H) Cereal C4: maize.
(I) Legume (pulses): all beans, including lentils and soybeans.
(J) Fruit: tropical and temperate fruits
(K) Tuber: tubers and roots (beet, carrot, cassava, and Irish and sweet potato).

MATERIALS AND METHODS: Stable-isotope ratio analysis

-A dried subsample of 1–2 mg was loaded into tin capsules for d13C and d15N analyses.
-Stable-isotope ratio analyses were conducted using a Finnigan isotope ratio mass spectrometer (Delta Plus isotope ratio mass spectrometer) interfaced with
an Elemental Analyzer (model 1110, Carla Erba, Milan, Italy) <Brazilian samples and a part of US samples), or 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) <US samples>.

The standard used for carbon is Pee Dee Belemnite (PDB), and the standard for nitrogen is atmospheric air. The precision of isotope ratio measurements (=SD?) was 0.15‰ and 0.3‰ for d13C and d15N, respectively.

RESULTS: Geographical patterns of contemporary omnivores reflected in d13C and d15N values of fingernails

δ13C／δ15N： no gender and age effect

SE-BRAZIL: -15.4（1.0）‰ <d13C>, 10.4 (0.7) ‰ <d15N>
Higher than
W-USA: -18.8（0.8）‰ <d13C>, 9.4 (0.6) ‰ <d15N>
W-EUROPE: -20.2（1.2）‰ <d13C>, 9.4 (0.8) ‰ <d15N>

W-USA d13C > W-EUROPE d13C

RESULTS: Omnivores vs. vegetarians from SE-BRAZIL and W-USA

Fig 1

Vegetarian: d13C/d15N == SE-BZL >W-USA

SE-BZL: d15N == vegetarian = omnivore
SE-BZL: d13C == vegetarian < omnivore
W-USA: d15N == vegetarian < omnivore
W-USA: d13N == vegetarian = omnivore

In SE-BZL: d15N*d13C=correlated (P<0.05)
In W-USA: d15N*d13C=correlated (P<0.05)

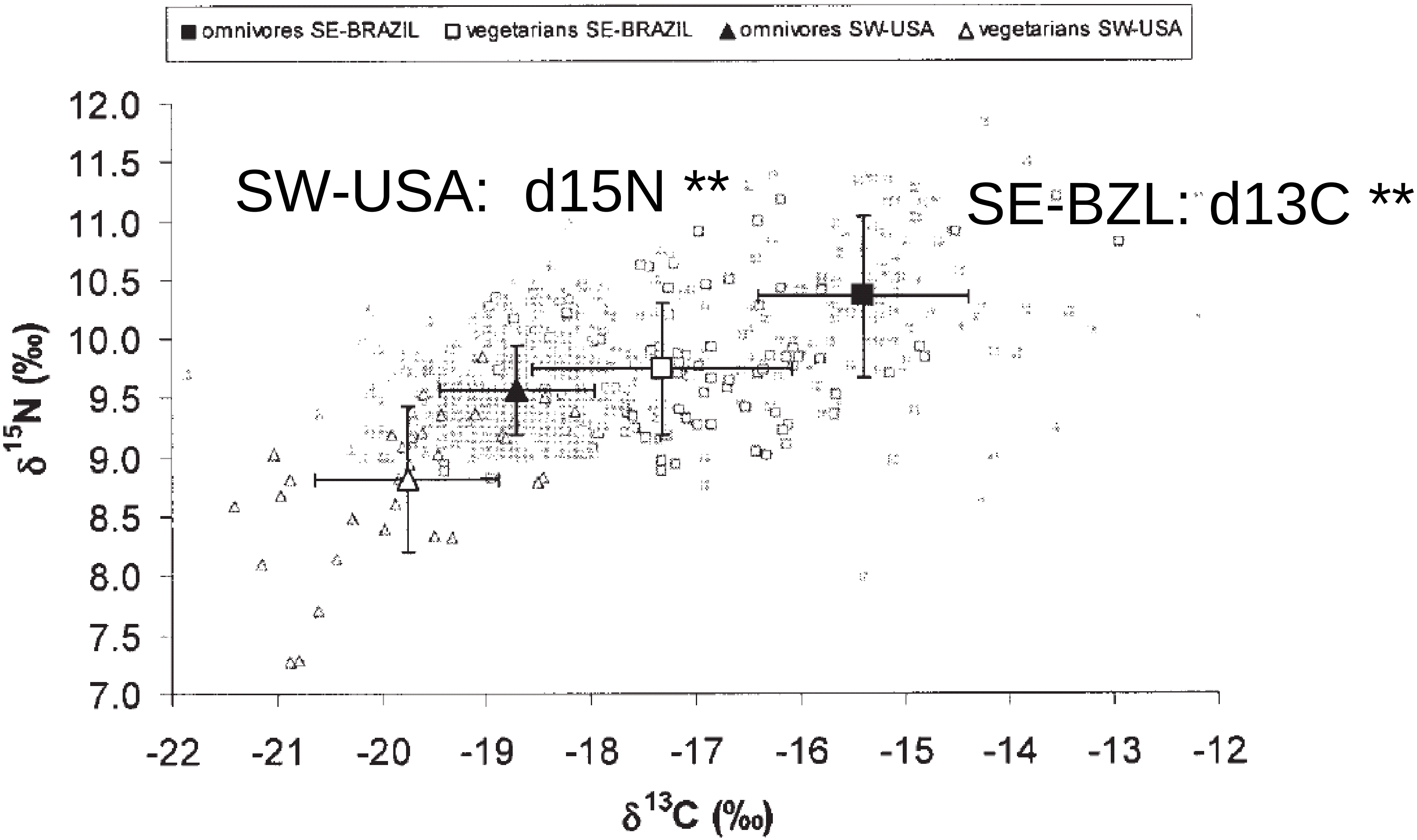


Fig. 1. Distribution of d13C and d15N values of fingernails (grey symbols) and mean 6 standard deviation (in black) for omnivores (solid symbols) and vegetarians (open symbols) from contemporary SE-Brazil and WUSA populations. Grey symbols correspond to 109 omnivores of SE-BRAZIL, 114 vegetarians of SE-BRAZIL, 263 omnivores of WUSA, and 51 vegetarians of W-USA who answered questionnaire.

RESULTS: Case study: Santarém, Amazon region

LAKE: cassava, fish
RIVER: in-btw
FOREST: cassava(C3), rice(C3), beans(C3),
maize(C4), wild animals/plants
CITY: diverse

Fig 2

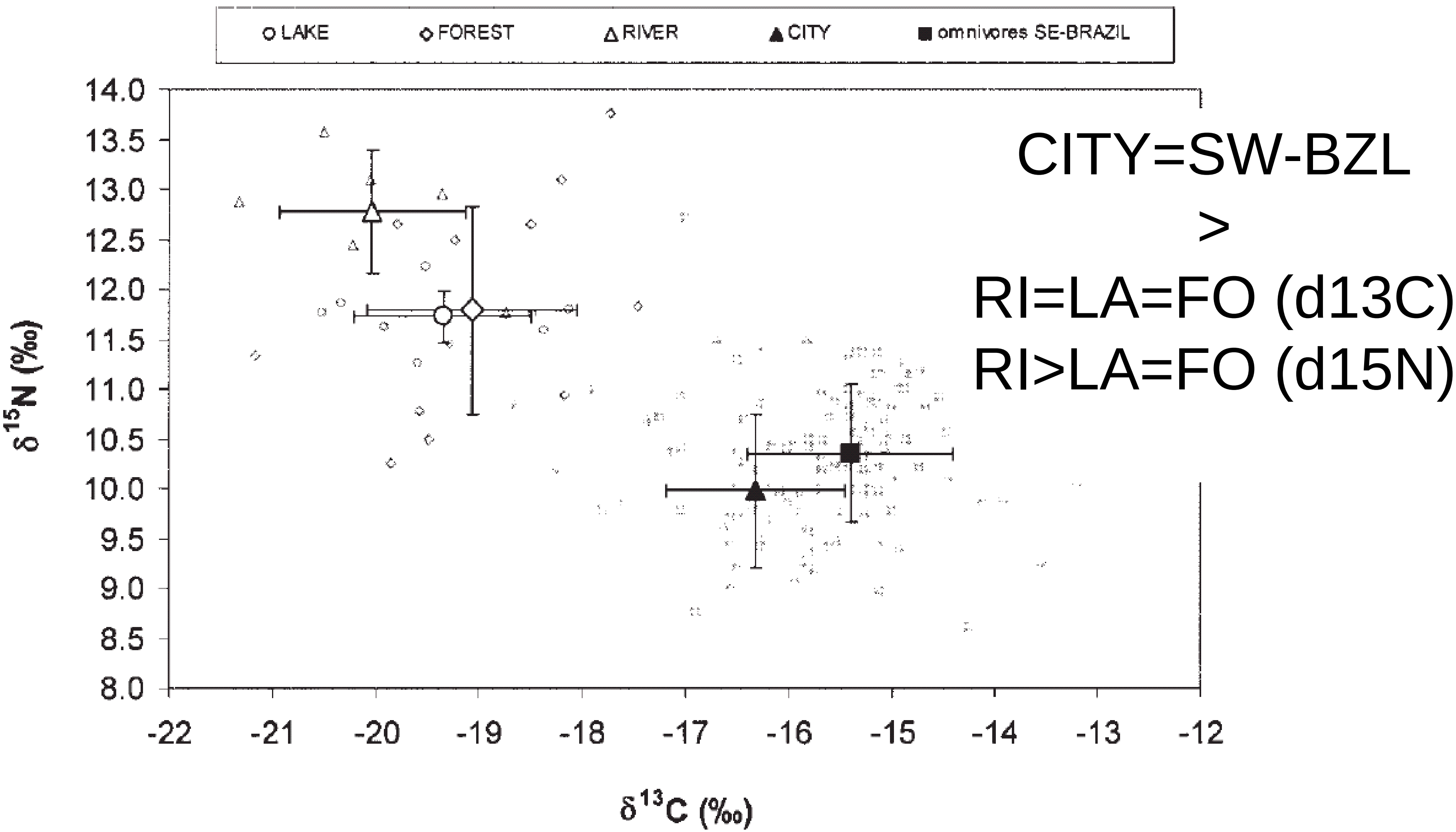


Fig. 2. Distribution of d13C and d15N values of fingernails values (grey symbols) and mean 6 standard deviation (in black) for urban SE-Brazil and residents in CITY of Santare´m (solid symbols) and more isolated communities in Brazilian Amazon region (open symbols). Grey symbols correspond to 109 SE-BRAZIL omnivores, 22 CITY, 6 RIVER, 15 FOREST, and 10 LAKE residents, respectively, who answered questionnaire.

RESULTS: Evaluating the isotopic contributions of food components to fingernails

All food samples in four categories:

TABLE 1. Average of C and N isotopic compositions for all C_3 and C_4 plants, animals and products, and seafood sampled independently[1]

Food	$\delta^{15}N$	$\delta^{13}C$
Plants C_3	2.9 ± 2.8 (151)[a]	–26.1 ± 1.9 (151)[a]
Plants C_4	1.0 ± 1.7 (16)[b]	–11.2 ± 0.6 (16)[b]
Animal and products	4.5 ± 2.3 (174)[c]	–16.8 ± 2.6 (174)[c]
Seafood	12.1 ± 2.8 (26)[d]	–19.2.0 ± 2.0 (26)[d]

[1] Values (‰) 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 ($P < 0.05$).

C C

TABLE 2. *C and N isotopic compositions of food items categorized according to FAO, obtained in supermarkets and restaurants in Piracicaba, São Paulo, Brazil and Salt Lake City, Utah*[1]

	Piracicaba δ^{15} N	Salt Lake City δ^{15} N	Piracicaba δ^{13} N	Salt Lake City δ^{13} N
Plants C_3[2]	4.2 ± 3.4 (43)[a]	2.4 ± 2.4 (108)[b]	−26.7 ± 2.5 (43)[a]	−25.9 ± 1.6 (108)[b]
Cereal C_3	3.6 ± 1.5 (7)[a]	2.7 ± 1.2 (34)[b]	−25.9 ± 2.3 (7)[a]	−25.1 ± 1.8 (34)[a]
Cereal C_4	2.2 ± 1.1 (3)[a]	0.7 ± 1.8 (13)[a]	−10.7 ± 0.5 (3)[a]	−11.3 ± 0.6 (13)[a]
Legume	1.7 ± 1.9 (13)[a]	1.5 ± 1.9 (13)[a]	−25.9 ± 1.5 (13)[a] =	−25.5 ± 1.2 (13) [a]
Tuber	5.0 ± 13.6 (6)[a] >	−1.2 ± 2.6 (17)[b]	−26.5 ± 1.0 (6)[a]	−26.5 ± 1.1 (17)[a]
Vegetable	6.1 ± 4.3 (14)[a]	4.5 ± 3.0 (16)[a]	−27.8 ± 3.5 (14)[a]	−27.3 ± 2.1 (16)[a]
Fruit	6.8 ± 2.9 (3)[a]	2.1 ± 2.4 (28)[b]	−26.9 ± 3.0 (3)[a]	−25.9 ± 1.1 (28)[a]
Animal and products[3]	5.0 ± 1.7 (47)[a] >	4.3 ± 1.1 (127)[b]	−14.7 ± 2.9 (47)[a]	−17.5 ± 2.0 (127)[b]
Beef	6.7 ± 1.2 (13)[a]	5.5 ± 0.8 (27)[b]	−10.4 ± 0.9 (13)[a]	−17.1 ± 2.4 (27)[b]
Poultry	2.7 ± 0.3 (6)[a]	3.5 ± 0.6 (41)[b]	−15.5 ± 0.8 (6)[a]	−17.0 ± 0.9 (41)[b]
Pork	4.2 ± 0.6 (12)[a] >	3.6 ± 0.7 (8)[b]	−17.6 ± 0.7 (12)[a]	−16.5 ± 1.4 (8)[b]
Processed meat	4.1 ± 1.8 (4)[a]	4.0 ± 0.8 (29)[a]	−16.8 ± 0.5 (4)[a]	−17.0 ± 1.1 (29)[a]
Dairy	6.0 ± 1.0 (7)[a]	5.1 ± 0.3 (18)[b]	−15.2 ± 1.4 (7)[a]	−20.6 ± 1.4 (18)[b]
Egg	4.8 ± 1.6 (5)[a]	5.3 ± 0.5 (4)[a]	−15.7 ± 0.3 (5)[a]	−18.3 ± 1.1 (4)[b]
Seafood	11.4 ± 2.1 (5)[a]	12.2 ± 3.0 (20)[a]	−17.3 ± 0.9 (5)[a]	−19.6 ± 1.9 (20)[b]

[1] Different letters in row indicate differences between cities, tested using one-way ANOVA and then *post hoc* test of Tukey ($P < 0.05$). Values (‰) are mean ± standard deviation; numbers in parentheses indicate sample size.
[2] Included legumes, vegetables, tubers, fruits, and C_3 cereals.
[3] Included beef, poultry, pork, processed meat, dairy, and chicken eggs.

d13C and d15N values differed by the regions

RESULTS: Calculating the potential impact of C4 plants in animal food items

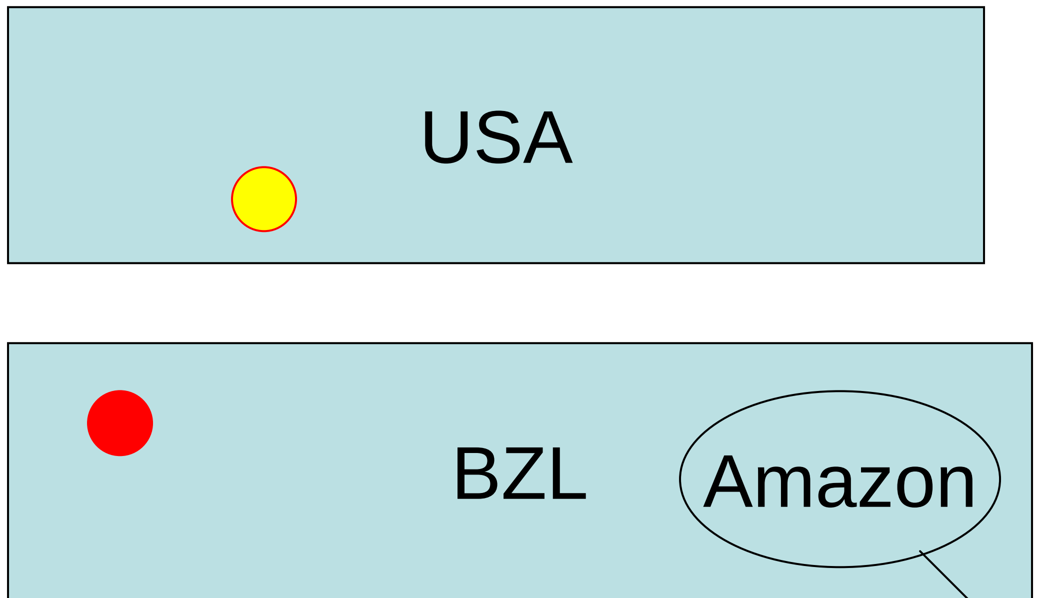
Relative contribution of C4 plants to the animal diets:

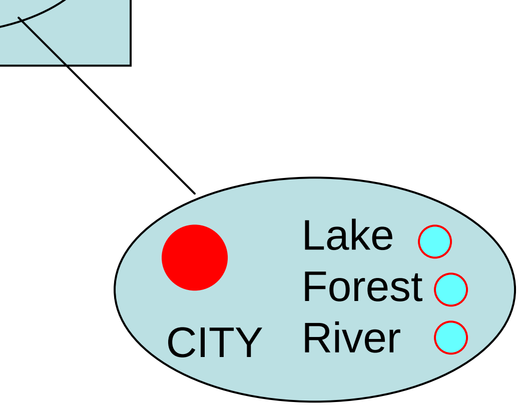
$$\%C_4 = (\delta^{13}C_{animal} - \delta^{13}C_{C3})/(\delta^{13}C_{C4} - \delta^{13}C_{C3}) \qquad (1)$$

where $\%C_4$ is the relative proportion of C_4 protein sources in the diet, $\delta^{13}C_{animal}$ is the average isotopic composition of *animal products* (−16.8‰) or any of the subcategories, and $\delta^{13}C_{C3}$ and $\delta^{13}C_{C4}$ 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,

%C4=75% (Brazil) and 58% (USA)
(cf. C4-1% of planet species, 25% of primary production)

DISCUSSION: Regional isotopic signals in a “global supermarket” environment!





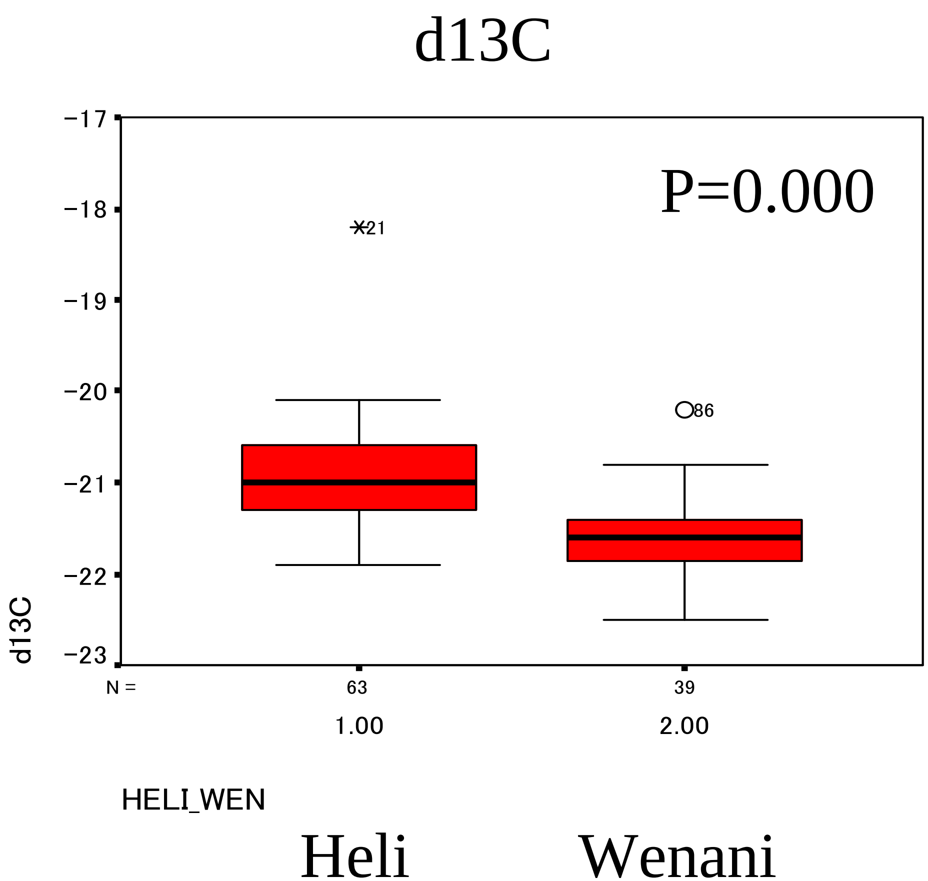
High d13C/d15N in SE-BZL?
Table 3

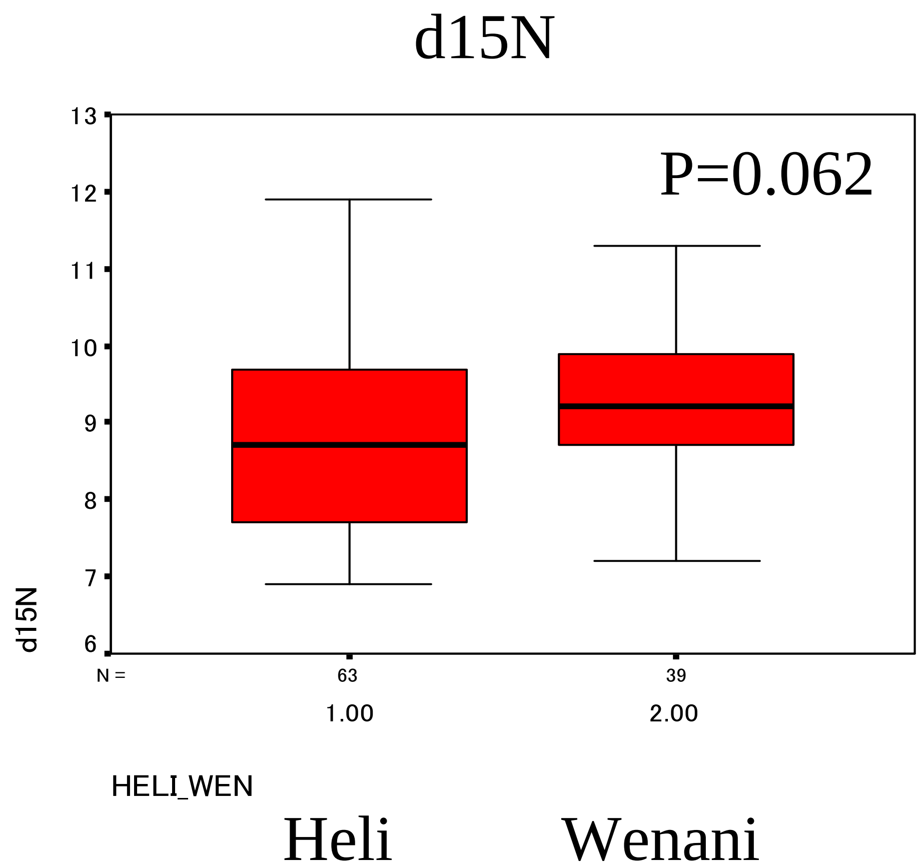
TABLE 3. Carbon and nitrogen isotope ratios for contemporary humans living in different regions of world[1]

Region	Local	Tissue	Diet[2]	δ^{13} C	δ^{15} N	N[3]	Reference[4]
USA	Chicago, IL	Hair	O	−16.4 ± 0.9	9.6 ± 0.6		1
USA	Chicago, IL	Hair	O	−16.6 ± 0.6	11.8 ± 0.4	8	2
Brazil	São Paulo	Hair	O	−14.5 ± 1.0		7	3
Germany	Munich	Hair	O	−20.4 ± 0.5	10.1 ± 0.3		1
Netherland		Hair	O	−20.4 ± 0.7	10.4 ± 0.4	5	3
Japan		Hair	O	−18.1 ± 0.5		23	3
Japan	Tokyo	Hair	O	−18.0 ± 0.8	10.3 ± 0.5		1
Japan	Tokyo, Akita	Hair	O	−18.2 ± 0.4	9.2 ± 0.4	42	4
China		Hair	O	−19.7 ± 0.9	9.7 ± 0.4	7	3
Korea		Hair	O	−19.1 ± 0.7	8.8 ± 0.6	10	3
UK	Oxford	Hair	O	−20.2 ± 0.7	8.7 ± 0.5	14	5
UK	Oxford	Hair	OLV	−21.0 ± 0.3	6.9 ± 0.5	6	5
UK	Oxford	Hair	V	−20.9 ± 0.8	9.8 ± 0.6	8	5
UK	Southwest England	Hair	O	−20.8 ± 0.4	8.5 ± 0.6	32	6
UK	Southwest England	Hair	OLV	−21.2 ± 0.3	6.7 ± 0.7	6	6
UK	Southwest England	Hair	V	−21.7 ± 0.1	9.2	3	6
Canada		Hair	O	−18.3	9.1	1	6
USA		Hair	O	−18.2	8.4	1	6
Germany		Hair	O	−20.4	8.4	1	6
Chile		Hair	O	−20.3	8.6 ± 0.8	2	6
UK	Oxford	Hair	O	−21.2 ± 0.5	9.3 ± 0.7	12	7
UK	Oxford	Nail	O	−21.4 ± 0.4	9.4 ± 0.6	12	7
USA	W-USA	Nail	O	−18.8 ± 0.8	8.8 ± 0.6	455	8
USA	W-USA	Nail	OLV	−19.8 ± 0.9	10.4 ± 0.7	35	8
Brazil	SE-BRAZIL	Nail	O	−15.4 ± 1.0	10.0 ± 0.7	155	8
Brazil	SE-BRAZIL	Nail	OLV	−16.9 ± 1.4	10.0 ± 0.8	118	8
Brazil	CITY of Santarém	Nail	O	−16.3 ± 0.9	11.8 ± 1.0	22	8
Brazil	FOREST	Nail	O	−19.1 ± 1.0	11.7 ± 0.3	15	8
Brazil	LAKE	Nail	O	−19.4 ± 0.9	12.8 ± 0.6	10	8
Brazil	RIVER	Nail	O	−20.0 ± 0.9	9.6 ± 0.8	6	8
W-EUROPE		Nail	O	−20.2 ± 1.2		35	8

[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 Pflieger, 2002; 7, O'Connell et al., 2001; 8, this study.

Cf. SHP in PNG, over 70%energy from sweet potato





DISCUSSION: Why are diets from urban regions of SE-BRAZIL and W-USA isotopically distinct?

“Global super market”

-More meat consumption in BZL? No
-Less use of ammonium nitrate fertilizer? Yes
  -US= 6 × BZL, relatively higher d15N in crops

DISCUSSION: The Santare´m case study

Nutritional study in two isolated community near LAKE (Murrieta and Dufour 2004)

25-34% energy = cassava
18-26% energy = fish

TABLE 4. Isotopic diet mass balance for Santarém isolated communities[1]

Food	Aracampina			São Benedito		
	Energy (%)	$\delta^{13}C_{food}$ (‰)	$\delta^{13}C_{tissue}$ (‰)	Energy (%)	$\delta^{13}C_{food}$ (%)	$\delta^{13}C_{tissue}$ (‰)
Root[2]	34.0	–26.5	–9.9	25.2	–26.5	–7.6
Fish[3]	17.5	–27.9	–5.4	26.0	–27.9	–8.3
Sugar[4]	11.5	–11.0	–1.4	11.5	–11.0	–1.4
Dairy	7.3	–15.2	–1.2	2.9	–15.2	–0.5
Cereal C_3	6.8	–25.9	–1.9	9.5	–25.9	–2.8
Oil[5]	4.6	–26.0	–1.3	2.6	–26.0	–0.8
Poultry	3.2	–15.5	–0.6	4.5	–15.5	–0.8
Fruit	2.5	–26.9	–0.7	1.2	–26.9	–0.4
Legume	1.3	–25.9	–0.4	1.3	–25.9	–0.4
Cereal C_4[6]	1.0	–10.7	–0.1	1.0	–10.7	–0.1
Beef	0.4	–10.4	0.0	0.9	–10.4	–0.1
Pork	0.4	–17.6	–0.1	0.9	–17.6	–0.2
Vegetable	0.0	–27.8	0.0	0.0	–27.8	0.0
Egg	0.0	–15.5	0.0	0.0	15.5	0.0
Animal fat	0.0	–12.0	0.0	0.0	–12.0	0.0
Total	90.5		–23.0	87.5		–23.4

[1] Food energy data from Murrieta and Dufour (2004). Averages of $\delta^{13}C_{food}$ were taken from Table 2. No fractionation was considered between diet and fingernail.
[2] $\delta^{13}C$ 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^{-1} day^{-1}) and energy provided by maize (167.8 calories per capita^{-1} 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.

Fraction Factor =3‰; agreed with the nail d13C in isolated communities

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

Species	Common name	$\delta^{13}C$	$\delta^{15}N$
Dasyprocta sp.	Agouti	–23.2	6.8
Tayassu sp.	Collared peccary	–23.4	5.6
Bradypus tridactylus	Sloth	–23.2	10.4
Colossoma macropomum[1]	Tambaqui	–27.9	9.8

[1] From Oliveira (2003).

??

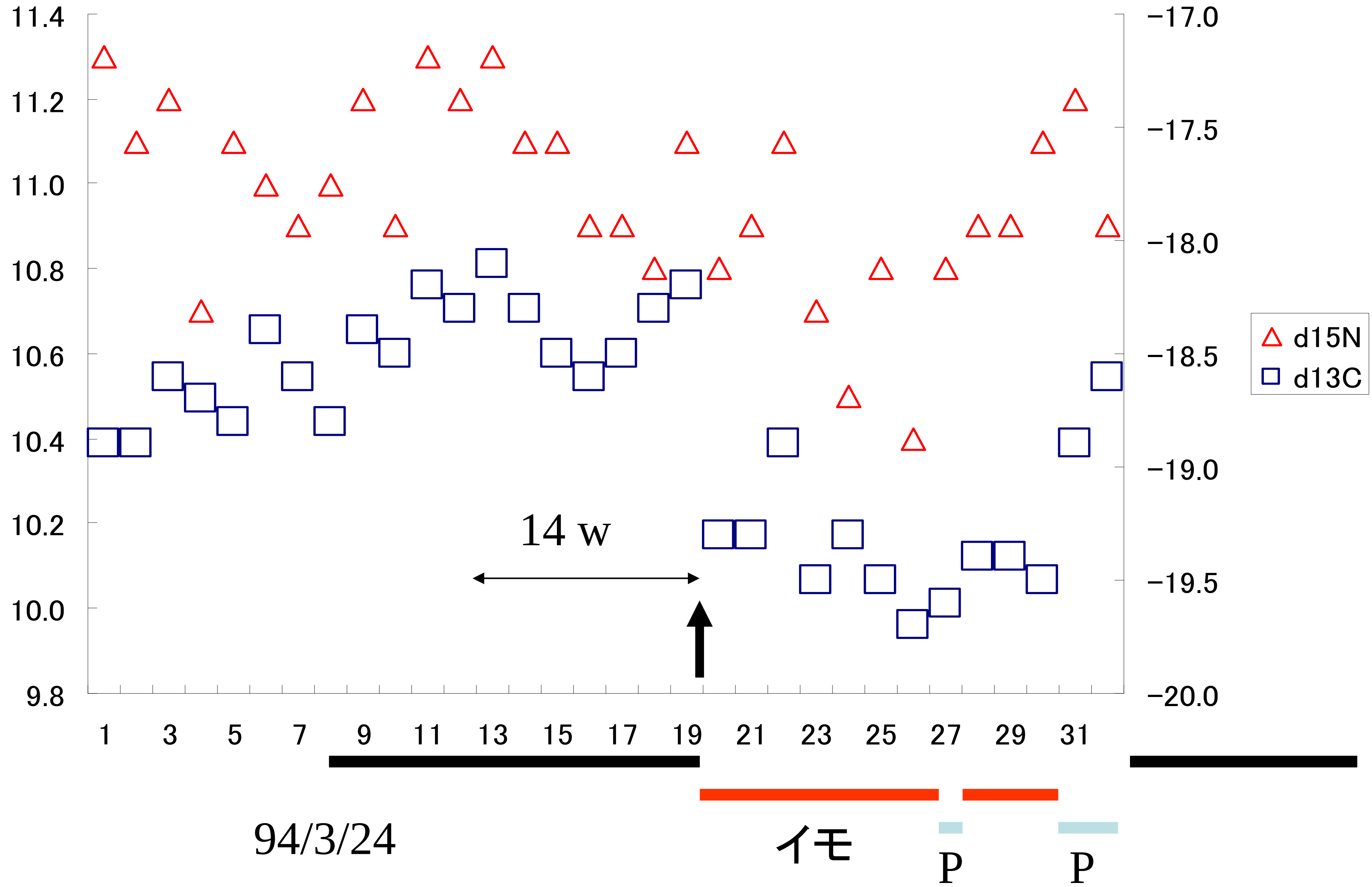
d15N was high in isolated communities, because …..

Conclusion

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 Santare´m 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
Santare´m recorded distinctive isotopic values, which were consistent with their diets and with the isotopic values of very local foods

Comments

- Pretreatment of samples
- Fingernails isotope ratios?
- Seasonality
- N metabolism;
- 2/3 of keratin from nonessential amino acid
- Fingernails recorded the diet of when?



Longitudinal change in d13C/d15N of a JPN student