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Carbon and nitrogen isotopic variability in bone collagen during the Neolithic period: Influence of environmental factors and diet

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ABSTRACT

Studies on Holocene periods in France and Liguria over the past 15 years provide an important isotopic database ($\delta^{13}C$ and $\delta^{15}N$) on human and animal bone collagen during the Neolithic period (ca. 5500 −3100 BC cal.; n = 573). The distribution of archaeological sites (n = 30) along a latitudinal transect from the Mediterranean to the Channel offers a broad data base reflecting a variety of environments and potential cultural practices. We propose a new insight into $\delta^{13}C$ and $\delta^{15}N$ data to understand the variability in both environment and human diet at the onset of farming. Statistical comparisons highlight significant geographical variation in both $\delta^{13}C$ and $\delta^{15}N$ ratios in most of the examined species and particularly in wild herbivores ($\Delta^{13}C = 3.8‰$; $\Delta^{15}N = 8.5‰$). Higher $\delta^{15}N$ and lower $\delta^{13}C$ ratios are found in samples from northern France. Conversely, lower nitrogen and higher carbon isotopic ratios are present in samples from the Mediterranean area. Results indicate the probable strong influence of natural factors impacting soil and plant isotopic ratios and passing this variation further on into the whole food chain. Our data indicate that the isotopic baseline depends on the local environmental particularities which must be taken into account in reconstructing human palaeodiets.

1. Introduction

Carbon and nitrogen stable isotopes ($\delta^{13}C$, $\delta^{15}N$)have been widely used for decades to study palaeoenvironments and palaeodiets (Peterson and Fry, 1987; Tieszen and Boutton, 1989; Hobson and Wassenaar, 1999; Schwarcz et al., 2010). These isotopic parameters record, among others, changes in the isotopic composition of plants and their consumers induced by changes in the chemical composition of the soils or the atmosphere occurring during climatic changes. Several studies have attempted to apply this tool to animal and human bones to infer climatic fluctuation sand their impact on ecology and human behaviour during the last 50,000 years in Europe (Iacumin et al., 1997; Drucker et al. 2000; van Klinken et al., 2000; Drucker and Célérier 2001; Richards and Hedges, 2003; Drucker et al., 2003; Drucker and Bocherens, 2004; Hedges et al., 2004; Drucker et al., 2011; Bocherens et al., 2014). For example, the extensive study by Richards and Hedges (2003) and Hedges et al. (2004) in Northwest Europe underlined important diachronic shift sin $\delta^{13}C$ of herbivore bone collagen. However, reconstructions of palaeodiets based on variation in isotopic composition (C,N) of collagen in animal or human bones do not indicate what comes from changes in climatic parameters and what comes from food choices.

Here, we present a compilation of carbon and nitrogen isotopic composition in the bone collagen of wild herbivores, domesticated herbivores, omnivorous or carnivorous species and humans from Liguria (North Western Italy) and France during the Neolithic period. In addition to the fact that a large isotopic data set is now available, it is particularly interesting to focus on the Neolithic period in these regions due to the following two circumstances:(1) there is no evidence for significant regional climate changes or shifts in isotopic composition and concentration of the atmospheric CO_2; and (2) human groups practiced and developed agriculture and herding differently depending on the local cultural and environmental conditions. Isotopic variation is discussed in relation to the expected environmental variability and potential changes in agricultural practices and human diet.

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2. Material and methods

Changes in climate parameters and in geochemical properties of soils induce changes in the isotopic composition of carbon and nitrogen in plants and, consequently in their consumers. The respective impact tof the different factors is summarized in the following subsections.

2.1. Carbon and nitrogen isotopic variation and environmental parameters

Variation in carbon isotopic composition in bone collagen reflects changes in vegetation and the relative proportion of C_3/C_4 plants in herbivore diet (van der Merwe and Vogel, 1978; DeNiro and Epstein, 1978). It also depends on the isotopic response of C_3 plants to changes in the isotopic composition and the concentration of CO_2 in the atmosphere (Farquhar et al. (1989a,b); O'Leary (1981), and Tieszen (1991). Precipitation and water availability are additional factors to reckon with (Wickmann 1952; O'Leary, 1981; 1988; Garten and Taylor, 1992; Schleser, 1995; Stewart et al., 1995; Heaton, 1999). The carbon isotopic composition of atmospheric CO_2 has been estimated to be 2‰ heavier/lighter relative to the period prior to the beginning of the industrial era (1750 AD) due to fossil fuel combustion (Revelle and Suess, 1957). On the other hand the carbon isotopic weight of the atmospheric CO_2 for glacial/interglacial transitions has been estimated to be 0.3‰ heavier/lighter relative to modern times (Leuenberger et al., 1992). These fluctuations have been demonstrated by measurements directly on plants (Farquhar et al., 1989a,b). CO_2 concentrations and carbon isotopic weight remained relatively stable between the last significant climate change at 8.5 ka (Harkness, 1972; Indermühle et al., 1999; Wagner et al., 2002) and the onset of the Industrial Revolution. A negative relationship between CO_2 concentrations and $\delta^{13}C$ in plants has been shown by several studies (Krishnamurthy and Epstein, 1990; Van de Water et al., 1994; Feng and Epstein, 1995; Pasquier-Cardin et al., 1999). All these authors agree with the coefficient established by Feng and Epstein (1995), i.e., a decrease of 2.0 ± 0.1‰ in plant $\delta^{13}C$ for an increase of 100 ppm in atmospheric CO_2. A decrease in water availability induces a closure of plant stomata and a reduction of CO_2 diffusion and consequently leads to lower carbon isotope fractionation. Field studies have shown that $\delta^{13}C$ values become higher with increasing aridity (Garten and Taylor, 1992; O'Leary, 1995; Schleser, 1995; Stewart et al., 1995; Heaton, 1999; Ferrio et al., 2003; Swap et al., 2004; Szpak et al., 2013). As many other environmental parameters affect the carbon isotopic composition of plants, some variability in the covariation between plant $\delta^{13}C$ and precipitation was observed in these studies. Nevertheless, it seems to be a consensus that for every 100 mm increase in precipitation there is a concomitant decrease of ~0.3‰ in$\delta^{13}C$ values in plants, a generally accepted guideline for palaeoenvironmental reconstructions (Hatté et al., 1998, 2001).

Variability in $\delta^{15}N$ in plants has multiple causes, regardless of the species considered (Handley and Raven, 1992). However, nitrogen isotopic variability in plants is strongly conditioned by soil $\delta^{15}N$ and the complexities of N-pathway in nature (Virginia and Delwiche, 1982; Handley and Raven, 1992; Amundson et al., 2003; Kendall et al., 2007). Plant nitrogen isotope composition is determined by the isotope ratio of the external nitrogen source (nitrate, ammonium – manuring - or atmospheric N_2 used by leguminous plants) and physiological mechanisms within the plant. Plant $\delta^{15}N$ is also related to the N content in soil or the residence time of N in an ecosystem or N cycle "openness," which depends on the temperature (Evans, 2007). The isotope ratio of source nitrogen is preserved during nitrogen absorption, assimilation and translocation. In leafy plants, the $\delta^{15}N$ of leaf tissues reflects that of the nitrogen source in the soil while in the pulses it reflects that of the atmosphere. Plant nitrogen isotopic composition is also strongly influenced by a series of environmental factors (Greenway and Munns, 1980; Heaton, 1987; Amundson et al., 2003).

Many studies have found a negative correlation between plant $\delta^{15}N$ values and local precipitation and/or water availability. These effects have been demonstrated at regional, meso-regional, continental and global scales (Austin and Sala, 1999; Handley et al., 1999; Amundson et al., 2003; Aranibar et al., 2004; Swap et al., 2004; Craine et al., 2009; Murphy and Bowman, 2009; Hartman and Danin, 2010; Ma et al., 2012; Szpak et al., 2013). Soil $\delta^{15}N$ variation is also conditioned by soil acidity (pH) with soil $\delta^{15}N$ ratios positively correlated to pH (Mariotti et al., 1980; Handley and Raven, 1992). A decrease in $\delta^{15}N$ in plants, and therefore in consumers' tissues, has been documented by Rodière in modern roe deer (Rodière, 1995; Rodière et al., 1996). The author recorded depleted $\delta^{15}N$ ratios (−2.4‰ to −0.4‰) compared to commonly documented values for this species in Europe (ca. 6.2 ± 0.5‰; Bocherens et al., 2005a, 2006). Plants consumed by roe deer indicate the same trend with low $\delta^{15}N$ ratios resulting from particularly low pH and N concentrations in forest soils (Rodière et al., 1996). Moreover, land use also affects the nitrogen composition of soils and plants. Research on modern soils shows that leached nitrate $\delta^{15}N$ is close to soil organic nitrogen $\delta^{15}N$, linked to pedological contexts (Sebilo et al., 2006). However, plants use nitrate, ammonium and dissolved organic nitrogen with possible preferences. Ammonium in soils is enriched in ^{15}N compared to nitrate and preferential plant uptake leads to differences between $\delta^{15}N$ ratios in plants and soils (Marshall et al., 2007). Sebilo et al. (2006) indicates environment-dependent $\delta^{15}N$ variation in soils, with low nitrate values (~0‰; nitrogen cycle performed in a closed system) recorded in forested areas and high nitrate values recorded in agricultural areas (~5‰; nitrogen cycle performed in an open system), mainly due to a more important denitrification process.

$\delta^{13}C$ and $\delta^{15}N$ values in plants are passed on by consumption to higher trophic levels, subject to ^{13}C and ^{15}N enrichment (DeNiro and Epstein, 1981; Ambrose and Norr, 1993). This enrichment between the diet and bone collagen is estimated at ~5‰ and 3–5‰ for carbon and nitrogen, respectively, with an additional enrichment of ca. 1‰ for bone collagen $\delta^{13}C$ in species from successive trophic levels (Ambrose and Norr, 1993; Bocherens and Drucker, 2003). This trophic level differentiation is mainly used in archaeology to assess the trophic level of an individual, and in particular, to measure the importance of animal protein intake as well as the role of marine resources in human diets (Hedges et al., 2007). On the other hand, C and N isotope variability in the food chain (wild and domesticated herbivores, omnivores and carnivores) provide an opportunity to understand the relative effects of climatic versus agricultural factors and of dietary practices on carbon and nitrogen isotopic variability in human bone collagen (van Strydonck et al., 2004). This is because wild herbivores reflect climatic parameters while domesticated herbivores and omnivores may reflect also changes inhuman agricultural practices and/or dietary preferences.

2.2. Past and present climatic parameters

The archaeological sites examined in this paper are spread out between 42 and 51° north latitude (DNL), covering several types of climate (Mediterranean, oceanic, semi-continental and their transition areas). The modern precipitation gradient between these sites is relatively low, ranging from 600 mm/yr in the areas bordering the Mediterranean Sea to 800 mm/yr in the North of France (Infoclimat 2001-2014). Most sites are located at low altitudes (0–400 m asl.), with only two reaching altitudes of almost 700 m asl., and within a few bioclimatic zones: thermo and meso-