



RESEARCH ARTICLE

Depositing skeletal remains in Czech and Moravian ossuaries and associated climatic variations

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Abstract

Samples of the bones of 47 individuals from 46 Czech and Moravian ossuaries were dated by the ¹⁴C method and analyzed for the collagen isotopic composition of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$). Most of the data for the ages of the remains corresponded to the cooler and damper periods described over the past 1000 years. Of the studied samples, the greatest number of remains corresponded to the Spörer (1400–1570), Dalton (1790–1830) and Wolf minima (1280–1350). One sample studied falls within the Maunder minimum (1645–1715). It can be assumed that these minima are connected with a reduced production of food and fodder, that may have initiated famines, epidemics and armed conflicts. Individual climatic minima showed positive correlations between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, indicating that the individuals studied consumed complementary plant or animal diets to different degrees. The elevated $\delta^{15}\text{N}$ values in our studied samples compared to the skeletal compositions of the population of the La Tène period (380–150 BC) and Germanic inhabitants in the territory of Bohemia (5th–6th centuries AD) and Great Moravia (9th–early 10th centuries AD) might reflect the effect of greater consumption of animal proteins or the proteins of omnivorous animals and fish, which compensated for the lack of plant foodstuffs during the colder periods.

The isotopic composition of carbon and nitrogen of the bone collagen for the Spörer and Dalton minima differs from the Wolf minimum. The younger minima show higher $\delta^{15}\text{N}$ values for a given $\delta^{13}\text{C}$ value.

Introduction

Cool and warm climatic fluctuations occurred in the period between the turn of the 1st millennium and the present time, in relation to solar activity, global mechanics, greenhouse gas levels or volcanic phenomena (e.g., Miller et al. 2012). These changes have been documented, e.g., by fluctuations in the ¹⁴C content of the atmosphere (Stuiver et al. 1998), the calculated solar flux (Schmidt et al. 2011), or proxy reconstruction and modelling of temperature trends (e.g., Jones et al. 1998). The following important climatic fluctuations have been recorded in this period (event – beginning – end): Oort minimum (1010–1050), Medieval maximum (temperature fluctuation 1100–1250), Wolf minimum (1280–1350), Spörer minimum (1400–1570), Maunder minimum (1645–1715), and also the Dalton minimum (1790–1830) and the Modern maximum (1950–2009) (e.g. Camenisch et al. 2016; Degroot 2018; Eddy 1976a; Usoskin et al. 2015). The period encompassing the last three cold fluctuations, i.e., 14–18th centuries, has been termed the Little Ice Age (LIA). However, the periods of the individual climatic minima can be defined in terms of various ranges. For example, the Spörer minimum has been defined as the period 1400–1510 (Eddy 1976b; Jiang and Xu 1986), 1420–1570 (Kappas 2009), or 1460–1550 (Eddy 1976a). Similarly, it can be assumed that the boundaries of the other minima



exhibited variations because, until the 18th century, the determined solar activity could have been accompanied by a substantial error (Vaquero et al. 2011).

Cool fluctuations are also demonstrated by other climate proxies (e.g., thermophilic algal shells, $\delta^{18}\text{O}$ speleothem, charcoal abundance in sediments, or the abundance of selected pollen (Degroot et al. 2021; Izdebski et al. 2022; Zonneveld et al. 2024). Cool fluctuations with social responses are also described in the 1st millennium AD (Zonneveld et al. 2024). This suggests a repetition of cold periods in both millennia and thus and the repetition of the climate thousand-year cycle, evidenced using different methods. Since we were also looking for support in dietary habits to repeat the climatic thousand-year cycle, we chose to compare the diet of the Lombards in the cold period of the 6th century AD and the diet of the population in La Tène (4th–1st BC) in the cold period of the 1st millennium BC, when it turned out that cereals of the C_3 photosynthetic cycle (wheat) were replaced by cereals of the C_4 photosynthetic cycle (millet).

Cold and damp fluctuations result in the reduction of agricultural production, which leads to a lack of food for humans and, especially, the impossibility of harvesting ripe and dry grain. The situation is similar with fodder for cattle with the impossibility of planting, drying and subsequently storing these products. Abnormal dampness complicates the extraction of salt as an essential conservation agent. This all leads to stagnation of economic growth, which is subsequently manifested in epidemics, famines, armed conflicts and a substantial reduction in the population. At the same time, it should be noted that pandemic-related climate fluctuations may have had devastating effects in some areas but may have been avoided in other areas (Izdebski et al. 2022).

Ossuaries are a priceless source of information for anthropological, bioarchaeological and paleopathological studies of skeletal material (e.g., Drozdová et al. 2018; Kostova et al. 2020; Matiegka 1896). If the remains are stored, they can preserve information contained in the contents of ^{14}C and $\delta^{13}\text{C}$, in the elemental composition or in other isotope systems for centuries. The material was usually stored in ossuaries after being removed from overcrowded graveyards after they are modified for other uses.

Together with other parameters (the trace element composition, the ratio of Sr isotopes), the ratio of the stable isotopes of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) are important proxy parameters for understanding historical human foodstuffs and determining the social status of individuals (Ambrose and DeNiro 1986; Bird et al. 2022; Le Huray and Schutkowski 2005; Schoeninger et al. 1983). The consumption of continental foodstuffs is manifested in the isotope composition of carbon ($\delta^{13}\text{C}$), and especially the various consumptions of C_3 and C_4 plants, which differ in their photosynthetic pathways and discrimination of ^{13}C during its fixation from CO_2 (Kortschak et al. 1965; Smith and Epstein 1971). There is discrimination in the isotopes of nitrogen between the heavier isotope (^{15}N) by 2–5‰ $\delta^{15}\text{N}$ between the individual levels of the trophic pyramid (e.g. Schoeninger and DeNiro 1984) and the dietary composition of the isotope composition of nitrogen, which is reflected especially in the ratio of the consumption of plant and animal proteins and, potentially, proteins from aquatic sources (e.g. Lee-Thorp 2008). However, the isotopic composition of dietary nitrogen can be influenced by the composition of crop derived proteins from cereals grown on fertilized areas. Manure application significantly increases $\delta^{15}\text{N}$ in cereal grains and this trend has been demonstrated since the Neolithic period (Bogaard et al. 2007, 2013; Dreslerová et al. 2021). Experiments show that in intensively fertilized fields, $\delta^{15}\text{N}$ in cereal grains can increase by up to 10‰ (Bogaard et al. 2007; Kanstrup et al. 2011). In paleodietary reconstructions, this should therefore be considered as important as animal protein consumption (Bogaard et al. 2013).

This work was carried out to determine the age of skeletal remains in selected Czech and Moravian ossuaries and discover how they are connected with climatic phenomena, epidemics, viral and bacterial infections and, potentially, armed conflicts in this area. In particular, we want to know, if the age of the studied bone samples does not lie in one of the periods whose climate was unfavorable for agriculture in the temperate zone due to temperature and precipitation.

Simultaneously, we studied the isotope composition of bone collagen ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) to determine the effect of climatic variations on the availability of plant and animal foods consumed by individuals

and compared their food with the populations living in the territory of Bohemia and Moravia. Alternatively, we wanted to determine whether the isotopic composition of nitrogen and carbon, i.e., the composition of the diet, is dependent on the period of death of a given individual. At the same time, it should be taken into account that there may have been warm spells during the individual minima, or that temperature minima may have occurred only in some areas and not in others. Alternatively, climatic events may have influenced activities quite different from those related to food production.

Material and methods

Bone samples (a total of 47 samples, Table 1, Figure 1) were collected in 2018–2019. The samples were preferentially taken from damaged skulls with healed or unhealed injuries (36 samples) and also from a tibia (5 samples), a finger (1 sample), a rib (1 sample), a pelvis (1 sample) and a femur (1 sample) and unidentified samples (2). The samples were collected at random in relation to the availability and undamaged sets in which they were deposited in the ossuaries. Thus, the collected samples need not represent skeletons of predominant ages or the period when the ossuaries were used to a greater degree.

The sex was estimated for the samples on the basis of the prominence of the superciliary arches and the temporal bones (Stloukal et al. 1999). The ages of the individuals were estimated according to the closing of the cranial sutures (Linc 1971; Stloukal et al. 1999).

¹⁴C dating of samples

The ages (¹⁴C) of the skeletal samples were determined in the Poznan Radiocarbon Laboratory by the AMS method. The ages were determined from the collagen separated from the individual samples in the same laboratory, according to the methodology originally described by Longin (1971) and adapted from Piotrowska and Goslar (2002). The samples were crushed to the <0.3 mm fraction. Subsequently, the bones were extracted in 0.5 M HCl for ca. 15 min, and the suspension was washed with water. After separation of the solution, the suspension was washed with 0.1 M NaOH. The solid phase was repeatedly washed with distilled water and then infused with HCl (pH = 3) at 80°C for ca. 12 hours. The obtained solution was separated from the residual solid phase by filtration, which was dried; the obtained collagen was transferred to a suitable glass vessel.

The ¹⁴C activity was measured by oxidizing to CO₂ in quartz ampoules with CuO and an Ag wool, the obtained CO₂ was reduced with hydrogen and Fe (as a catalyst) over graphite. The formed mixture of graphite and Fe was prepared for measurements on a special target holder in an Ar protective atmosphere (Czernik and Goslar 2001).

The actual activity of ¹⁴C was determined using a Compact Carbon AMS spectrometer (National Electrostatics Corporation, USA). The measurement was performed by comparing the intensities of the ionic beams of ¹⁴C, ¹³C and ¹²C measured for each sample and for standard samples (modern standard: “Oxalic Acid II” and standard of ¹⁴C-free carbon: “background”). In each AMS run, 30–33 samples of unknown age were measured, alternating with measurements of 3–4 samples of the modern standard and 1–2 background samples. When organic samples are dated, the background is represented by coal, while, for carbonate samples, the background is represented by the IAEA C1 sample (Goslar et al. 2004).

The ¹⁴C age and radiocarbon curve were calibrated using IntCal20 (Reimer et al. 2020). The results were presented at a probability level of 95.4%. All ages reported hereafter are calibrated years of Anno Domini (AD).

Determination of δ¹³C and δ¹⁵N in collagen samples

The elementary and stable isotopic compositions of nitrogen and carbon in the collagen samples were determined using a Thermo Flash 2000 elemental analyser connected to a Thermo Delta V Advantage

Table 1. Locations, sample type, sex, estimated age of the individual, ^{14}C age, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and assignment to the climatic minimum

Sample name	Sample type	Sex	Age (years)	Cal age (AD)**	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	Climatic minimum	
Zruč No. 2	Skull sample	Unknown	20–40	1033–1179	8.60	–18.05	Oort	1010–1050
Žehuň 1	Skull sample	Male	20–40	1263–1310	11.00	–19.04	Wolf	1280–1350
Žehuň 8 TL	Skull sample	Male	Unknown	1298–1371	10.73	–19.07	Wolf	1280–1350
Kašperské hory	Skull sample	Unknown		1295–1404	10.71	–19.65	Wolf	1280–1350
Broumov	Skull sample	Male	20–40	1341–1396	11.55	–19.30	Wolf	1280–1350
Nížkov	Finger phalanx	Unknown	?	1307–1363	8.69	–19.36	Wolf	1280–1350
Nížkov II TYF	Tibial fragment	Unknown		1295–1404	10.59	–19.36	Wolf	1280–1350
Sedlec No. 1	Skull sample	Male	40–60	1297–1406	12.85	–19.09	Wolf	1280–1350
Skřípěl No. 1	Skull sample	Male	40–60	1276–1321	11.13	–19.64	Wolf	1280–1350
Kotouň No. LB17	Skull sample	Male	60+	1276–1321	9.01	–19.77	Wolf	1280–1350
Malín 1P1 TYF	Tibia	Unknown	?	1300–1372	10.45	–19.63	Wolf	1280–1350
Žehuň 10	Skull sample	Male	20–40	1404–1450	12.20	–19.34	Sporer	1400–1570
Kolín	Skull sample	Unknown	?	1408–1454	12.47	–19.29	Sporer	1400–1570
Losenice	Bone-left pillar	Unknown	?	1433–1522			Sporer	1400–1570
Mikulov	Skull sample	Male	20–30	1465–1638	13.32	–19.39	Sporer	1400–1570
Budyně 16	Skull sample	Male	40–50	1470–1640	12.27	–19.29	Sporer	1400–1570
Budyně 170	Skull sample	Male	20–30	1470–1640	12.84	–19.42	Sporer	1400–1570
Hrádek	Ribs	Unknown	?	1483–1646	13.78	–19.35	Sporer	1400–1570
Mouřenec	Femur	Unknown	?	1484–1648	10.81	–20.15	Sporer	1400–1570
Letařovice	Tibia	Unknown	?	1489–1604	9.77	–20.14	Sporer	1400–1570
Vamberk No. 8	Skull sample	Male	?	1489–1604	11.39	–19.75	Sporer	1400–1570
Smečno No. 1	Skull sample	Male	40–60	1395–1447	11.24	–19.21	Sporer	1400–1570
Kouřim No. 1	Skull sample	Male	60+	1419–1495	11.41	–19.32	Sporer	1400–1570
Křtěnov No. 1	Skull sample	Female	15–20	1490–1637	9.51	–20.34	Sporer	1400–1570
Řesanice No.1	Skull sample	Male	20–40	1450–1526	11.79	–19.85	Sporer	1400–1570
Kotouň PA TYF	Tibia	Unknown	20–25	1505–1596	12.21	–19.66	Sporer	1400–1570
Mnichov No. 1	Skull sample	Male	60+	1490–1646	11.70	–19.49	Sporer	1400–1570
Mnichov mummy	Skull sample	Child	1 or 2 years	1499–1600	15.31	–18.88	Sporer	1400–1570
Broumov II, No. 13	Skull sample	Male	20–40	1388–1434	11.87	–19.54	Sporer	1400–1570

(Continued)

Table 1. (Continued)

Sample name	Sample type	Sex	Age (years)	Cal age (AD)**	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	Climatic minimum	
Žehun3	Skull sample	Male	20–30	1721–1818	11.95	–19.34	Dalton	1790–1830
Náklo	Pelvis	Female	?	1726–1814	12.59	–19.35	Dalton	1790–1830
Třebívlice	Skull sample	Male	20–30	1726–1815	11.23	–19.85	Dalton	1790–1830
Zdislavice	Skull sample	Unknown	?	1720–1819	12.00	–19.70	Dalton	1790–1830
Lukavice	Tibia			1807–1928	9.71	–20.21	Dalton	1790–1830
Plumlov No. 2	Skull sample	Male	50–60	1810–1924	11.67	–19.94	Dalton	1790–1830
Páleček No. 2	Skull sample	Male	60+	1800–1941	12.42	–19.21	Dalton	1790–1830
Páleč No. 1	Skull sample	Male	50–60	1800–1941	12.65	–19.27	Dalton	1790–1830
Velíš No. 3	Skull sample	Male	40–60	1810–1919	11.38	–20.29	Dalton	1790–1830
Bohuslavice No. 1	Skull sample	Male	20–40	1799–1942	11.52	–20.57	Dalton	1790–1830
Červený Kostelec No. 3	Skull sample	Male	20–40	1801–1938	11.14	–20.34	Dalton	1790–1830
Lidéřovice No. 3	Skull sample	Male	60+	1799–1942	11.01	–19.35	Dalton	1790–1830
Korouhev	Unknown			1671–1779	10.42	–19.66	Maunder	1645–1715
Mělník	Skull sample			1717–1785	11.85	–19.64	Outside clim. min.	
Třešť	Skull sample	Male	30–40	1717–1784	10.59	–19.44	Outside clim. min.	
Putim	Skull sample	Unknown	?	1717–1782	10.70	–19.76	Outside clim. min.	
Kostelec	Skull sample	Male	20–25	1219–1284	11.30	–18.76	Outside clim. min.	
Chlum sv. Máří No. 1	Skull sample	Male	20–40	1222–1285	8.62	–20.06	Outside clim. min.	



Figure 1. Map of sampled Bohemian and Moravian ossuaries.

(Thermoscientific, Germany) isotope ratio mass spectrometer in a Continuous Flow IV system in the isotopic laboratory of Institute of Geochemistry, Mineralogy and Mineral Resources, Charles University. Samples wrapped in tin capsules were combusted and the released gases (CO_2 , N_2) split in a GC column were transferred to the MS source through a capillary. The isotope ratios are reported as delta (δ) values and expressed relative to VPDB for $\delta^{13}\text{C}$ and to atmospheric nitrogen for $\delta^{15}\text{N}$. The raw $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were normalized to the scale using a multiple-point linear regression based on certified international reference materials IAEA-CH-6, IAEA-CH-3 and IAEA-600 (International Atomic Energy Agency, Vienna) for carbon and IAEA-N-2, IAEA-N-1 and IAEA-NO-3 (International Atomic Energy Agency, Vienna) for nitrogen, run during the same sequence. The analytical precision, expressed as the long reproducibility for the homogenous standards, was within $\pm 0.2\text{‰}$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Results and discussion

The collected samples were taken primarily from injured and unhealed skulls (36 samples), where only 3 samples (Budyně 16,170 and Broumov II-13, Table 1) of injured skulls exhibited subsequent healing; samples were also taken from a tibia, rib, pelvis, fingers and femurs.

Table S1 gives an estimate of the number of skeletons (individuals) in the individual ossuaries. The largest sampled ossuaries were those in Sedlec (50,000 individuals), Kolín, Mělník and Mikulov, which contain the remains of approximately 10,000 individuals. In contrast, the smallest collections were those in Kostelec nad Ohří (50–100 individuals) and Budyně nad Ohří (160 individuals), Mnichov (50–100 individuals) and Liděřovice (50–100 individuals). The radiometric ages of most of the samples lie in the period between 1219 and 1928. Figure 2 shows the individual samples and their radiometric ages (before present (BP) and ages (AD) based on the calibration model (Reimer et al 2020). From the age values, the definition of the individual minima and the calibration curve showing the plateau or decline in the atmospheric ^{14}C value, it can be seen that most of the samples correspond to the fundamental

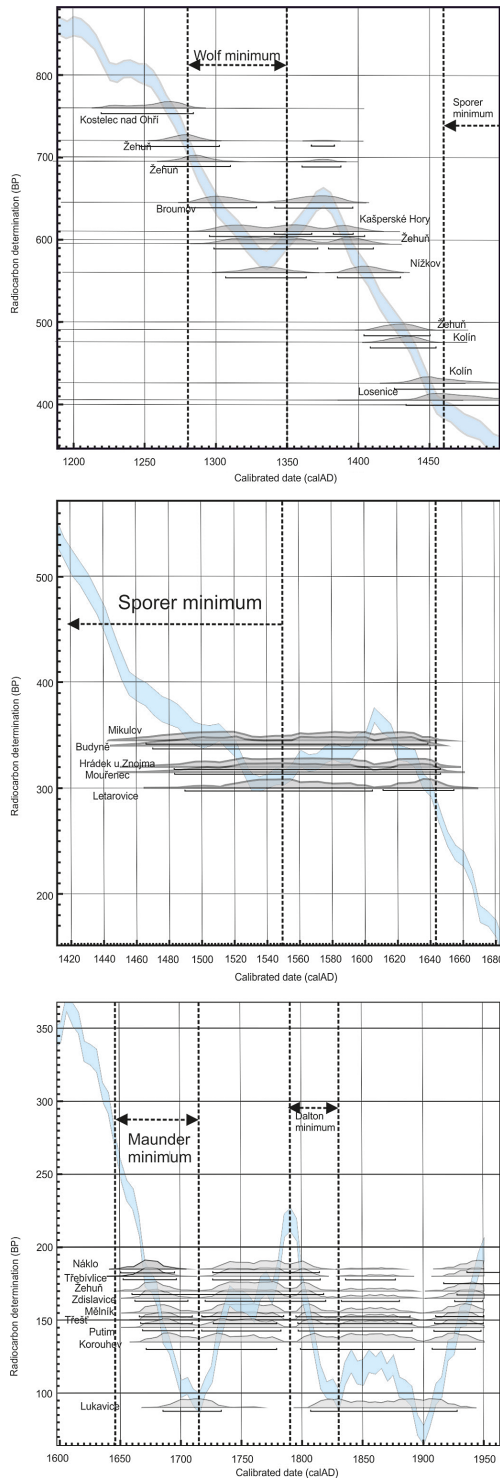


Figure 2. ^{14}C dating of deposited skeletal material from ossuaries in the Czech Republic.

climatic minima that have been observed in the northern hemisphere, i.e., the Wolf, Spörer, Maunder and Dalton minima.

The minimum and maximum ages of some samples approach the interval of the temperature minima, but do not exactly correspond to their spans. These applies to the sample from a skull from Mělník (1717–1785), the sample from a skull from Třešť (1717–1784) and the sample from a skull from Putim (1717–1782). The lower limits of the ages of these samples approach the end of the Maunder minimum (1645–1717) and the upper limits approach the beginning of the Dalton minimum (1790–1830). Because the minima themselves have variously defined spans (e.g., Eddy 1976a, 1976b; Jiang and Xu 1986; Kappas 2009), it can be assumed that the duration of the effect of these fluctuations lasted beyond their determined intervals.

Bones dated to the time of the Wolf minimum (1280–1350) were identified in the ossuaries in Kostelec nad Ohří (skull), Žehuň (skull), Kašperské hory (skull), Broumov (skull) and Nížkov (finger bone), Sedlec (skull), Skřípel (skull) Kotouň (skull), Malín (tibia) and Chlum sv. Máří (skull) (Figure 2). Epidemics occurring in the Wolf minimum are documented in the Zbraslav Chronical (Durynský and Žitavský, 1305–1339), but their etiology is not known. A plague epidemic appeared, originally in isolated patches, in the period from 1250 to the beginning of the 14th century; after 1312, a related plague epidemic appeared in Germany and subsequently, in 1318, in Bohemia (Kahudová 1967). The large scale “plague epidemics” on a large scale were caused by a compromised immunity of the whole population, which occurred in connection with famines. Famines are described in the Chronicle of Cosmas to the beginning of the 12th century, and in the Zbraslav Chronicle (Durynský and Žitavský, 1305–1339) almost until the middle of the 14th century.

The infection associated with famines was paleopathologically identified through the signatures of typhoid lesions on tibial planes as typhoid fever (*Salmonella typhi*) and was documented in the ossuaries in Nížkov, Malín, Sedlec and Kotouň. In all of them, dating pointed to the year 1345 ± 30 (Smrčka et al. 2020)

The bubonic plague (Yersin plague) had the greatest intensity in Europe in 1348–1351; accompanying phenomena such as high prices and hunger were recorded, not only in Bohemia, but also in neighbouring countries (Kahudová 1967). The bacteria *Yersinia pestis* caused pandemics in waves that returned in cycles until the beginning of the 18th century (Rascovan et al. 2019; Rasmussen et al. 2015). The end of the Wolf Minimum is linked to the Black Death pandemic, which killed tens of millions of people in Europe and Asia (Aberth 2021). However, this epidemic had significant regional variations, while in some areas we observe a decline in agriculture, in others an increase in agricultural production is documented (Izdebski et al. 2022).

The cold fluctuation designated as the Spörer minimum (1400–1570) witnessed increased deposition of skeletal material in Czech and Moravian ossuaries in Kolín, Velká Losenice, Mikulov, Budyně, Hrádek u Znojma, Annín, Letařovice, Vamberk, Smečno, Kouřim, Křtěnov, Řesanice, Kotouň and Mnichov (near Karlovy Vary) (in 14 ossuaries a total of 18 dated ¹⁴C samples, Figure 2).

The coldest decade of the Spörer minimum is defined as the period after 1440 (Camenisch et al. 2016). Plant production decreased in a number of European countries as a result of long winters and frequent precipitation. This resulted in a sharp increase in prices, stagnation of trade and an increase in the number of armed conflicts (Camenisch et al. 2016). It is apparent that more people died during the Spörer minimum than in the previous century. It was similarly cold in the 16th century when poor harvests and hunger reduced the resistance of the population to infection. In this period, plague was apparently a factor in Bohemia in the years: 1502, 1505, 1507, 1520–1521, 1530–1531, 1542, 1551–1553, 1553–1555, 1557–1558, 1561–1563, 1564, 1566–1569, 1571–1573, 1580, 1581–1582, 1584–1585, 1592 and 1597–1599. In 1582, 30,000 people died in Prague, corresponding to half of the inhabitants, while 20,000 people died in the other Czech cities (Chlumská et al. 1965; Fialová et al. 1998). Most influenza epidemics were followed by bacterial plague epidemics, mostly following influenza (1561 and 1584). These influenza epidemics had common symptoms—fever, stomach pains, diarrhea and nasal bleeding (Short 1890). Major eruptions of Hekla (1510) and Etna (1510 and 1580) also occurred in this period (Sigurdsson et al. 1999).

An increased amount of skeletal material was also deposited in nine Czech and Moravian ossuaries in the Maunder minimum (1645–1715) (Figure 2). There is no doubt that a substantial portion of the increase in remains from this period occurred as a result of armed conflicts associated with the Thirty-Year War. However, this fluctuation also occurred during the coldest century of LIA (17th century) and, simultaneously, the greatest number of cold decades in the northern hemisphere occurred in this century: 1691–1700, 1601–1610, 1641–1650 (Jones et al. 1998). Consequently, there was an increase in the number of bacterial infections, especially plague, and also famines (1677, 1684, 1692–1696). For example, the famine of 1692–1696 in Bohemia was less drastic than that in France, where it grew to terrifying proportions and led to social unrest (Fialová et al. 1998). The famine was exacerbated by a locust invasion in 1693 (Mašková-Janotová and Tošnerová 2017). This period witnessed one of the largest eruptions of Etna in 1669 (Sigurdsson et al. 1999). Two influenza epidemics also occurred in the 17th century, in April of 1658 (Willis 1890) and in the autumn of 1675 (Sydenham 1890). A new kind of fever affecting the brain and nerves appeared in the summer of 1658 (Willis 1890). Another major eruption of Etna occurred in 1675 (Sigurdsson et al. 1999), followed by widespread bacterial dysentery infections (1677). Further influenza epidemics occurred in 1688 and 1693 (Molyneaux 1890). Plague epidemics occurred again in Bohemia in 1602, 1604, 1606–1607 to 1613, 1616, 1620, 1622–1623, 1632, 1655, 1665 and 1679–1680 and affected 70% of children under 15 years of age. Some were of only local extent, such as that in 1665, which occurred at the same time as the well-known London plague, the last one in Western Europe. However, a demographic crisis resulted from the epidemic in 1679–1680, when 12 thousand people died in Prague, i.e., one third of the inhabitants of Prague (Černý 2014; Chlumská et al. 1965; Fialová et al. 1998).

Ages corresponding to the Dalton minimum (1790–1830) were found for the skull samples from Třebívlice, Žehuň, Zdislavice, Plumlov, Páleček, Páleč, Velíš, Bohuslavice, Červený Kostelec, Liděřovice, the pelvis sample from the ossuary in Náklo and the tibia sample from the ossuary in Lukavice (Figure 2). The Dalton minimum is fundamentally similar to the Maunder minimum. Nonetheless, solar activity was not as weak during the Dalton minimum and the climate was affected more by volcanic activity (Wagner and Zorita 2005). The war of the Bavarian succession between Prussia and Austria took place in Bohemia at this time (Stellner 1998). In particular, the north-western front of this conflict with a fortified line between Ústí nad Labem and Milešovka Mountain could have affected the skeletal remains in the ossuary in Třebívlice. The other ossuaries cannot be associated with this conflict. Several influenza epidemics also occurred in the 19th century (in the spring of 1803, in June 1831, in April 1833, in January 1837, in October 1847 and from December (of 1888) to April of 1889). The influenza epidemics were followed by bacterial infections (dysentery in 1831 and cholera in 1837). The joint symptoms of the influenza epidemics in the 19th century were diarrhea, headache, nasal bleeding, loss of taste, pressure on the sternum, pneumonia or dangerous bronchial inflammation and erysipelas (Falconer 1890).

Climatic fluctuations play a much more important role in the history of civilization than was thought in the past (Zhang et al. 2007). Especially cooling can lead to collapse of up to 80% of the agroecosystem (Zhang et al. 2011), which consequently loses the ability to feed the human population. During cold and wet periods, preindustrial civilization was not capable of dealing with problems connected with a shorter vegetation period, failure to ripen and impossibility of harvesting grain and drying feedstuffs and difficulty in obtaining salt as a conservation agent by evaporating sea water (Galloway 1986; Lucas 1930; Zhang et al. 2006). Cold and wet periods establish conditions for and initiate famines, epidemics, migration, armed conflicts and morbidity (e.g., Grolle 1997).

For the 1500–1800 AD period, Zhang et al. (2011) compared the temperatures in the northern hemisphere determined from tree ring widths and correlated them with events in society. Mild phase 1 (1500–1559), Cold phase (1560–1660) and Mild phase 2 (1664–1800) were identified in this period, with temperature fluctuations by 0.43σ , -0.59σ and 0.24σ , respectively. In these periods, they studied the ratio of grain yields to seed, the price of grain, magnitude of agricultural production, wage index, human height, number of conflicts and the number of epidemics. During the cold phase, all the indicators of growth and wellbeing decreased (the production of grain decreased by 28% and the price

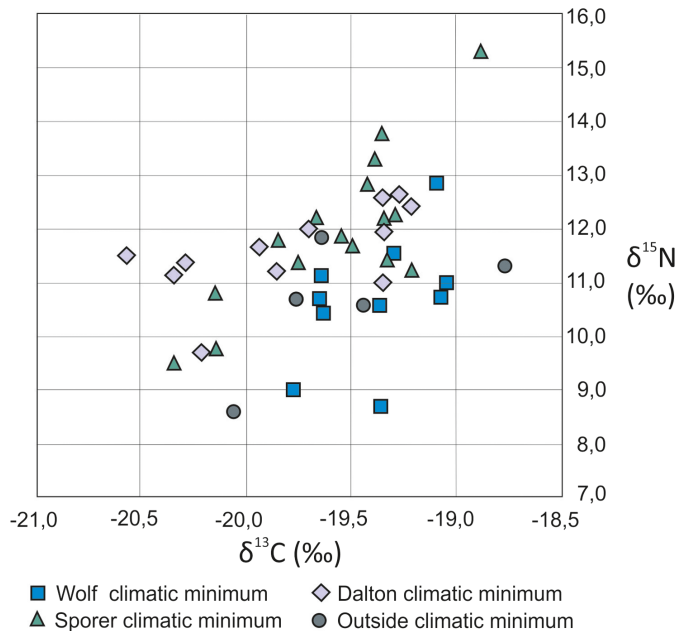


Figure 3. The isotopic composition of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in dependence on the individual cold periods.

of grain increased by 133%). As soon as resources for a human or animal population decrease, elimination competition occurs until the group size is reduced for the remaining resources to be sufficient (Chu and Lee 1994, 1997). This is not different in human populations. In cold periods, the number of armed conflicts increased by 46% and there was a simultaneous 126% increase in famines and 205% increase in the number of epidemics (Zhang et al. 2011). Other authors have pointed out that some societies were especially sensitive to climatic fluctuations, while others were less affected and were strengthened by these negative experiences. In any case, socioeconomic bonds, the relevant institutions, culture and human behaviour are strengthened or weakened by the effects of climatic fluctuations on human society (Degroot 2018).

Although the correlations in these and other references need not indicate causality and connections must be sought in other demographic and anthropogenic factors, conflicts, famines and excessive mortality predominate during periods of climate change (Slavin 2016) and may be manifested in the deposition of skeletal remains in Bohemian and Moravian ossuaries.

The isotope compositions of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in the collagen of the studied bones are depicted in dependence on the various periods in Figure 3 and are shown in Table S2. Table S3 gives the collagen composition of the bones in relation to the estimated sex of the individuals. The isotopic compositions of carbon and nitrogen are similar in the groups divided according to sex. Women have an average of $\delta^{13}\text{C} = -19.91\text{‰}$, $\delta^{15}\text{N} = 11.2\text{‰}$ ($n=3$), while men have an average of $\delta^{13}\text{C} = -19.53\text{‰}$, $\delta^{15}\text{N} = 11.58\text{‰}$ ($n=20$); the bones of undetermined sex have an average of $\delta^{13}\text{C} = -19.57\text{‰}$, $\delta^{15}\text{N} = 10.54\text{‰}$ ($n=40$). A child's bones from the ossuary in Munich have a remote value of ($\delta^{13}\text{C} = -18.88\text{‰}$, $\delta^{15}\text{N} = 15.31\text{‰}$). The higher $\delta^{15}\text{N}$ value is caused by nursing (Fuller et al. 2006). Some of the samples for carbon and nitrogen isotopic compositions were measured in replicates, so their numbers do not correspond to the number of ^{14}C -dated bones.

It is apparent in the dependences of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Figure 3, Figure S2) that the data correspond to a trend of positive correlations of both variables, where lower values of $\delta^{13}\text{C}$ correspond to lower $\delta^{15}\text{N}$, i.e., individuals that had a higher content of vegetable protein in their diets (i.e., more negative $\delta^{13}\text{C}$ values) and simultaneously had lower consumption of animal proteins, which have higher $\delta^{15}\text{N}$ values.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ dependencies for bone samples from each minimum are shown in Figure S2. The figure shows the tightest dependence of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for samples from the Spörer minimum (correlation coefficient $R = 0.8046$), followed by samples from the Dalton minimum ($R = 0.6041$), and then by samples from the Wolf minimum ($R = 0.4769$). The entire data set shows a smaller correlation coefficient ($R = 0.2289$).

It is apparent from Figure 3 that the samples with low $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values have carbon isotopes affected by greater consumption of plant proteins.

While $\delta^{13}\text{C}$ reflects the contents of C_3 and C_4 plants in the diet (in the bones we studied, the only possible source of C_4 is millet), $\delta^{15}\text{N}$ reflects a higher content of plant and animal proteins or the character of the animal proteins, whose $\delta^{15}\text{N}$ reflects the position of the source in the trophic chain. The higher $\delta^{15}\text{N}$ value in the collagen samples could be caused by greater consumption of animal proteins or by the content of proteins with higher $\delta^{15}\text{N}$ values (e.g., animal proteins, unweaned herbivores, or fish). At the same time, some isotopic work shows that the influence of animal proteins is overestimated when studying diet composition, and the fact that increased ^{15}N in collagen may occur due to the consumption of cereals grown in fields intensively subsidized with stable manure is underestimated (e.g. Bogaard et al. 2013; Dreslerová et al. 2021).

The connection between the contents of the two isotopes in dependence on the age and correspondence to the individual cold periods is demonstrated by analysis of the main component (PCA, Figure S3) and analysis of the scatter. It follows from PCA analyses that the axis of the main components has a right-left diagonal dependence of $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ and the second main component is perpendicular to it (Figure S3). Subsequent analysis of the scatter demonstrated that bones from the Wolf, Spörer and Dalton periods do not have the same parameters ($p = 0.0002$) and thus the composition of the bones from the Wolf minimum is different from the bones from the Spörer and Dalton minima. These two minima cannot be separated on the basis of the carbon and nitrogen isotopic composition and lower consumption of animal products can be assumed at the Wolf minimum. On the other hand, changes could have occurred in agriculture during the younger cold periods, causing the increase in the $\delta^{15}\text{N}$ value. This shift could be caused by better fertilization, which increases the $\delta^{15}\text{N}$ value for the fertilized plants and then in the related consumers (Bogaard et al. 2007; Kanstrup et al. 2011; Szpak et al. 2012). Izdebski et al. (2022) used pollen distributions showing intensive or stagnant agriculture from dated geochemical archives (lake sediments and wetlands) to demonstrate land use changes after the 1347–1352 plague epidemic (end of the Wolf Minimum). They found that while in some areas the epidemic had devastating effects on the population and its farming (central Germany, central Italy, Scandinavia) by contrast, in other parts of Europe (e.g. Bohemia, the Baltic) there was a signal (pollen content) documenting an increase in cereal production. This jump in the rural economy could be related to the observed difference between the C and N isotopic composition of collagens of individuals from the Wolf minimum and the younger minima.

Le Huray and Schutkowski (2005) provide an analysis of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the bone collagen in the population living in the present-day Czech Republic, giving the composition of the collagen of the inhabitants of the La Tène period in Kutná Hora and Radovesice (380–150 BC; Figure S4), while Plecerová et al. (2020) describe the bones of the Moravian population living in the 5th–6th centuries AD (Figure S5), and Halffman and Velemínský (2015) analysed the bones of the inhabitants of Great Moravia (9th–early 10th century AD) (Figure S6). Salesse et al. (2013) give an analysis of the bones of individuals buried in the St. Benedict cemetery in Prague in the 15–18th century (Figure S7).

The population of La Tène period of Kutná Hora and Karlov (380–150 BC; Le Huray and Schutkowski 2005) exhibited differences in the nitrogen isotope compositions, especially in relation to the accessories in the graves. Individuals buried with swords, shields and spears exhibited higher $\delta^{15}\text{N}$ values and it can thus be assumed that they consumed more animal proteins. In comparison with the sample from ossuaries and the population of the La Tène period (380–150 BC; Figure S4), a generally lower $\delta^{15}\text{N}$ can be observed and thus a lesser consumption of animal products. At the same time, most of the samples of the La Tène period bones have higher $\delta^{13}\text{C}$ values than the samples from ossuaries and

several samples exhibit values of $\delta^{13}\text{C} > -18\text{‰}$, probably as a result of consumption of C_4 plants, in this case millet (Le Huray and Schutkowski 2005).

The bones of the Moravian Lombard population (5th–6th centuries AD) studied by Plecerová et al. (2020) are also different from the populations of the ossuaries. Samples of the bones of Lombard population have a similar $\delta^{13}\text{C}$ composition to the samples from ossuaries (with the exception of three samples with $\delta^{13}\text{C} > -18\text{‰}$ documenting the consumption of C_4 plants), but lower values of $\delta^{15}\text{N}$ (Figure S5), probably due to the effect of lower consumption of animal proteins. The greatest difference between the studied ossuary samples is apparent in comparison with the bone population of Great Moravia (9th–early 10th centuries AD) (Halffman and Velemínský 2015). The bones of the inhabitants of Great Moravia have higher $\delta^{13}\text{C}$ values (mean males -17.8‰ PDB; females -17.9‰ PDB) and simultaneously lower $\delta^{15}\text{N}$ values (mean male 10.3‰ ; mean female 9.7‰) compared with the bones of the studied ossuaries (Figure S6). The diet of the tested population contained the plant proteins of C_3 and C_4 plants (millet), the plant proteins of legume plants and the animal proteins of mammals, poultry and fish (Halffman and Velemínský 2015).

The compositions of the isotopes of carbon and nitrogen in the collagen of the tested bones is closest to the composition of the collagens of individuals buried in the St. Benedict cemetery (Prague, 15th–18th centuries AD; Salesse et al. 2013). The isotopic compositions of the collagens of the bones in this cemetery and in the ossuaries are depicted in Figure S7. Samples were taken from the individual graves (IG) and three mass graves (MG1, MG2 and MG3). In only one case did the samples in this work differ in their compositions of $\delta^{13}\text{C}$ (IG -19.6‰ and MG1 -20.0‰), but in all cases in their values of $\delta^{15}\text{N}$ (IG vs MG1, MG2 a MG3). The average $\delta^{15}\text{N}$ values in the samples from IG attained 12.2‰ , while MG $10.3\text{--}9.2\text{‰}$. Our tested samples had average values similar to the low values of $\delta^{13}\text{C}$ (mean $= -19.57\text{‰}$, $n = 52$). For example, Ogrinc and Budja (2005) give $\delta^{13}\text{C}$ for wheat and rye as -25‰ . Bird et al. (2022) present values of the equivalent composition of the diets in the range of -31 to -19‰ for the entire set ($n=1298$). A value for the $\delta^{13}\text{C}$ difference of 4.8‰ and simultaneously 1.9‰ in relation to the preindustrial atmosphere is common for the calculation of collagen in the diet (Bird et al. 2022). This difference is manifested for both domestic fauna and humans. For example, for the Mikulčice Kostelisko–Czech Republic (9th–early 10th c. AD) locality, Halffman and Velemínský (2015) give an average collagen composition for cows, sheep, pigs and horses of -20.3 ; -20.5 ; -20.4 and -20.3‰ . Because the $\delta^{13}\text{C}$ isotopic composition of the bones varies in the range -20.3 to -18.7‰ , the equivalent composition of the diet has values of -27 to -25.4 and thus corresponds to the consumption of the grains of C_3 plants, their isotopic composition is lighter compared to that of seeds (e.g., Bird et al 2022).

Elevated $\delta^{15}\text{N}$ values (mean $= 11.36\text{‰}$, $n = 52$) of the collagen of the studied bones can be explained by greater consumption of animal proteins or proteins with elevated $\delta^{15}\text{N}$ values that are exhibited by unweaned herbivores, poultry or fish (Reitsema et al. 2013). It was common to consume fish in medieval Europe. E.g., Reitsema et al. (2013) give values of $\delta^{15}\text{N}$ for freshwater fish in the range $6.6\text{--}12.1\text{‰}$ (mean 9.87‰ ; $n = 15$). Dufour et al. (1999) give a range for freshwater fish of $\delta^{15}\text{N}$ $7\text{--}14.9\text{‰}$ while France (1995) states values of $4\text{--}15\text{‰}$ for freshwater fish, $6\text{--}13\text{‰}$ for estuary fish and $7\text{--}14\text{‰}$ for anadromous fish.

After 1300, the distribution of salted and dried fish (herrings and cod) spread across European medieval Europe. They were transported from coastal areas to the interior and became a cheaper alternative to freshwater fish; they were consumed especially by the poorer members of society (Adamson 2004). While freshwater fish mostly do not exceed values of $\delta^{15}\text{N} = 15\text{‰}$, in saltwater fish this parameter can be as large as 20‰ (France 1995). Where the shift in the $\delta^{15}\text{N}$ values of the diet vs collagen is usually given as $2\text{--}5\text{‰}$ (Hedges and Reynard 2007; Schoeninger and DeNiro 1984) and the average composition in the bones investigated here reaches a value of 11.36‰ ($n = 52$), then it is probable that this isotopic composition could also be affected by a substantial content of fish in the diet. At the same time, it cannot be overlooked that elevated $\delta^{15}\text{N}$ values may be related to a crop derived diet produced by the practice of intensive fertilization affecting the nitrogen isotopic composition of plant proteins.

Conclusions

The ossuaries in the Czech Republic (Figure 1) have not dated bone material. Thanks to radiocarbon dating, they have been temporally classified (Figure 2), including in individual deposits.

Samples of skeletal remains collected from 46 Bohemian and Moravian ossuaries were of an age corresponding to cold climatic fluctuations in the past millennium, i.e., the Spörer, Maunder, Wolf and Dalton minima. It is possible that climatic events, associated with limited solar activity, influenced the production of plant food. Long-term dietary restrictions evolved into famines, resulting in increased mortality of 90% during solar minima but only 10% during times of increased solar activity. The population was in a demographic crisis, especially in the Medieval Cold Minima, when the Little Ice Age began. However, this fact does not necessarily mean that the bones of the studied individuals are related to the climate and its influence on agriculture and food processing and storage. The samples include individuals that died during armed conflicts, especially as a result of skull injuries, as well as individuals who survived these injuries or whose cause of death is not clear.

The values of the compositions of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ have a linear dependence, indicating a predominant source of proteins. Simultaneously, the compositions of the bone collagen in the studied individuals exhibit differences in the isotopic compositions of C and N, especially between the Wolf minimum and remaining minima. These differences could be caused by the better management in smaller municipalities and as a consequence of the availability of plant proteins with a higher content of the heavier isotopes of nitrogen or greater availability of animal proteins.

The studied bones have different compositions than the populations of the La Tène period in Kutná hora and Radovesice (380–150 BC), Moravian Lombard population (5th–6th centuries AD) and the inhabitants of Great Moravia (9th–early 10th centuries AD) and are closest to the population of the 15–18th century skeletons buried in the St. Benedict cemetery in Prague.

Supplementary material. To view supplementary material for this article, please visit <https://doi.org/10.1017/RDC.2024.71>

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