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Life history of the individuals buried in the St. Benedict cemetery (Prague, 15th-18th centuries): Insights from 14C dating and stable isotope (δ¹³C, δ¹⁵N, δ¹⁸O) analysis

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ABSTRACT

Funerary practices and bioarchaeological (sex and age) data suggest that a mortality crisis linked to an epidemic episode occurred during the fifth phase of the St. Benedict cemetery in Prague (Czech Republic). To identify this mass mortality episode, we reconstructed individual life histories (dietary and mobility factors), assessed the population’s biological homogeneity and proposed a new chronology through stable isotope analysis ($\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$) and direct radiocarbon dating. Stable isotope analysis was conducted on the bone and tooth enamel (collagen and carbonate) of 19 individuals from three multiple graves (MG) and 12 individuals from individual graves (IG). The $\delta^{15}N$ values of collagen and the difference between the $\delta^{13}C$ values of collagen and bone carbonate could indicate that the IG individuals had a richer protein diet than the MG individuals or different food resources. The human bone and enamel carbonate and $\delta^{18}O$ values suggest that the majority of individuals from MG and all individuals from IG spent most of their lives outside of the Bohemian region. Variations in $\delta^{18}O$ values also indicate that all individuals experienced residential mobility during their lives. The stable isotope results, biological (age and sex) data and eight $^{14}C$ dates clearly differentiate the MG and IG groups. The present work provides evidence for the reuse of the St. Benedict cemetery to bury soldiers despite the funeral protest ban (1635 AD). The Siege of Prague (1742 AD) by French-Bavarian-Saxon armies is identified as the cause of the St. Benedict mass mortality event.
INTRODUCTION

Until the end of the eighteenth century, mortality crises were recurrent events that instigated important demographic, social, economic and cultural changes in European societies (Beauvalet-Boutouyrie, 2007). According to historical documents, war, famine, infectious disease and natural disasters were typically the main causes of the high mortality rates (Bulst and Delort, 1989; Walter and Schofield, 1991). Research on past mortality crises, particularly those of epidemic natures, is relatively new in the fields of archaeology and physical anthropology and requires elaborate multidisciplinary work (Castex, 2008; Castex et al., 2008).

The extensive St. Benedict cemetery, located in the Old Town district of Prague (Czech Republic), was excavated as part of a rescue archaeology contract in 1971; this excavation yielded 845 skeletons and a large number of artifacts that are mostly associated with human remains (Martinec, 1971). Based on archaeological and architectural evidences, five phases of inhumations dating from the 11th to the 18th centuries were identified by archaeologists (Ječný and Olmerová, 1988; Martinec, 1971). The fifth phase includes 462 individuals (Ječný and Olmerová, 1988; Martinec, 1971) and was the only one that presented multiple graves (MG) (a multiple grave is a single grave containing several individuals buried at the same time; Leclerc and Tarrête, 1988). An initial exhaustive analysis of the human remains from the five phases was undertaken to understand the morphology, demography and pathology of the population living in Prague from the Middle Ages to the early modern period (Hanáková and Stloukal, 1988). This study did not attempt to relate the type of inhumation, individual (IG) versus multiple grave, or the nature of the mortality, attritional versus mass mortality (Hanáková and Stloukal, 1988). A new mortality profile obtained from a study of 120 individuals buried in 20 MG, half of the total number of MG, showed a very selective
composition of the population (Castex et al., 2007). Adolescent and young adult males were clearly over-represented, suggesting that a catastrophic event was the cause of the mortality of the MG individuals. The absence of lethal and traumatic injuries to the bones excluded violent deaths resulting from an act of war, massacre or natural disaster (Castex et al., 2007). A mortality crisis linked to an epidemic disease was thus hypothesized to be the cause of the presence of the MG during the fifth phase of St. Benedict cemetery (Castex et al., 2007). The current chronology based on archaeological findings and the evolution of the buildings, such as the St. Benedict Church dates the fifth phase to between 1635 and 1792 AD. During this period, the Order of Canons Regular of Prémontré (the “Premonstratensian Order”) was the landowner of the site. Historical sources report two episodes of mass mortality, which occurred in 1639 AD (during the Thirty Years War) and in 1742 AD (during the Siege of Prague). There is no indication, however, of the burial locations for these two events, and it is not currently possible to identify which event caused the mortality crisis evidenced by the MG at the St. Benedict cemetery.

Stable isotope analysis conducted on human tissues preserved in archaeological sites can be used to characterize individual life histories with respect to dietary, environmental and geographical differences (Knudson, 2009; Müldner et al., 2011). Investigating the relationship between life history reconstruction and archaeological evidence (bioarchaeological information and funerary practices) can improve our knowledge of the history of the St. Benedict site. The isotope values of carbon and nitrogen (δ^{13}C and δ^{15}N) from human tissues are used to reconstruct diet. The δ^{13}C values differ between plants that use C_3 and C_4 photosynthetic pathways, and between terrestrial and marine resources (Smith and Epstein, 1971). The natural variations of δ^{13}C values among plants and animals are maintained in the tissues of their consumers because carbon is incorporated in the food chain with an isotopic offset at each trophic level. Thus, the δ^{13}C value of a consumer’s collagen (δ^{13}C_{coll}) is
generally enriched by 5‰ relative to consumed plants and by 1‰ relative to consumed animals (DeNiro and Epstein, 1978; van der Merwe and Vogel, 1978; van Klinken et al., 2000). A fractionation between the δ¹³C values of structural carbonate in bone (δ¹³C_{sc(bone)}) or tooth enamel (δ¹³C_{sc(enamel)}) and diet of approximately 12‰ has been determined for pigs and appears to be suitable for generalization to humans (Harrison and Katzenberg, 2003; Passey et al., 2005; Schwarcz, 2006). The δ¹³C_{col} values mainly reflect the carbon in the dietary protein, whereas the δ¹³C_{sc(bone)} or δ¹³C_{sc(enamel)} values represent an average of the carbon inputs from the whole diet i.e. from the primary macronutrients, proteins, carbohydrates and lipids (Ambrose and Norr, 1993; Jim et al., 2004; Tieszen and Fagre, 1993). The difference between δ¹³C_{sc(bone)} and δ¹³C_{col} (Δ¹³C_{sc-col(bone)}) mainly reveals information regarding the δ¹³C values of the protein and non-protein portions in the diet (e.g., Ambrose and Norr, 1993; Tieszen and Fagre, 1993). δ¹⁵N values in bone collagen (δ¹⁵N_{col}) have been used to investigate the importance of marine protein in past human diets (Chisholm et al., 1982; Schoeninger et al., 1983), as well as to infer the trophic position by considering the step-wise enrichment of approximately 3-5‰ between the consumer’s diet and its tissues (Bocherens and Drucker, 2003; DeNiro and Epstein, 1981; Schoeninger and DeNiro, 1984). However, the δ¹⁵N_{col} values can be affected by many factors such as pregnancy (Fuller et al., 2004), breastfeeding (Fuller et al., 2006), growth (Trueman et al., 2005), diseases (Katzenberg and Lovell, 1999), starvation (Mekota et al., 2006), field manuring (Bogaard et al., 2007) and arid environment conditions (Thompson et al., 2005). The geographic origin and potential movements of humans during their lifespans can be inferred from the oxygen isotope analysis (δ¹⁸O) of structural carbonate in bone (δ¹⁸O_{sc(bone)}) and tooth enamel apatite (δ¹⁸O_{sc(enamel)}) (Schwarcz et al., 1991; Sealy et al., 1993; White et al., 1998). Diachronic changes of residence, particularly between the childhood age and the few last years before the death, can be studied through the difference between δ¹⁸O_{sc(bone)} and δ¹⁸O_{sc(enamel)} values (Δ¹⁸O_{sc(bone-enamel)}). Oxygen in the
tissues is in equilibrium with the body’s water oxygen pool, which derives primarily from drinking water, inhaled vapor water and food sources (Longinelli, 1984; Luz et al., 1984). Drinking water stems from surface water (e.g., lakes and rivers) and groundwater (e.g., wells and springs) sources that are fed by precipitation (e.g., rain and snow) and water from the catchment area. The δ^{18}O values of the river waters predominantly reflect the δ^{18}O values of the mean annual recharge and, to a lesser extent, the local precipitation (Bowen et al., 2005; Kendall and Coplen, 2001). The δ^{18}O values of the environmental waters (e.g., surface water, groundwater and precipitation) are linked to climate and physical factors, such as altitude, latitude and distance from the coast (Dansgaard, 1964; Gat, 1996) leading to predictable variations over a continuous landmass.

The present study aims to identify the mass mortality episode that resulted in the death of the MG individuals during the last inhumation phase of the St. Benedict cemetery by comparing the reconstructed life histories of the IG and MG individuals and refining the current dating of the two inhumation types. The homogeneity and identification of local Bohemian individuals and foreigners were examined using stable isotope analyses (i.e., δ^{13}C, δ^{15}N and δ^{18}O) conducted on different tissues (i.e., bone collagen, bone carbonate and enamel carbonate), archaeological data, and direct ^{14}C dating of human bone collagen.

MATERIALS AND METHODS

Sample collection

A total of 31 individuals buried in the fifth occupation phase of the St. Benedict cemetery in Prague were selected. This sample was composed of 12 individuals from IG, 9 individuals from multiple grave 1 (MG1), 9 individuals from multiple grave 2 (MG2), and 1
individual from multiple grave 3 (MG3) (Fig. 1). The skeletons were preserved in their
integrity and bones did not present macroscopic alteration. The individuals buried in IG were
randomly selected, whereas those from MG1, MG2 and MG3 (MG1-2-3) were selected
according to the location and size of the grave. The three MG were located to the northwest of
the St. Benedict Church (Fig. 1). The MG1 and MG2 contained the largest number of
skeletons of all graves in this sector. Individuals were sexed by combining primary and
secondary sexual diagnoses using the pelvic bones (Brůžek, 2002; Brůžek et al., 2005; Murail
et al., 2005) and others bones such as the cranium, mandible and femur (Castex et al., 1993;
Martin, 1914; Murail et al., 1999). Different methods were used for estimating age-at-death
for immature individuals (Moorrees et al., 1963a,b; Birkner, 1980; Scheuer and Black, 2000)
and adults (Owings-Webb and Suchey, 1985; Schmitt, 2002). Our sample consisted of 5
females, 20 males and 6 individuals of unknown sex (Table 1). With the exception of 3
immature individuals, all of the individuals were adults (Table 1). For each individual, we
sampled the permanent second molar (M2) and a piece of the cortical bone from the femur.
This sampling strategy allowed for a diachronic approach given that the M2 forms between 3
and 8 years of age and is not remodeled once it is formed (Moorrees et al., 1963a), whereas
the femur undergoes constant remodeling, with a complete bone replacement time of 10 to 30
years (Hedges et al., 2007; O'Connell et al., 2001). All the skeletons were curated in the
Department of Anthropology at the National Museum in Prague.

No fauna were found at the St. Benedict site; therefore, no direct comparison between
contemporaneous human and animal isotopic values could be performed to estimate the
proportions of the different food resources and the isotopic values of the drinking water.
Moreover, there were no environmental isotopic data available in the literature for the early
modern period of the Czech Republic to provide the local range of isotope values. Three teeth
from cows, two teeth from pigs and one tooth from a goat were selected from the site of the
fortified gate of the Old Town district in Prague (13th-14th centuries) to estimate the local range of environmental $\delta^{18}O$ values.

**Bone and enamel apatite preparations and analysis**

Tooth calculus, cancellous bone and the superficial layer of compact bone were removed using a tungsten carbide dental tool to eliminate the possible presence of adsorbed diagenetic carbonates. Enamel powder was recovered from the crown using a diamond drill bit. For each sample, approximately 10 mg of enamel powder was treated as described by Balasse et al. (2002). This powder was first soaked in 2-3\% NaOCl solution (0.1 ml solution/mg sample) at room temperature for 24 h to oxidize the organic matter and was subsequently rinsed five times with distilled water. The remaining fraction was treated with 0.1 M CH$_3$COOH for 4 h to eliminate exogenous and adsorbed carbonate and was then rinsed five times with distilled water. Next, the enamel powder was either freeze-dried for 90 min at -87°C or oven-dried for 18 h at 65°C. The purification process resulted in an average loss of 30\% of the initial sample weight. The bone samples were ground to a powder using either an electric grinder or an agate mortar and pestle. Chemical bioapatite purification was performed on 15-25 mg of bone powder using the same protocol as were used to purify the tooth enamel, except that the bone powder was treated with 1 M CH$_3$COOH for 1 h to eliminate exogenous adsorbed carbonate. The purification process resulted in an average loss of 78\% of the initial sample weight. Bone and enamel apatite preparations were conducted at the Muséum National d’Histoire Naturelle (MNHN) in Paris. Apatite powder (580-630 μg) was weighed and reacted with 100\% H$_3$PO$_4$ at 70°C in a Kiel IV device interfaced to a Delta V Advantage (Thermo Scientific) isotope ratio mass spectrometer at the Service de Spectrométrie de Masse Isotopique at the MNHN (SSMIM) to determine the $\delta^{13}C$ and $\delta^{18}O$ values. Over the period of
analysis of these carbonate samples, 46 analyses of the SSMIM internal carbonate standard (Marble LM) were analyzed and provided a mean $\delta^{13}$C value of $2.13 \pm 0.02\%$ (theoretical $\delta^{13}$C value normalized to NBS-19 = $2.13\%$) and a mean $\delta^{18}$O value of $-1.84 \pm 0.09\%$ (theoretical $\delta^{18}$O value normalized to NBS-19 = $-1.83\%$). The analytical precision within each run, measured from 6 to 8 analyses of the standard Marble LM, varies from 0.01 to 0.02\% for $\delta^{13}$C and from 0.04 to 0.07\% for $\delta^{18}$O.

Bone collagen extraction and analysis

The cleaned bone was ground to a powder with an electric grinder to obtain a grain size of 0.7 mm. Collagen was extracted following Longin’s (1971) protocol modified by Brown et al. (1988) and Bocherens et al. (1991). Approximately 200 mg of powdered bone was soaked in 20 ml of 1 M HCl solution for 20 min at room temperature for decalcification and then filtered through a MF-Millipore 5 $\mu$m filter. The residue was soaked into 0.125 M NaOH for 20 h to remove potential contamination of fulvic and humic acids. After filtration, it was soaked into 0.01 M HCl (pH = 2) in a sealed tube at 100°C for 17 h for solubilization, filtered again and freeze-dried at -87°C for 24 to 36 h. Bone collagen extraction was conducted at the MNHN. Samples (0.4-0.5 mg) were combusted using an Elemental Analyser Flash 2000 coupled to a Delta V Advantage (Thermo Scientific) isotope ratio mass spectrometer at the SSMIM for $\delta^{13}$C and $\delta^{15}$N analysis. Over the sample run, 8 samples of the IAEA-600 international standard were analyzed and provided a mean $\delta^{13}$C value of $-27.77 \pm 0.34\%$ and a mean $\delta^{18}$O value of $1.00 \pm 0.02\%$.

Diagenetic alteration indicators
Several indicators have been developed to assess potential diagenetic alterations or contaminations of the bone collagen. The atomic C:N ratio (DeNiro, 1985), the collagen yield from the whole bone (wt %) (Ambrose, 1990) and the percent of carbon (%C) and nitrogen (%N) in the extract (Ambrose, 1990) were used to check the state of the collagen preservation. The atomic C:N ratio of unaltered bone collagen ranges from 2.9 and 3.6 (DeNiro, 1985). Fresh bone contains approximately 22 wt % collagen, which consists of 34 to 43% of carbon and 11 to 16% of nitrogen (van Klinken, 1999). The $\delta^{13}C_{\text{col}}$ and $\delta^{15}N_{\text{col}}$ values remain constant until bone collagen yields fall to less than 1% of the total bone mass (Dobberstein et al., 2009). The percent of carbon and nitrogen in unaltered bone collagen should exceed 13% and 4.8%, respectively (Ambrose, 1990). Unlike for bone collagen, there is no well-established indicator to assess the preservation of structural carbonate in bone and enamel apatite (Shin and Hedges, 2012). However, different tests can be performed to detect potential diagenetic effects on structural carbonate isotopic values. First, based on the assumption that bone apatite is more sensitive to recrystallization and highly susceptible to postmortem changes in comparison with enamel apatite a pairwise comparison of $\delta^{13}C_{\text{sc}}$ (bone) and $\delta^{13}C_{\text{sc}}$ (enamel) values and of $\delta^{18}O_{\text{sc}}$ (bone) and $\delta^{18}O_{\text{sc}}$ (enamel) values can be done for detecting a preferential effect on bone structural carbonates. Second, the $\delta^{13}C_{\text{sc}}$ (bone) and $\delta^{18}O_{\text{sc}}$ (bone) values can be compared with the bone collagen yields to assess the influence of collagen loss on structural carbonate.

**Radiocarbon dating**

Eight human bone collagen samples from IG and MG (Table 6) were prepared for AMS $^{14}$C dating at the AMS C-14 Labor Erlangen (Germany) and the Oxford Radiocarbon Accelerator Unit (England) using routine collagen extraction procedures (Bronk Ramsey et
al., 2000; Longin, 1971). Dates were calibrated using the IntCal09 calibration curve and Cal Rev 6.1.0 software (Reimer et al., 2009; Stuiver and Reimer, 1993).

**Statistical analysis**

The statistical R package (version 2.10, R Development Core Team, 2010) and the software Statistica© 7.1 were used for statistical computations and graph generations. We used non-parametric Mann-Whitney U tests to determine whether our groups of IG, MG1, MG2 and MG3 samples came from the same population. Additionally, we used multivariate analysis of variance (MANOVA), decision tree learning and mixed-data factor analysis (MDFA) to test for homogeneity and the distance between the IG, MG1, MG2 and MG3 groups across all of the qualitative and quantitative variables.

**RESULTS**

**Sample preservation**

The bone collagen atomic C:N ratios show low variability, ranging from 3.1 to 3.3 (mean = 3.2 ± 0.1) (Table 2). The carbon and nitrogen concentrations in bone collagen range from 38.5 to 43.2% (mean = 41.3 ± 1.3%) and from 13.7 to 16% (mean = 15.1 ± 0.6%), respectively (Table 2). The bone collagen yields show a large variability, ranging from 4.2 to 22.2 % (mean = 14.1 ± 6.1%) (Table 2). There is a very low correlation (R² = 0.37) and no correlation (R² = 0.1) between collagen yield and the δ¹⁸Osc (bone) and the δ¹³Csc (bone) values, respectively. Pairwise comparisons between the δ¹³Csc (bone) and δ¹³Csc (enamel) values (Fig. 2) as well as between the δ¹⁸Osc (bone) and δ¹⁸Osc (enamel) values do not show any trend of variation.
(Fig. 3). For the whole sample set, bone samples do not present more homogeneous $\delta^{18}O$ and $\delta^{13}C$ values than enamel samples (Figs. 2-3).

**Stable isotope results**

**Human bone collagen**  
The mean bone collagen values for all of the groups combined are $-19.7 \pm 0.5\%$ and $10.7 \pm 1.6\%$ for $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$, respectively (Table 1). The mean $\delta^{13}C_{col}$ values are $-19.6 \pm 0.4\%$ for IG, $-20.0 \pm 0.6\%$ for MG1 and $-19.6 \pm 0.6\%$ for MG2. The $\delta^{13}C_{col}$ value is $-19.7\%$ for MG3 (Fig. 4). Only IG and MG1 are significantly different with regard to the $\delta^{13}C_{col}$ value (Mann-Whitney $= 0.04$) (Table 4). The mean $\delta^{15}N_{col}$ values are $12.2 \pm 0.9\%$ for IG, $10.3 \pm 0.7\%$ for MG1 and $9.2 \pm 1.2\%$ for MG2. The $\delta^{15}N_{col}$ value is $9.3\%$ for MG3 (Fig. 4). All of the groups are significantly different with regard to the $\delta^{15}N_{col}$ value (Mann-Whitney $p < 0.05$) (Table 4). The mean bone collagen values for MG1-2-3 are $-19.8 \pm 0.6\%$ and $9.7 \pm 1.1\%$ for $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$, respectively.

**Human bone apatite**  
The mean bone carbonate values for all of the groups combined are $-12.5 \pm 1.3\%$ and $-4.7 \pm 0.7\%$ for $\delta^{13}C_{sc (bone)}$ and $\delta^{18}O_{sc (bone)}$, respectively (Table 1). The mean $\delta^{13}C_{sc (bone)}$ values are $-13.5 \pm 1.3\%$ for IG, $-12.2 \pm 0.6\%$ for MG1 and $-11.5 \pm 0.9\%$ for MG2. The $\delta^{13}C_{sc (bone)}$ value is $-12.4\%$ for MG3 (Fig. 5). There are significant differences for the $\delta^{13}C_{sc (bone)}$ value between IG and MG1 (Mann-Whitney $= 0.02$) and between IG and MG2 (Mann-Whitney $= 0.00$), but there is no difference between MG1 and MG2 (Table 4). The mean $\delta^{18}O_{sc (bone)}$ values are $-4.2 \pm 0.7\%$ for IG, $-5.0 \pm 0.5\%$ for MG1 and $-4.8 \pm 0.5\%$ for MG2 (Fig. 5). The $\delta^{18}O_{sc (bone)}$ value is $-6.0\%$ for MG3. Only IG and MG1 are significantly different with regard to the $\delta^{18}O_{sc (bone)}$ value (Mann-Whitney $=$
0.00) (Table 4). The mean bone carbonate values for MG1-2-3 are -11.9 ± 0.8‰ and -5.0 ± 0.5‰ for δ¹³C (bone) and δ¹⁸O (bone), respectively.

**Human enamel apatite** The mean enamel carbonate values for all of the groups combined are -13.2 ± 0.7‰ and -4.8 ± 0.9‰ for δ¹³C (enamel) and δ¹⁸O (enamel), respectively (Table 1). The mean δ¹³C (enamel) values are -13.6 ± 0.4‰ for IG, -12.9 ± 0.6‰ for MG1 and -12.8 ± 0.9‰ for MG2. The δ¹³C (enamel) value is -12.9‰ for MG3 (Fig. 6). There are significant differences for the δ¹³C (enamel) value between IG and MG1 (Mann-Whitney = 0.01) and between IG and MG2 (Mann-Whitney = 0.01), but there is no difference between MG1 and MG2 (Table 4). The mean δ¹⁸O (enamel) values are -4.0 ± 0.5‰ for IG, -4.9 ± 0.7‰ for MG1 and -5.5 ± 0.7‰ for MG2 (Fig. 6). The δ¹⁸O (enamel) value is -5.8‰ for MG3. There are significant differences for the δ¹⁸O (enamel) value between IG and MG1 (Mann-Whitney = 0.01) and between IG and MG2 (Mann-Whitney = 0.00), but there is no difference between MG1 and MG2 (Table 4). The mean enamel carbonate values for MG1-2-3 are -12.9 ± 0.7‰ and -5.3 ± 0.7‰ for δ¹³C (enamel) and δ¹⁸O (enamel), respectively.

**Animal enamel apatite** The δ¹⁸O (enamel) values range from -10.3 to -7.3‰. The mean δ¹⁸O (enamel) value is -8.3 ± 1.1‰ (Table 3).

**Comparison between tissues** The mean difference between δ¹³C values measured in bone carbonate and collagen (Δ¹³C (bone-collagen)) is 6.1 ± 1.5‰ and 7.9 ± 0.9‰ for IG and MG1-2-3, respectively (Table 1). The mean difference between δ¹³C values measured in bone and enamel carbonate (Δ¹³C (bone-enamel)) is 0.1 ± 1.5‰ and 1.0 ± 1.0‰ for IG and MG1-2-3, respectively (Table 1). The mean difference between δ¹⁸O values measured in bone and
enamel carbonate ($\Delta^{18}$O_{sc (bone-enamel)}) is -0.2 ± 0.9‰ and the 0.3 ± 0.6‰ for IG and MG1-2-3, respectively (Table 1).

**Statistical comparisons**

MG1, MG2 and MG3 are not significantly different from each other according to the isotopic dataset (MANOVAs, p > 0.05). Therefore, they form a single group (MG1-2-3). MG1-2-3 is significantly different from IG (MANOVA, p < 0.05). There are no significant differences between the three MG with respect to age and sex (MANOVAs, p < 0.05). However, MG1-2-3 is distinct from IG with respect to age (younger or older than 30 years) and sex (MANOVAs, p > 0.05) (Table 5). The decision tree learning analysis highlights the separation between MG1-2-3 and IG, as well as the homogeneity within each group regarding the isotopic variables. The MDFA performed on the isotopic and bioarchaeological (sex and age) data confirms the distinction between MG1-2-3 and IG (Fig. 7). The first three components explain 71.4% of the variance, and the most significant and correlated variables are the $\delta^{18}$O_{sc (bone)}, $\delta^{13}$C_{sc (enamel)}, $\delta^{18}$O_{sc (enamel)} and $\delta^{15}$Ncol values and sex.

**$^{14}$C dates**

Calibrated intervals at 2σ (95.4% confidence) fall in the ranges of 1476-1662, 1030-1208 and 1449-1635 cal AD for H36, H193 and H270, respectively, who were buried in the three IG. The intervals are 1685-1954, 1659-1952 and 1683-1954 cal AD for H60, H83 and H797, respectively, from MG1 and 1668-1953 for H75 from MG2 (Table 6). In addition, although the individual H421 from a different IG provides a calibrated radiocarbon date of 1486-1950 with a confidence interval of 95.4% (Table 6), 94% of this probability distribution ranges between 1486 and 1669 cal AD.

**DISCUSSION**
Sample diagenesis

All the bone collagen indicators used to assess the influence of the diagenesis on the stable isotope values show that the bone collagen samples from the St. Benedict cemetery are well preserved. Regarding structural carbonate that is considered as highly susceptible to diagenetic alteration, the lack of correlation between the $\delta^{13}C_{sc\ (bone)}$ or $\delta^{18}O_{sc\ (bone)}$ values and the collagen yield indicates that collagen loss has no influence on its isotopic values. An important isotopic exchange between human remains and diagenetic fluids from the burial environment is expected to erase part the natural isotopic inter-individual variability (related to difference in life histories and age) by impressing the values of the diagenetic pole (Lecuyer et al., 2003; Amiot et al., 2004). Because bone is less resistant to diagenesis than enamel, the biogenic signal of bone structural carbonate is more prone to be replaced by that of the diagenetic pole. In fact, the inter-individual variability of structural carbonate values is similar to that of enamel and there not tendency in the isotopic difference in $\delta^{13}C$ and $\delta^{18}O$ between the two tissues. Although it is not possible to totally exclude postmortem changes, these results indicate that $\delta^{13}C_{sc}$ and $\delta^{18}O_{sc}$ values of bone and enamel were not significantly affected by diagenesis and can be used for reconstructing life histories of the St. Benedict cemetery individuals.

Life history of the St. Benedict population

The $\delta^{13}C$ values for collagen and carbonate, as well as the $\delta^{15}N$ values for collagen are used to provide insight into human diet. All the individuals from the St. Benedict cemetery have relatively low $\delta^{13}C_{col}$ values, which is typical of European diets based mostly on C3 plants (e.g., cereals, fruits and vegetables). The relatively high $\Delta^{13}C_{sc-col\ (bone)}$ values (> 7‰)
exhibited by the individuals from MG1-2-3 indicate that the protein source is $^{13}$C-depleted compared to the whole diet (Kellner and Schoeninger, 2007), suggesting a diet predominantly composed by a mixture of $C_3$ and $C_4$ energies (i.e. non-protein components of diet) as well as $C_3$ protein (Fig. 8). In contrast, the relatively low $\Delta^{13}$C$_{sc-col}$ (bone) values (< 7‰) exhibited by the IG individuals indicate that the protein component of their diet is more $^{13}$C-enriched than the whole diet (Kellner and Schoeninger, 2007), suggesting a diet comprising mainly $C_3$ energy and $C_3$ protein (Fig. 8). There is a significant negative relationship ($p < 0.01$) between $\Delta^{13}$C$_{sc-col}$ (bone) and $\delta^{15}$N$_{col}$ values, such that the MG group has more depleted $\delta^{15}$N$_{col}$ values and more enriched $\Delta^{13}$C$_{sc-col}$ (bone) values than the IG group (Fig. 8). Despite this difference, most IG and MG individuals present relatively high $\delta^{15}$N$_{col}$ values. High $\delta^{15}$N$_{col}$ values can be explained by either a high reliance on animal proteins or the consumption in smaller quantities of proteins with relatively high $\delta^{15}$N values. Unweaned terrestrial herbivores (Hedges and Reynard, 2007), herbivores feeding on $C_3$ plants grown on soils fertilized by manure (Boogard et al., 2007; Stevens et al., 2012) and aquatic resources (Vika and Theodoropoulou, 2012) exhibit high $\delta^{15}$N values. Although both marine and freshwater resources show high $\delta^{15}$N values, freshwater resources have usually lower $\delta^{13}$C values (Dufour et al., 1999; France, 1995). Because the IG individuals do not present high $\delta^{13}$C values, the preferential consumption of freshwater resources is more likely (Fig. 8).

Alternatively, relatively high $\delta^{15}$N$_{col}$ values of the IG individuals might result from a nutritional or physiological stress (Fuller et al., 2005). However, a short-term dietary change caused by an episode of starvation is unlikely to be identified in the isotopic values of adult cortical bone collagen (Beaumont et al., 2013). Bone undergoes constant turnover and its isotope composition reflect average diet over approximately a decade (Hedges et al., 2007). Only regular dietary stress due to chronic illness may modify significantly the $\delta^{15}$N$_{col}$ values (Katzenberg and Lovell, 1999). IG were randomly selected in the St. Benedict cemetery and it
is unlikely that they all shared the same chronic illness. In addition, there was no evidence of pathological lesions on the studied skeletons. Low protein intakes over life could explain the lowest $\delta^{15}N_{col}$ values exhibited by MG individuals. Nevertheless, a rich protein diet based on immature herbivores, herbivores consuming manured crops or freshwater resources is suggested for most individuals from the St. Benedict cemetery.

Comparison of the bone and enamel carbonate $\delta^{18}O$ values and $\delta^{18}O$ values from the environmental water sources was used to study the residential mobility across the lifespan and to identify local and foreign-born individuals (Keenleyside et al., 2011). The $\delta^{18}O_{bc}$ (bone) and $\delta^{18}O_{en}$ (enamel) values are relatively homogeneous within each grave type. IG individuals have higher $\delta^{18}O_{bc}$ (bone) and $\delta^{18}O_{en}$ (enamel) values than MG1-2-3 individuals (Fig. 9).

$\Delta^{18}O_{bc}$ (bone-enamel) absolute values range from 0 to 1.5‰. Large intra-individual variation in $\delta^{18}O$ values for most individuals from IG and MG1-2-3 indicate that individuals ingested water from different geographical sources throughout their lives, or more precisely between the childhood age and the few last years before the death, and suggest diachronic changes of residence. The $\delta^{18}O$ values of drinking water ($\delta^{18}O_{dw}$) were estimated and compared to the local environmental $\delta^{18}O$ range for the Bohemian region. We used the equation of Chenery et al. (2012) to derive the $\delta^{18}O_{dw}$ values from the $\delta^{18}O$ values in bone and enamel carbonate. The $\delta^{18}O_{dw}$ values derived from the enamel carbonate range from -7.8 to -5.1‰ (mean = -6.1 ± 0.8‰) for IG and from -10.0‰ to -5.9‰ (mean = -8.1 ± 1.2‰) for MG1-2-3. The $\delta^{18}O_{dw}$ values from the bone carbonate range from -7.7 to -4.7‰ (mean = -6.3 ± 1.1‰) for IG and from -9.3 to -6.0‰ (mean = -7.6 ± 0.9‰) for MG1-2-3 (Table 7). Although the spatial distribution of the $\delta^{18}O$ values in meteoric water ($\delta^{18}O_{mw}$), groundwater ($\delta^{18}O_{gw}$) and surface waters is well-known for Western and Southern Europe (Longinelli and Selmo, 2003; West et al., 2010), no regional-scale distribution or isopleths of $\delta^{18}O_{mw}$ and $\delta^{18}O_{gw}$ are available for Central Europe. However, the $\delta^{18}O_{gw}$ values near Prague can be estimated from a compilation
of different works (Jiráková et al., 2010; Möller et al., 1998; Noseck et al., 2009; Pačes and Šmejkal, 2004; Šilar and Šilar, 1995), and the δ¹⁸Oₘₚ values can be estimated using the high-resolution global and regional mapping model OIPC (http://www.waterisotopes.org; Bowen, 2011; Bowen and Wilkinson, 2002). According to these data sources, the local δ¹⁸Oₕ values range from -11.2‰ to -8.6‰ with a mean value of -9.8 ± 0.6‰ and an annual mean δ¹⁸Oₘₚ value of -8.9‰ ± 0.4‰. This range of values falls into the range of European regions, such as Bohemia (between -12‰ and -7‰), Saxony (between -10‰ and -8‰) and France (between -13‰ and -4‰) (Fig. 9). Additionally, we used the equation developed by Amiot et al. (2004) (for all mammals excepted marsupials) and the δ¹⁸Oₑ values of the six animals from the fortified gate of the Old Town district in Prague to define the environmental δ¹⁸O values of the Prague region (Table 3). The δ¹⁸Oₑ values range from -10.3 to -8.0‰ (mean = -9.1 ± 1.1‰) for cattle and from -7.7 to -7.3‰ (mean = -7.5 ± 0.3‰) for pigs whereas the δ¹⁸Oₑ value of goat is -7.7‰. The faunal δ¹⁸Oₑ values are consistent with the defined local range of environmental δ¹⁸O values (Fig. 9). With the exception of H62 and H84, all of the individuals from MG1-2-3 have δ¹⁸Oₑ values that fall within the range of local δ¹⁸O values, whereas IG individuals have higher δ¹⁸Oₑ values than the local range. Although it is not possible to determine precisely their countries of origin because these regions are not discriminated by their δ¹⁸O environmental values, the individuals from IG can be identified as foreigners and may be native to Western Europe or the Mediterranean coast (http://www.waterisotopes.org; Bowen, 2011; Bowen and Revenaugh, 2003) (Fig. 9). Finally, the range of Δ¹⁸Oₑ (bone-enamel) values (from -1.5 to 1.3‰ for IG and from -0.8 to 1.5‰ for MG1-2-3) for IG and MG1-2-3 individuals indicates that these individuals moved across different regions within the Western and Mediterranean part of Europe before they died in Prague.

Chronology of inhumation
Radiocarbon dating of human bone collagen indicates that IG individuals date from 1030 to 1669 AD, whereas MG1-2-3 individuals date from 1659 to 1954 AD. There is no overlap between the age ranges of MG1-2-3 (H60, H75, H83 and H797) and the age ranges of IG (H36, H193 and H270). We note that H193 predates H36, H270 and H421 and is most likely from an older occupation phase. Moreover, we know that the St. Benedict site was desecrated and demolished in 1792 (Ardura, 1995). There is a small overlap of approximately 10 years between H421 (IG) and H83 (MG1). These data suggest two distinct inhumation phases for IG (15th-17th centuries AD) and MG1-2-3 (17th-18th centuries AD).

**History of the fifth occupation phase of St. Benedict cemetery**

Stable isotope analysis ($\delta^{13}$C, $\delta^{15}$N, $\delta^{18}$O) and $^{14}$C dating challenge previous knowledge regarding the history of the last phase of use of the St. Benedict cemetery. According to historical sources, St. Benedict was used as a cemetery until the early 17th century (Ardura, 1995). Premonstratensian chronicles and parish registers make no mention of inhumations in the cemetery after the settlement of the Premonstratensian canons at the St. Benedict site and the funeral protest ban in 1635 (DJ IV 1, 1637-1736; DJ IV 2, 1736-1785). However, the radiocarbon dates of MG1-2-3 contradict the historical data and suggest that the St. Benedict cemetery was briefly reused between the 17th and 18th centuries. Several buttons from clothing were found with the MG1-2-3 skeletons (Libor Jun, unpublished data), and they were identified as belonging to military uniforms, most likely dating from the 17th and 18th centuries (Bleckwenn, 1984; Karger, 1998). The presence of soldiers in St. Benedict cemetery is further supported by the selective composition of the MG population, as it was composed of young adult males (Castex et al., 2007). $^{14}$C dates support the hypothesis that an episode of
mass mortality was caused by the siege of Prague. The siege of Prague occurred during the
War of the Austrian Succession when the French-Bavarian-Saxon armies took control of
Prague between 1741 and 1742. The population suffered considerable stress (Anonymous,
1863; Major, 1904; Vlnas, 2002). The Habsburg forces besieged the city and placed an
embargo on foodstuffs and fodders (Macek, 1984). The lack of essential nutrients and medical
care, as well as poor hygienic conditions, was the breeding ground for the development of
infectious diseases and epidemics. The Premonstratensian College, which is named the
Norbertinum, was converted into a military infirmary to take care of dying soldiers (DJ IV 1,
1637-1736; DJ IV 2, 1736-1785). Given the large number of soldier bodies found in MG, we
suggest that they were buried in the St. Benedict cemetery in spite of the funeral protest ban
of 1635. Isotopic data support the various origins of the soldiers as belonging to different
army corps from France and Saxony.

Our results suggest residential mobility within the European region for all of the
individuals, even the IG individuals who were hypothesized to be of local origin. Prague was
a cultural and commercial hub in Central Europe during the 15th and 16th centuries
(Theinhardt et al., 2005). According to parish registers, people migrated to Prague for
personal and professional reasons (Fialová, 2006a; Grulich, 2007). The Old Town district of
Prague accommodated a large number of foreign merchants, particularly in the complex of
buildings surrounding the courtyard called the Ungelt, which is located approximately 1 km
from St. Benedict. The Ungelt served as a meeting and storage place for traders from Europe
and Asia (Bělina et al., 1995; Fialová, 2006b). Moreover, houses in the Old Town district of
Prague were owned by important political and religious figures from Czech and other
European regions, who came to the King’s court for different missions with their escorts and
staff. When deaths occurred, the bodies were generally buried in the closest neighborhood
cemetery (Bělina et al., 1995). Therefore, it is conceivable that the individuals from the IG
who were identified as foreign to the Bohemian region could have been merchants or
members of the aristocracy originating from Western or Southern Europe. Unfortunately, due
to the overlap of environmental isotopic values from dietary and water sources, it is not
possible to identify more precisely their places of origin within Europe.

CONCLUSION

The combination of stable isotope analysis, biological data, new $^{14}$C dates and
archaeological evidence provides new insight into the role of the St. Benedict cemetery during
the fifth occupation phase. Stable isotope analysis reveals differences in diet and drinking
water between skeletons buried in MG and IG. Isotopic data also suggest that the majority of
the individuals may have spent most of their lives outside of the Bohemian region. The
heterogeneity of the St. Benedict population and the distinction between the two grave types
are supported by radiocarbon dating and archaeological materials, such as the presence of
military buttons. The skeletons found in MG were buried despite the ban on funerals in the St.
Benedict cemetery in 1635. The Siege of Prague by the French-Bavarian-Saxon armies (1742)
may have caused an epidemic that resulted in the mass mortality of young soldiers.
Consequently, this mortality may have generated simultaneous burials in the St. Benedict
cemetery that is adjacent to the Norbertinum. This paper highlights the importance of stable
isotope analysis in early modern studies, as it provides additional independent information to
that of historical written sources.

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