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Subadult Dietary Variation at Trino Vercellese, Italy, and Its Relationship to Adult Diet and Mortality

Laurie J. Reitsema,[1]* Giuseppe Vercellotti,[2] and Rosa Boano[3]

[1]*Department of Anthropology, University of Georgia, Athens, GA 30602*
[2]*Department of Anthropology, The Ohio State University, Columbus, OH 43210*
[3]*Department of Life Sciences and Systems Biology, University of Torino, 10123, Italy*

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ABSTRACT

Objectives: Early-life nutrition is a predisposing factor for later-life outcomes. This study tests the hypothesis that subadults from medieval Trino Vercellese, Italy, who lived to adulthood consumed isotopically different diets compared with subadults who died before reaching adulthood. We have previously used a life history approach, comparing dentine and bone of the same adult individuals ("subadults who lived"), to elucidate dietary variation across the life span. Here, we examine diets of "subadults who died" from the same population, estimated from subadult rib collagen, to explore whether dietary behaviors of subadults who lived differed from those of subadults who died.

Methods: Forty-one subadults aged six months to 14.5 years were studied through stable carbon and nitrogen isotope analysis of collagen.

Results: Individuals were weaned by age 4 years, with considerable variation in weaning ages overall. Post-weaning, diets of subadults who died comprised significantly less animal protein than diets of subadults who lived. Isotopic values of the two oldest individuals, 13.5 and 14.5 years, show the same status-based variation in diet as do adults from the population.

Conclusions: Our results suggest that incorporating animal protein in diet during growth and development supported medieval subadults' ability to survive to adulthood. Isotopic similarities between adults and older subadults suggest "adult" dietary behaviors were adopted in adolescence. Stable isotope evidence from subadults bridges a disparity between ontogenetic age categories and socioculturally meaningful age categories in the past, and sheds light on the underpinnings of health, mortality, growth, and disease in the bioarchaeological record. Am J Phys Anthropol 160:653–664, 2016. © 2016 Wiley Periodicals, Inc.

Early-life nutrition and living conditions are predisposing factors for mortality, longevity, and later-life health outcomes. Individuals who are nutritionally stressed during development experience growth perturbation and may be "primed" for either greater susceptibility to stress, or for thrifty phenotypes and greater resilience to stress later in life (Bogin et al., 2007). Life history theory appreciates adult conditions as possible products of trade-offs made earlier in life (Hill and Kaplan, 1999). In bioarchaeology, a life history approach can be taken at the level of the individual, taking advantage of knowledge of tissue formation times and the etiology of pathological conditions to query living conditions of individuals at life stages preceding death (Bell et al., 2001; Schroeder et al., 2009; Beaumont et al., 2015), or at the level of the population, by considering differences in life experiences of survivor and nonsurvivor cohorts at select age points (Beaumont et al., 2015; Sandberg et al., 2014).

Diet can play both supporting and leading roles in mortality and stress. This study explores whether diet during early life could have been a factor in subadult mortality at medieval Trino Vercellese, Italy (8-13th century AD). In particular, we test the hypothesis that diets of children from Trino Vercellese who lived to adulthood differed from diets of children who died before reaching adulthood. In a previous study, we compared diets of children and adults using a longitudinal, life history approach, comparing dentine and bone of the same individuals ("subadults who lived") to explore variation in diet throughout the life span (Reitsema and Vercellotti, 2012). Here, we take a cross-sectional approach, and examine diets of "subadults who died" from the same population, estimated from subadult rib collagen, to know whether the dietary behaviors of subadults who lived truly differed from those of subadults who died. Beyond the physiological ramifications of early life nutrition, age-related variations in diets are telling of social relationships and cultural norms of behavior, and we explore dietary variations of subadults of different ages up to 14.5 years to better understand the significance of life history stages in past populations.

The carbon and nitrogen used to build the collagen of human teeth and bones chiefly are derived from the pools of carbon and nitrogen atoms in dietary protein (DeNiro and Epstein, 1978, 1981; Schoeninger and

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*Correspondence to: Laurie J. Reitsema, Department of Anthropology, University of Georgia, 250 Baldwin Hall, Jackson St., Athens, GA, 30602, USA. E-mail: reitsema@uga.edu

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Moore, 1992; Ambrose and Norr, 1993; Tieszen and Fagre, 1993). Stable carbon and nitrogen isotope ratios of some food categories vary systematically – for example, the stable carbon isotope ratios ($\delta^{13}C$) of plants using differing photosynthetic pathways (C_3 versus C_4 plants) (Vogel and van der Merwe, 1977; van der Merwe and Vogel, 1978; O'Leary, 1981), or of fish from aquatic environments with different carbon sources (Katzenberg and Weber, 1999), and the stable nitrogen isotope ratios ($\delta^{15}N$) of foods from different trophic levels in a food web (Minagawa and Wada 1984; Schoeninger and DeNiro, 1984; Schoeninger, 1985). As a result, it is possible for bioarchaeologists to back-track from a consumer's tissue stable isotope signatures to the stable isotope signatures of its foods.

Bioarchaeologists capitalize on the different turnover rates of various skeletal tissues and the different representation of macronutrients in these tissues to create a longitudinal record of diet and diet change in an individual's lifetime (Sealy et al., 1995; Bell et al., 2001; Reitsema and Vercellotti, 2012). Teeth form once, in youth, and, excepting secondary and tertiary dentine, do not remodel, but bones remodel continually throughout the lifetime (Hedges et al., 2007; Tsutaya and Yoneda, 2013). As a result of such differences in modeling and remodeling rates, teeth record a snapshot of early life diet at discrete time periods depending on age of tooth formation, whereas bones are continually updated in their isotopic record, and represent a period of several years, as in the case of still-growing bones, to more than 30 years preceding death, in the case of individuals whose bone moderling and remodeling has slowed (Libby et al., 1964; Stenhouse and Baxter, 1979; Hedges et al., 2007; Tsutaya and Yoneda, 2013). Therefore, a comparison of teeth and bones from an adult individual may reveal a change in diet has occurred during the life span (Sealy et al., 1995; Lamb et al., 2014).

Isotopically, breast milk is unlike other foods consumed by members of the same population, because it derives from the highest trophic position of that population's food web – the members themselves. Both the $\delta^{13}C$ and the $\delta^{15}N$ ratios of a mother's breast milk are different from the ratios of her own diet (Fuller, 2003). Weaning therefore may be monitored using an isotopic approach (Fogel et al., 1989), which has opened up a wide range of hypothesis-testing regarding human biocultural adaptation and demography (Katzenberg et al., 1996; Schurr, 1997, 1998; Wright and Schwarcz, 1998; Giuffra and Fornaciari, 2013; Sandberg et al., 2014; Beaumont et al., 2015).

Trino Vercellese is a medieval and postmedieval site complex located in northern Italy consisting of the Church of San Michele and its associated cemetery and fortified settlement (Fig. 1) (Negro Ponzi Mancini, 1999). Excavations by the University of Torino in the 1980s recovered a total of 749 individuals from inside the church and in the surrounding cemetery, including 585 adults and 103 subadults, which have been the subjects of previous bioarchaeological examination (Masali et al., 1995; Celoria, 1999; Girotti and Doro-Garetto, 1999; Porro et al., 1999; Vercellotti et al., 2011; Reitsema and Vercellotti, 2012). Most of the burials ($n = 688$), including those that are the subjects of this study, date to the 8-13th century, whereas fewer are postmedieval. Individuals buried within the church have been interpreted to represent elite members of the community, whereas individuals interred in the cemetery are believed to be lower-status individuals (for a detailed review of this evidence, see Vercellotti et al., 2011).

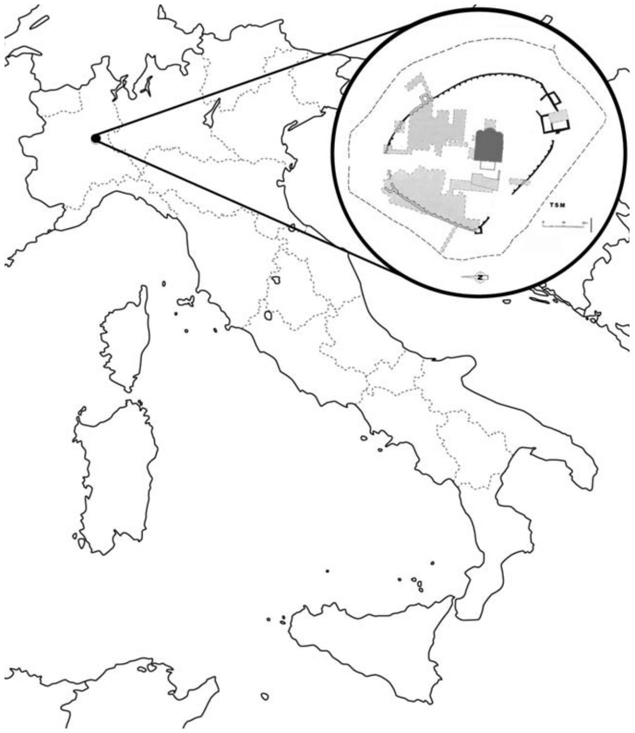

Fig. 1. Map showing the location of Trino Vercellese (black solid circle) and the planimetry of the site (modified from Negro Ponzi Mancini, 1999). In the site plan, the dark gray area represents the Church of San Michele, the light gray area represents the cemetery outside the church, and the black line indicates the fortification walls.

Zooarchaeological, paleobotanical, and stable isotope ratio evidence from Trino Vercellese point to a diet based on cultivated cereals, including the C_4 plant millet (*Setaria italica* and *Panicum miliaceum*) (Reitsema and Vercellotti, 2012), legumes, and aromatic plants (Accorsi et al., 1999), livestock including swine, cattle, sheep/goats, and domestic fowl (Ferro, 1999), wild ungulates, including red deer (*Cervus elaphus*) and roe deer (*Capreouls capreouls*), and, to a lesser extent, fish. This diet appears to have accompanied relatively good living conditions for the population, as indicated by low prevalence of skeletal stress indicators (Harris lines, metabolic disorders, nonspecific periostitis, linear enamel hypoplasia) (Celoria, 1999; Girotti and Doro-Garetto, 1999) and only moderate growth disruptions (Porro et al., 1999; Vercellotti et al., 2011) among the adult population.

In an earlier study of diet of the medieval population from Trino Vercellese, we compared stable isotope signatures in dentine and bone of adults to interpret diet changes over the life span (Reitsema and Vercellotti, 2012). We concluded that diets of women and children may have been constrained and "buffered," owing to the similarity of their isotopic signatures to those of high-status individuals, and the fact that women's diets (regardless of status) did not change greatly over the life span. Low-status males, on the other hand, experienced a change in diet during life, exhibiting greater variation, somewhat less animal protein consumption, and greater consumption of C_4 plants in adulthood. Low-status males

TABLE 1. Medieval life-history stages, terms, and humoral associations

Life history phase[a] and Latin[a] and Italian terms[b]	Age ranges and landmarks	Humor and associations[a]
Infancy/*infantia*: "*bambini*"	Birth to age 7, permanent teeth	Blood
Childhood/*pueritia:"fanciulli"*	Age 7-14, sexual maturity	Hot and moist Spring
Adolescence/*adolescentia*: "*giovani*" "*giovanetti*"	Age 15-28, Facial hair and skeletal maturity	Red gall
Youth/*iuventus*: "*giovani*"	Age 28–50	Hot and dry Summer
Maturity (in judgment)/*gravitas*	Age 50–70	Black gall Cold and dry Autumn
Extreme old age/*senectus*, terminating in *senium*	Age 70+	Phlegm Cold and moist Winter

[a] Life history phases described in the *Etymologiae Sive Origines* by Isidore of Seville, from Sears (1989: pages 9, 13, 25-28, 61).
[b] Taddei, 2002.

also exhibit greater signs of developmental stress and poorer oral health (Girotti and Doro-Garetto, 1999), and lower body mass (Vercellotti et al., 2011) than the rest of the adult population.

Use of the term buffering presupposes that people enacted a behavior that was beneficial, or perceived to be beneficial in some manner. In the present study, our first objective is to test the hypothesis that the dietary variation of "subadults who lived" (the adult dentine isotopic record) was, in fact, beneficial, in terms of survivorship, in comparison to the diets of "subadults who died" (the subadult bone isotopic record). If the diets of subadults who died exhibit variation outside the range of "buffering" observed in adult dentine, it would lend support to the interpretation that the population did in fact safeguard children's diets (Reitsema and Vercellotti, 2012). This would in turn suggest that the buffering behavior was a successful strategy aimed at improving subadult survival.

We explore the weaning process at Trino Vercellese with bone collagen stable isotope data from infants and young children. Weaning is a risky transition for infants (Lewis, 2007; Horta and Victoria, 2013). Breast milk is gradually removed from diet, and along with it, a reliable source of macronutrients and immunological benefits. Simultaneously, supplemental foods are introduced, exposing the infant to a new pathogen environment. The stressful nature of weaning in the past is attested to by incidence of enamel hypoplasia at times coincident with the introduction of supplemental foods and with the cessation of breastfeeding (Katzenberg et al., 1996). We explore ages of exclusive breastfeeding, introduction of supplemental foods, and cessation of breastfeeding, at Trino Vercellese.

A third goal of this study is to examine post-weaning childhood diet through comparisons of subadult and adult bone isotopic signatures. Did the diets of (weaned) youths at Trino Vercellese (the subadult bone isotopic record) differ from diets of adults (the adult bone isotopic record)? Diet is a mundane, daily element of an individual's existence, and one that may differ across life history stages (Prowse et al., 2005; Nehlich et al., 2011). Measured isotopically, diet reflects sociocultural aspects of how infants, children, and adolescents were treated in past societies, and may be interpreted alongside ontogenetic ages toward characterizing the meanings of life history stages in the past. Based on the portrayals of children in historic iconography, French art historian Philippe Ariès famously opened a discussion in the 1960s concerning children in the medieval period, arguing that until modern times, children were perceived as little more than miniature adults (Ariès, 1962). Critical evaluation of this notion has repeatedly shown that the medieval world did in fact recognize childhood and other life history phases (Sears, 1986; Eisenbichler, 2002). Information recovered historically regarding these phases, including medieval humoral associations, are presented in Table 1. Information about diets of children offers a complementary line of evidence into a discussion of to what extent children were treated merely as miniature adults in pre-modern times, versus treated differently in acknowledgment of childhood as a socially delineated phase (Ariès, 1962; Sofaer Derevenski, 2000; Orme, 2001).

MATERIALS AND METHODS

This study expands on previous bioarchaeological work at Trino Vercellese (Masali et al., 1995; Celoria, 1999; Girotti and Doro-Garetto, 1999; Porro et al., 1999; Vercellotti et al., 2011; Reitsema and Vercellotti, 2012). An earlier study of paleodiet of adult individuals from Trino Vercellese reported isotopic data from rib and second molars (M2) (Reitsema and Vercellotti, 2012). M2 crown dentine forms between approximately age 5.5 and 8.5 years (AlQahtani et al., 2010; Moorrees et al., 1963). Because medieval records indicate a recommended weaning age of two years (Shahar, 1990), the isotopic signature of M2 was expected to reflect postweaning childhood diets at Trino. Used in consultation with historical and archaeobotanical lines of evidence, the stable isotope data suggested a diet comprising chiefly C_3 protein, some C_4 protein (millet) and with perhaps small contributions from freshwater fish (Montanari, 1988; Nisbet, 1999; Reitsema and Vercellotti, 2012). In the present study, ribs of subadults are studied isotopically, and compared to the adult rib and tooth data. Henceforth, the term "subadults" refers to individuals under the age of 18 years. The subadult sample comprises individuals aged six months to 14.5 years, as estimated from dental eruption and indicators from the skeleton (Buikstra and Ubelaker, 1994).

Our first objective is to assess age of weaning through a cross-sectional examination of subadult bone stable isotope values. We do so chiefly to know which subadults to exclude from comparisons between the isotopic values of subadult bone and adult M2 dentine, dentine forming after weaning. Our second objective is to examine

TABLE 2. Results

Sample ID	Age	Status	$\delta^{13}C_{VPDB}$ (‰)	$\delta^{15}N_{Air}$ (‰)	Nitrogen (%)	Carbon (%)	C/N ratio	%Coll
Human subadults								
188-D	0.5	High	−18.3	11.5	11.1	31.6	3.3	8.5
188	0.5	High	−19.0	10.1	12.5	35.9	3.3	4.8
173-C^{a3}	0.8	High	−17.9 ± 0.03	10.8 ± 0.2	7.6 ± 0.3	22.2 ± 0.2	3.4 ± 0.2	10.9
192^{a4}	0.8	High	−16.7 ± 0.0	11.6 ± 0.2	11.1 ± 0.3	32.4 ± 0.8	3.4 ± 0.2	17.5
133	1	High	−18.6	10.0	7.1	20.8	3.4	7.3
188-A	1.5	High	−19.0	8.1	8.3	25.5	3.6	10.5
190	1.5	High	−18.0	8.0	5.3	16.6	3.6	10.4
194	1.5	High	−19.5	9.9	10.8	30.7	3.3	6.9
385	1.5	Low	−17.7	11.8	8.5	24.5	3.4	11.1
446	1.5	Low	−18.7	11.7	9.9	27.9	3.3	13.1
142^{a2}	2	High	−18.5 ± 0.0	11.6 ± 0.2	11.0 ± 0.2	31.6 ± 0.1	3.3 ± 0.0	10.0
168	2	High	−18.4	10.9	6.9	19.2	3.2	10.1
173-A	2	High	−18.6	10.1	10.4	30.7	3.4	5.8
4	2.5	High	−19.5	10.5	12.1	33.9	3.3	6.9
42	2.5	High	−19.7	9.5	8.2	23.2	3.3	8.0
176-1	3	High	−17.1	11.4	9.7	27.1	3.2	6.4
179-B	3	High	−18.8	8.7	11.6	33.4	3.4	9.8
188-B	3	High	−19.3	9.7	7.7	23.1	3.5	6.7
34-A	3	High	−21.0	8.1	7.0	21.7	3.6	7.4
35	3	High	−19.2	11.1	10.4	30.3	3.4	5.7
189a 4^{47}	3.5	High	−17.6 ± 0.0	11.0 ± 0.2	9.4 ± 0.2	27.3 ± 0.1	3.4 ± 0.1	5.7
103	4	High	−20.2	8.6	9.4	27.6	3.4	7.9
288	4	Low	−19.6	7.8	12.3	34.8	3.3	9.3
420	4	Low	−19.3	7.8	9.5	26.0	3.2	9.6
50^{a2}	4	High	−19.7 ± 0.0	8.4 ± 0.1	11.7 ± 0.2	33.3 ± 0.2	3.3 ± 0.0	10.9
161	4.5	High	−19.3	10.3	7.9	22.8	3.4	8.6
S-547	4.5	Low	−20.4	3.7	0.9	1.8	2.5	3.3
155^{a4}	5	High	−19.7 ± 0.4	9.9 ± 0.3	8.7 ± 0.2	25.4 ± 0.8	3.40	7.1
32	5	High	−19.9	8.4	9.9	28.3	3.3	8.5
141	5.5	High	−19.2	7.7	9.8	26.7	3.2	7.7
185	6	High	−18.8	9.2	11.1	30.2	3.2	8.2
211^{a2}	7	Low	−19.7 ± 0.6	9.0 ± 0.1	9.8 ± 0.1	27.5 ± 0.2	3.3 ± 0.0	9.1
239	7	Low	−18.9	8.5	11.0	30.7	3.3	14.7
107	8	High	−19.7	8.3	10.4	29.4	3.3	8.3
11	8	High	−19.0	9.1	9.6	27.8	3.4	9.0
455/459^{a2}	8	Low	−19.6 ± 0.0	8.8 ± 0.2	9.9 ± 0.0	28.1 ± 0.1	3.3 ± 0.1	8.4
90	8	High	−19.8	8.9	10.1	28.4	3.3	6.7
269	9	Low	−20.0	8.4	9.2	27.2	3.4	8.1
47	9	High	−20.6	9.0	11.8	33.8	3.3	6.9
34-B	13.5	High	−20.4	8.8	12.1	34.4	3.3	9.6
364	14.5	Low	−17.6	8.2	13.4	36.9	3.2	18.6
Terrestrial Fauna								
482-A	Artiodactyl (other than pig)		−17.4	4.8	9.7	27.4	3.3	4.5
S-18-C	*Bos*		−19.7	6.4	9.1	26.1	3.3	3.7
S-409-P	*Sus*		−21.0	4.5	10.7	29.3	3.2	5.6
S-343-M	Mammal		−18.1	6.2	15.0	40.3	3.1	8.6
499-M	Mammal		−22.2	6.7	4.6	12.7	3.3	3.6
17-M	Mammal		−17.2	5.2	11.9	31.7	3.1	8.1

[a] Numbers appearing in superscript represent the number of replicates that were run for a sample. Means ± 1 sd are given.

selective mortality and early-life diet, for which we compare subadult bone to adult dentine isotopic values. Our final objective is to characterize age-related variation in post-weaning diet, for which we compare subadult bone and adult dentine isotopic values, to adult bone isotopic values.

Animal bones from Trino Vercellese also are analyzed in the present study. Whenever possible, faunal stable isotope analyses should accompany human paleodiet reconstructions, because faunal stable isotope ratios may vary considerably with different environments, husbandry, and land management practices (e.g., Bogaard et al., 2007; Fraser et al., 2011). For example, $\delta^{15}N$ ranges of 8-9‰ and $\delta^{13}C$ ranges of 4‰ or higher are observed in Hartman et al. (2013), Makarewicz (2014), Makarewicz and Tuross (2012), Reitsema et al. (2013), and Stevens et al. (2013). The zooarchaeological remains available for study from Trino Vercellese were highly fragmented, and only two of the available animal bones could be identified to the genus level (*Sus* and *Bos*). Another animal bone was identified as belonging to an artiodactyl other than *Sus*. Three other animal bones were identified only as mammalian.

Collagen was extracted from ribs of 41 subadults and from six animal bones at the University of Georgia Bioarchaeology and Biochemistry Laboratory using the whole-bone protocol outlined in Reitsema and Vercellotti (2012) (after Richards and Hedges, 1999). Between 0.400 and 0.700 mg of dry, powdered bone collagen for each sample was analyzed on a Costech Elemental Analyzer coupled to a Finnigan MAT Delta Plus XL Isotope Ratio Mass Spectrometer at the University of Georgia Center for Applied Isotope Studies. Eight samples were run in replicate. Results of replicate analyses are shown in

TABLE 3. Summary statistics

	δ^{15}N (‰)			δ^{13}C (‰)		
	Median	Range	*r*	Median	Range	*r*
Subadults ($n = 41$)	9.2	7.5–11.8	−0.514	−19.2	−21.0 to −16.7	−0.545
High-status subadults ($n = 32$)	9.6	7.5–11.6	−0.453	−19.2	−21.0 to −16.7	−0.608
Low-status subadults ($n = 9$)	8.5	7.8–11.8	−0.371	−19.3	−20.0 to −17.6	−0.258
Adult dentine ($n = 30$)	9.4	6.7–12.0		−19.4	−20.1 to −17.6	
Adult bone ($n = 28$)	9.1	8.1–11.8		−19.3	−19.9 to −17.4	

Adult dentine and bone data from Reitsema and Vercellotti (2012). Results of Spearman's correlations between stable isotope ratios and subadult age are indicated with r values.

Table 2. Stable isotope ratios are expressed as a permil (‰) ratio of one of an element's isotopes to another in relation to a standard of known abundance (Vienna Pee Dee Belemnite for δ^{13}C and AIR for δ^{15}N). Both carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope ratios are reported to the nearest 0.1‰ according to the equation [$\delta = (R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}} \times 1{,}000$]. Stable carbon ($\delta^{13}$C = permil deviation of the ratio of ^{13}C:^{12}C relative to the Vienna Peedee Belemnite Limestone standard) and stable nitrogen (δ^{15}N = permil deviation of ^{15}N:^{14}N relative to the ambient inhalable reservoir, AIR) isotope measurements were made where the standard deviation of repeated measurements of the acetanilide standards (n = 8) was 0.04‰ for δ^{13}C and 0.25‰ for δ^{15}N, and of the protein standards ($n = 8$) was 0.27‰ for δ^{15}N and 0.05‰ for δ^{13}C.

Statistical analyses

To evaluate and compare stable isotope signatures among status and age subsamples, we applied the Mann–Whitney or Kruskal–Wallis tests, depending on the number of groups compared, and Spearman's correlations. These nonparametric tests are applicable even when the distribution of the data departs from normality. Statistical analyses were performed with MYSTAT (comparisons) and SPSS Software (correlations). In the following sections, *P*-values are considered statistically significant if less than or equal to 0.05.

RESULTS

Assessment of collagen preservation quality

We evaluate collagen quality by comparing the collagen content in archaeological bone (%coll) and the carbon and nitrogen content in collagen (%C and %N) to previously-reported well-preserved archaeological and modern bone (DeNiro, 1985; Ambrose, 1990; van Klinken, 1999). Collagen content of bone ranged from 3.3%-18.6% (Table 2). For comparison, values of 1% (van Klinken, 1999) or 3.5% (Ambrose, 1990) up to 22% are considered well-preserved. The bone with the lowest %coll value (S-547-R; 3.3%) partially disintegrated during demineralization, which is a sign of poor collagen preservation (Garvie-Lok, 2001). This individual's %C and %N values were unusually low (1.8% and 0.9%, respectively), giving an anomalous C:N ratio of 2.5. These values fall outside the parameters accepted for well-preserved, biogenic collagen (DeNiro, 1985; Ambrose, 1990), and this individual is excluded from statistical analyses and discussion below. The rest of the samples yielded collagen of acceptable quality. From the final sample of 40 subadults' rib collagen, C:N values range from 3.1 to 3.6, %C values range from 16.6% to 36.9%, %N values range from 5.3% to 13.4%, and %coll values range from 4.8% to 18.6%. Animal bones exhibit lower %coll values of 3.6%–8.6% but exhibit acceptable %C values of 12.7%–40.3%, %N values of 4.6%–15.0%, and C:N values of 3.1-3.3. No animal bones are excluded from the subsequent analyses and discussion as their collagen was deemed well-preserved.

Stable isotope results

Results are presented in Table 2, with summary data presented in Table 3. Replicates of the same sample differed, on average, by 0.1‰ for δ^{13}C, 0.1‰ for δ^{15}N, 0.5% for %C and 0.1% for %N. Standard deviations of replicate analyses are shown in Table 2.

DISCUSSION

Reconstructing diet

Animal bones from Trino Vercellese facilitate paleodiet reconstructions by establishing an interpretive baseline for humans. Animals from Trino Vercellese exhibit δ^{13}C values ranging from −22.2‰ to −17.2‰, and δ^{15}N values ranging from 4.5‰ to 6.7‰, which is in-line with the faunal stable isotope values reported for the Bronze Age in Northern Italy used as the baseline in the previous paleodiet study of Trino Vercellese (the site of Moreto: δ^{13}C = −20.1 ± 1.2‰; δ^{15}N = 4.5‰; Olmo di Nogara: δ^{13}C = −16.6‰, δ^{15}N = 6.4 ± 1.0‰) (Tafuri et al., 2009). Faunal δ^{13}C values at Trino Vercellese suggest some animals consumed C_4 plants.

A diet-tissue offset of approximately 3-5‰ is estimated for δ^{15}N values in food webs (Bocherens and Drucker, 2003; Hedges and Reynard, 2007; O'Connell et al., 2012). Adults and older subadults at Trino Vercellese exhibit δ^{15}N values ranging from 6.7‰ to 12.0‰. These human values are approximately one trophic position above local terrestrial herbivore values, with some variation above and below. Freshwater fish consumption may help explain some of the higher adult δ^{15}N values on the order of 11–12‰ reported previously (Reitsema and Vercellotti, 2012). In general, diet at medieval Trino Vercellese comprised C_3/C_4 plants and varying amounts of terrestrial and, to a lesser extent, aquatic animal protein.

A cross-sectional view on weaning from subadult bone values

Before comparisons may be made between childhood diets of survivors and non-survivors, subadults who are still breastfeeding must be excluded from the non-survivor subadult cohort. In this section, we identify which age group bears a weaning signal, so that

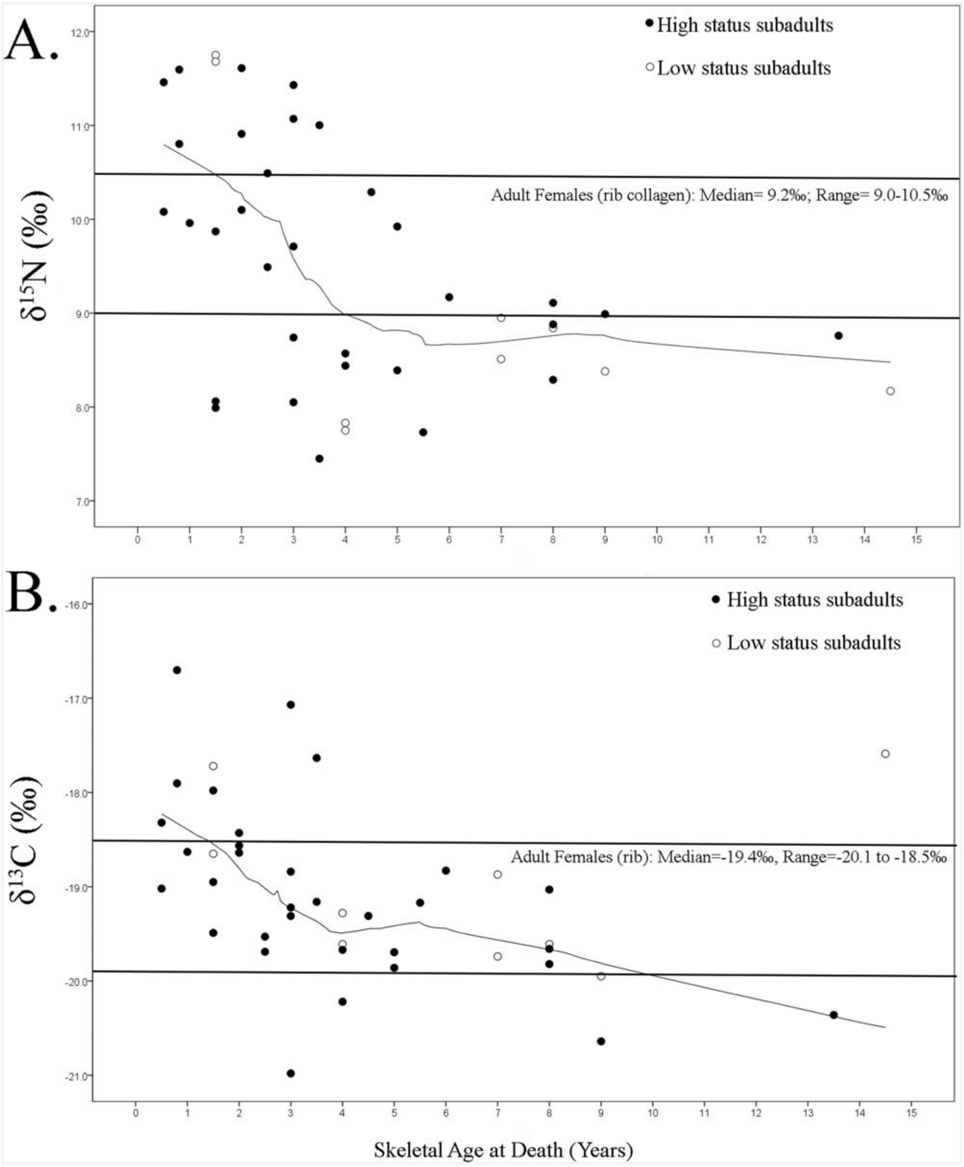

Fig. 2. Stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope data from the cross-sectional study of subadult bone collagen. Horizontal lines represent the range of adult female rib collagen values from the population (Reitsema and Vercellotti, 2012). A *loess* smoothing line is overlaid, after Prowse et al. (2008).

individuals in this group may be excluded from a discussion of selective mortality.

Following Prowse et al. (2008), we plot subadult $\delta^{15}N$ and $\delta^{13}C$ values by age, and overlay a *loess* smoothing line to evaluate the ages at which stable isotope ratios make conspicuous declines (Fig. 2). $\delta^{15}N$ values show a pattern of decline until approximately age 5.5 years, but the steepest decline is between ages two and four years. $\delta^{13}C$ values, which are more sensitive to the introduction of grains, vegetables, and other plant foods (Fuller et al., 2006; Reitsema, 2012), show a steady decline until four years of age, with little variation in slope. After age 5.5 years, a restricted range of childhood foods appears to have been consumed by all subadults. This post-weaning "childhood diet" appears to have been C_3-based and relatively lower in animal protein than the adult diet(s). Considering these patterns, in subsequent discussions, the subadult sample is divided into a breastfeeding/weaning group younger than age four years, and a weaned group ages four years and older.

Both $\delta^{15}N$ and $\delta^{13}C$ values vary considerably. Several individuals up to age 3.5 years exhibit $\delta^{15}N$ values of approximately 11‰ or higher (compared to the adult population mean of $9.2 \pm 0.8‰$; $n = 28$), high enough to suggest breast milk remained part of their diets. Several young individuals (ages 1.5-3.5+) exhibit $\delta^{15}N$ values of approximately 8‰, low enough to suggest breast milk was not a part of their diets. Infants who are not breast-fed do not benefit from immunological boosts and ready access to a reliable nutrient source, and are at heightened risk of physiological stress (Katzenberg et al., 1996). Therefore, some variation in infant feeding may be related to selective mortality. Cultural norms about what is appropriate for subadults may interfere with growth, development, and even survival (Hampshire et al., 2009; Stinson et al., 2012; Bourbou et al., 2013). Human culture, combined

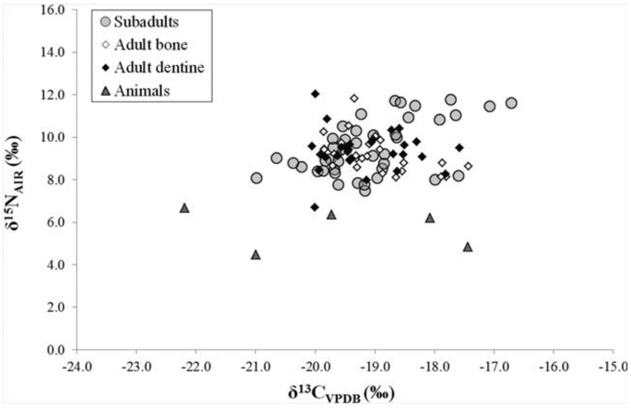

Fig. 3. Stable carbon (δ^{13}C) and (δ^{15}N) isotope data from bone collagen of subadults and fauna from Trino Vercellese (present study) in comparison to previously reported stable isotope data from dentine and bone collagen of adults from the same population (Reitsema and Vercellotti, 2012).

with a prolonged childhood stage during which children no longer are breast feeding, but still depend on others for food (Stinson et al., 2012), come together to make humans uniquely vulnerable to culturally-influenced mis-steps in infant feeding practices. Decisions regarding the appropriate duration of breastfeeding and the types of foods suitable for consumption by infants may have jeopardized infant survival at Trino Vercellese.

Physiological stress may explain isotopic variation among the subadult cohort. Stress can induce a state of negative nitrogen balance during which human tissues become ^{15}N-enriched (Fuller et al., 2005; Reitsema et al., 2013; Beaumont et al., 2015). Conversely, positive nitrogen balance (growth) leads to comparatively lower δ^{15}N ratios in tissues. Previous work has shown how nitrogen balance may affect the δ^{15}N ratios of fetuses and breast feeding infants, in particular (Fuller et al., 2004, 2005, 2006; Beaumont et al., 2015; Reitsema and Muir, 2015). Subadults in particular may be prone to showing isotopic effects of nutritional stress because bone is remodeling more rapidly during these years, unlike bones of adults which are more inert and less susceptible to isotopic variations from stress episodes. It may be the case that infants who appear to be have breaast fed for longer than usual are in fact children of nutritionally stressed mothers or are themselves nutritionally stressed, all of which would result in relatively high δ^{15}N values. However, relatively low prevalence of skeletal stress at Trino Vercellese does not readily indicate a population experiencing stress severe enough to occasion negative nitrogen balance.

Six subadults aged nine months to 3.5 years exhibit δ^{13}C values that are, on average, 2‰ higher than those of adult females from Trino, which is greater than the expected diet-tissue offset of 1‰ for breast feeding infants (Fuller et al., 2006). This may indicate the use of millet-based or marine paps as weanling foods. Marine fish may be unlikely ingredients of weanling foods here, given the inland location of Trino Vercellese and the lack of evidence for marine resource consumption in the adult population, but millet was cultivated in this time and region, and could have been used as a weanling gruel. In a review of weanling foods advised to medieval and Renaissance mothers, only vegetal, C_3 paps and mush appear on the list (Giuffra and Fornaciari, 2013), and millets and other small grains were regarded as less desirable starches in this region of Italy (Nada Patrone, 1981). However, our isotopic evidence suggests millet may have been fed to subadults, despite these stigmas. If millet was provided to subadults, its consumption declines over the course of childhood, from six months onward (Fig. 2B). The higher-than-expected subadult δ^{13}C values also may stem from consumption of protein from animals foddered on C_4 plants. Some animals do exhibit evidence of having eaten C_4 plants (Fig. 3), but the absence of a correlation between the stable carbon and nitrogen values of subadults counterindicates animals as the source of human ^{13}C enrichment.

A survey of isotopic evidence for weaning in agricultural societies indicates breastfeeding rarely persists past age three years (Bourbou et al., 2013; Howcroft, 2013; Fulminante, 2015). Stable isotope evidence of Roman period subadults in Italy (Isola Sacra) indicates infants likely were weaned by 2-2.5 years of age (Prowse et al., 2008), which is supported by evidence from enamel hypoplasia for weaning stress commencing in the first year of life (Barbiera and Dalla-Zuanna, 2009). During the 4th through 9th centuries, the ages of peak enamel hypoplasia affliction are later, at ages 3-5 years, suggesting weaning ages became older through time (Barbiera and Dalla-Zuanna, 2009). Our isotopic case study lends support to a preliminary interpretation that weaning occurred later in life in the medieval period in Italy than in the Roman period. Longer weaning can effect a longer inter-birth interval (Stinson et al., 2012), and indeed, paleodemographic evidence suggests populations experienced slow but steady growth ca. AD 600-1300, compared with more rapid growth in the Roman period, ca. 200-0 BC (Lo Cascio and Malanima, 2005; Barbiera and Dalla-Zuanna, 2009). In general, populations in the medieval period may have been more well-fed and healthier than they were in the Roman period (Montanari, 1988; Steckel, 2004; Koepke and Baten, 2008; Barbiera and Dalla-Zuanna, 2009). Late weaning in the medieval period may help explain the paradox of why apparently healthier living conditions were not accompanied by rapid population gains, but additional evidence is warranted.

Diet and selective mortality: Comparing subadult bone and adult dentine values

A main objective of this study is to assess whether there are differences in the diets of subadults who died, and subadults who lived (i.e., adults, estimated from dentinal isotopic data). If the diets of subadults who died exhibit variation outside the range of "buffering" observed in adult dentine, it would lend support to the interpretation that diet is related to subadult mortality in this population. We restrict this section to discussion of subadults aged four years and older, and adult M2 dentine.

Contra the hypothesis that subadults who died would exhibit greater isotopic variation than "buffered" adults who survived, the isotopic ranges of older subadults are narrower than those of adult dentine (7.7-10.3‰, compared to 6.7-12‰) (Fig. 4). The δ^{15}N values of subadults who died between the ages of four and 14.5 years are significantly lower than the δ^{15}N values of adult M2 dentine, i.e. subadults who lived (Mann-Whitney U: $p = 0.001$). Contrastingly, the δ^{15}N values of adult bone, i.e., subadults who lived, are *not* significantly lower than

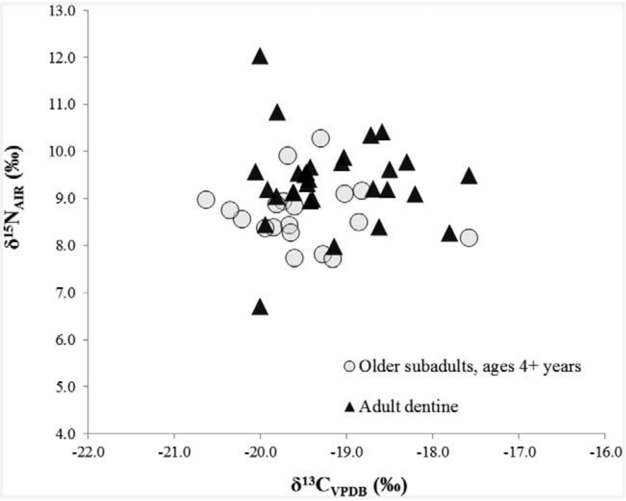

Fig. 4. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope data from bone collagen of post-weaning subadults and second molar dentine from adults from Trino Vercellese.

$\delta^{15}N$ values of dentine from the same individuals ($P = 0.214$) (Fig. 3). Lower $\delta^{15}N$ values of subadults who died while still young suggest consumption of relatively more plant-based protein, and relatively less animal-derived protein (including dairy) compared to subadults who lived to adulthood. The implication of higher $\delta^{15}N$ values among subadults who lived, compared with subadults who died, is that animal protein and/or prolonged nursing predisposes individuals for survival to adulthood. Indeed, breastfeeding and protein in infant and early childhood diet both are implicated in positive health outcomes (for a review from an isotopic perspective, de Luca et al., 2012). Animal-sourced foods have a crucial role for child growth and development because they provide both high-quality protein and several important nutrients, including vitamin A, vitamin B12, folate, iron, and iodine (Black et al., 2008). The consumption of animal-source foods has been shown to be directly associated with linear growth outcomes in human populations in the developing world (Allen et al., 1992; Marquis et al., 1997; Leonard et al., 2000).

It should be noted that approximately half of the adult dentine values (including both sexes and status groups) appear identical to the subadult bone values in terms of the protein composition of their diets (Fig. 4), meaning individuals adopting this type of diet were certainly not condemned to die an early death. Nevertheless, individuals who consumed the types of higher-protein diets indicated by the adult dentine isotope data appear to have been more likely to live to adulthood.

Explaining age-related variation at Trino

The preceding discussion pertains to subadult bone and adult dentine values, addressing the question, did individuals who lived to adulthood consume different diets as subadults compared to individuals who died as subadults? In addition to considering subadult bone data to answer this question about selective mortality, we also consider subadult bone data as a window into age-related dietary variation. The following discussion refers to adult and post-weaning subadult bone values, to compare the diets of children and the diets of adults. The key difference between this and the preceding section is that here, we are considering adult *bone* values, not dentine values, and these bone values reflect diets in adulthood, not adults' diets during their childhoods. Age-related variation in diet has been explored previously only among "survivors," using dentine compared to bone from the same adult individuals (Reitsema and Vercellotti, 2012). The diets of subadults may not represent the "typical" subadult diet of Trino Vercellese (Wood et al., 1992), but they are nevertheless diets of subadults in this population, and we discuss older subadult diets accordingly, based on the present evidence.

Subadults aged 4–14.5 years (present study) and adults who died above the age of 18 years (Reitsema and Vercellotti, 2012) consumed different diets. Lower $\delta^{15}N$ values of subadult bones compared to adult bones (Mann-Whitney U: $P = 0.002$) suggest these subadults consumed less animal protein than did adults. The $\delta^{13}C$ values of these subadult bones also are lower than those of adult bones (Mann-Whitney U: $P = 0.025$), suggesting millet may not have been typical in (older) subadult diets at Trino Vercellese (see above discussion for millet as a possible weanling gruel). However, previously reported, dentine stable isotope values of adults – also indicative of subadult diet – are not significantly different than bone stable isotope values for the same adults (Reitsema and Vercellotti, 2012). That is, subadults who attained adult ages had seemingly similar diets in childhood and in adulthood (excepting low-status males). Thus, we detect at least two dietary regimes for subadults at Trino.

How do we explain the existence of subadult diets that differ from adult patterns? Among other possible explanations, in the medieval period, concepts of humors informed to some extent what someone should eat given his or her age, sex, and individual temperament. The humoral system was widespread in northern Italy during the medieval period and was disseminated and reinforced through the activities of politicians, doctors, and even clergy, via penitentials, thus "extend[ing] well beyond medical texts and/or advice for the wealthy" (Pilsworth, 2011). In general, the humoral system instructed that a person's ideal diet should be well-balanced, but at times should be deliberately skewed in one humoral direction or another to correct episodic imbalances. An imbalance left uncorrected could result in disease. Foods and their associated humoral qualities, along with recommendations for different life history stages, seasons, and times of day, are documented in the *Tacuinum Sanitatis in Medicina*, written by the Iraqi physician Ibn Butlan (d. 1038 AD) and reproduced throughout the medieval period in various manuscripts, some of which are extant. Spencer (1984), for example, provides a useful translation of an extant 14th century Latin copy owned by the Cerruti family of Verona, and includes a list of many of the foods of cultural and economic significance in northern Italy. For example, cold and dry foods on this list include millet, pickled fish, and sorghum; cold and moist foods include prawns, egg whites, and spinach. Hot and moist foods include veal, egg yolks, and fresh figs; hot and dry foods include roast meat, wine, and dried figs.

A simplistic expectation that may be devised based on the humoral system is that cold and dry foods will be greater in diets of subadults, who are hot and moist by humoral nature. Coincidentally, cold and dry foods include three of a few available high-^{13}C resources:

(pickled) fish, millet and sorghum. Rather than subadults exhibiting high-^{13}C rib collagen, in the present study we find evidence that consumption of C_4 protein and fish may have been lower among subadults, compared to adults. It is possible that subadults were given foods that were consistent with, rather than palliative for, their hot and moist humoral nature. It also is possible the humoral system's dietary recommendations were not diligently observed. Alternatively, widespread though it was, the humoral system may not be noticeable in stable isotope signatures not necessarily because the system was ignored, but because it was complex and varied, designed to be flexible and changing in response to the changing disposition of people, and because the ingredients to correct imbalances do not all sort under category divisions of marine, freshwater, C_3 and C_4. Stable isotope signatures in human bone reflect chronic, habitual dietary choices, and it is possible that the humoral system was more varied than what may ever be isotopically detected.

The oldest member of the subadult cohort, T-364, aged 14.5 years, raises questions about age-related variation at Trino Vercellese. This low-status individual, of indeterminate sex, exhibits a δ^{13}C value that is considerably higher than the other post-weaning subadult δ^{13}C values (−17.6‰, versus −19.4 ± 0.8‰ [$n = 40$]), and that is similar to values of low-status males from the adult cohort (−18.4 ± 0.7‰ [$n = 10$]) (Reitsema and Vercellotti, 2012). Diets of low-status adult males at Trino were unusual compared to the rest of the population, in that they comprised more millet, considered a less-desirable grain in parts of northern Italy (Nada Patrone, 1981). We previously have interpreted differences in millet consumption between low-status adult males and the rest of the adult population as representative of broader lifestyle differences affecting food access, perhaps involving low-status males tending to consume foods outside households, and/or laboring away from households. The youngest low-status male in the adult cohort to exhibit this "millet" signature was 20 years old. The new subadult data indicate that this dietary behavior and possible lifestyle difference were shared by individuals younger than 14.5 years of age. The next oldest individual in the Trino subadult sample is a high-status individual aged 13.5 years, who exhibits a low δ^{13}C value of −20.4‰, which plots comfortably in the range of previously-reported high-status adult values. The stark contrast between the two adolescents in the present study, one high-status and one low-status, suggests subadults participating in status-appropriate "adult" behaviors in pubescent years.

A basic six-stage conception of life history stages held during the medieval period mirrored the days of Creation, depicted in Table 1, alongside 15th century Italian terms for several of the stages. This six-stage scheme was espoused ca. AD 600 in *Etymologiae Sive Origines* by Isidore of Seville (Sears, 1986), a book so widely used in medieval education that the number of extant copies today is surpassed only by the number of extant copies of the Bible. Within this scheme, delineation of age points within the medieval view on the life cycle was flexible, but legally and conceptually, age 14 years was a turning point in childhood. At age 14 in Italy, subadults were believed to have lost their innocent nature. Around age 14 years, subadults assumed "the beginning of the intellectual capability of action" (Taddei, 2002). At age 14, subadults lost their juridicial immunity, and were advised by sumptuary law to henceforth employ a different manner of dress (Sebregondi, 2002; Taddei, 2002). In Italy around this time, it was common practice for middle-class parents to send their teenage sons into the work force of a broader world (Eisenbichler, 2002). Although terminology for life history stages is characteristically fluid across cultures and throughout time, in pre-modern Italy, the term *fanciulli* frequently referred to children younger than 14 years, whereas the term *giovani* referred to subadults older than age 14. Age 14 years was thus a relatively firm turning point in the medieval life cycle.

Archaeological perspectives on children have tended to be limited and even reductionist, focusing on children as passive, not active participants in shaping the archaeological record, and perpetuating modern, western-world cultural conceptions of life history stages (Perry, 2005; Sofaer Derevenski, 2000). Stable isotope evidence from individuals at different ontogenetic stages sheds light on the intersection of ontogenetic age and sociocultural conceptions of a person's age. If subadults as young as 13.5 and 14.5 years of age were consuming "adult" diets particular to their status groups, it would suggest these individuals, still quite young by today's standards, embodied certain behaviors shared with adults in their communities.

Isotopic evidence from Trino Vercellese assists interpretations of the existence and significance of a "medieval childhood" (Ariès, 1965; Orme, 2001). Evidence for a medieval childhood comes from the significantly lower δ^{15}N values of subadults over the age of four years compared to the values of adults, which point to a different childhood diet (but see above discussion concerning selective mortality). Evidence that *older* subadults and adults were not perceived as being so very different comes from the values of the two oldest subadults, whose diets are remarkably "adult" in the interpretive context of the overall sample. Extrapolating from dietary behaviors to daily life, subadults around 14 years of age may have been viewed as much the same as adults. This finding warrants additional study of more individuals in this age range to further assess age-related dietary turning points in the medieval life cycle.

CONCLUSION

This study explored the issue of selective mortality at medieval Trino Vercellese, Italy (8-13th century), specifically examining whether individuals who died at young ages consumed different foods in their youth than did subadults who lived. Our comparisons show that subadults who lived consumed diets during youth that were more isotopically varied, but in general were higher in animal protein, than were diets of subadults who died. We interpret the data to mean that diets higher in animal protein predisposed some subadults for a better chance of survival, and interpret subadult-adult isotopic differences as an example of selective mortality. The differences in subadult and adult diets, though linked to selective mortality, nevertheless represent age-related variation in diet. Many individuals consumed lower-protein diets as subadults, including all those reported in the present study and approximately half of those in the previously-reported survivor cohort (Reitsema and Vercellotti, 2012). Other subadults consumed high-protein diets, including approximately half of the previously-reported survivor cohort. The parallel existence and persistence of multiple child feeding practices

– one that seems to have supported survival and one that seems not to have supported survival in about half the cases – underscore the cultural diversity of medieval society, and offer a case-study in the imperfection of cultural adaptation, when viewed from an evolutionary lens.

This study also assessed the weaning process of subadults. As expected, both δ^{15}N and δ^{13}C values are high initially, and decline during the first years of life. Breastfeeding ceased by approximately age four years. Assessing weaning age at Trino is complicated by high variation in stable isotope values of subadults under the age of four years, particularly for δ^{15}N, which is likely to be linked to some subadults' premature mortality. The two oldest individuals of the subadult cohort offer evidence that adolescents consumed foods that were isotopically similar to the foods consumed by adults. Speculatively, any special or different diets that children received at Trino had changed by adolescence (approximately by age 13-14 years) into a more "adult" pattern. Our effort to reconcile a longitudinal and a cross-sectional approach to dietary variation offers answers and raises questions about selective mortality and past food ways.

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