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CARBON AND NITROGEN ISOTOPES: DIETS OF BRAZILIAN PREHISTORIC HUMANS

ISÓTOPOS DE CARBONO E NITROGÊNIO: OS HÁBITOS ALIMENTARES DE HUMANOS PRÉ-HISTÓRICOS BRASILEIROS

ISOTOPOS DE CARBONO Y NITRÓGENO: LOS HÁBITOS ALIMENTARIOS DE LOS HUMANOS PREHISTÓRICOS BRASILEÑOS

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ABSTRACT

13C and 15N analysis of remaining collagen in prehistoric human bones is the most important method to determine ancient human diets. Although this technique has provided information about eating habits of various ancient peoples of Africa, Europe and North America, few studies of this type were developed in Brazil. The objective of this study is to present and discuss the results of chemical analysis of 13C and 15N performed in nine samples from Brazilian old human bones, in order to obtain information about the diet of these primitive men. The results indicated that three skeleton excavated in Minas Gerais belonged to consumers of plants the C3 group and were hunter-gatherers. The other five skeletons of Minas Gerais could not be assessed. The individual whose bones were found in Piauí probably already practiced agriculture e had an additional source of protein in their diet. This made him occupy a higher trophic level in the food chain than those of Minas Gerais.

Keywords: Prehistoric Diet. Carbon 13. Nitrogen 15. Archaeometry.

RESUMO

A análise de 13C e do 15N do colágeno remanescente em ossos humanos pré-históricos constitui o mais importante método para se determinar dietas humanas antigas. Embora essa técnica tenha fornecido informações sobre hábitos alimentares de vários povos antigos da África, Europa e América do Norte, poucas pesquisas dessa natureza foram desenvolvidas no Brasil. O objetivo deste trabalho foi apresentar e discutir os resultados das análises químicas de 13C e de 15N realizadas em amostras de nove esqueletos humanos pré-históricos brasileiros, visando obter informações sobre a dieta alimentar desses homens primitivos. Os resultados indicaram que três esqueletos escavados em Minas Gerais pertenceram a consumidores de vegetais do grupo C3 e eram caçadores-recoletores. Os outros cinco esqueletos de Minas Gerais não puderam ser avaliados. O indivíduo cuja restos esqueléticos foram encontrados no Piauí provavelmente já praticava agricultura e tinha uma fonte adicional de proteína em sua alimentação. Isto o fazia ocupar um nível trófico mais elevado na cadeia alimentar que os de Minas Gerais.

Palavras-chave: Dieta Pré-histórica. Carbono 13. Nitrogênio 15. Arqueometria.

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RESUMEN
El análisis de $\delta^{13}C$ y $\delta^{15}N$ en el colágeno presente en huesos humanos prehistóricos constituye el método más importante para determinar la dieta de los humanos antiguos. Si bien esta técnica ha proporcionado información sobre los hábitos alimenticios de diversos pueblos antiguos de África, Europa y Norteamérica, se ha desarrollado poca investigación de este tipo en Brasil. El objetivo de este trabajo fue presentar y discutir los resultados de los análisis químicos de $\delta^{13}C$ y $\delta^{15}N$ realizados en muestras de nueve esqueletos humanos prehistóricos brasileños, con el fin de obtener información sobre la dieta de estos hombres primitivos. Los resultados indicaron que tres esqueletos excavados en Minas Gerais pertenecían a consumidores de plantas del grupo C3 y eran cazadores-recolectores. Los otros cinco esqueletos de Minas Gerais no pudieron ser evaluados. El individuo cuyos restos óseos se encontraron en Piauí probablemente ya practicaba la agricultura y contaba con una fuente adicional de proteínas en su dieta. Esto lo ubicaba en un nivel trófico superior en la cadena alimentaria que los individuos de Minas Gerais.

Palabras clave: Dieta Prehistórica. Carbono 13. Nitrógeno 15. Arqueometría.

1 INTRODUCTION

In recent decades, archaeologists have focused their scientific interests on reconstructing the dietary history of ancient populations. This knowledge is key to understanding many aspects related to the development of human culture: subsistence strategies, social stratification, gender roles, technology, and pathologies (AL-AKHRAS *et al*., 2025; WEBER *et al*., 2025; EGLITE, MOHM, and DIERKING, 2022; GUISERIX *et al.*, 2024).

Since the change in the dietary habits of populations throughout their lifespan constitutes the main point in the study of prehistoric human diets, it becomes fundamental, in the study of these populations, to analyze the possible sources of their food, which can be plant or animal. These diets may have influenced the alteration of the morphology and behavior of human societies (WEBER *et al*., 2025; VARALLI, MOGGI-CECCHI, and GOUDE, 2022).

Chemical analyses of bones have provided new ways to understand ancient dietary habits. From studies of collagen and trace elements, components of bone tissue, it is possible to obtain important information about the dietary habits and social behavior of ancient humans (AL-AKHRAS *et al.*, 2025; EGLITE, MOHM and DIERKING, 2022; LARSEN, 2022).

However, it is necessary to identify the chemical changes that occurred in these bones during the burial period (diagenesis). These changes can be assessed through the results of chemical analysis of the trace elements contained in the bones. Another important piece of data for analyzing the degree of diagenesis in buried bone tissue is the collagen content, a macromolecule (protein) formed by a large number of amino acid molecules and which makes up about 20.00 to 20.40% of bone tissue (KARSDAL, 2023; VAN DER MERWE, 1982).

From this perspective, the objective of this work was to present and discuss the results obtained from the chemical analyses of carbon (C) and nitrogen (N) isotopes remaining in the bone collagen of prehistoric Brazilian humans. Samples from 09 (nine) skeletons were analyzed: 08 (eight) excavated at archaeological sites in Minas Gerais (Buritizeiros, 01 sample, Santana do Riacho I, 05 samples, and Santana do Riacho III, 02 samples) and 01 (one) at the São Miguel do Tapuio site in Piauí.

2 METODOLOGICAL PROCEDURES

A well-established technique in bioarchaeology is the use of stable carbon and nitrogen isotope analyses to reconstruct ancient diets. This technique is based on the

principle that the isotopic signatures of consumed foods are reflected in the tissues of consumers (GARCÍA-MORENO; CORTIZAS and LÓPEZ-COSTAS, 2022).

In a terrestrial ecosystem, the ratio of stable carbon isotopes (13C/12C = δ13C) is determined by the photosynthetic process used by a plant (VAN DER MERWE, 1982). The two photosynthetic processes explored in isotopic research are known as C3 and C4. Trees, shrubs, forest plants, that is, uncultivated plants, are part of the C3 group or the Calvin Pathway and have δ13C values between -20.00‰ and -34.00‰. The δ13C values of plants from the C4 group or the Hatch-Slack Pathway, characteristic of cultivated plants (corn, sugarcane, for example), range from -9.00‰ to -16.00‰ (DENIRO and EPSTEIN, 1981; LIMA, 2019; BRITTON *et al*., 2023).

Consequently, consumers of C3 plants have a carbon isotopic composition that is quite different from those consumers of C4 plants (DENIRO and EPSTEIN, 1981). From the chemical study of bone collagen, it is possible to determine the values of δ13C and δ15N and detect the types of vegetables consumed by the individuals to whom the studied bones belonged. An example was the work of Styring and his collaborators (2022). They observed that the δ13C values for goat grass (*Setaria italica*, a type of maize) and rice (*Oryza sativa*) were, on average, -11.80‰ and -26.00‰, respectively, and were able to differentiate between rice and maize consumers.

The application of stable carbon isotopes in the study of human diets arose almost by accident. Determining δ13C from a skeleton found at an Iron Age archaeological site in Phalaborwa in the Transvaal Lowveld, South Africa, Van der Merwe (1982) obtained the value -10.00‰, believing it to be a farmer. However, Vogel (1983) reports that all grasses in the Transvaal Lowveld region were C4 plants.

After this first determination, Van der Merwe and Vogel (1983) made hundreds of measurements on human skeletons from selected sites in the Americas and Africa. Since then, many researchers have contributed to enriching the body of information on the isotopic composition of plants, animals, and humans.

Thus, according to Van der Merwe (1982), there are three different types of plant-based human diets, which are shown in Table 1.

The nitrogen in bone collagen is directly obtained from proteins and therefore reflects only the protein sources consumed in the individual's diet (CRAIG *et al*., 2010). Stable nitrogen isotopes (15N and 14N) can be useful in determining the trophic level of foods consumed, since there is an enrichment of 3.00 to 4.00 ‰ in δ15N (15N/14N) values between

trophic levels in the food chain (DENIRO and EPSTEIN, 1981). This is true for both terrestrial and marine food chains (RICHARDS, 2020).

Table 1

The three different types of plant-based diets, according to Van der Merwe. Source: Van der Merwe (1982)

Approximate value of $\delta^{13}C$	Diet composition
- 21,40‰	100% C_3 plants
- 7,40‰	100% C_4 plants
-14,40‰	50,00% C_3 plants and 50,00% C_4 plants

Source: Van der Merwe (1982).

The δ15N values of terrestrial plants are generally 4.00‰ lower than those of marine plants, so animals inhabiting oceans and seas have higher δ15N values than terrestrial ones. Thus, stable nitrogen isotopes are used to determine both the relative contribution of plant and animal resources, and that of terrestrial and marine food sources in the diet (PEZO-LANFRANCO and COLONESE, 2022).

In general, the δ15N value can determine whether the type of food is of marine origin, when it is above +13.00‰, or terrestrial, when it is below +6.00‰. If the δ15N values are around +10.00‰, it can be assumed that this food came from freshwater systems. These relationships are directly linked to the biogeochemical nitrogen cycle (DENIRO and EPSTEIN, 1981; RICHARDS, 2020).

However, to determine reliable values of δ13C and δ15N, it is necessary to obtain a purified collagen sample (SCHOENINGER, 2014). In order to verify whether the collagen sample was sufficiently pure or not to allow its use in dietary analyses, DeNiro (1985), studying bones burned by cooking or cremation, suggested that the carbon-nitrogen atomic ratio (C/N) of the sample should be measured. If this parameter is between 2.90 and 3.60, a characteristic range for collagens from recent bones, the sample retains its δ13C and δ15N value equal to that of the living being. Samples whose C/N atomic ratios fall outside this range may not reflect the composition of the individual's diet (CUNHA *et al*., 2022; DENIRO, 1985).

In addition to the problem of bone burning, there is also the problem of contamination by humic and fulvic acids, which are high molecular weight chemical compounds found in soil, soluble in water and with an atomic composition similar to that of collagen. Thus, to assess

ancient diets from excavated skeletal remains, it is important to know the C/N ratio, which indicates whether or not diagenesis has occurred (SCHOENINGER, 2014).

DeNiro and Weiner (1988) recommend that collagen obtained from bone be subjected to the analytical procedure of Infrared Spectrometry. If the spectrum obtained retains the characteristics of good quality collagen, isotopic analysis will reveal reliable δ13C and δ15N values for studies of ancient dietary habits.

Studies conducted by Lambert and his collaborators (MAYS, 2021) concluded that different types of skeletal bones (femur, rib, fibula, skull, maxilla, vertebra, tibia, ilium, undefined bone fragments, etc.), of compact or spongy nature, can be used interchangeably in these analyses. For DeNiro (1985), it is sufficient and necessary that the results of the analyses performed by Infrared Spectrometry and the C/N ratio guarantee that it is collagen that presents characteristics of being well preserved.

2.1 OBTAINING BONE SAMPLES

The 8 (eight) samples found at sites in Minas Gerais were kindly provided by the Natural History Museum of the Federal University of Minas Gerais (UFMG). The sample from the São Miguel do Tapuio site, in Piauí, was kindly provided by the Department of Archaeology and Conservation of Rock Art of the Federal University of Piauí (UFPI).

2.2 PREPARATION OF BONE POWDER

Each of the 9 (nine) samples was cleaned with distilled water using a very delicate brush. Then, it was placed in an oven at 50°C for 1 hour. After this time, it was crushed using a porcelain mortar and pestle and passed through a 100 Mesh sieve. The resulting bone powder was again placed in an oven at 50°C for another 30 minutes. After cooling to room temperature, the pulverized bone mass was stored in a sterilized and clean container.

2.3 COLLAGEN EXTRACTION

Collagen extraction from each of the 09 (nine) bone samples was conducted using the modified Longin method (LIMA *et al.*, 1995; BIZ and BORGES, 2025). Five successive washes of 150.00 mg of powdered bone were performed with 10.00 mL of 1.00 mol/L HCl each, followed by centrifugation with removal of the supernatant. The precipitate was washed with ten 10.00 mL portions of distilled water, in order to eliminate all excess concentrated hydrochloric acid and raise the pH to approximately 7.00, and placed under reflux at 95°C for

10 hours in 50.00 mL of 10^{-3} mol/L HCl. At the end of this time, a new filtration was performed in a sintered glass funnel (porosity 1), and the filtrate was concentrated in a rotary evaporator to an approximate volume of 3.00 mL and lyophilized. In the end, a gelatin-like material was obtained, identified as collagen, which was characterized by infrared spectrometry (DENIRO and WEINER, 1988).

2.4 ESPECTROS NO INFRAVERMELHO (IV) DO COLÁGENO EXTRAÍDO

They were recorded using spectrophotometers Mattison Instruments FTIR 3000 (Chemistry Department, ICEx, UFMG) and FTIR BOMEN from the Ezequiel Dias Foundation (Belo Horizonte – MG). The spectra were obtained in the range of 400 to 4000 cm^{-1}. All 9 (nine) samples of materials identified as collagen were analyzed in KBr (potassium bromide) pellets.

2.5 ANÁLISE DOS ISÓTOPOS ESTÁVEIS

Each of the 9 (nine) samples was analyzed using an ANCA-SL/20-20 mass spectrometer (IRMS) from Europa Scientific at the Stable Isotopes Laboratory of the Center for Nuclear Energy in Agriculture (CENA), in Piracicaba, São Paulo. All isotopic results were obtained using Vienna-Pee Dee Belemnite (VPDB) as a standard for carbon and air for nitrogen (AHN and PARK, 2023). The δ values were defined as:

$$13C \text{ or } 15N = [(Rsample/Rstandard)-1]x1000 \quad (1)$$

where R= 13C/12C or 15N/14N

2.6 C/N RATIO

The percentages of carbon (C) and nitrogen (N) elements were obtained in a PERKIN ELMER 2400 CHN elemental analyzer from the Chemistry Department, ICEx, UFMG, using a known standard compound for equipment calibration. The C/N atomic ratio was calculated for each sample using the following formula:

$$C/N = 1.17\ (\%C/\%N)$$

where 1.17 is the quotient between the atomic mass of nitrogen (14.00u) and carbon (12.01u) (LIMA, 2019).

3 RESULTS AND DISCUSSION

Table 2 presents the results obtained from the chemical analyses of the remaining collagen contained in the samples of the 09 (nine) human skeletons studied.

Table 2

Results of the chemical analyses of the collagen contained in the samples of prehistoric Brazilian human bones. Source: prepared by author

Order number	Skeleton identification	% of Collagen	C/N	$\delta^{15}N$	$\delta^{13}C$
01	Sítio Buritizeiros - MG Burial I – 6,000 to 7,000 years BP	2,00	4,19	+12,86	-20,99
02	Sítio Santana do Riacho I - MG Burial II – 8,000 to 10,000 years BP	1,80	**3,26**	+13,74	-20,09
03	Sítio Santana do Riacho I - MG Burial III – 8,000 to 10,000 years BP	1,74	**3,45**	+14,50	-22,87
04	Sítio Santana do Riacho I - MG Burial Va – 8,000 to 10,000 years BP	2,69	8,58	+15,36	-22,32
05	Sítio Santana do Riacho I - MG Burial IX – 8,000 to 10,000 years BP	1,66	21,16	+11,68	-22,40
06	Sítio Santana do Riacho I - MG Burial XI – 8,000 to 10,000 years BP	1,38	**3,60**	+14,84	-22,35
07	Sítio Santana do Riacho III - MG Burial V – 2,000 to 3,000 years BP	10,98	4,05	+9,80	-20,56
08	Sítio Santana do Riacho III - MG Burial VI – 2,000 to 3,000 years BP	11,58	3,72	+7,63	-18,82
09	Sítio São Miguel do Tapuio 2,000 to 2,500 years BP	32,19	**3,06**	+18,39	-16,78

Source: prepared by author.

When comparing the results of the percentage of collagen extracted from ancient bones (Table 2) with those obtained by Lima (2019) for current bones (84.98 to 98.24%), it is noticeable that the values of the former are very small in relation to the latter. Probably, this difference is due to the fact that the prehistoric samples underwent diagenetic processes throughout the period in which they were buried, significantly decreasing the percentage of bone organic matter.

Only sample 09, from the skeletal remains found in São Miguel do Tapuio-PI, showed a significant collagen content (32.19%). This can be explained by the fact that this skeleton reveals a relatively short burial period, 2,000 to 2,500 years, and by having been buried in soil with alkaline characteristics, which are less favorable to the occurrence of diagenetic processes (DENIRO, 1985; LIMA, 2019).

However, the existence of evidence of these alterations in ancient bone tissue does not necessarily mean changes in the chemical composition of collagen molecules, that is, the atomic contents of carbon, nitrogen and hydrogen, the most abundant elements in this molecule, are not modified to the level of compromising the quality of the organic material remaining in the buried bone (CURTO *et al*., 2024).

For the collagen extracted from samples 01, 04, 05, 07 and 08 (Table 2), the C/N atomic ratio values were outside the DeNiro range (1985), characteristic of well-preserved collagens (2.90 - 3.60). In turn, infrared spectrometry of the materials identified as collagen extracted from these samples (Figure 1, spectra 01, 04, 05, 07, and 08, respectively), revealed absorption bands characteristic of other compounds that are not collagens.

Therefore, the organic part of the samples of these skeletons identified as collagen most likely underwent diagenetic processes of such intensity that they compromised its quality. Thus, the 13C and 15N values (Table 2) do not reflect true information about diets (DENIRO 1985). Although they were determined, the 13C and 15N values of these five skeletons cannot be used to assess the dietary habits of the individuals to whom they belonged.

Table 2 shows that the C/N atomic ratio values of the collagen extracted from samples 02, 03, 06, and 09 were 3.26, 3.45, 3.60, and 3.06, respectively, and are within the range (2.90-3.60) predicted by DeNiro (1985) for well-preserved collagens. Furthermore, infrared spectrometry of the organic part identified as collagen in these samples revealed identical spectra for all of them (Figure 1, spectrum 09), showing only the absorption bands characteristic of the collagen molecule (LIMA, 2019).

Therefore, the 13C and 15N values of samples 02, 03, 06 and 09 (Table 2) were used in this work to evaluate the dietary habits of the individuals to whom these skeletons belonged.

For these 04 (four) samples, the 13C values obtained from the bone collagens were -20.09‰, -22.87‰, -22.35‰ and -16.78‰ (Table 2), respectively, indicating that these individuals consumed vegetables from the C3 group when alive, characteristic of non-cultivated plants. Based on these findings, it can be said that these bones likely belonged to individuals who had hunter-gatherer habits.

These studies corroborate the results found by Cornero, Neves and Prous (1999) who, when evaluating caries in the permanent dentition of skeletons from the Santana do Riacho I

group, concluded that they belonged to individuals from a population consisting of hunter-gatherers.

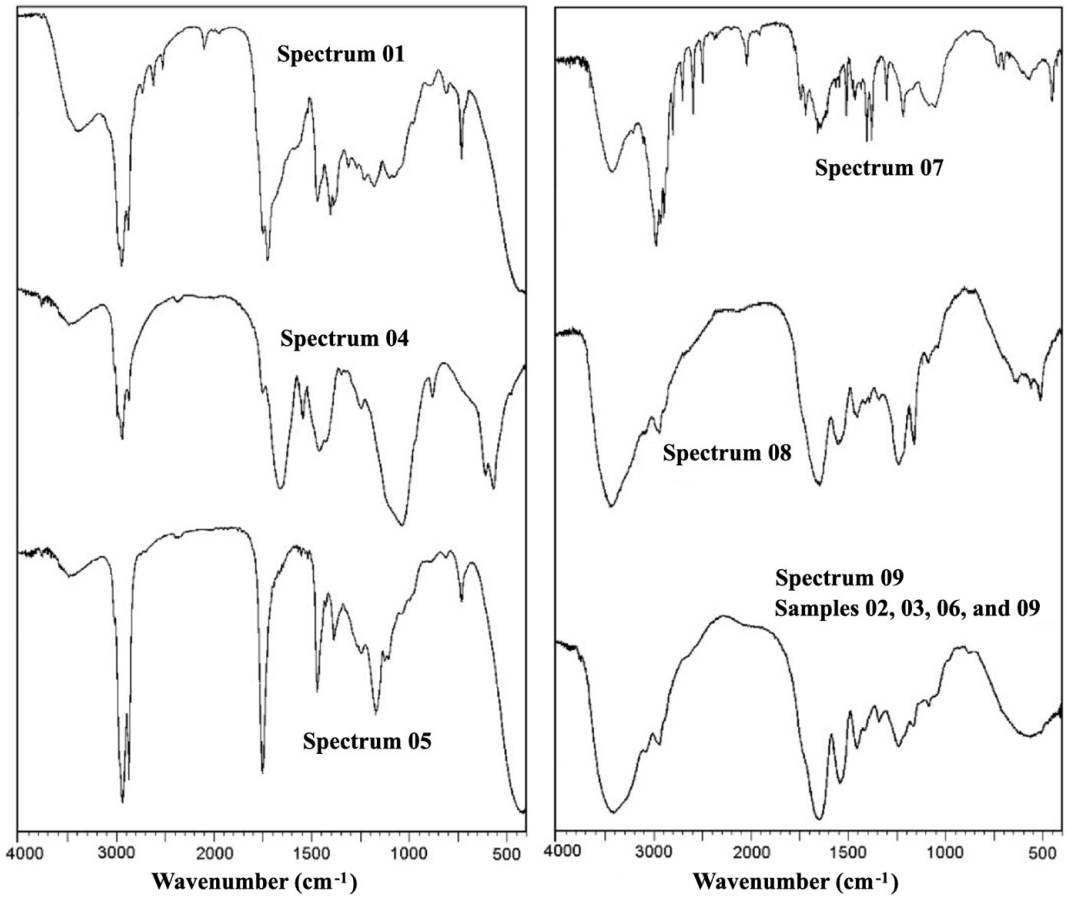
However, the value of -16.78‰ obtained for 13C of sample 09 (São Miguel do Tapuio, Table 2) reveals a difference of 4.99‰ in relation to the average value (-21.77±1.48‰) of the other three samples excavated in Santana do Riacho I. Thus, based on studies carried out by Van der Merwe and Vogel (1983) when analyzing a large number of ancient skeletons, it can be assumed that this individual would have already included in his diet about 25 to 30% of C4 type vegetables, that is, cultivated plants.

The δ15N values obtained for the 4 (four) samples were +13.74‰, +14.50‰, +14.84‰ and +18.39‰ (Table 2), indicating that the individuals to whom these bones belonged regularly consumed protein from animal or marine sources.

For sample 9 (nine), from São Miguel do Tapuio, the value +18.39‰ is 4.03‰ higher than the average value (+14.36±0.56‰) of the 3 (three) samples from Sítio Santana do Riacho I. This suggests that the individual from Piauí regularly consumed protein from an additional source (CRAIG *et al*., 2010; DEWAR *et al*., 2010), therefore, he occupied a higher trophic level in the food chain than the three individuals from Minas Gerais.

Figure 1

Infrared spectrometry results of the material extracted from the bone samples and identified as collagen



Observations: spectra 01, 04, 05, 07 and 08 refer to samples 01, 04, 05, 07 and 08, respectively. The spectra of samples 02, 03, 06 and 09 were identical, therefore only the spectra of sample 09 (Spectrum 09) were reproduced here.
Source: Prepared by the authors.

In addition to this observation, the 15N values of the 04 (four) samples, above +13.00‰, according to well-established studies in the literature (CRAIG *et al.*, 2010; DENIRO and EPSTEIN, 1981; DEWAR *et al.*, 2010; RICHARDS, 2020; VAN DER MERWE, 1982; VOGEL, 1983; PEZO-LANFRANCO and COLONESE, 2022), are indicative of a diet based on food of marine origin. This finding raises a question: how could these individuals inhabit the interior of the continent and present 15N values characteristic of consumers of food of marine origin?

An answer that might satisfy this question would be the possibility of error in the analyses. However, this assumption is very unlikely to be true, since the internationally recognized Stable Isotope Laboratory of the Center for Nuclear Energy in Agriculture (CENA)

at the University of São Paulo (USP), where they were carried out, uses cutting-edge equipment and has a tradition of precision and accuracy in the results of this type of analysis. Therefore, the possibility of errors in isotopic analyses would be ruled out.

A logical answer, which would clarify the inconsistency between the 15N values indicating a diet of marine origin and the fact that these individuals do not inhabit coastal regions, would be attributed to the epicontinental seas that existed millions of years ago, in the Cretaceous period of the Mesozoic era, in the region that extends from the north to the south of Brazil, including the current states of Piauí and Minas Gerais (CARVALHO, 2014; GALLO, 2015; SILVA, 2022).

As these seas disappeared, they left behind a fairly high nitrogen isotopic ratio (δ15N) 'imprinted' on the soil. Since these atoms (15N and 14N) do not disintegrate, as they are stable, this ratio remains practically intact (CARVALHO, 2023). Absorbed by the plants that grew and developed in the region, it reached the trophic level occupied by these individuals, indicating that they would have consumed food of marine origin.

In addition to this hypothesis, which finds support in the presence of these ancient epicontinental seas (CARVALHO 2014; GALLO, 2015; CARVALHO, 2023; SILVA, 2022), there is no archaeological evidence that could suggest a different interpretation for the results of the δ15N values obtained from these skeletons.

4 FINAL CONSIDERATIONS

Stable isotope analyses of human remains excavated from archaeological sites in Minas Gerais and Piauí provided valuable and important information, which allowed us to make assumptions and hypothesize about the diet of individuals who inhabited these regions more than 2000 years ago.

The δ13C data indicated that, of the 8 (eight) skeletons excavated from sites in Minas Gerais, 3 (three) belonged to individuals who consumed a diet based on plants from the C3 group, characterizing them as individuals who had hunter-gatherer habits. The data relating to the other 5 (five) skeletons from Minas Gerais could not be used to assess the type of diet of the individuals to whom they belonged, as the C/N values indicated the occurrence of diagenetic processes that compromised the quality of the remaining collagen in these bones. The δ13C value of the skeletal remains found in Piauí indicated that this individual was also a hunter-gatherer; however, they probably already practiced agriculture.

The nitrogen data from these bones could not be used to characterize the type of diet of the individuals to whom they belonged. Since they inhabited inland regions of the South American continent, the δ15N values obtained, indicating a diet of marine origin, are actually due to the isotopic signature left by the epicontinental seas when they disappeared millions of years ago.

The individual whose skeletal remains were found in São Miguel do Tapuio, Piauí, had an additional source of protein in their diet, occupying a higher trophic level in the food chain than those from Minas Gerais.

The most likely hypothesis is that the results obtained in this work cannot be compared with the studies of Van der Merwe (1982) and Vogel (1983), which were carried out with human bones excavated from North American and African sites. This is due to the possibility that the soil and climate, as well as the customs of the primitive peoples who lived in the regions comprising the current states of Minas Gerais and Piauí, presented peculiarities not contemplated by the chemical parameters described in the literature. This, however, can only be clarified after a systematic study of the results of chemical analyses of a much larger number of samples of ancient bones from different Brazilian regions.

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