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Isotopic analysis in teeth of contemporary brazilians with known diet and geolocation and its forensic value for human identification

Análise isotópica em dentes de brasileiros contemporâneos com dieta e geolocalização conhecidas e seu valor forense para identificação humana

Análisis isotópico en dientes de brasileños contemporáneos con dieta y geolocalización conocidas y su valor forense para la identificación humana

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Abstract
Dental anthropological data may provide valuable information about an individual's life, including age at death, health, signs of violence, ancestry, diet features, intentional modifications, and geographic origin. These pieces of information may be further detailed by diet and residential mobility estimates provided by isotopic analyses. The use of isotope data in the modern population is strongly dependent on the diverse origin of food supply, thus requiring an updated and location-specific database. In this study, we have analyzed the isotopic ratio of strontium, carbon, and nitrogen in enamel and dentin collagen of third molars individuals from three main cities in Brazil. Besides the isotope data, we have also surveyed their diet in adolescence habits to develop models to be applied for forensic studies in the Brazilian territory. We show that the forensic value of the place of origin estimation based 87Sr/86Sr levels is reduced in these highly urbanized samples. Among the outliers, most individuals are older than the mean age (25.1) or had water from wells in childhood. The sample's diet has a robust C4 presence and mean trophic levels consistent with the declared high frequency of chicken, meat, and dairy products, also compatible with the high consumption of sugar, rice, and beans in the local culture. Individuals with special diets, either by choice or therapeutic needs, were potential outliers among the group. Our results also contribute to the international human tissues isotopic database and can contribute for human identification of Brazilians or foreigners with distinct isotopic signatures.
Keywords: Human identification; Stable isotopes; Strontium isotopes; Diet; Geographic location; Forensic anthropology.

Resumo
Dados odontológicos podem fornecer informações valiosas sobre a vida de um indivíduo, incluindo idade, saúde, sinais de violência, ancestralidade, dieta, modificações intencionais e origem geográfica. Essas informações podem ser mais detalhadas pela estimativa de dieta e mobilidade residencial, fornecidas por análise isotópica. O uso de dados isotópicos na população moderna é fortemente relacionado à origem diversificada dos alimentos, exigindo, portanto, um banco de dados atualizado e específico para o local. Neste estudo, analisamos a razão isotópica de estrôncio, carbono e nitrogênio no colágeno da dentina e esmalte de terceiros molares de indivíduos de três grandes cidades do Brasil. Além dos dados de isótopos, também foram coletados dados de hábitos alimentares para o desenvolvimento de

modelos a serem aplicados em estudos forenses em território brasileiro. Os resultados mostraram que o valor forense da estimativa de local de origem com base nos níveis 87Sr/86Sr é reduzido nessas amostras altamente urbanizadas. Entre os outliers, a maioria dos indivíduos tem idade superior à média (25,1) ou consumiu água de poço na infância. A dieta da amostra tem uma presença robusta de C4 e níveis tróficos médios relacionados à alta frequência de frango, carne e laticínios na dieta declarada, compatíveis também com o alto consumo de açúcar, arroz e feijão na cultura local. Indivíduos com dietas especiais, por opção ou necessidade terapêutica, foram potenciais outliers entre o grupo. Os resultados também contribuem para o banco de dados isotópicos internacionais de tecidos humanos, contribuindo para identificação humana de brasileiros, ou estrangeiros com assinaturas isotópicas distintas.
Palavras-chave: Identificação humana; Isótopos estáveis; Isótopos de estrôncio; Dieta; Localização geográfica; Antropologia forense.

Resumen
Los datos dentales pueden proporcionar información valiosa sobre la vida de una persona, incluida la edad, la salud, los signos de violencia, la ascendencia, la dieta, las modificaciones intencionales y el origen geográfico. Esta información se puede detallar más mediante la estimación de la dieta y la movilidad residencial, proporcionada por el análisis isotópico. El uso de datos isotópicos en la población moderna está estrechamente relacionado con el origen diverso de los alimentos, por lo que requiere una base de datos actualizada y específica de la ubicación. En este estudio, analizamos la proporción de isótopos de estroncio, carbono y nitrógeno en el colágeno de la dentina y el esmalte del tercer molar de individuos de tres grandes ciudades de Brasil. Además de los datos isotópicos, también se recopilaron datos sobre hábitos alimentarios para el desarrollo de modelos para ser aplicados en estudios forenses en territorio brasileño. Los resultados mostraron que el valor forense de la estimación del lugar de origen basado en los niveles de 87Sr / 86Sr se reduce en estas muestras altamente urbanizadas. Entre los valores atípicos, la mayoría de las personas son mayores que el promedio (25,1) o consumieron agua de pozo en la infancia. La dieta de la muestra tiene una presencia robusta de C4 y niveles tróficos medios relacionados con la alta frecuencia de pollo, carne y productos lácteos en la dieta declarada, también compatible con el alto consumo de azúcar, arroz y frijoles en el cultivo local. Los individuos con dietas especiales, por elección o necesidad terapéutica, eran posibles valores atípicos entre el grupo. Los resultados también contribuyen a la base de datos internacional de isótopos de tejidos humanos, contribuyendo a la identificación humana de brasileños o extranjeros con firmas isotópicas distintas.
Palabras clave: Identificación humana; Isótopos estables; Isótopos de estroncio; Dieta; Localización geográfica; Antropología Forense.

1. Introduction

Teeth can provide valuable information about an individual's health and life habits. For instance, based on detailed analyses one may infer the age at death, general health, signs of violence, biological distance from other groups, oral pathologies such as caries and periodontal diseases, dietary habits, and different cultural practices, such as intentional dental modifications (Hillson, 1996; Koussoulakou, Margaritis & Koussoulakos, 2009; Scott and Turner, 2000). This kind of approaches can be explored both in archaeological and forensic contexts, with the addition of all possible therapeutic and aesthetic procedures, with different dental materials in contemporary groups. Dental *antemortem* data include forms, notes, image exams, plaster models, and photographs (Charangowda, 2010; Delattre, 2007; Pretty & Sweet, 2001).

Isotopic analysis of human remains is an auxiliary method that was initially used in archeology, and later in forensic sciences. The most important chemical elements in living organisms are hydrogen, carbon, nitrogen, and oxygen. Their isotopic ratios, together with strontium, can be determined in different tissues such as bones, teeth, hair, and nails. In most instances, these isotopic ratios depend on dietary characteristics and individual location, always considering the period of the biological tissue formation (Makarewicz & Sealy, 2015). By comparing different isotopic composition among different tissues and individuals, various studies have assessed different anthropological data of great use for human identification and habits (Eerkens et al, 2014; Bastos et al, 2015). The forensic use of isotopes for human identification requires a consistent modern database of the isotopic composition of hair, nails, and teeth in a contemporary population (Juarez, 2008; Alkass et al, 2011; Alkass et al, 2013; Kamenov & Curtis, 2017; Warner et al, 2018). The multicenter availability of isotopic could be applied to unidentified corpses and enhance the chances of estimating place of origin, residential mobility, and diet particularities.

The strontium $^{87}Sr/^{86}Sr$ ratio found in different types of rocks and soils, formed in different geological periods, varies due to the decay of ^{87}Rb to ^{87}Sr at a half-life of 4.88×10^{10} years. Together with calcium, strontium enters the biosphere and, in contrast to the isotopes of light elements (e.g., carbon, nitrogen, and oxygen), the isotopic ratio of strontium ratio does not change as this element is incorporated into the different tissues of an organism. Thus, food and water intake from a given location imprint a specific isotopic signature of $^{87}Sr/^{86}Sr$ in a living organism, which may be useful for studying their provenance (Budd et al, 2004; Faure, 1986). This kind of approach has been largely applied to dental enamel to study geolocation in childhood or adolescence. According to the chronology of tooth mineralization used for analysis, dental enamel forms in the first decades of life and is not remodeled afterward. Combining the results of different tissues allows addressing residential mobility and its relationship to demographic, pathological, cultural, social, and forensic history (Kamenov & Curtis, 2017; Price, Burton & Bentley, 2002; Bentley et al, 2003; Katzenberg, 2008).

Carbon ($\delta^{13}C$) and nitrogen ($\delta\ ^{15}N$) stable isotopes provide relevant information about the diet habits of an individual. The isotopic values of $\delta^{13}C$ in soft and hard tissues, such as enamel or dentine, are related to diet composition and, particularly, to the intake of terrestrial C3 (as potato, beans, wheat, and rice) or C4 (maize, sorghum and sugar cane) plants. Marine food has values superimposed to C4 plants (Katzenberg, 2008; Fry, 2006; Meier-Augenstein, 2010) since their carbon source includes CO_2 and bicarbonate dissolved in the water and debris from the terrestrial flora. The usual diet composition, including the amount of marine species, C3, and C4 plants, and protein from animals fed by these species directly influence in the isotopic ratio $^{13}C/^{12}C$ that will be present in human tissues (Howland et al, 2003; Kellner and Schoeninger, 2007; Lee-Thorp and van der Merwe, 1991; Schoeninger & DeNiro, 1984). Nitrogen isotopes are also related to the diet since they vary according to the trophic level of the individual. The $d^{15}N$ increases between 2 and 6‰ at each stage of the food chain (Schwarcz & Schoeninger, 1991). For instance, carnivores have $\delta^{15}N$ higher than herbivores, which have higher values than plants and other autotrophic organisms (Schoeninger & DeNiro, 1984; Schwarcz & Schoeninger, 1991; O'Connell and Hedges, 1999). Nitrogen isotopes are essential tools to address the trophic level and food chain studies. The combination of carbon and nitrogen isotopes allows differentiating diets rich in C4 plants from those rich in marine animals (Bastos et al, 2015). On studying tooth elements, the $\delta\ ^{13}C$ and $\delta\ ^{15}N$ from the detin collagen refer to the protein component of the diet. The $\delta^{13}C$ from the enamel represents the average isotopic composition of all carbon sources ingested (Bastos et al, 2015; Burt & Amin, 2014).

In this paper, we present of strontium, carbon, and nitrogen isotopic data in enamel and dentin collagen of contemporaneous individuals from three large cities in Brazil. We analyzed the third molars and compared the results with an anthropological survey of the subjects that included information about age, gender, residential mobility, diet, and socioeconomic conditions during childhood and adolescence aim to expand models for the exploration of the context in Brazilian territories. By generating new data, this paper is also intended to contribute to the international isotopic database for human identification purposes.

2. Methodology

Sampling

The sample consisted of third molars of individuals from Brasília, Florianópolis, and Rio de Janeiro, three large Brazilian cities apart from 744 to 1,316 km from each other (Figure 1). Subjects with third molar extraction planned for dental reasons unrelated to the research donated their teeth and filled out a survey about diet and residence from childhood to youth.

Figure 1 – Location and linear distance between the selected target-cities – Brasília, Rio de Janeiro, and Florianópolis.

Source: Google Maps.

All selected subjects filed the following criteria: Brazilian nationality, minimum age of 14 years, place of birth, and residence in one of the target cities during the tooth's mineralization period, healthy third molar donated. We initially had a total of 139 teeth from 94 individuals. After applying the selection criteria, 19 individuals' teeth were excluded. Also, partial exclusions involved three crowns and two roots. After selecting a third molar tooth from each subject, we got 75 samples for the study. We are not able to specify know the number of patients who denied participating in the study, since this information was not provided by the surgeons that invited patients that fit the inclusion criteria without recording rejection.

Complementary data collection

Besides the teeth, we have applied to each subject a Questionnaire on Diet and Geolocation in Adolescence (QDGA). Based on discursive and multiple-choice questions, this questionnaire asked questions about the place of birth and residential mobility up to the age of 18, diet habits, dietary restrictions, and socioeconomic information as education and monthly income. The diet section included questions about the approximate frequency of consumption of the following items: rice, wheat derivatives, cassava, and yam; corn and derivatives; beef or pork; chicken meat; fish and their origin - salted or sweet water; milk and milk products; and sugar and sugar cane derivatives. Each participant estimated how often they used to ingest each item, using the categories "never," "rarely," "occasionally," "frequently," or "daily". Each group was coded with a value from 0 to 4, respectively. Regarding sugar, the participants chose the approximate amount of sugar intake, with the options "none", "little", "average", and "a lot", which also were coded with numerical values from 0 to 3.

Participants reported their usual diet during adolescence, which is the age of forming the third molars. Therefore, there was a limitation in the use of detailed and standardized nutritional questionnaires, which use recent memory and record ingested content with a 24hs recall. The survey also extracted information about water sources, primary sources of food, and possible dietary restrictions, such as allergies, diabetes, lactose intolerance, and personal options, including vegans and vegetarians, with a detailed description of the limitation and its chronology.

Sample preparation

After removing calculus and cleaning the teeth with a periodontal curette (SS White Duflex, Rio de Janeiro) and ultrasound device (Dabi Atlante, Ribeirão Preto, SP), the elements were dried with gauze and stored in a coded plastic envelope. The enamel was removed with diamond tip PM-06 (KG Sorensen, São Paulo, Brazil), wearing out a shallow groove around it, on the cervical half of the crown, being conditioned in a microtube with at least 20mg of enamel.

Collagen extraction were performed following previous studies reported in the literature (Ambrose, 1990; Bastos et al, 2016) [29, 30]. The roots were initially immersed in NaOH 0.1M for 24 hours, to neutralize humus acid. After rinsing with distilled water, immersion was started in HCl 0.2M for seven days, with daily replacement of HCl, and cutting the material into small pieces to increase the contact surface. After rinsing, the samples were immersed for 24 hours in chloroform, methanol, and water (2:1:0.8) to remove lipid residues. The collagen pseudomorphs were then rinsed and dried for about 8 hours in an oven at 50ºC.

Isotopic analyzes

For analyzes of $\delta^{13}C$ and $\delta^{15}N$ of collagen, aliquots between 500 and 650µg of each sample were weighed in tin crucibles for measurement in a mass spectrometer. Samples were introduced in the automatic sampler Thermo Scientific MAS 200R; the N_2 and CO_2 resulting from the samples were then sent to the ion source of Thermo Scientific Delta V IRMS where they were ionized and accelerated. In all runs, International reference standards IAEA601, NIST8542, UIW, USGS 41 were also analyzed.

For the strontium analyses, about 20mg of each sample was weighed and transferred to a Teflon beaker. The chemical treatment was initiated by the addition of 2ml of concentrated nitric acid (HNO_3). Samples were cold digested for about 90 minutes. A 2ml aliquot of each sample was placed in a 2ml Eppendorf micro-tube for centrifugation for 10 minutes. Then, a 1 ml aliquot of this solution was submitted to chromatography separation over SR-B50-A resin (Eichrom) using 2.9 M nitric acid as eluent. After total evaporation of the acid phase, the residue was taken up with 5 ml of 3% nitric acid, and the isotope ratio was determined by a Neptune Multicollector ICP-MS (Neptune, Thermo Scientific). Samples were analyzed together with the standard NIST SRM 987.

Data treatment

Data were tabulated in Excel spreadsheets (Microsoft, Washington, USA) and statistically analyzed using Statistical Package for Social Sciences (SPSS) (IBM, New York, USA). The data were evaluated using descriptive and inferential statistics: normality test (Shapiro-Wilk), and means comparison tests (Student's T-test, MANOVA test). After the analysis of the Levene test, we performed the Tukey Post-test. Pearson's correlation test was also performed, and, in cases where there was a statistically significant correlation between the variables, Linear Regression analysis was performed sequentially to verify the relationship between these variables and the coefficient of determination of age in function of the $^{87}Sr/^{86}Sr$ and $d^{15}N$ values. All used IBM SPSS Software (22.0) using a significance level of 5% in all tests.

Ethical aspects

We submitted this research project to an ethical appraisal to the Committee of Ethics Research from the Center for Philosophy and Human Sciences of the Federal University of Rio de Janeiro. The project was approved by report number 1.561.481.

3. Results

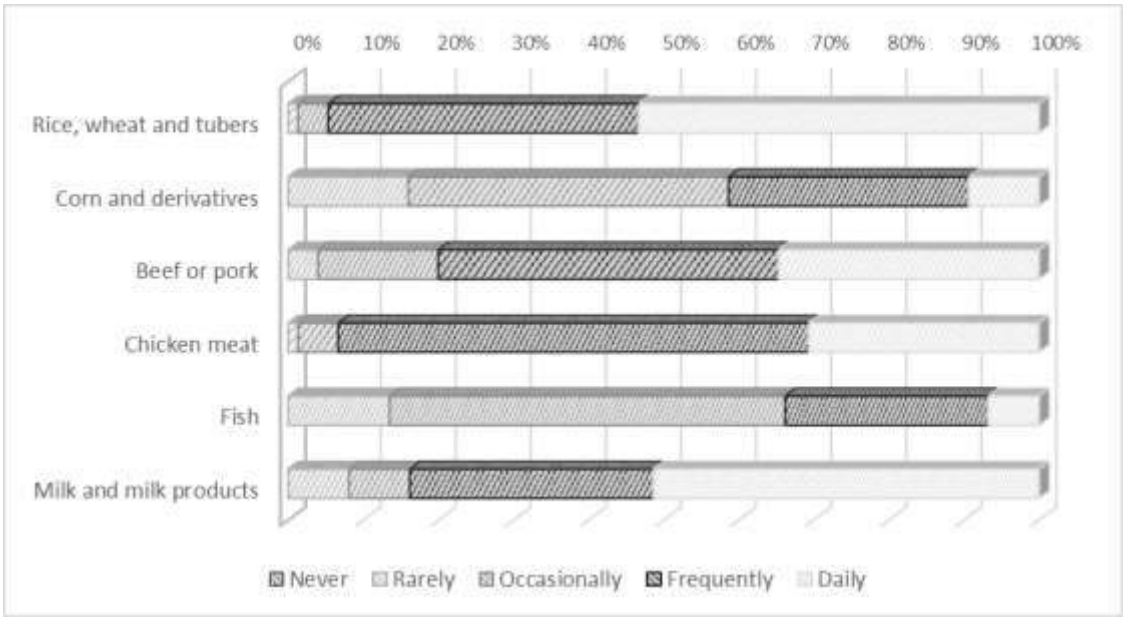
The sample consisted of 75 individuals, mean age of 25.1 (±8.4) years old, with 34 (45.3%) men and 41 (54.7%) women. As for socioeconomic data, the most significant part (36.0%) of the sample had complete graduation, and family monthly income of approximately 2 to 5 minimum wages[1] (Table 1). Based on the questionnaire responses, Figure 2 exhibits the average consumption of food intake reported.

Table 1 – Distribution of sample groups by sex, age, and socioeconomic pattern.

	Brasília	Florianópolis	Rio de Janeiro	Total
N	29	13	33	75
Sex	N (%)	N (%)	N (%)	N (%)
Male	13 (44.8)	5 (38.5)	16 (48.5)	34 (45.3)
Female	16 (55.2)	8 (61.5)	17 (51.5)	41(54.7)
Age				
Mean	24.1	25.8	25.8	25.1
SD	7.7	8.0	9.0	8.4
Median	20.9	22.4	23.2	22.4
Minimun	16.0	17.5	17.3	16.0
Maximun	48.9	41.2	58.8	58.8
Education:	N (%)	N (%)	N (%)	N (%)
Elementary school	3 (10.3)	1 (7.7)	4 (12.1)	8 (10.7)
High school	10 (34.5)	4 (30.8)	13 (39.4)	27 (36.0)
Graduation	11 (37.9)	5 (38.5)	10 (30.3)	26 (34.7)
Post-graduation	5 (17.2)	2 (15.4)	6 (18.2)	13 (17.3)
unknown	0 (0.0)	1 (7.7)	0 (0.0)	1 (1.3)
Monthly income:	N (%)	N (%)	N (%)	N (%)
up to 2 m.w.*	7 (24.1)	1 (7.7)	9 (27.3)	17 (22.7)
2 – 5 m.w.	10 (34.5)	7 (53.8)	15 (45.5)	32 (42.7)
over 5 m.w.	11 (37.9)	4 (30.8)	9 (27.3)	24 (32.0)
unknown	1 (3.4)	1 (7.7)	0 (0.0)	2 (2.7)

* minimum wage. Source: Authors.

Figure 2 – Percentage distribution of the estimated frequency of intake, per food group.



Source: Authors.

1 Brazilian minimum wage in 2020: R$ 998.00, which is equivalent to US$ 185,86 (in February 2021)

The $^{87}Sr/^{86}Sr$ ratios from the enamel varied between 0.71015 and 0.71566, with a mean of 0.71207 and a standard deviation of 0.00102. Dentin collagen samples presented a mean $\delta^{13}C$ value of -15.06 (between -16.66 and -13.40) and a standard deviation of 0.73. They have a mean $\delta^{15}N$ value of 11.45 (between 9.83 and 14.56), and a standard deviation of 0.79. Table 2 exhibits the results of $\delta^{13}C$, $\delta^{15}N$, and $^{87}Sr/^{86}Sr$ and shows that there is no significant difference in the values of $^{87}Sr/^{86}Sr$ among the samples from Rio de Janeiro and Brasília. There are also no significant differences in $\delta^{13}C$ among all three groups.

Table 2 – $\delta^{13}C$, $\delta^{15}N$, and $^{87}Sr/^{86}Sr$ values distributed by sample group.

			$^{87}Sr/^{86}Sr$	$\delta^{15}N_{collagen}$	$\delta^{13}C_{collagen}$
Sample	Brasília	N	23	27	27
		Mean	0.7121257	11.1341	-14.9556
		Median	0.7121900	11.0500	-14.9600
		Minimum	0.71015	9.83	-16.62
		Maximum	0.71551	12.43	-14.11
		Std. Deviation	0.00108803	0.61763	0.65750
	Florianópolis	N	9	11	11
		Mean	0.7114489	11.5345	-15.3600
		Median	0.7114400	11.4000	-15.3600
		Minimum	0.71063	10.50	-16.58
		Maximum	0.71267	13.03	-14.03
		Std. Deviation	0.00055026	0.73316	0.71179
	Rio de Janeiro	N	31	30	30
		Mean	0.7122100	11.6977	-15.0453
		Median	0.7120700	11.5350	-15.0150
		Minimum	0.71087	9.91	-16.66
		Maximum	0.71566	14.56	-13.40
		Std. Deviation	0.00103175	0.87067	0.79955
	Total	N	63	68	68
		Mean	0.7120705	11.4475	-15.0606
		Median	0.7119600	11.4100	-15.0300
		Minimum	0.71015	9.83	-16.66
		Maximum	0.71566	14.56	-13.40
		Std. Deviation	0.00102037	0.79071	0.73441

Source: Authors.

The Pearson's correlation test indicates a significant correlation (Sig. <0.05) between the following parameters: age and $\delta^{15}N$; age and $^{87}Sr/^{86}Sr$, sample group and $\delta^{15}N$; and $\delta^{15}N$ and $^{87}Sr/^{86}Sr$. Some significant correlations among reported consumed foods were also found – between rice and meat, meat and chicken, and other – but are not explored in the results, since they are not related to isotopic values, but contributed to the understanding of the subjects' food profile and the interpretation of the results. However, it must be mentioned that the studied sample has higher purchasing power and education level than the average values of the Brazilian population, with 17.4% of Brazilians having completed higher education. Table 3

presents the correlation between isotope data, sample, age, and socioeconomic variables (number of cohabitants, education, and monthly income).

Table 3 – Correlations between isotopic variables, sample, age, and socioeconomic data.

		Sample	Age	Cohab	Education	Income	$^{87}Sr/^{86}Sr$	$\delta^{15}N$	$\delta^{13}C$
Sample	Pearson Correlation	1	0.138	-0.313*	-0.133	-0.135	0.057	0.386**	-0.007
	Sig. (2-tailed)		0.329	0.024	0.346	0.339	0.686	0.005	0.961
Age	Pearson Correlation	0.138	1	-0.069	0.166	0.307*	0.614**	0.708**	0.142
	Sig. (2-tailed)	0.329		0.628	0.240	0.027	0.000	0.000	0.316
Cohab.	Pearson Correlation	-0.313*	-0.069	1	-0.014	0.151	0.145	-0.113	-0.119
	Sig. (2-tailed)	0.024	0.628		0.924	0.284	0.304	0.425	0.402
Education	Pearson Correlation	-0.133	0.166	-0.014	1	0.491**	0.195	0.108	0.208
	Sig. (2-tailed)	0.346	0.240	0.924		0.000	0.166	0.445	0.140
Income	Pearson Correlation	-0.135	0.307*	0.151	0.491**	1	0.546**	0.165	0.201
	Sig. (2-tailed)	0.339	0.027	0.284	0.000		0.000	0.242	0.153
$^{87}Sr/^{86}Sr$	Pearson Correlation	0.057	0.614**	0.145	0.195	0.546**	1	0.416**	0.215
	Sig. (2-tailed)	0.686	0.000	0.304	0.166	0.000		0.002	0.126
$\delta^{15}N$	Pearson Correlation	0.386**	0.708**	-0.113	0.108	0.165	0.416**	1	0.234
	Sig. (2-tailed)	0.005	0.000	0.425	0.445	0.242	0.002		0.096
$\delta^{13}C$	Pearson Correlation	-0.007	0.142	-0.119	0.208	0.201	0.215	0.234	1
	Sig. (2-tailed)	0.961	0.316	0.402	0.140	0.153	0.126	0.096	

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).
Source: Authors.

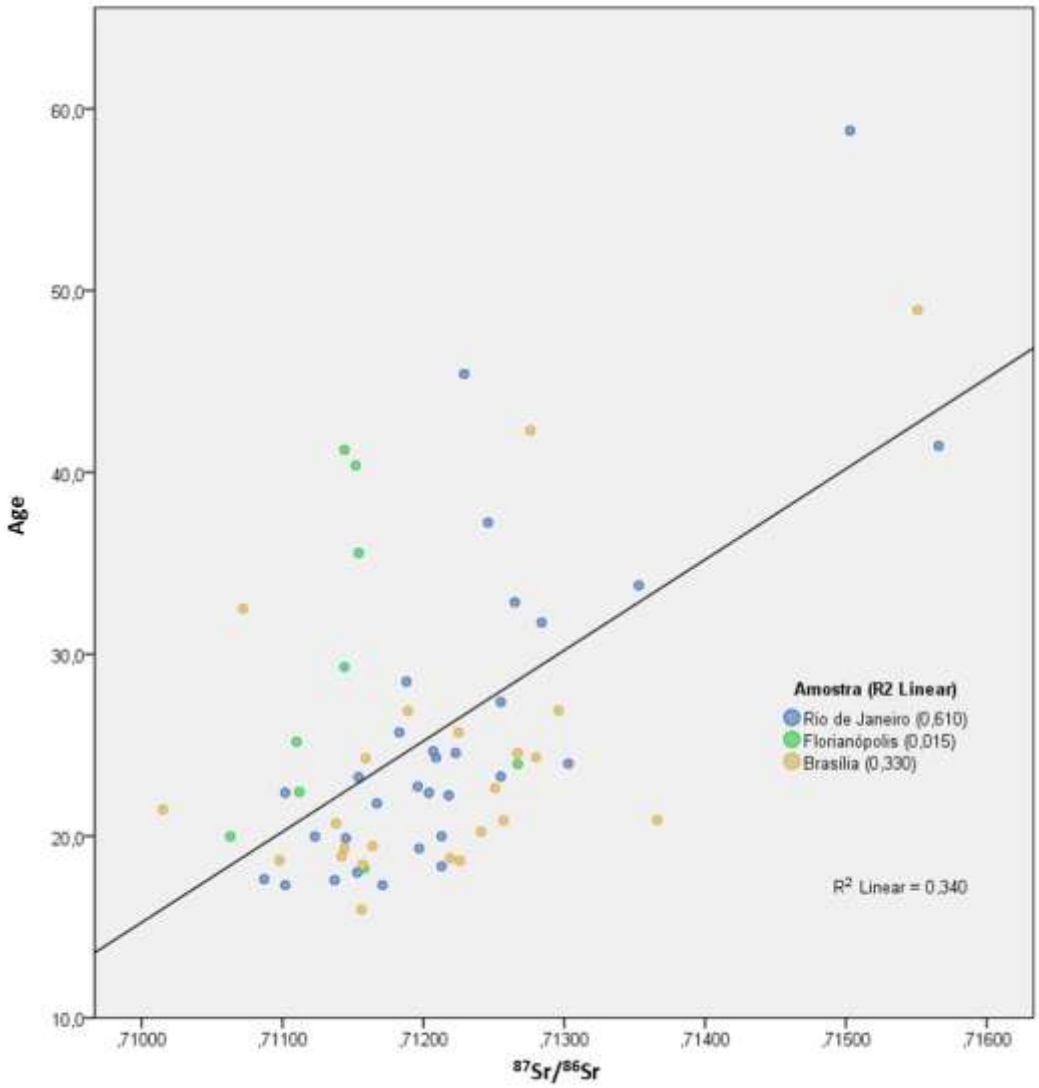
The statistical treatment revealed a positive correlation between 87Sr/86Sr and age (Pearson correlation = 0.579, Sig = 0.002), and δ 15N (Pearson correlation = 0.403, Sig = 0.032) and age for samples from Brasília. It also revealed correlation between δ 15N and age (Pearson's correlation = 0.441, Sig. = 0.011) for samples from Florianópolis; and between 87Sr/86Sr and age (Pearson's correlation = 0.780; Sig. = <0.001); and δ 15N and age (Pearson's Correlation = 0.800; Sig. = <0.001) for sample from Rio de Janeiro.

The high values of δ13C indicate a significative influence of C4 plants in the base of the protein source chain. Also the δ15N data suggest that, in average, the trophic level of the diet is mainly terrestrial. There is, however, no significant difference in the declared dietary habits among the individuals from the three cities. According to the diet reported in the survey, there was a correlation between the consumption of meat and chicken (Sig. <0.001), fish and chicken (Sig = 0.001), and chicken and milk (Sig <0.005). Among the outliers, most are above the average age value, and others reported the consumption of well water and food restriction, such as lactose intolerance.

Regarding strontium, three participants had an isotopic ratio above 0.714, all aged over 40 years, and two of them reported having consumed well water during the adolescence. For δ 15N, the three highest values (above 12.5 ‰) included individuals over 40 years old that reported the use of well water. One of them further related household production as the sole source of supply. The subject with the lowest δ 15N value also reported the well water consumption, but around the sample mean age. The lowest $\delta^{13}C$ values refer to individuals who declared the use of domestically produced food and another that reported low corn and fish consumption. The most negative carbon values were found in participants with milk and salt restriction.

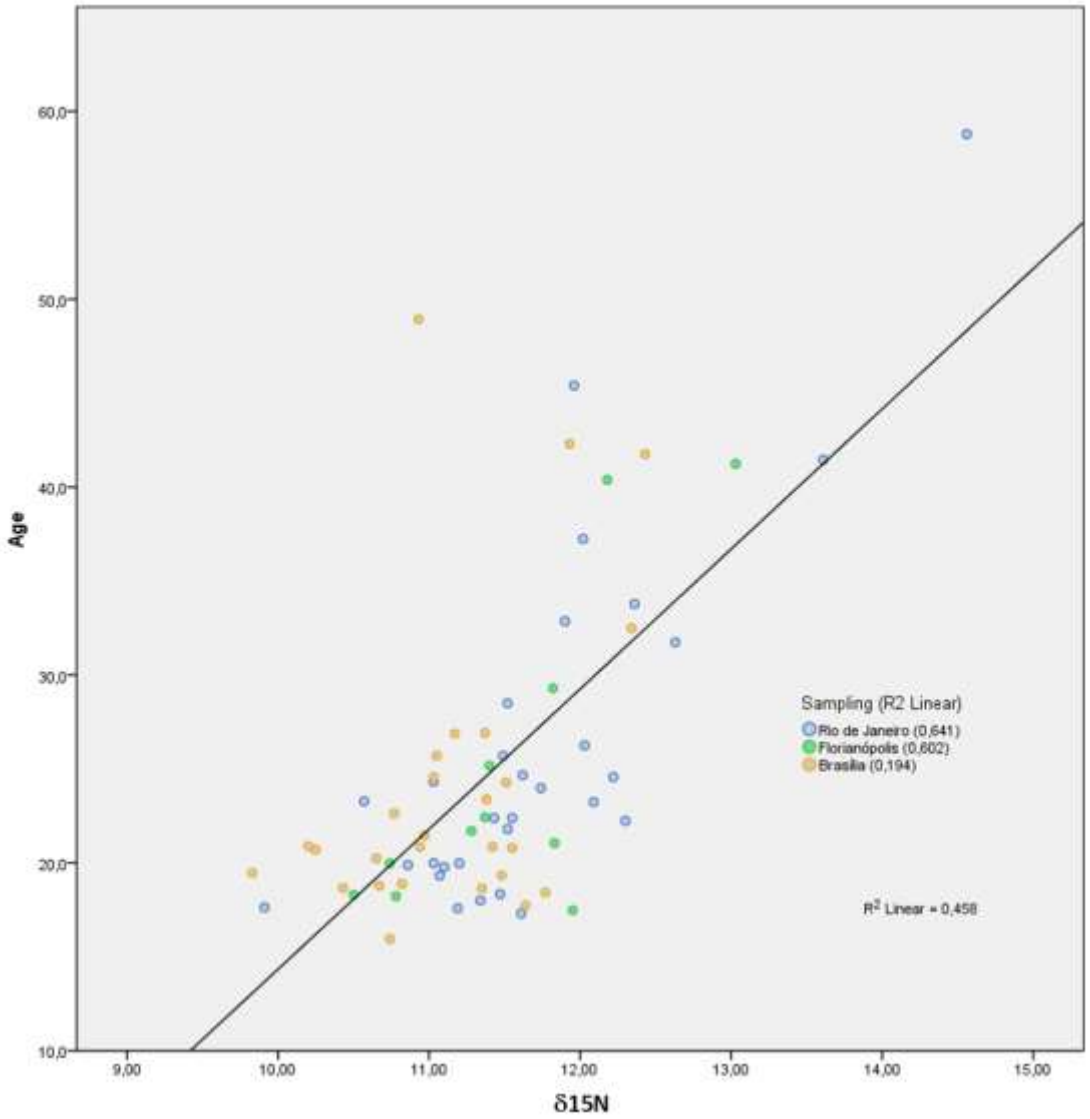
Based on the δ15N and 87Sr/86Sr data, which showed a significant difference among the samples, a discriminant function was tested to predict the place of origin of the subjects. It revealed that 63.2% of the cases could be correctly classified for the whole sample. The group from Brasília, however, presented a higher success rate (68.2%). Linear regression analysis between the 87Sr/86Sr ratio and the subject's age has shown a higher coefficient of determination in the sample of Rio de Janeiro (R2=0.610), followed by Brasilia (R2=0.330). The entire samples exhibited an R2 value of 0.340 (Figure 3). The linear regression analysis between δ15N values and age showed a higher coefficient of determination in Rio de Janeiro (R2=0.641), followed by Florianópolis (R2=0.602), and an R2 value of 0.458 for all samples (Figure 4).

Figure 3 – Linear regression analysis from age and values of $^{87}Sr/^{86}Sr$.



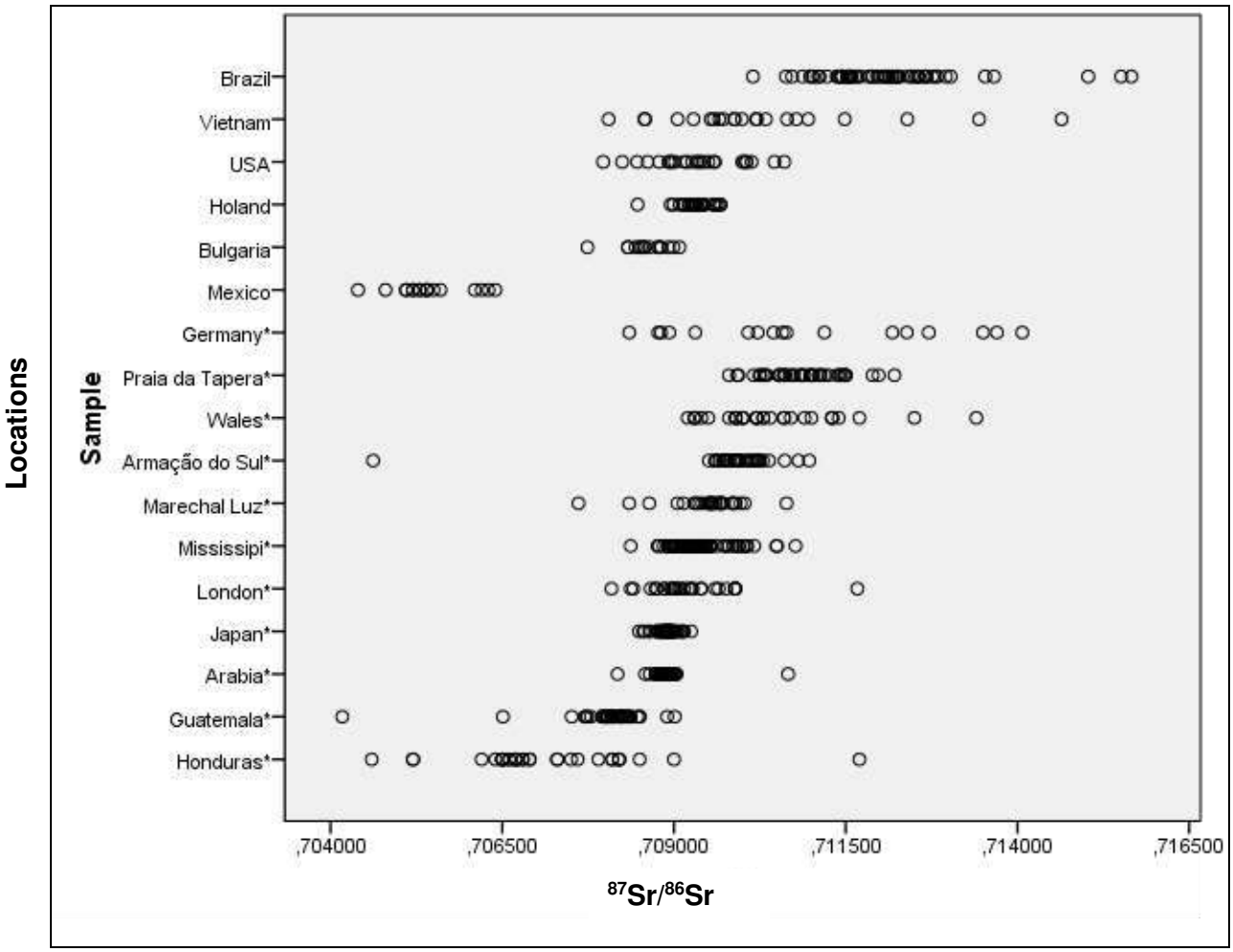
Source: Authors.

Figure 4 – Linear regression analysis from age and values of $\delta^{15}N$.



Source: Authors.

Figure 5 – Scatter plots of values of $^{87}Sr/^{86}Sr$ of the unified sample compared to results found in the literature.



*: archaeological sample
Data source: Vietnam and USA (Regan, 2006); Holand (Font et al, 2015); Bulgaria (Kamenov and Curtis, 2017); Mexico (Juarez, 2008); Germany (Knipper et al, 2016); Praia da Tapera (Bastos et al, 2015); Wales (Hermer et al, 2013); Armação do Sul (Oppitz, 2015); Marechal Luz (Bastos, 2009); Mississippi (Slater, Hedman and Emerson, 2014); Japan (Kusaka et al, 2012); Arabia (Gregoricka et al, 2017); Guatemala (Wright, 2012); Honduras (Price et al, 2014). Source: Authors.

4. Discussion

Diet is the most important control on the carbon, nitrogen, and strontium isotopic composition of tissues. For instance, the isotopic composition of dentin collagen depends on the source of protein intake. While the $\delta^{15}N$ values vary according to the protein's trophic level, the $\delta^{13}C$ values depend on the consumption of C3 and C4 food source (Schoeninger and DeNiro, 1984; Beaumont and Montgomery, 2016). Various studies have used isotopic analyses to address changes in food sources, allowing inference of the demographic dynamics, resource origin, food sharing, and new food insertion into distinct groups (Eerkens et al, 2014; Benson et al, 2006; Beaumont et al, 2013; Bourbou et al, 2013).

This kind of approach has also been applied as an auxiliary tool in forensic investigations, in which circumstance human identification bases on food profiles ($\delta^{13}C$ and $\delta^{15}N$) and geolocation ($^{87}Sr/^{86}Sr$). Since tissues have different turnover times, isotopic data may also provide chronological information, allowing the investigation of geographic origin, residential mobility, and food changes. However, dental enamel and dentin collagen provide dietary e geographic data about childhood and adolescence, since they are the only tissues that do not remodel (Font et al, 2012; Kamenov and Curtis, 2017).

In general, this study indicated that there is a large overlap among the isotopic values of these three large cities in Brazil. The similarity in the values revealed that there is not a significant isotopic difference in food intake among these cities, reflecting the low regional food diversification in this part of the country. Despite the similarities, there are some noticeable aspect and correlations between the isotopic data and the individuals' surveys that suggest potential use of these methods for human identification for forensic purposes in Brazil.

Evolution of food consumption as revealed by isotopes

In the last decades, social inclusion public policies have succeeded in reducing poverty and malnutrition in Brazil, especially between 1996 and 2007. This new scenario raised the frequency of overweight and obesity in the country, which is now up to three times higher than that of malnutrition (Conde and Monteiro, 2014). Recent studies indicate that ultra-processed food – such as snacks, cereal bars, instant noodles and ready or semi-ready meals – represent 21.5% of total energy intake in the Brazilian population. Rice and beans are the most frequently consumed C3 plants, accounting for 22.9% of the total energy intake. Other in natura food like red meat, milk, poultry, and fish, represent 10.0, 5.4, 4.9, and 1.7%, respectively (Louzada et al, 2015). The products with the highest daily consumption among the analyzed sample were "rice, wheat and derivatives, cassava or yam" (53.3%), and "milk and derivatives" (51.4%), according to the questionnaire.

The difference in profile between young and old individuals is related to a significant change in dietary patterns in the Brazilian population. The ultra-processed food industry has become increasingly crucial in Brazilian diet. Essential variations in indicators of food household availability were observed in the metropolitan areas of the country in the period from 1974-2003, with a decline in the consumption of staple foods such as rice and beans, and an increase in the intake of beef (+ 22%), chicken meat (+ 100%), sausages (+ 300%), milk and dairy products (+ 36%), biscuits and soft drinks (+ 400%), and ready-to-eat meals (+ 80%) (Conde and Monteiro, 2014; Louzada et al, 2015; Levy-Costa et al, 2005; Feferbaum et al, 2012; Monteiro et al, 2010; Souza et al, 2013). This change in profile agrees with the strong presence of C4 derived carbon in our data, as well as the high consumption of chicken, meat, and dairy products. The production of protein-rich food is also substantially based on C4 derived carbon, such as grass and corn (Martinelli et al, 2020). Moreover, sugar is commonly present in many commercial products that may have not been considered by the participants. Our isotopic data reiterates the evidence that although the Brazilian population has rice and beans consumption, C3 derived carbon does not represent the primary source of carbon on their diet. The exception to the present-day diet pattern is subjects with food restriction that generally avoid ultra-processed products. The isotopic data of this sample plot as outliers show that isotopes may be powerful tools to identify them.

Industrialization has also affected the strontium isotopic composition of the population as revealed by the positive correlation between $^{87}Sr/^{86}Sr$ of our data. Younger individuals tend to consume food produced in more diversified places and to have a higher consumption of meat and processed products during the mineralization of their dental elements. Participants with ages above the sample average, in turn, had in general high strontium isotopic ratios and lower $\delta^{13}C$ values. Their higher consumption of local products may explain this pattern. In terms of strontium isotopes, the public water supply system tends to homogenize the strontium of a population. Consumers in a large geographical area will have access to the same source of drinking water. This buffer effect was less effective for older individuals, which in some instances had well water as main supply during their childhood and adolescence. This is the case, for example, of the outliers observed in this study.

The secondary dentin layer, with its continuous lifelong apposition that makes its relative portion directly proportional to the individual's age, should also be considered a remote hypothesis. Thus, this layer of dentin, used as a parameter for age estimation (Cameriere et al, 2013), contains elements related to diet throughout adult life, and not only in childhood, as can be affirmed in relation to primary dentin. Future studies with representative samples of different age groups would be useful to

measure the effect of secondary dentin on the collagen values δ ^{13}C and δ ^{15}N. Once known and measured, this possible bias must be considered in every isotopic research that includes adult teeth, which are, in most archaeological contexts, the only available material. Archaeological studies usually use bones such as ribs for carbon and nitrogen collagen isotopic analyses, but teeth are less affected by diagenesis, and are a more reliable source for inorganic strontium, carbon and oxygen analysis.

δ^{13}C, δ^{15}N and ^{87}Sr/^{86}Sr profile of the Brazilian population

The comparative analysis of the ^{87}Sr/^{86}Sr values did not show a significant difference between Brasília and Rio de Janeiro, different from the expected due to their position regarding the coast. Both regions have formations with close geo-chronologies, which does not imply having the same proportion of rubidium. One must also consider the remarkable presence of marine strontium in Rio de Janeiro. Florianopolis, that is also a coastal city, presented narrower strontium variation, but its results overlap those found in Rio de Janeiro and Brasilia.

Although these values were not specific and with isotopic signature among the target cities, comparing the sample as a single group with international data showed a significant difference between the Brazilian sample and all other groups, including the USA and other countries in Latin America - neighboring countries with contemporary data available in the literature. This finding reaffirms the forensic value of the technique as a possible auxiliary method in the human identification process, since a Mexican or a Honduran individual, for example, would be readily found among multiple human remnants in Brazil, as shown in Figure 5.

The use of isotopic analysis as an auxiliary resource for human identification requires regularly updated databases from local population samples. However, these values are sensitive to the degree of urbanization, agricultural dynamics, water supply system, trade relations, local culture, and personal preferences. Therefore, relevant data to isotopic values must be recorded from the selected sample, such as age, income, water source, possible dietary restrictions, and diet at the time of the analyzed tissue formation.

5. Conclusion

The forensic value of the place of origin estimation through ^{87}Sr/^{86}Sr levels was shown to be reduced in the highly urbanized sample as the one selected for the study. Among the outliers, individuals older than the mean age and those who reported well water consumption in adolescence were the most frequent cases. The discriminant function analysis considering the values of ^{87}Sr/^{86}Sr and δ^{15}N obtained a success rate in sample group prediction of 63.2%. The method can also be used as an auxiliary resource in the investigation of place of origin, which presents accuracy in distinguishing individuals from distant places, as seen in the comparison of the group with international samples.

According to the isotopic ratios of carbon and nitrogen found in collagen, the sample's diet has an important C4 component and high trophic level, which is consistent with the high consumption of red meat, amount of sugar in industrialized products, and increasing intake of chicken meat, as reported in the literature. People with special diets, either by choice or therapeutic needs were potential outliers among the group, which can be interpreted as greater control of consumed industrialized products and less average distance between subject and food's point of origin.

The authors suggest new research to verify the effect of urbanization, diet industrialization, educational levels and water supply on isotopic indicators, and to evaluate the forensic accuracy of these tools in groups of different locations, degrees of urbanization, ages, and dietary compositions. In cases of human identification, a peculiar dietary profile, whether by choice or therapy, such as vegan, vegetarian, allergic, bulimic or anorexic can be an important individualizing feature, increasing chances of success. Therefore, individuals within these groups or with migration history should be studied to

evaluate the accuracy of isotopic analysis in these cases. Among the 139 teeth received at the beginning of the present paper, some special cases were selected for further investigations, which could not be performed due to the loss of the material during the fire at the National Museum, Rio de Janeiro.

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