

Nitrogen and Strontium Isotopes as Tools for the Reconstruction of Breastfeeding Practices and Human Behavior – A Neolithic Collective Grave in Bronocice (Poland)

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ABSTRACT

Isotopic analyses are often used in biological anthropology and bioarcheology, in studies of ancient human populations. Such analyses in anthropology have been used to study migration patterns, the nutrition strategies of prehistoric populations and the weaning of infants. The main objective of this work was to investigate patterns of breastfeeding and weaning in Neolithic populations at Bronocice in Poland using nitrogen stable isotopes. Additionally, strontium isotope analysis was conducted to determine if the individuals from the collective grave (Burial XIII, Pit 36-B1) at Bronocice were of local origin. The samples consisted of skeletal remains from individuals buried in the collective grave during the early Funnel Beaker-Baden phase (3300-3100 BC). Two models have been used for reconstructing precisely the age at the start and end of weaning (Schurr's model and WARN model). The results suggest that weaning began in the first year of life and ended at about 3 years of age.

Key words: Strontium, Carbon, Nitrogen, Breastfeeding practices, Neolith.

Introduction

Infancy and early childhood is one of the most critical stages of human biological development, since the body is extremely vulnerable to all kinds of diseases. The extremely dynamic development of a young individual needs a large quantity of nutrients and an immune system to protect the body against pathogens. In this period, mother's milk represents the most appropriate nutrition for a child. A change in diet causes stress, since a reduction in the frequency of breastfeeding reduces the passive immune protection for the child against pathogens and increases the risk of death in infancy¹⁻². These observations are indirectly supported by numerous paleopathological studies of children's skeletons which exhibit a wide range

of malnutrition symptoms, infectious diseases or the occurrence of non-specific indicators of stress. These individuals did not survive early childhood³⁻⁸.

Age of weaning is variable and often a protracted process during which the diet of the infant changes from mother's milk to other foods⁹. It depends on variability between individual infants, the duration of mother's lactation, the state of her health and biocultural conditions. At Kaminaljuyu, Guatemala (400 BC) breastfeeding ended when the child was approximately 4 years of age¹⁰⁻¹¹. At Kellis, Egypt (0-500 AD), at Weinigumstadt, Germany (500-800 AD) and at Wadi Halfa, Nubia, Sudan (500-1500 AD) weaning occurred at approx. 3 years of age^{5,12-14}. In other populations, for example, at Waharam Percy, England

(900–1600 AD), at Wetwang Slack, England (400–100 BC AD) and Krakow, Poland (800–1100) weaning occurred a little earlier, at about 2.5 years of age^{15–17}.

Investigations of weaning in relation to nutritional status suggest that shorter breastfeeding periods correlate with a higher incidence of some types of diseases within communities^{5,18}. Another frequently observed phenomenon is gender favoritism with males being weaned later than females^{19–20}. Nitrogen isotopes (¹⁴N and ¹⁵N) are components of the amino acids, which are the building blocks of all proteins. Their ratio is expressed as $\delta^{15}\text{N}$ where:

$$\delta^{15}\text{N} = \left(\frac{{}^{15}\text{N}/{}^{14}\text{N}_{\text{sample}}}{{}^{15}\text{N}/{}^{14}\text{N}_{\text{standard}}} - 1 \right) \times 1000\text{‰}$$

The $\delta^{15}\text{N}$ value in bones and teeth reflect proteins consumed by an individual during life as there is a trophic level shift of +3 to +5‰, so that consumption of resources from a high trophic level in the food chain will result in higher $\delta^{15}\text{N}$ values in the consumer's bone collagen²¹. The consequence of this trophic effect is a higher $\delta^{15}\text{N}$ value, of 2–3‰, in the bone tissue of children compared to the adult bone tissue, for which the only protein source was human milk²². As already mentioned the weaning process involves a gradual introduction of other food and a gradual decrease in breast milk. The process of weaning will be recorded as a decrease in the value of $\delta^{15}\text{N}$ isotope, within the organic part of children's bones and teeth, until the values are similar to those of their mothers (assuming that weaned children and adults sharing the same diet)^{1,2,3}.

A reconstruction of the process of weaning infants in a group requires that its members are of common origin. This applies particularly to the females who are potential mothers^{24–25}. Therefore, whether and which individuals are of local origin is important in considering the results of isotope studies^{16–26}. The environmental isotopic background is the starting point for research into the origin of the individuals in a group.

Strontium isotopes unlike those of oxygen, carbon or nitrogen, are transferred without the ⁸⁷Sr/⁸⁶Sr ratio measurably changing from the weathering of rock mineral through soils and soil water into the biosphere and food chains²⁷. In bone, strontium substitutes for calcium in the crystal lattice of the apatite^{28–30}. The ratio of ⁸⁷Sr/⁸⁶Sr in rocks and minerals vary depending on the type of bedrock and the geological age, which is closely related to the Rb/Sr ratio in minerals and the initial concentration of ⁸⁷Rb, which gradually decays into a ⁸⁷Sr (half-life ~ 4.7 x 10¹⁰ years)^{31–33}. Consequently the measured isotope ratio in the tissue of living organism's should correspond directly to that in their environment^{34–38}. This relationship is not obvious, because the strontium in organisms' tissue reflects all of its sources in the region. A technique commonly used to determine local ratios of ⁸⁷Sr/⁸⁶Sr is to establish these values in the hard tissue of animals found with human remains^{31,37,39–41}. We use local fauna, especially domestic animals for this purpose, since their food comes from the same region as that consumed by humans. Additionally, data obtained from animal remains have relatively low standard deviations and coefficients of variation^{31,42–43}.

Studies of prehistoric populations use the ⁸⁷Sr/⁸⁶Sr ratio which enables verification of hypotheses concerning the mobility of human and animal populations. The geographical diversity of this isotopic ratio has been examined by such scholars as Budd *et al.* (2004)⁴⁴, Bentley (2006)⁴⁵, White *et al.* (2007)⁴⁶, Gregoricka (2013)⁴³. The utility of strontium isotope studies has been demonstrated by Mitchell and Millard (2009)⁴⁷ in a study of Europeans who migrated to Jerusalem during the crusades: not only were Mitchell and Millard able to distinguish natives from immigrants, they also identified the parts of Europe from which the latter came. Similarly, the original home was identified for the Copper Age human remains known as Ötzi, found in the Ötztal Alps. In addition, from isotopic analysis we know that in the latter years of his life he was mobile or the food he consumed, came from different geographical locations^{48–50}. The main objective of this work was to reconstruct patterns of breastfeeding by analyzing the $\delta^{15}\text{N}$ values in bone collagen. Additionally, this study verifies on the basis of the analysis of strontium isotopes, that the individuals recovered from the collective grave at Bronocice are of local origin⁵¹.

Material and Methods

Archaeological background

The State University of New York at Buffalo and the Institute of Archaeology and Ethnology, Polish Academy of Sciences conducted a cooperative archaeological project at Bronocice, Świętokrzyskie province, between 1974 and 1978. Excavation exposed a large settlement area in which burials and a large number of pits were located. These features spanned 1200 or 1300 years of occupation and were associated with the successive Funnel Beaker, Lublin-Volhynian, Funnel Beaker-Baden and Corded Ware cultures. Human remains included articulated skeletons from burials and isolated bones from pits. Funnel Beaker material appeared in the Bronocice region around 3900 BC and disappeared around 3300 BC. Funnel Beaker-Baden material dates to ca. 3300 BC and Corded Ware material to ca. 2600/2500 BC.

Between 3300 and 2900/2800 BC, the settlement was occupied by Funnel Beaker-Baden people. At this time Funnel Beaker ceramics began to incorporate Baden culture motifs. Also around 3300 BC economic, demographic and social changes in the upper basin of the Vistula loess areas contributed to the abandonment of many large Funnel Beaker settlements, and resulted in the increased mobility of the population. A few large settlements, including Bronocice, continued to be inhabited. The Funnel Beaker-Baden settlement expanded to 26 ha at Bronocice and then contracted to 17 ha. The rising importance of cattle and sheep husbandry suggests that more people were practicing herding instead of agriculture.

During the 1974 field season, the remains of 17 individuals dating to the early Funnel Beaker-Baden phase (3300–3100 BC) were uncovered in the collective Burial XIII (Pit 36-B1). This pit was roughly circular at the top,

140 x 130 cm in size. It measured 130 cm in depth and its profile was rectangular. The remains of 17 individuals were found at a depth of 90–130 cm. At this level, flint artifacts, animal bones, a boar's tusk pendant, mussel bead necklaces, pieces of burnt clay and the remains of three Funnel Beaker-Baden vessels were also found.

The burial included four adults, one juvenile and 12 children. The adults included two women about 18 years old, two men between 18 and 25 years of age. The twelve children ranged in age from six months to 10 years and a juvenile, approx. 15 year-old individual had undetermined sex.

The burial was carefully arranged. Most of the individuals were placed in a circular pattern around the periphery of the pit. The oldest male was placed in the center of the burial in a flexed position. The archaeological evidence indicates that all individuals were placed in the burial pit at the same time. The archaeological aspects of the Bronocice project have been described in numerous publications^{38,52–66}.

Skeletal remains

For isotopic analysis the samples consisted of femurs and ribs from 17 individuals recovered from the collective grave at Bronocice (southeastern Poland) (Figure 1)^{51,67–68}. Twelve cases were taken from children aged 6 months to 10 years, and five from adults aged 15 years or older (Table 1). To determine the local base level of isotopes we used animal bones (5 cattle and 4 sheep) from Bronocice, dating to the same phase as the human remains (Table 2).

Samples for isotopic analysis were prepared using a diamond saw. The resulting fragments were purified using an ultrasonic bath and dried. Then, we reduced the sample to bone powder by using a ball mill MM200 (Retsch).

Stable isotopes analysis

Strontium was extracted from the cortical bone of human and animal remains from the excavated site using established preparation methods at the Scottish Universities Environmental Research Centre in Scotland (SUERC). First, bone samples were cleaned by rinsing in an ultrasonic bath with deionized water and dried overnight. Subsequently, samples were ground and transferred to the clean laboratory where ~ 90 mg of powdered sample were weighed into Teflon beakers and leached in 1M acetic acid for 30 min. Then each sample was evaporated to dryness and combusted at 500 ° C in clean ceramic beakers for 8 hours to remove the organic fraction of the bone. Then, 10–30 mg of ash were weighed into clean Teflon beakers and digested in 1 ml of 8M HNO₃ on a hotplate (150 ° C). The dissolved samples of bones were loaded onto columns containing cleaned Sr-spec[®] resin (Triska International). After several washes with 3M HNO₃ (to remove the Ca and Rb), the strontium was eluted from the resin with 0.01 M HNO₃ into clean Teflon beakers and dried down on a hotplate. The remaining samples were then ready for the measurement by thermal ionization mass spectrometry (TIMS) together with samples prepared from SRM 987.



Fig 1. The collective human grave excavated at Bronocice (southeastern Poland).

Isotopes of nitrogen were extracted from the organic fraction of bone according to the protocol of Bocherens (1997)⁶⁹. 10 ml of 1M hydrochloric acid (HCl) were added to about 0.3 g of milled bone. After 20 min. 10 ml of 0.001M HCl were added and heated to 100° C for about 17h in closed tubes until complete dissolution of the bone. The resulting solution was centrifuged in tubes with Amicon ultra filters, and then the samples were freeze-dried. Isotope measurements were again made at SUERC. The isotopic compositions were expressed using the 'δ' (delta) values (δ¹⁵N) with the international reference being atmospheric nitrogen (AIR)⁶⁹.

All results were analyzed statistically by using Statistica 12 package. Reconstruction of the weaning process was conducted using software packages, Mathematica and R package WARN respectively.

Results

Analysis of diagenetic changes

Due to the fact that the analyzes were performed using bone, a thorough analysis of this tissue was carried out in terms of diagenetic changes both within the organic and mineral parts. The atomic ratio of carbon to nitrogen (C/N) was used to establish degree of diagenetic changes occurring *post mortem* in the organic part of the bone. According to some scholars^{70–72}, unchanged samples during the retention in the soil are in the range 2.9–3.6. All results beyond this range should be excluded from further analysis. The C/N samples were obtained simultaneously with the measurement of isotopic ratios of nitrogen at the Scottish Universities Environmental Research Centre, East Kilbride.

TABLE 1.
CHARACTERISTIC OF INDIVIDUALS BURIED IN COLLECTIVE GRAVE IN BRONOCICE AND RESULTS OF ISOTOPIC ANALYSIS

Grave no.	Age	Sex	Bone type	⁸⁷ Sr/ ⁸⁶ Sr	± 2SE ⁸⁷ Sr/ ⁸⁶ Sr	C/N	δ ¹⁵ N
Infans I							
XIII-11	6 months	?	rib	0,70922	0,00002	3,2	14,3
XIII-17	6-9 months	?	rib	0,70924	0,00002	3,3	15,6
XIII-9	1,5	?	rib	0,70925	0,00002	–	–
XIII-16	3	?	rib	0,70923	0,00002	3,2	12,5
XIII-7	3	?	rib	0,70923	0,00002	–	–
XIII-2	4	?	rib	0,70925	0,00002	3,3	9,9
XIII-5	4	?	rib	0,70925	0,00002	3,2	11,3
XIII-10	5	?	rib	0,70920	0,00002	3,2	10,9
Infans II							
XIII-14	6	?	rib	0,70914	0,00002	3,3	10,9
XIII-12	7	?	rib	0,70924	0,00002	3,3	10,8
XIII-15	8	?	rib	0,70927	0,00002	3,2	10,9
XIII-3	10	?	rib	0,70936	0,00002	–	–
Adults							
XIII-13	15	?	femur	0,70922	0,00002	3,4	10,3
XIII-1	17	F?	femur	0,70936	0,00002	3,2	11,1
XIII-4	18	M?	femur	0,70924	0,00002	3,2	11,1
XIII-8	20	F?	femur	0,70924	0,00002	4,9	10,1
XIII-6	25	M	femur	0,70905	0,00002	3,2	11,5

TABLE 2.
RESULTS OF STRONTIUM ISOTOPE ANALYSIS OF ANIMAL
SAMPLES FOUND IN BRONOCICE

ANIMALS SAMPLES			
Sample no	Animal species	⁸⁷ Sr/ ⁸⁶ Sr	± 2SE ⁸⁷ Sr/ ⁸⁶ Sr
1KR	Cow	0,709384	0,00002
2KR	Cow	0,70882	0,00002
3KR	Cow	0,70922	0,00002
4KR	Cow	0,709042	0,00002
5KR	Cow	0,709567	0,00002
10W	sheep	0,709459	0,00002
30W	sheep	0,709237	0,00002
40W	sheep	0,709131	0,00002
50W	sheep	0,709984	0,00002

The obtained values of C/N ratio are within the range of 3.2 – 4.9 (Tab. 1). One sample was above the upper limit of normal 3.6. Therefore, sample XIII / 8 (C/N = 4.9) was excluded from the further analysis. The remaining

C/N values were in the range of 3.2 to 3.4. Three specimens (Nos: XIII-3, XIII -7 and XIII-9) lacked sufficient collagen to establish δ¹⁵N values.

In turn, FTIR analysis of bone samples allowed the assessment of the degree of preservation of the inorganic fraction in bones. It should be noted, that there have been studies that would suggest that bone samples will take on the Sr ratio of the surrounding burial environment and that tooth enamel is preferable because the Sr does not exchange^{44,47}. However, for strontium analysis we used cortical fraction of bone which is considered to be less subject to diagenesis and we had very strict cleaning procedure of those samples using ultrasonic bath and chemical treatment (acetic acid). Additionally, FTIR analysis of those skeletons did not reveal diagenetical changes in mineral fraction of bones⁵¹. This allows us to assume that the results of ⁸⁷Sr /⁸⁶Sr reflected intravital values of analyzed individuals.

Determination of local origin and analysis of specimens

To assess the origin of the individuals in the sample, it was necessary to determine, on the basis of animal remains, the range of available strontium in the Bronocice region (Fig. 2). The ⁸⁷Sr /⁸⁶Sr ratio calculated for 9 animal

bones yielded a mean and standard deviation of 0.70931 ± 0.00034 . For two standard deviations the range is $0.70864 - 0.70999$. This was used as a basis to determine the origin of the Neolithic individuals (Tab.2). This is a good way to illustrate variations in the strontium isotope in the environment, even if the sample of animals is small^{31,73–74}.

Soils in Poland yield an average $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.709, but depending on the type and age of bedrock the value may range between 0.7069 and 0.7123. For soils on loess subsoil, values range from 0.7085 – 0.7105⁷⁵. For surface water the range is 0.7078–0.7096 throughout Poland, including that from the Vistula River (0.7095). Additionally the $^{87}\text{Sr}/^{86}\text{Sr}$ of locally-grown wheat are 0.7090–0.7.106 and butter from Polish dairy cattle is 0.7088⁷⁶.

In this region of Poland there is a wide range of $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in soils, due mainly to the diversity of the underlying geological structures. Unusually high $^{87}\text{Sr}/^{86}\text{Sr}$ ratios have been noted in some regions⁷⁷. Thus, the use of fauna and flora samples is considered to be an accurate method of determining local variations in strontium isotope ratios.

In order to determine the origin of the 17 individuals from the collective grave, the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios were measured. Bone samples from the 17 individuals were compared to previously determined $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in the Bronocice region. It was found that the ratios measured in these individuals was in the range of 0.70905 to 0.70936 (Tab. 1, Fig.2) and fell within that regional values. We can conclude that the individuals buried in the collective grave came from the Bronocice region. These results confirm conclusions drawn from the analysis of stable oxygen isotopes⁵¹.

Weaning

The analyses discussed above make it possible to study the patterns of breastfeeding. The results of the stable nitrogen isotope analysis for the individuals from the col-

lective grave are shown in Table 1 and Figure 3. The juveniles were divided into three age groups: the first group was of individuals under 1 year of age (individual number: XIII-11 and XIII-17), the second group – children aged between 1 and 6 years, i.e. *infans I* (Nos: XIII-2, XIII-5, 10-XIII, XIII-16), and the third group - children aged 6–14 years, i.e., *infans II* (Nos: XIII-12, XIII-14 -and XIII-15). A separate group consisted of adults (Nos: XIII-1, XIII-4 and XIII-6) and juvenile individual No. XIII-13, who at the time of his death was approximately 15 years of age.

The $\delta^{15}\text{N}$ value in the bones of children ranged from +9.9 to +15.6 ‰ (Mean = 11.74, SD = 1.85) (Tab.1, Fig. 3). Samples were placed in the sequence indicated by the age groups described above. The results from children were compared with the nitrogen isotopic variation found in the group of four individuals (including a juvenile and adults) whose range is +10.3 to +11.5 ‰. Figure 2 shows higher $\delta^{15}\text{N}$ levels in the three youngest individuals (XIII-11, XIII-17 and XII-6) compared to the other juveniles and in the adults. Two infants (XIII-11, XIII-17) showed values of $\delta^{15}\text{N}$ above the upper limit of variation in adults by 4.1 and 2.8 ‰. respectively. Our results indicate that the two youngest children in the collective grave, were likely breastfed until their death. In the child aged 3 (XIII-6), this difference was only 1 ‰. Bearing in mind the fact that the bone remodeling process is very rapid in children, one can assume that this child had consumed food other than breast milk for more than half a year, but it cannot be concluded that the process of weaning was completed. In the case of the other juveniles, the $\delta^{15}\text{N}$ values are close to adult values, which leads to the conclusion that at the time of death they had been weaned.

Modeling process of weaning

In the next step of the analysis we tried to reconstruct the process of weaning in the group of children on the basis of a mathematical model described by Schurr (1997)⁷⁸ using Mathematica 10 software.

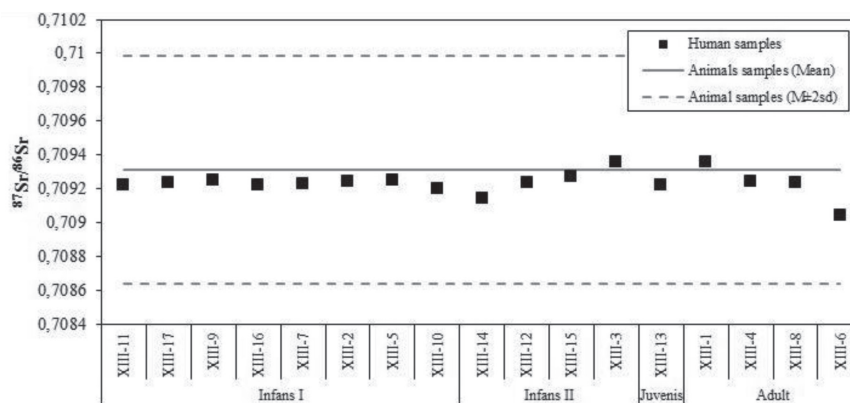


Fig 2. $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in the bones of Bronocice individuals. The variation of in ratio in local environment is based on animal bone samples (black lines).

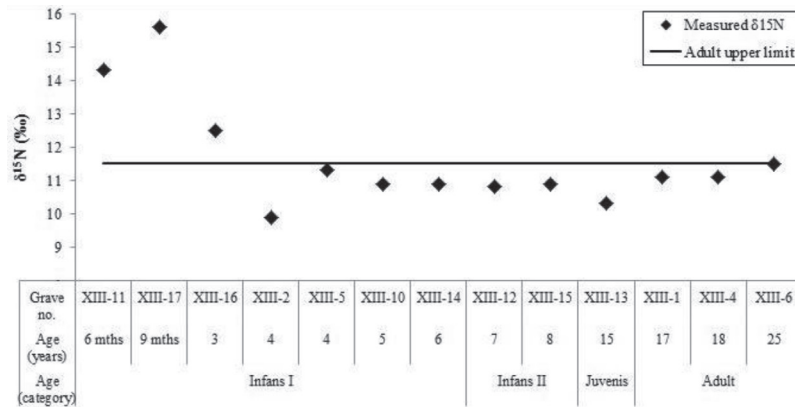


Fig 3. $\delta^{15}N$ bone collagen values for children buried in Bronocice.

In terms of food consumption, early childhood can be divided into two stages: breastfed and weaned. The Schurr (1997)⁷⁸ model proposes two curves, one for the breastfeeding period, in which $\delta^{15}N$ levels increase and one for the weaning process, in which levels decrease. Infant weaning is not a single event. It consists first, of the time when other food is introduced into the child's diet, the second is the time when that food starts to replace mother's milk, and the third period is the time at which the infant's diet corresponds to that of the adult. The model assumes that the age profiles of $\delta^{15}N$ values provide information on the beginning and end of the process of weaning, as well as the rate of weaning. To plot the weaning process a non-linear exponential function was used^{23,78}: $\delta^{15}N_x = \Delta e^{-R_w(x-x_w)} + \delta^{15}N_c$, where $\delta^{15}N_c$ is the average level of the stable isotope in the bones of weaned children, Δ is the difference between $\delta^{15}N$ in the child and the mother's milk, R_w is the degree of change in the proportion of the isotope dependent on the degree of tissue remodeling and x_w is the age at which food is included in the diet. For individuals aged 5–18 from Bronocice, $\delta^{15}N_c$ was +10.86 ‰. The best fit regression line is presented in Figure 4 and was obtained by assuming that Δ is 3.3 (this ratio has a value 3–3.6⁷⁹). The model-calculated value of R_w is 0.64 and x_w lies between 0.79 and 1.02. These results suggest that the start of weaning occurred in the first year of life, and ended between 3 and 4 years of age, when $\delta^{15}N$ values are within error of the adult values. It should be noted that the rate of weaning was quite fast, as indicated by the slope of the regression line between the ages of 1 and 3 years.

As mentioned earlier Schurr's model also allows one to identify the stage of feeding children mother's milk using a modified Weibull function. However, the collective grave at Bronocice yielded few children whose biological age was in the first months of life. This precluded any observation of $\delta^{15}N$ levels associated with the breastfeeding stage. The model proposed by Schurr suffers from a number of deficiencies, the most serious one is that the subadult bone turnover rate is not considered. However, bone turnover rate depends on an individual's age. Tsutaya and Yoneda (2013)²⁴ showed that bone collagen turnover is high in

early infancy – during the first year of life, the integrated turnover rate was estimated to be 1.588. In subsequent years of age, the turnover rate gradually reduces until about at 20 years of age, it reaches 0.13. Therefore, Tsutaya and Yoneda (2013)²⁴ proposed an alternative model of the weaning process, taking into account the dynamic of changes in turnover rate of bone during childhood. The model was distributed as the R package WARN (Weaning Age Reconstruction with Nitrogen isotope analysis) (Tsutaya & Yoneda, 2013)²⁴.

Based on the simulation, using the WARN package, we obtained four weaning parameters that result in maximum probability density (the Maximum Density Estimators – MDEs) such as: the ages at the start (t1) and end (t2) of weaning, enrichment factor between the infant and mother (E) and $\delta^{15}N$ value of collagen synthesized entirely from weaning foods ($\delta^{15}N_{wn}$ food) as well as posterior probabilities and joint probability. The results are presented Table 3 and Figure 5

The results obtained from the simulation of the weaning process using the WARN model are similar to those obtained by the Schurr's model. It shows that the introduction of weaning food to the child's diet in the population from Bronocice followed around the end of the first year of life but breastfeeding continued until 3 years old.

TABLE 3.
MAXIMUM DENSITY ESTIMATORS (MDES) FOR INDIVIDUALS FROM COLLECTIVE GRAVE IN BRONOCICE CALCULATED USING WARN MODEL

	MDEs	Posterior probability
T1	0,8	0,068
T2	2,8	0,22
E	4,3	0,1
Wnfood	10,65	0,18

Joint probability = 0,02

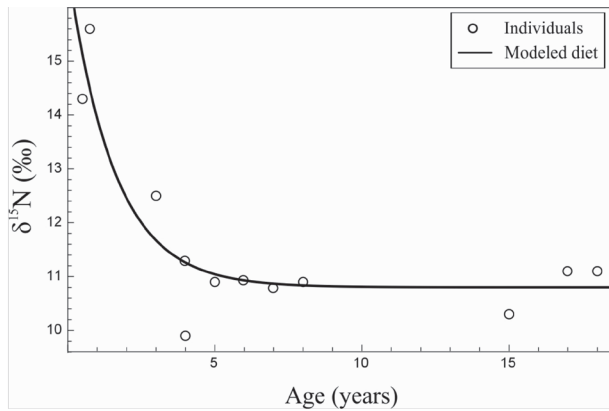


Fig 4. Modelling of the weaning process based on $\delta^{15}\text{N}$ values in the bones of children buried in Bronocice (Schurr's model).

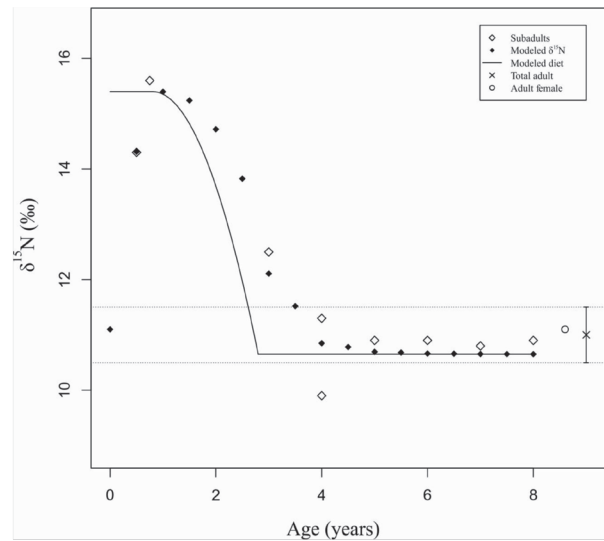


Fig 5. Simulation of the weaning process based on $\delta^{15}\text{N}$ values from bone collagen in the bones of children buried in Bronocice (WARN model).

Discussion and Conclusion

There are few data on breastfeeding and weaning from the Neolithic. The results obtained in this study are consistent with the current knowledge. In Poland, analyses of carbon and nitrogen isotopes has been done at some Neolithic and early Bronze Age sites such as Koszyce (Globular Amphorae culture, first half 3rd M BC), Pełczyska (c.2400–2200 BC) and Nowe Kichary (Mierzanowce culture, 2200–1700 BC) and it has shown that the diet of those children was enriched with food other than mother's milk from about 6 months of age, and that complete weaning occurred at about 3 years of age^{80–81}. Studies of children in the Meuse Basin area (Belgium, Middle, 4300–3000 BC and Late Neolithic, 3300–1700 BC) have revealed that weaning occurred earlier, at about 2 years of age⁸². Similar results were obtained at Neolithic sites in Turkey such as Çayönü (Aceramic-Ceramic Neolithic, 8500–6000 BC), Asikli Höyük (Aceramic Neolithic, 8500–4500 BC), and Çatalhöyük (Early Neolithic 8300–7400 BP)^{18,83}.

The study of infant feeding practices in relatively recent non-industrial societies indicates that the first introduction of liquids other than breast milk often followed soon after birth, and only rarely at approx. 6 months of age⁸⁰. There are differences between hunter-gatherer, herding and farming cultures in this respect. Hunter-gatherer societies introduce liquid and solid foods into the child's diet earlier, but these children were no longer fed their mother's milk at about 4 years^{84–85}. In farming cultures, complete weaning occurs at about 3 years^{81,86}. The study of weaning in hunting-gatherer cultures as against other cultures and non-hunting-gathering by Tsutayai and Yoneda (2013)²⁴ showed no difference in the length of the breastfeeding period, but rather significant differences in the food offered at weaning. Non-hunter-gatherers fed food

to children with a lower trophic level than did the hunters-gatherers, probably indicating the greater quantity of plants in their diets. It should be noted, however, that there is considerable diversity between groups, even those with the same subsistence strategies. Supplementary food to mother's milk during the Neolithic period was similar to that eaten by adults: cereal products, meat and the milk of animals. There is great variation in the post-weaning diets of different cultures, depending on locally available resources, such as fish, and also on traditions and cultural norms concerning children's nutrition. It does not seem that breast milk could be the only source of food for children, because it is low in bioavailable iron⁸⁷. Analyses of calcium isotopes do not unequivocally show that the Neolithic transition resulted in increases in milk within the diet of children⁸⁸. Balass and Tresset (2002)⁸⁹ analyzing the age of weaning in Neolithic cattle, observed that prehistoric calves were fed cow's milk for shorter periods than is the case with the modern cattle. Either the lactation period in primitive breeds was shorter or humans were diverting cow's milk for their own consumption.

The Funnel Beaker-Baden people who were buried in the collective grave at Bronocice were a homogenous group in terms of origin. By comparing the strontium isotope ratios ($^{87}\text{Sr}/^{86}\text{Sr}$) of individuals with those of animals which indicate the local Sr isotope values, we infer that this group is indigenous. This confirms and complements the observations made by Szostek *et al.* (2014)⁵¹, based on analysis of stable oxygen isotopes in bones and teeth. Based on a mathematical model of nitrogen isotopes ($\delta^{15}\text{N}$), allowed us to characterize the process of weaning in children. We conclude that probably the introduction of complementary foods began when the child reached 1 year of age. Infants were sustained on mixed diets until about the age of 3, after which the child was completely weaned.

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IZOTOPI DUŠIKA I STRONCIJA KAO ALATI ZA REKONSTRUKCIJU POSTUPAKA DOJENJA I LJUDSKOG PONAŠANJA – NEOLITSKI KOLEKTIVNI GROB U BRONOCICAMA (POLJSKA)

SAŽETAK

Izotopske analize se često koriste u biološkoj antropologiji i bioarheologiji, u istraživanjima starih ljudskih populacija. Takve analize antropologije korištene su za proučavanje uzoraka migracija, prehrambenih strategija pretpovijesnih populacija i prestanka dojenja kod dojenčadi. Glavni cilj ovog rada bio je istražiti obrasce dojenja i odbića u neolitiku populacije u Bronocicama u Poljskoj pomoću stabilnih dušikovih izotopa. Osim toga, provedena je analiza stroncijskog izotopa kako bi se utvrdilo jesu li osobe iz kolektivnog groba (pokop XIII, Pit 36-B1) na Bronocice-u lokalnog podrijetla. Uzorci su se sastojali od skeletnih ostataka pojedinaca ukopanih u zajedničkoj grobnici tijekom rane Faille Beaker-Baden faze (3300-3100 prije Krista). Dva su modela korištena za rekonstrukciju točnog doba na početka i kraja odbića (Schurrov model i model WARN). Rezultati sugeriraju da je odbiće počelo u prvoj godini života i završilo oko 3. godine života.

