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Palaeodiet Reconstruction in a Woman With Probable Celiac Disease: A Stable Isotope Analysis of Bone Remains From the Archaeological Site of Cosa (Italy)

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ABSTRACT Stable isotope analysis in the reconstruction of human palaeodiets can yield clues to early human subsistence strategies, origins and history of farming and pastoralist societies, and intra- and intergroup social differentiation. In the last 10 years, the method has been extended to the pathological investigation. Stable isotope analysis to better understand a diet-related disease: celiac disease in ancient human bones was carried out. To do this, we analyzed the nitrogen and carbon isotopic composition of human ($n = 37$) and faunal ($n = 8$) bone remains from the archaeological site of Cosa at Ansedonia, on the Tyrrhenian coast near Orbetello (Tuscany), including the skeletal remains of a young woman (late 1st century–early 2nd century Common Era [CE]) with morphological and genetic features suggestive of celiac disease. We compared the young woman's isotopic data with those of other individuals recovered at the same site but from two later time periods (6th century CE; 11–12th century CE) and with literature data from other Italian archaeological sites dating to the same period. Her collagen $\delta^{13}C$ and $\delta^{15}N$ values differed from those of the samples at the same site, and from most but not all of the contemporary sites. Although the woman's diet appears distinct, chronic malnutrition resulting from severe malabsorption of essential nutrients due to celiac disease may have affected the isotopic composition of her bone collagen. Am J Phys Anthropol 154:349–356, 2014.

Celiac disease is an immune-mediated disorder characterized by chronic inflammation of the small intestine and systemic manifestations precipitated by exposure to dietary gluten in genetically predisposed individuals (Ludvigsson et al., 2013). Considered part of a spectrum of gluten-related disorders, celiac disease is caused by a reaction to the gluten protein found in wheat and other gluten-containing grains. Chronic inflammation is due to the activity of a subgroup of lymphocytes that play a key role in the immune system, particularly in the adaptive immune system: T helper cells that express the surface protein CD4 and are referred to as $CD4^+$ T cells. The ingestion of wheat gluten and similar proteins in barley and rye activates a T-cell $CD4^+$ immune response resulting in chronic inflammation and villous atrophy of the small intestine, with impaired absorption of nutrient (Koning, 2005). Characteristic symptoms of celiac disease are chronic diarrhea, weight loss, neurologic symptoms, dermatitis herpetiformis, delayed puberty, osteoporosis, infertility, vitamin and protein deficiencies, and elevated liver enzyme levels (Green, 2005; Krupa-Kozak, 2014), all of which are correlated with a myriad of autoimmune diseases (Cataldo et al., 1997; Cronin and Shanahan, 1997). The prevalence of celiac disease is higher in women (Hischenhuber et al., 2006) and in persons with genetic disorders such as Down syndrome and Turner syndrome (Bettendorf et al., 2006; Shamaly et al., 2007).

The first description of the malabsorptive symptoms consistent with celiac disease was recorded by Aretaeus of Cappadocia, a Greek physician living between the 1st and 2nd centuries BCE (Adams, 1856). While a role for carbohydrates had long been suspected, the link between celiac disease and the gluten component, specifically the storage proteins rich in glutamine found in cereal grains such as wheat, barley, and rye, was not made until the 1950s by Dutch pediatric researchers (Dicke et al., 1953).

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The etiology of celiac disease is multifactorial, involving both genetic and environmental factors, and more than one factor is necessary for the disease to manifest in a person. Although the HLA genotype is associated with celiac sufferers, the gene does not cause the disease on its own (Margaritte-Jeannin et al., 2004; Liu et al., 2005; Bourgey et al., 2007). It has been estimated that only 2%–5% of at-risk gene carriers develop the disease (Schuppan et al., 2009). Previously thought a rare illness, celiac disease is now recognized as one of the most common genetic disorders (incidence of 1% in newborns) among populations with a long history of agriculture, such as those that settled Europe and the Near East (Gasbarrini, 2008; Sams and Hawks, 2013).

Here, we investigated by means of isotope analysis the case of a young woman (late 1st century– early 2nd century Common Era [CE]) found in a tomb at the archaeological site of Cosa, whose skeletal remains showed morphological and genetic features suggestive of chronic malnutrition consistent with celiac disease (Gasbarrini et al., 2010, 2012). Supporting our hypothesis is a particular set of morphological features, including very frail physique, short stature (140–145 cm) (Oliver et al., 1978), abnormally small teeth and thinning of diaphyseal compact bone, as well as several pathological signs, such as cribra orbitalia, enamel hypoplasia, and hip dysplasia. Taken together, they suggest that the woman suffered from chronic nutritional stress during growth, resulting in abnormal development (Gasbarrini et al., 2010). Possible causes are metabolic disease or poor diet. Poor diet seems unlikely given the tomb type and richly decorated funeral artifacts found inside, which suggest that the woman belonged to a wealthy family. We believe metabolic disease is a more likely probability given that the woman has the genetic haplotype (HLA DQ 2.5) associated with the highest risk of celiac disease (Gasbarrini et al., 2012).

We compare her isotopic data with those obtained from the other individuals recovered from the same site but from two later periods: 6th century CE and 11th–12th century CE (Fentress and Bodel, 2004). Since other skeletal remains of the Imperial Roman period have not been found in Cosa, we also compare the data for the young woman with the published data for various different Italian archeological sites dating from the same period.

The best indicators for palaeodiet reconstruction are the ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) stable isotopes in collagen. Stable isotope ratios are expressed as parts per mil (‰) by the delta (δ) notation, relative to a standard reference material: atmospheric N_2 (ambient inhalable reservoir [AIR]) for nitrogen (Mariotti, 1983) and the Vienna-PeeDee belemnite (V-PDB) marine limestone for carbon (Coplen, 1996). Stable isotope signatures provide information about diet: nitrogen is obtained from dietary protein whereas carbon in bone collagen is preferentially obtained from dietary protein (Ambrose and Norr, 1993; Tieszen and Fagre, 1993), but it can also be derived from other dietary components such as carbohydrates and lipids, particularly when diets are low in protein (Jim et al., 2006). Because collagen is constantly resorbed and reformed, the isotopic composition of collagen provides a good proxy for reconstructing the diet of the last 10–15 years of an individual's life (Hedges et al., 2007). Furthermore, since one of the main processes responsible for fractionation in nature is photosynthesis, carbon isotope compositions ($\delta^{13}C$) are particularly suited to determining the consumption of plants with different photosynthetic pathways (i.e., C_3 versus C_4) (Smith and Epstein, 1971; Vogel, 1980; O'Leary, 1981; Griffith, 1992). Also, stable carbon isotopes are useful to distinguish between terrestrial and marine diets (Chisholm et al., 1982; Walker and DeNiro, 1986). Importantly, the carbon stable isotope increases in the trophic chain by small increments of ≈1‰ (van der Merwe, 1982).

Another food source is freshwater fish which has lower $\delta^{13}C$ values as compared with marine fish and values similar to terrestrial data. This could be due to environmental influences such as interactions between the bicarbonate dissolved in freshwaters and other carbon sources, for example, soil and rock bicarbonate and organic carbon from waste products and decomposition (Katzenberg, 2000; Krigbaum, 2003). Nitrogen compositions are used to distinguish between different trophic levels in a food web because a increases in $\delta^{15}N$ values (∼3‰–5‰) occur at each trophic level (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984) which is probably associated with the excretion of more ^{14}N in urea (terrestrial environments) or ammonia (marine environments) and, consequently, with the use of more ^{15}N in tissue synthesis (Schoeninger and Moore, 1992).

Furthermore, the fractionation of stable isotopes can be altered by metabolic and physiological changes due to starvation and disease (Katzenberg and Lovell, 1999; Mekota et al., 2006; Olsen et al., 2014). Specifically, $\delta^{15}N$ and $\delta^{13}C$ values might be modified by metabolic and physiological changes during severe malnutrition. During starvation, $\delta^{15}N$ values reflect gluconeogenesis in which glucose is produced from non-carbohydrate sources such as pyruvate, lactate, and alanine (Voet and Voet, 1995). Thus, through catabolism, the nitrogen source is consumer tissue (Hobson and Clark, 1992; Hobson et al., 1993; Gannes et al., 1997). This biochemical mechanism involves an additional enrichment in ^{15}N since this source of nitrogen has already been enriched relative to diet, resulting in trophic level fractionation between diet and consumer tissue similar to the breastfeeding effects as found by Fogel et al. (1989). A consequence of this process is the increase in tissue $\delta^{15}N$ values.

Previous research has highlighted that during starvation or nutritional stress $\delta^{13}C$ values might be subject to change (Hobson and Clark, 1992; Hobson et al., 1993; Fuller et al., 2004, 2005). During nutritional stress, the use of body proteins, such as muscle tissue, leads to an increase in $\delta^{13}C$ values. The increase in $\delta^{13}C$ values depends not only on the trophic effect but also on additional enrichment due to the fractionation factor from plant food to consumer protein (+3‰ for muscles, and +5‰ for collagen) (Lee-Thorp et al., 1989).

Finally, since celiac disease is a nutrition-related disorder associated with malabsorption of nutrients and, therefore, with nutritional stress, we analyzed stable carbon and nitrogen isotopes in bone collagen in order to investigate the diet of the young woman and to shed light on how diet-related disorders could change the stable isotopic composition of bone tissue.

MATERIALS AND METHODS

Samples

Bone samples were recovered from the archaeological site of Cosa located near Ansedonia, a village on the

Fig. 1. Map of the Cosa archaeological site (Ansedonia, Tuscany, Italy).

Fig. 2. The skeletal remains of a young woman of the Imperial Roman period from Cosa.

Tyrrhenian Sea coast, in southwestern Tuscany, 140 km northwest of Rome (Fig. 1).

Cosa was founded in Roman times in 273 BCE. Never a truly prosperous city, its economy was mainly based on the cultivation of wheat and olives. Following its decline during the 4th and 5th centuries CE, commerce and trade resumed in the early 6th century and new buildings were constructed (Fentress and Bodel, 2004), among which was a church built over the Forum ruins. The area was still inhabited during the 11th and 12th centuries CE, as documented by a medieval church and cemetery in the Forum area.

Long bones were collected from 37 human and 8 faunal specimens. The skeletal remains of the young woman were dated to the Imperial Roman period (late 1st century–early 2nd century CE), according to the grave goods and the burial architecture (Fig. 2).

In 1998, the "alla cappuccina" type tomb (covered by 10 large terracotta plain tiles arranged in a pitched roof) was found near Cosa. Excavations were carried out by the Superintendence of Archaeological Heritage of Tuscany, and the skeleton was assigned code number 0175 by the Laboratory of Archeology and Anthropology of the Superintendence. The grave goods consisted of personal jewelry: one golden and two bronze rings decorated with gems, two golden earrings, and one golden button. Ceramic artifacts were found near the feet: a poculum, a lucerna, and a censer cup (Agricoli et al., 2013). All skeletal regions were present, but many bones were incomplete, either eroded at the ends or lacking some smaller bones (vertebrae, hand bones, feet). Bone texture was fragile because of the chemical and physical characteristics of the soil.

Female sex was determined on the basis of morphological features and grave goods; the age at death (between 18 and 20 years) was estimated on the basis of molar teeth development (Ubelaker, 1989) and the stages of union of epiphyses (Scheuer et al., 2000). Four humans (2 adults: 1 female and 1 male, and 2 children) were found in the IX D North area which is comprised of the cemetery of a 6th century CE church cemetery built in the area of the Forum (Fentress and Bodel, 2004).

Thirty-two humans (1 child and 31 adults of both sexes), 4 *Cervus elaphus*, 2 *Ovis aries*, and 2 *Bos taurus*, were found in the Forum II area, encompassing a later medieval cemetery (between the 11th and 12th centuries CE) also in the area of the Forum.

Collagen extraction and stable isotope analysis

One-gram fragments of the long bones were collected with a dentist's drill. The bone surface was removed by scraping, and the cortical samples were pulverized using a pestle and mortar. Collagen was extracted using a modified Longin method (Brown et al., 1988). About 0.5–1 g of powdered bones was demineralized in 0.6 M HCl for 2–4 days at 4°C. The acid was removed and the remaining residue was gelatinized with 1 mM HCl at 65°C for 24 h and ultrafiltered (30 kDa MW cut-off; Millipore, Billerica, MA) and then lyophilized. To evaluate extraction efficiency, collagen extraction was carried out simultaneously on modern, homogenized bovine bone powder of known isotopic composition which served as a reference control.

For each collagen extract 0.8–1.2 mg of collagen were weighed, put into tin capsules, and analyzed in triplicate by continuous-flow isotope ratio mass spectrometry (CF-IRMS) at the Institute of Environmental Geology and Geoengineering, CNR Rome, Italy. The reliability of stable isotopic analyses of collagen partly depends on the degree of collagen preservation. To test reliability and to exclude contamination from exogenous carbon and nitrogen sources, the samples were assessed against established criteria (DeNiro, 1985; Ambrose, 1990; van Klinken, 1999).

Statistical analyses

Dietary variability between the two historical periods was evaluated and differences between the sexes and across six different age groups (0–6; 7–12; 13–19; 20–29; 30–39; 40–50 years) were compared using the Kolmogorov–Smirnov test run on PAST software version 2.08b (Hammer et al., 2001) only for the samples from the Forum II area because of the scarcity of samples of the IX D North area.

RESULTS AND DISCUSSION

Stable carbon and nitrogen isotope analysis

Biological information, C and N percentage content, quality indicators, collagen yields and collection sites of the samples are listed in Table 1.

TABLE 1. Biological information, results of stable isotope analysis of collagen and quality indicators of the samples

Sample	Species	Sex	Age	%C	%N	C:N	δ^{13}C V-PDB (‰)	δ^{15}N AIR (‰)	% Collagen	Area
Young woman	*Homo sapiens*	F	18–20	41.7	14.7	3.3	−19.5	10.7	5.4	
Bu. 4	*H. sapiens*	I	14–18	41.9	15.4	3.2	−18.4	7.0	7.1	Forum II
Bu. 6	*H. sapiens*	M	25–35	47.3	16.1	3.4	−17.8	9.3	9.1	Forum II
Bu. 7	*H. sapiens*	M	25–35	40.5	14.5	3.2	−18.9	6.1	3.4	Forum II
Bu. 13	*H. sapiens*	F	25–45	45.9	16.8	3.2	−18.2	9.9	10.4	Forum II
Bu. 15	*H. sapiens*	I	13–20	42.2	15.3	3.2	−18.2	8.1	5.2	Forum II
Bu. 17	*H. sapiens*	I	8–12	44.5	16.4	3.2	−16.4	7.9	3.9	Forum II
Bu. 18	*H. sapiens*	F	35–45	44.5	16.2	3.2	−15.9	7.4	6.4	Forum II
Bu. 22	*H. sapiens*	I	10–15	42.0	14.2	3.4	−17.4	8.9	5.8	Forum II
Bu. 25	*H. sapiens*	I	1.5–3	44.2	16.5	3.1	−18.3	8.0	7.9	Forum II
Bu. 29	*H. sapiens*	F	14–20	41.6	14.7	3.3	−17.8	9.5	1.3	Forum II
Bu. 32	*H. sapiens*	M	35–45	44.6	16.1	3.2	−17.6	7.6	5.7	Forum II
Bu. 37	*H. sapiens*	F	I	37.3	12.5	3.5	−18.4	7.7	2.5	Forum II
Bu. 38	*H. sapiens*	F	30–40	48.4	17.2	3.3	−17.3	8.8	8.4	Forum II
Bu. 39	*H. sapiens*	M	Adult	46.3	15.6	3.5	−17.8	9.1	4.6	Forum II
Bu. 41	*H. sapiens*	M	17–25	44.9	16.4	3.2	−17.4	9.0	9.2	Forum II
Bu. 42	*H. sapiens*	M	30–45	44.0	15.8	3.2	−17.2	8.0	6.2	Forum II
Bu. 45	*H. sapiens*	I	9–14	46.1	15.5	3.5	−18.2	6.8	7.7	Forum II
Bu. 55	*H. sapiens*	I	10–15	45.3	15.8	3.3	−18.2	8.1	5.6	Forum II
Bu. 56	*H. sapiens*	F	Adult	45.5	16.3	3.2	−17.8	6.6	5.4	Forum II
Bu. 59	*H. sapiens*	F	35–50	43.0	15.1	3.3	−17.3	8.9	1.4	Forum II
Bu. 60	*H. sapiens*	F	18–23	47.6	16.0	3.5	−18.5	8.0	8.8	Forum II
Bu. 62	*H. sapiens*	I	7–12	44.4	15.8	3.3	−18.1	8.4	2.2	Forum II
Bu. 63	*H. sapiens*	M	18–25	43.5	15.8	3.2	−17.8	8.9	5.1	Forum II
Bu. 66	*H. sapiens*	F	20–30	41.8	15.4	3.2	−17.4	8.9	6.6	Forum II
Bu. 68	*H. sapiens*	F	45–60	47.1	16.0	3.4	−17.9	7.8	8.4	Forum II
Bu. 69	*H. sapiens*	M	35–50	39.3	14.5	3.2	−17.7	10.0	2.8	Forum II
Bu. 77	*H. sapiens*	F	30–40	43.6	15.4	3.3	−18.2	8.9	2.3	Forum II
Bu. 78	*H. sapiens*	F	16–23	41.8	14.4	3.4	−17.4	10.0	4.0	Forum II
Bu. 80	*H. sapiens*	F	18–25	42.2	15.1	3.2	−17.9	9.4	4.6	Forum II
Bu. 85	*H. sapiens*	F	20–30	44.0	15.3	3.3	−17.7	8.9	4.9	Forum II
Bu. 86	*H. sapiens*	M	Adult	45.5	16.6	3.2	−17.7	8.9	9.3	Forum II
Bu. 88	*H. sapiens*	M	25–35	49.8	17.5	3.3	−17.1	9.6	7.3	Forum II
US 13	*Cervus elaphus*			45.4	16.6	3.2	−19.9	6.7	9.5	Forum II
US 13 (2)	*Cervus elaphus*			33.0	11.1	3.5	−20.2	4.6	5.9	Forum II
US 20	*Ovis aries*			42.8	15.2	3.3	−19.8	7.5	2.6	Forum II
US 22	*Ovis aries*			49.5	16.7	3.4	−19.8	3.9	7.0	Forum II
US 22 (2)	*Cervus elaphus*			38.8	14.2	3.2	−20.2	4.8	5.8	Forum II
US 24	*Bos taurus*			36.6	13.4	3.2	−20.0	5.6	3.9	Forum II
int SW Q5	*Bos taurus*			45.1	15.4	3.4	−19.4	5.4	4.2	Forum II
int N5	*Cervus elaphus*			47.7	16.8	3.3	−20.6	3.4	4.8	Forum II
Bu. 22	*H. sapiens*	I	7.5–9.5	45.7	16.3	3.3	−18.6	7.3	3.6	IX D North
Bu. 23	*H. sapiens*	F	25–40	51.5	17.5	3.4	−18.1	9.8	10.1	IX D North
Bu. 28	*H. sapiens*	I	8–10	49.3	17.3	3.3	−18.3	8.2	4.8	IX D North
Bu. 31	*H. sapiens*	M	20–30	46.6	16.5	3.3	−17.4	10.6	5.9	IX D North

M: male; F: female; I: indeterminate.

All 37 human and 8 faunal bone remains yielded collagen of sufficient quality (Table 1), >1% (van Klinken, 1999) to obtain acceptable data for carbon and nitrogen isotopes, and almost all samples yielded collagen of satisfactory quality: carbon content (C%) between 15.3 and 47.0 and nitrogen content (N%) between 5.5 and 17.3 (Ambrose, 1990); only a few samples fell outside these ranges (Table 1). All samples showed an atomic C:N ratio between 3.1 and 3.5, which was within the acceptable range (2.9–3.6) as proposed by DeNiro (1985). According to van Klinken (1999), badly preserved bones not only show values outside the carbon and nitrogen ranges but also unacceptable C:N ratios in combination with low collagen yields. Therefore, we considered all our samples as well-preserved.

In general human diet in the Mediterranean area is characterized by low δ^{13}C values (−20‰) and δ^{15}N values between 6‰ and 10‰ (Craig et al., 2006). Freshwater and marine contributions to diet are indicated by nitrogen values higher than 10‰ and marine foods also tend to have carbon isotope values greater than −18‰.

Isotopic results for human and faunal remains at Cosa. The δ^{13}C content of the young woman was very low (−19.5‰), whereas the δ^{15}N value was high (10.7‰). For the human remains from the IX D North area, the mean δ^{13}C was $-18.1‰ \pm 0.5‰$ (range, −18.6‰ to −17.4‰) and the mean δ^{15}N was $9.0‰ \pm 1.5‰$ (range, 7.3‰ to 10.6‰) (Table 1). For the human remains from the Forum II area, the mean δ^{13}C was $-17.7‰ \pm 0.6‰$ (range, −18.9‰ to −15.9‰) and the mean δ^{15}N was $8.4‰ \pm 1.0‰$ (range, 6.1‰–10.0‰). The δ^{13}C values for the faunal remains (range, −20.6‰ to −19.4‰; mean, $-20.0‰ \pm 0.4‰$) were fairly homogeneous and characteristic of C3 plant consumers; the δ^{15}N values showed a greater variation (range, 3.4‰–7.5‰; mean, 5.2‰–1.4‰).

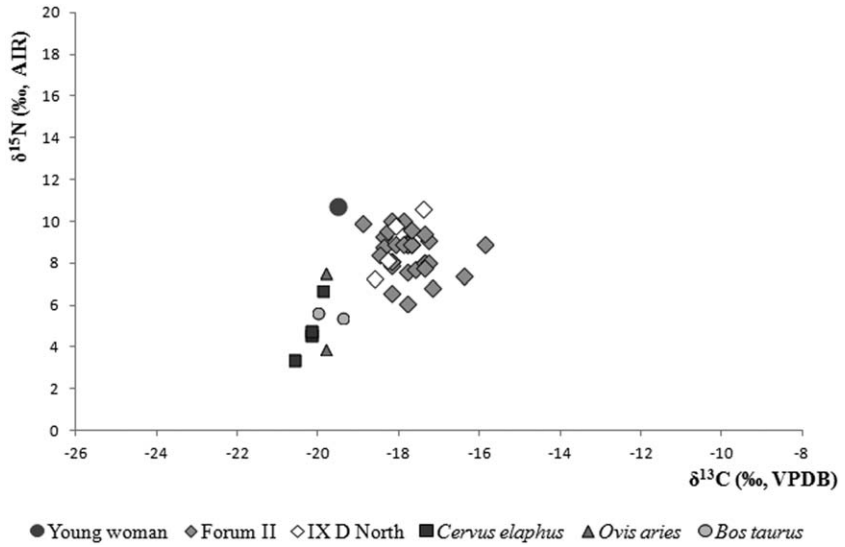

Fig. 3. Plot of $\delta^{13}C$ and $\delta^{15}N$ values from bone collagen samples analyzed in this study.

In general, the isotopic results might suggest that the woman was a C3 plant consumer and/or a consumer of terrestrial animals who consumed C3 plant foods. Moreover, her position in Figure 3 could confirm that she was mainly a terrestrial meat eater (Hedges and Reynard, 2007), although freshwater fish in her diet cannot be ruled out as a possibility either (Rutgers et al., 2009).

The $\delta^{13}C$ data, of Forum II area samples, indicated a mixed diet of terrestrial C3 and C4 or marine sources. The average difference of 3.2‰ in $\delta^{15}N$ between the human and animal remains suggests a relatively large proportion of animal protein consumption or a possible contribution from freshwater fish to the human diet (Muldner and Richards, 2007).

The IX D North area data are very similar to those obtained from the human remains of the Forum II area (Fig. 3). Taken together, the stable isotope results suggest that the diet of the inhabitants of the Forum II and IX D North areas consisted of a complex mixture of vegetables, animal proteins, and some fish food (Fig. 3). Comparison of the isotopic values between males and females of the Forum II area showed no significant difference in either $\delta^{13}C$ (Kolmogorov–Smirnov, $D = 0.3$; $P = 0.5$) or $\delta^{15}N$ (Kolmogorov–Smirnov, $D = 0.2$; $P = 0.9$), indicating no dietary differences between the sexes. Comparison between the datasets of the two historical periods (Forum II and IX D North areas) showed no statistical difference in either $\delta^{13}C$ (Kolmogorov–Smirnov, $D = 0.5$, $P = 0.2$) or $\delta^{15}N$ (Kolmogorov–Smirnov, $D = 0.4$, $P = 0.6$). Comparison across the different age groups was performed only for the Forum II samples, due to the limited number of samples from the IX D North area. Also in this case no statistical differences were found in either stable isotope $\delta^{13}C$ or $\delta^{15}N$. These findings suggest that the ancient inhabitants of Cosa did not substantially change their dietary habits over the centuries but rather maximized the different geographical environmental resources.

The isotopic data for the young woman differed from those of the samples from the Forum II and IX D North areas (Fig. 3). Her $\delta^{13}C$ composition (−19.5‰) was lower than two standard deviations of the average of the samples from Forum II (−17.8‰ ± 0.6‰) and IX D North (−18.1‰ ± 0.5‰). Her $\delta^{15}N$ composition (10.7‰) was higher than two standard deviations of the average of the Forum II group (8.5‰ ± 1.0‰) but less than two standard deviations of the IX D North area group (9.0‰ ± 1.5‰). Taken together, the results make her stand out isotopically (Fig. 3). But since she dates to an earlier time period, this shift could have been due to increased C4 plant and marine food consumption in subsequent periods or perhaps even to the nitrogen and carbon balance in nutritional human stress.

Comparing results across Imperial Roman period sites. In order to determine whether these observed variations were related to nutritional stress or to the temporal differences (500–1,000 years), we plotted the young woman's stable isotope results ($\delta^{15}N$, $\delta^{13}C$) with other published data on Imperial Roman populations from Velia (Craig et al., 2009), Isola Sacra (Prowse et al., 2004), three different areas from Rome (Killgrove and Tykot, 2013), and the St. Callixtus catacombs (Rutgers et al., 2009) (Fig. 4).

The young woman's nitrogen values are very similar to both Isola Sacra and St. Callixtus, while her carbon values are very similar to Velia and St. Callixtus. Therefore, it seems that she falls within the range established for contemporary sites, although there does seem to be somewhat of a difference across these sites in that some have more of a marine dietary component. Altogether, the woman is certainly most similar to St. Callixtus, for which, as reported by Rutgers et al. (2009), the results might suggest a large use of freshwater fish in the diet of this Christian community.

Celiac disease and bone collagen isotopic compositions. Young woman isotopic data might also reflect the effects of the disease process itself. Consistent with the particular set of morphological bone features, she might have suffered from chronic malnutrition due to celiac disease, which produces a chronic inflammation and villous atrophy of the small intestine, thus reducing the absorption of nutrients. This might have led to

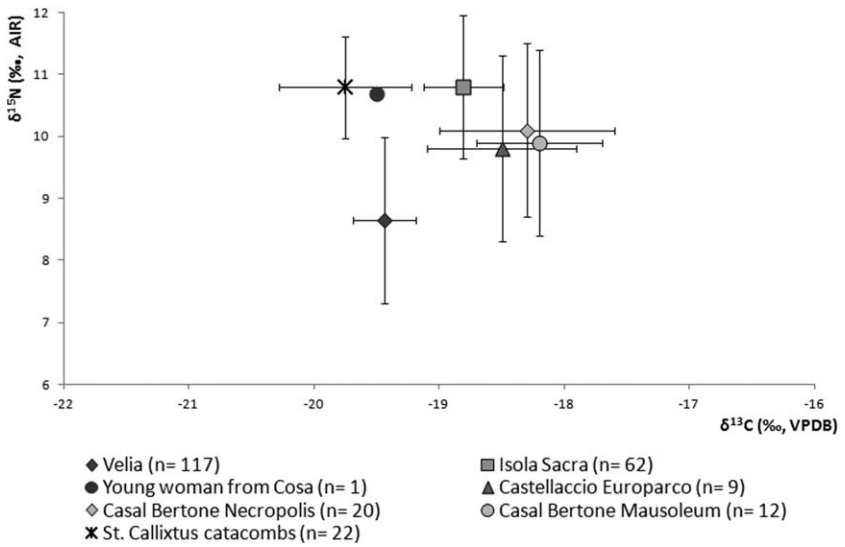

Fig. 4. Plot of $\delta^{13}C$ and $\delta^{15}N$ mean values of published data from other Imperial Roman period populations as compared with those of the young woman from Cosa. The error bars are standard deviations.

catabolization of skeletal muscle tissue, since muscle mass is the body's largest reservoir of nitrogen. The use of body proteins might have produced the trophic level effect, manifesting with high $\delta^{15}N$ values in the tissues (Hobson et al., 1993; Oelbermann and Scheu, 2001; Gaye-Siessegger et al., 2004; Fuller et al., 2005) and could have increased the $\delta^{13}C$ values (Hobson and Clark, 1992; Hobson et al., 1993; Fuller et al., 2004, 2005). Therefore, the high $\delta^{15}N$ values could be explained in two possible ways. First, the large proportion of animal proteins or the contribution of freshwater fish to her diet increased the value of $\delta^{15}N$. Second, malabsorption of nutrients, due to the disease, promoted the consumption of body proteins and changes in physiology. On the basis of the woman's morphological and genetic features (Gasbarrini et al., 2012), the latter explanation seems to be more likely.

CONCLUSION

Using stable isotope analysis we wanted to gain a better understanding of the dietary habits of the ancient Cosa populations, and of a young woman in particular, who showed morphological and genetic features suggestive of chronic malnutrition consistent with celiac disease. Her isotope data suggest that she might have followed a different diet, based mainly on freshwater fish, though cereals were most likely not completely eliminated from her food intake, or that she suffered from malabsorption of nutrients due to her illness. It cannot be excluded that she was unable to absorb dietary nutrients normally and that this nutritional stress could have led to altered biochemical processes, thus altering the isotopic composition of her bone collagen.

Stable carbon and nitrogen isotope analysis can be usefully applied to make inferences about diet-related illnesses and subsistence practices in ancient populations. In order to investigate starvation in archaeological samples, more data on isotopic analysis for diet-related diseases in modern patients are needed to elucidate the effects of malabsorption on stable isotope fractionation in bone collagen. Our results underline the importance of combining stable isotope data with osteological findings so as to avoid erroneous reconstruction of the nutritional habits of ancient humans exposed to nutritional stress or affected by malabsorption.

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