Title: Diet and social status in the Lejasbitēni Iron Age population from Latvia

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Key words: Carbon and nitrogen isotope analysis; ancient DNA analysis; Viking Age; Baltic region

Declarations of interest: none

Highlights:

- Significant dietary differences were observed between male and female individuals
- Evidence for increased social differentiation was consistent with Viking Age
- Childhood diet had no relation to social status in adults

ABSTRACT

This study reports the first dietary stable isotope data from Iron Age in Latvia. Archaeological, osteological, genetic, and stable isotope data from the Lejasbitēni cemetery were used to study gendered differences in childhood diet expressed in stable isotope ratios with social status expressed in grave goods, in this population from the 7th – 10th centuries CE.

Carbon and nitrogen isotope analysis showed significant differences in childhood diet between male and female gendered individuals, indicating that gender might have been a key factor for dietary differences in children. There were no significant dietary differences within the gender groups in adults of differential social status expressed in grave goods, suggesting no link between childhood diet and social status in adulthood, although the sample size was very small. A change towards a more hierarchical society was observed in the later period of the cemetery, expressed in the appearance of more elaborately furnished burials, rare grave goods, and a new burial tradition. All these changes were contemporary with the development of the Viking Age in Northern Europe, and thus possibly signified external cultural influence.

Ancient DNA analysis showed that gender as expressed by grave goods corresponded with biological sex in two individuals with the highest quality aDNA, while biological sex could not be confirmed in the other five tested individuals.

1. INTRODUCTION

This research is based on the population of Lejasbitēni cemetery (7th – 10th centuries CE), which is one of the few fully excavated Iron Age cemeteries in Latvia. Lejasbitēni cemetery was located near the modern-day town of Aizkraukle, on the right bank of the river Daugava, approximately 90 m

from the river (Figure 1). Daugava was one of the major river trade routes in the region during the Viking Age, providing direct access from the Baltic Sea up to the Dnieper and Volga trade routes (Atgāzis, 2001: 264-66). During the period in question, several ethnic groups lived in the territory of modern-day Latvia, and ancient Latgalians inhabited vast territories in the east (Vilcāne, 2018: 59 - 84).

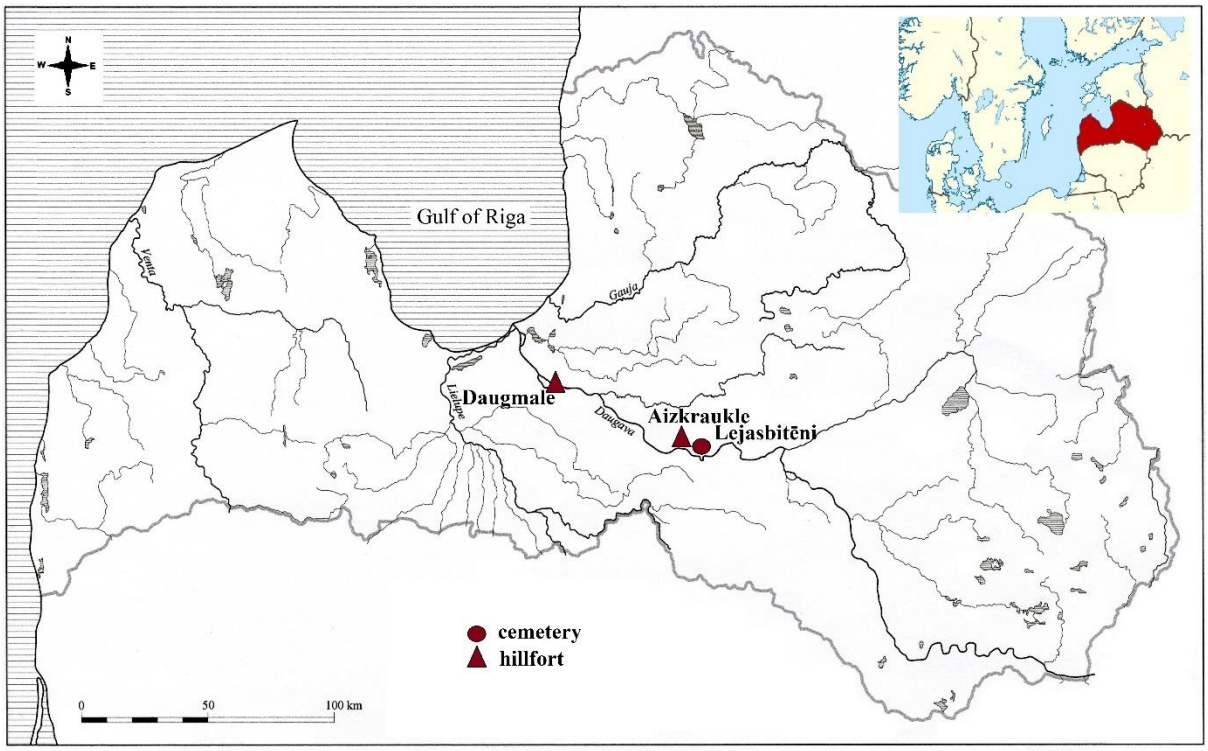
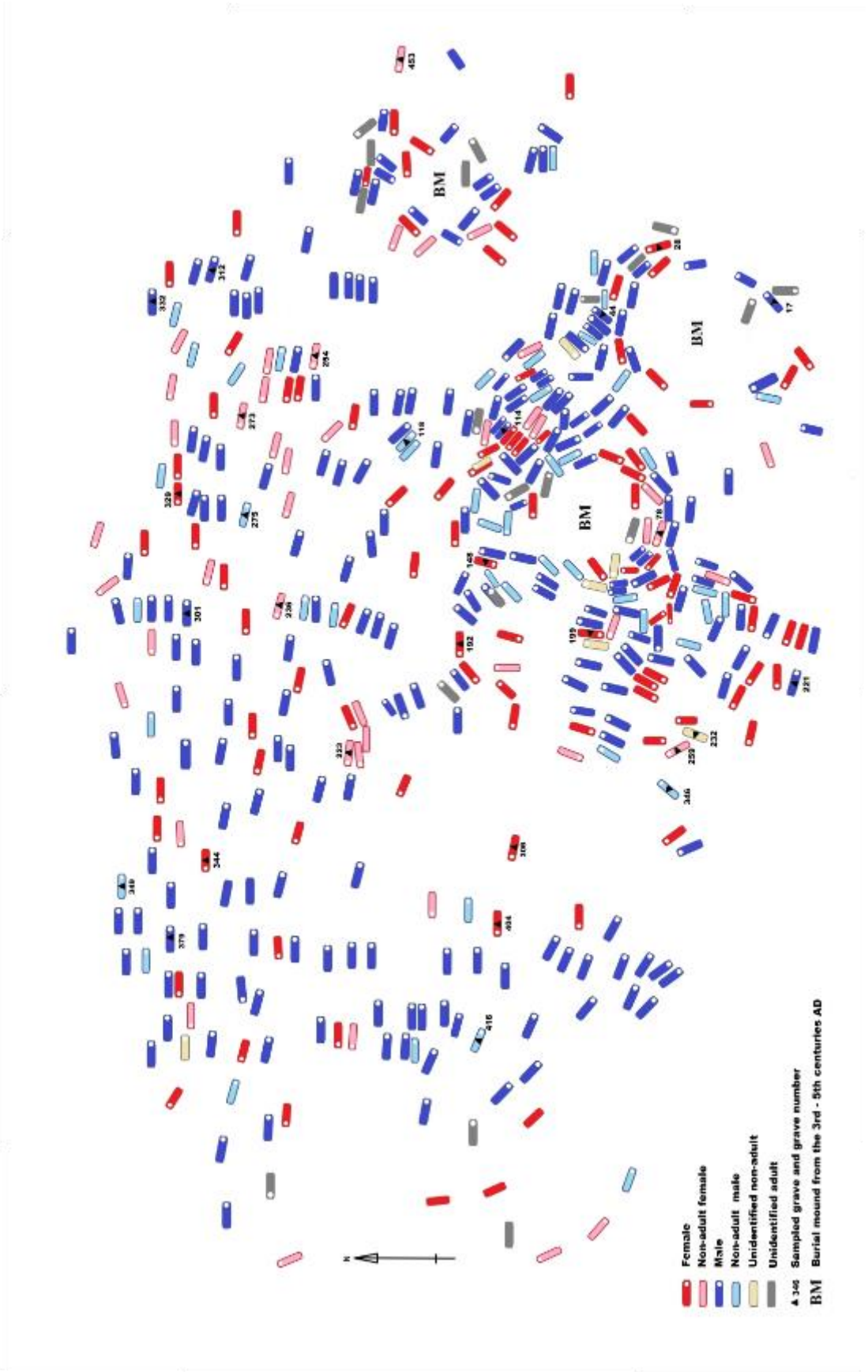


Figure 1 Map of Latvia showing the Lejasbitēni cemetery and the comparative archaeological sites with faunal isotope data. The map in the upper right corner shows the geographic location of Latvia

The period discussed in this study, between the 7th – 10th centuries CE corresponds to the second half of the Middle Iron Age, and the Late Iron Age in Latvia (Vasks, 2018: 28 - 38). This period partly coincides with the Viking Age in Northern Europe (around 750 – 1050 CE, Brink and Price, 2008). The main aim of this study is to evaluate if the development of the Viking Age brought about changes in social status as expressed by burial traditions and grave goods, and diet, in the Lejasbitēni population.

To achieve this, archaeological (burial traditions, grave goods), and osteological (biological sex and age), genetic (ancient DNA analysis), and biochemical (carbon and nitrogen stable isotope analysis) data, was used.

The cemetery was dominated by flat inhumation burials, which were organised around burial mounds from the chronologically earlier period, the 3^{rd} – 5^{th} centuries CE. The first burials from the 7^{th} century AD were arranged around the mounds, but later burials from the 9^{th} – 10^{th} centuries CE were placed in rows (Figure 2). The cemetery had been disturbed by historic and modern farming activities, as well as grave robbers.


BM
BM
BM
Female
Non-adult female
Male
Non-adult male
Unidentified non-adult
Unidentified adult
▲ 346 Sampled grave and grave number
BM Burial mound from the 3rd - 5th centuries AD

77

Figure 2 Plan of the Lejasbitēni cemetery showing burials by gender, and burials sampled for isotope analysis, redrawn and supplemented by A. Vilcāne from V. Urtāns (1970: 69; Figure 5). The oldest burials in the cemetery are concentrated around the three burial mounds (BM).

1.1 Gender and social status in the Lejasbitēni cemetery, as expressed by grave goods and as used for analysis in this research

In Latgalian cemeteries, it was common practice to bury male gender individuals with their heads to the east, and female gender individuals with their heads to the west (Urtāns, 1970: 68; Radiņš, 1999: 25 – 27; Šnē, 2002: 225 – 227; 243 - 247). Certain types of grave goods are also thought to represent male and female gender. Male and female gender burials at Lejasbitēni might correspond to the biological sex of the interred in many cases, as has been shown by high rates of correlation between biological sex and gender in Danish Iron Age burials, which also includes the Viking Age (Sellevold et al., 1984). Without osteological or biomolecular analysis, however, in this study individuals will be referred to according to their gender as expressed by grave goods and grave orientation. This approach by no means implies that there were only two gender categories in the Lejasbitēni population and accepts that gender might have been a complex concept, as has been pointed out previously (Arnold, 2006: 138–140; Moen, 2019). A more detailed study about this subject is outside the scope of the current research.

With regard to evaluating individual social status in the study of Iron Age populations from Latvia, grave goods have traditionally been used as a guide, applying qualitative and quantitative methods of analysis (Radiņš, 1999: 131 – 133; Šnē, 2002: 247 – 274). This study is using a similar approach but acknowledges that grave goods might not have necessarily belonged to the deceased during their lifetime. This is because burial does not represent the identity of the individual, but rather how that individual was seen by the community burying them (Parker Pearson, 1993; 1999). Accordingly, the meaning of grave goods can vary considerably, and their relevance to social status of the individual

105 remains only an assumption. As Carr (1995) has suggested, it is possible that only particular types of
106 grave goods can be regarded as representative of an individual's social status.
107
108 1.2 Ancient DNA and carbon and nitrogen isotope analysis
109
110 Ancient DNA analysis was employed as a small pilot study to aid interpretation of those burials
111 selected for dietary isotope analysis which had been disturbed or contained too little skeletal and/or
112 archaeological material in order to determine the gender of the individual. The other objective was
113 to aid osteological analysis in assessing the correlation between gender and biological sex in this
114 population.
115
116 Carbon and nitrogen stable isotope analysis was primarily used to achieve the main aim of this study,
117 so as to provide an insight into the diet of the Lejasbitēni population, and to study any differences in
118 diet between and within gender groups, as well as to assess if there was any link between childhood
119 diet and social status in adulthood. Previous archaelogical studies have shown that there can be a link
120 between higher social status as expressed in burial location and/or grave goods, and differential access
121 to resources, even though this can vary between age and sex groups (Błaszczyk et al., 2021; MacKinnon
122 et al., 2019). The wider importance of this new dataset is that to date, there is no published dietary
123 stable isotope data from any Iron Age population in Latvia, and few from the other Baltic States
124 (Bliujienė et al., 2020), resulting in a regional gap in knowledge. This research will thus provide much
125 needed comparative Baltic Iron Age dietary isotope data.
126
127 In archaeological studies, the application of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analysis has
128 become a useful and reliable tool for reconstructing diet. Diet and nutritional status is recorded
129 during the deposition of new bone and other body tissues (D'Ortenzio et al., 2015; Fuller et al., 2006)

130 from where this information can be extracted by measuring $\delta^{13}C$ and $\delta^{15}N$ values in collagen
131 (Schoeninger et al., 1983).
132
133 Carbon stable isotope ($\delta^{13}C$) analysis is mainly used to detect the presence of foodstuffs based on
134 plants with different photosynthetic pathways in the diet: C_3 plants, which grow worldwide and have
135 yielded values between -35 ‰ and -20 ‰, and C_4 plants which mainly grow in tropical regions, with
136 values between -14 ‰ and -9 ‰ (Katzenberg, 2008: 423). In diets dominated by C_3 plants, $\delta^{13}C$
137 analysis can detect the contribution of marine resources, which have $\delta^{13}C$ values between -5.0 ‰
138 and -17.0 ‰ (Schoeninger and DeNiro, 1984). $\delta^{13}C$ values in skeletal tissue are around 1 ‰ higher
139 than in diet (ibid.). Nitrogen stable isotope ($\delta^{15}N$) analysis provides information about the sources of
140 protein in the diet and shows the individual's position in the food chain (Hedges and Reynard, 2007).
141 In skeletal tissue, a dietary shift to a higher trophic level will be expressed as an increase of 2-5 ‰
142 (ibid.).
143
144 In order to obtain a dietary average for a population, bone is usually the preferred medium, as it has
145 a turnover rate between 10 and 25 years, depending on the skeletal element and the age of the
146 individual (Hedges et al., 2007). To enable comparability, the same skeletal element should be
147 sampled in all individuals (Beaumont, 2020). In this study, poor preservation of skeletal material and
148 the lack of post-cranial bones prevented the sampling of fragments from the same bone for bulk
149 bone collagen analysis, especially in children. To retain consistency, teeth were sampled instead for
150 bulk dentine isotope analysis. Teeth form at a constant rate and dentine, once formed, does not
151 remodel (Dean and Scandrett, 1995), locking in dietary information for the period of tooth formation
152 (Beaumont and Montgomery, 2016, Fuller et al. 2003). Unlike bone, which can cease to grow in
153 response to physiological stress (Beaumont et al., 2018; Beaumont, 2020), dentine keeps forming
154 during undernutrition (Elamin and Liversidge, 2013) and has been proven to record a variety of
155 factors, including breastfeeding, weaning, in-utero stress, and periods of dietary deficiency

(Beaumont and Montgomery, 2016; Beaumont et al., 2018; Crowder et al., 2019; King et al., 2018a). During breastfeeding, the child's $\delta^{15}N$ values rise one trophic level above the person breastfeeding them and rise of approximately 1 ‰ is also observed in $\delta^{13}C$ values (Fogel et al., 1989). A rise in $\delta^{15}N$ values in the body can also be caused by physiological and nutritional stress (Mekota et al., 2006; Reitsema and McIlvaine, 2014). The ability of dentine to record short-term dietary and physiological changes makes it a valuable tool in tracing individual isotopic life histories through incremental dentine studies. Analysing dentine collagen in a single measurement loses this detailed record, but the obtained data provide a very useful insight into the childhood diet of a population over a known period of time in their lives (King et al., 2018b; Whitmore et al., 2019).

2. MATERIAL

The Lejasbitēni cemetery was excavated in 1961–1964, led by V. Urtāns. In total, 453 burials were excavated in an area measuring approximately 7000 m^2, and according to grave goods the estimated date of the cemetery was between 7th and 10th centuries CE (Urtāns, 1965). The burials can roughly be divided into two chronological periods, Period 1 (7th – 8th centuries CE), and Period 2 (9th – 10th centuries CE). The chronology of the site is supported by radiocarbon (^{14}C) dates measured at the Poznan Radiocarbon Laboratory (Supplementary Material A, Table 1S). When the site was excavated, it was common archaeological practice to only collect skulls from cemeteries. This strategy thus limited osteological analysis.

The archaeological material has not been published in its entirety, but the documentation of the excavations, as well as all artefacts are curated, and are available for scientific research at the National History Museum of Latvia. The skeletal material from Lejasbitēni is curated at the Repository of Bioarchaeological Material, Institute of Latvian History, University of Latvia.

3. METHODS

182 3.1 Evaluation of archaeological data, and estimation of social status and gender

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184 The documentation from the original excavation was evaluated with regard to the types of burial
185 (flat burials with and without stones over them). The gender of each individual (male or female) was
186 estimated based on grave orientation and grave goods in Latgalian cemeteries. Social status was
187 estimated by grave goods (Table 1). It was based on the number of artefact types (NAT) in
188 undisturbed burials, counting each artefact type as one, following the categorisation developed by
189 Hedeager (1980, 1992). Accordingly, a higher NAT indicates a higher social status, as it could indicate
190 access to certain resources, and vice versa (ibid.; Šnē, 2002: 248-251). A male gender burial with
191 grave inventory typical of high social status, as categorised in this study, is shown in Figure 3.

192

193 Table 1 Criteria for the estimation of social status according to grave goods in the Lejasbitēni
194 population

Group	**Social status category**	Description of gender-specific grave goods and NAT			
		Male gender	**NAT**	**Female gender**	**NAT**
I*	Highest	Rich grave inventory including rare items (weapons or jewellery) the making of which require considerable skill (e.g., double edged swords, axes with ornamented bronze band around the shaft, owl fibulae), and clothing items decorated with bronze ornaments	6-7	Rich grave inventory including rare items uncharacteristic for female gender burials (e.g., axes with ornamented bronze band around the shaft), and woollen shawls with bronze ornaments	6-8
II	High	Rich grave inventory including weapons (1-2 spearheads, an axe, a broad battle knife), jewellery (crossbow fibulae, armbands, rings), a belt with fittings, and sometimes tools (a knife and an awl)	4-6	Rich grave inventory including jewellery (1-2 metal headbands, 1-2 neck rings, 1-2 necklaces from spirals, bells, ribbed tin rings, or cowrie shells, 2 pins, 2-4 armbands, 2-3 rings), sometimes an axe, and tools (a sickle and an awl)	4-7

III	Low	Selected items including an axe, a knife, an item of jewellery, etc.	1-3	Selected items including a necklace from spirals or ribbed tin rings, a pin, 2 armbands, a ring, etc.	1-3
IV*	Lowest	No items	0	No items	0

*No skeletal material from this group was available for isotope analysis, except the non–adult burial 416

Figure 3 Artefacts from a male grave No 435 at Lejasbitēni cemetery. The artefacts are stored in the National History Museum of Latvia (LNVM A11912:56-68). Photograph by A. Vilcāne

3.2 Age and sex estimation

The skeletal material available for macroscopic analysis was very limited, both due to the excavation strategies at the time of the excavation, and also poor preservation of the bones. Where possible, biological sex determination in adult individuals was based on the morphological characteristics of the skull and mandible (Buikstra and Ubelaker, 1994: 16-21). Age in adults was determined mainly by assessing skeletal fusion, including ectocranial and endocranial suture closure (Buikstra and Ubelaker, 1994: 21-26). The age in non-adults was determined by dental eruption (Schour and Massler, 1941) and/or development (AlQahtani et al., 2010). Individuals were considered non-adults if their biological age was less than 19-20 years (Buikstra and Ubelaker, 1994: 9).

3.3 Ancient DNA analysis

Ancient DNA analysis was primarily aimed to determine the biological sex of non-adult individuals who were also included in the dietary stable isotope analysis. Accordingly, seven individuals were selected for ancient DNA analysis. The methods used for ancient DNA analysis are given in Supplementary Material B.

3.4 Sampling strategy for dietary stable isotope analysis

For dietary isotope analysis, individuals were selected according to chronological periods, their gender, age, and social status according to grave goods (Table 4). In the female group, samples from the roots of the first permanent molar (upper or lower) were collected from nine adults and six non-adults, and in the male group, from eight adults and four non-adults, while samples from the roots

of deciduous second molars (upper and lower) were taken from two children, one from each gender group. Considering the lack of remodelling in dentine collagen, dietary isotope data from most people refers to the period of time when they were aged between approximately three and ten years (or the age at death in some children), while in two children with the second deciduous molars the data refers to the age between 10.5 months and three-and-a-half years (AlQahtani et al., 2010). All teeth were photographed prior to analysis to retain future reference.

Details on sample preparation and analysis for carbon and nitrogen stable isotope analysis are given in Supplementary Material C. Statistical analysis of the isotope results was calculated with the Mann-Whitney test using Vassar Stats Website for Statistical Computation (http://vassarstats.net/index.html).

4. RESULTS

4.1 Age, sex, and gender estimation

Twelve of the 453 excavated burials contained too little skeletal material to estimate an age (adult or non-adult), biological sex, or gender, so these burials were excluded from further analysis. Among the remaining individuals, there were 343 adults (77.78%) and 98 non-adults (22.22%) (Table 2). Of the adults, 230 were of male gender, and this was twice the number of female gender burials (103). In 10 adult individuals, gender could not be determined. Biological sex was only possible to estimate in 30% of adult individuals, mainly according to skull fragments. With regard to the non-adult individuals, the distribution of male and female gender was roughly 50:50, both overall and by age groups (Table 2). Six individuals could not be assessed for gender. There were no children younger than three years of age (Table 3).

Table 2 Lejasbitēni individuals by gender (n = 441)

Gender	%	Number of individuals
Adult males	52.15	230
Adult females	23.36	103
Adults	2.27	10
Non-adult males	10.43	46
Non-adult females	10.43	46
Non-adults	1.36	6
Total	100	441

252

253 Table 3 Lejasbitēni non-adults by age group and gender

Age group	Males	Females	Total
0 - 1	0	0	0
1 - 4	5	3	8
5 - 9	17	19	36
10 - 14	7	9	16
15 - 19	8	7	15
Non-adult (0-18)	9	8	17
Total	46	46	92

254

255 4.2 Results of ancient DNA analysis

256

257 Sequencing yielded 15 – 26 million reads per sample. After processing, the reads aligning to human
258 reference sequence hg38 were less than 2% for all samples. MapDamage authentication showed
259 the damage patterns characteristic of ancient DNA for burials 114, 346, 349 and 416, while reads
260 from burials 223, 232 and 236 did not show aDNA damage patterns. Burials 114 and 416 had more
261 than 80’000 and 110’000 reads aligning to GRCh38 and Ry ratios 0.0901 and 0.0844, respectively,
262 which are consistent with XY chromosomes and male sex. Burials 346 and 349 had 13524 and
263 13313 reads aligning to GRCh38, respectively, which is at limit of necessary number of reads for sex
264 determination using Ry, leading to very large standard error (Figure 1S, Supplementary material B)
265 and not allowing unambiguous sex determination.

266

267 4.3 Results of the carbon and nitrogen isotope analysis

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Carbon and nitrogen stable isotope results were processed after obtaining ancient DNA results to include individuals whose biological sex and gender was unknown or unclear upon sample selection due to insufficient contextual information and/or osteological material (Table 4).

272 Table 4 Individuals selected for carbon and nitrogen stable isotope analysis, and results of the carbon and nitrogen stable isotope analysis

Burial #	Gender	Sex	Age (years)	Sample	Age of root formation (years)	Period	Status	%N	$\delta^{15}N$ ‰ AIR	%C ‰ PDB	$\delta^{13}C$	C:N atomic
28	F	F	20 - 25	M^1	3.5-9.5	1	II	15.27	9.26	41.24	-22.71	3.2
78	F	?	18 - 20	M^1	3.5-9.5	1	III	14.28	9.69	40.51	-23.10	3.3
148	F	?	50 - 60	M^1	3.5-9.5	1	III	12.85	9.62	40.95	-23.30	3.7
192	F	F	25 - 30	M_1	3.5-10.5	1	II	14.29	9.59	41.86	-22.17	3.4
199	F	F	25 - 30	M_1	3.5-10.5	1	III	15.04	8.81	41.63	-22.61	3.2
223	F	?	9-10	M_1	3.5-10.5	2	III	15.21	9.21	42.21	-21.74	3.2
236	F	?	9-10	M^1	3.5-9.5	2	II	15.42	8.80	43.19	-22.81	3.3
254	F	?	7-8	M_1	3.5-8.5	2	III	15.53	8.75	42.90	-23.63	3.2
259	F	?	9-11	M_1	3.5-10.5	1	II	15.51	9.59	41.57	-22.62	3.1
273	F	?	15 - 17	M^1	3.5-9.5	2	II	15.44	9.77	43.63	-23.11	3.3
306	F	F	25 -30	M^1	3.5-9.5	2	III	15.55	9.56	42.58	-22.79	3.2
329	F	F	18 - 20	M^1	3.5-9.5	2	II	14.38	9.37	43.13	-23.26	3.5
344	F	F	45 - 50	M_1	3.5-10.5	2	II	15.45	10.04	42.73	-23.17	3.2
404	F	F	50 -60	M_1	3.5-10.5	2	III	15.10	9.39	42.55	-22.37	3.3
453	F	?	12-15	M^1	3.5-9.5	1	II	15.29	9.46	42.98	-22.96	3.3
17	M	?	>30	M^1	3.5-9.5	1	II	13.68	10.95	38.08	-22.35	3.2
44	M	M	40 - 50	M^1	3.5-9.5	1	II	15.40	10.09	42.45	-22.90	3.2
114	M	M^{**}	>30	M^1	3.5-9.5	1	III	15.63	9.55	42.87	-22.68	3.2
118	M	?	12-13	M^1	3.5-9.5	1	II	14.23	9.99	40.28	-22.16	3.3
221	M	M	45 - 50	M^1	3.5-9.5	1	III	15.51	9.32	43.13	-22.21	3.2
232*	?	?	4-5	m^2	0.9-3.5	1	III	15.23	11.50	43.29	-22.21	3.3

275*	M	?	8-10	m_2	0.9-3.5	2	II	15.46	10.58	42.56	-22.79	3.2
301	M	M	50 - 60	M^1	3.5-9.5	2	III	15.04	10.17	42.84	-22.49	3.3
312	M	M	50 - 60	M_1	3.5-10.5	2	III	15.21	10.31	42.19	-22.45	3.2
332	M	M	55 - 65	M_1	3.5-10.5	2	II	15.45	11.39	42.79	-21.97	3.2
346	M	?	9-10	M_1	3.5-10.5	2	III	14.68	10.07	42.48	-23.20	3.4
349	M	?	10-11	M_1	3.5-9.5	2	III	14.26	11.03	42.19	-22.84	3.5
379	M	M	20 - 25	M_1	3.5-10.5	2	II	15.76	10.81	41.80	-22.29	3.1
416	M	M**	10-11	M_1	3.5-10.5	2	I	15.34	10.37	42.54	-22.11	3.2

*-Deciduous tooth sampled; F- female; M-male; **-biological sex determined by ancient DNA analysis; M^1-upper first molar; M_1-lower first molar; m^2-deciduous upper second molar; m_2-deciduous lower second molar; I-highest social status; II-high social status; III-low social status; 1-Period 1 (7^{th}-8^{th} centuries CE); 2-Period 2 (9^{th}-10^{th} centuries CE)

The C:N ratio of sample from burial 148 was outside the acceptable range, and the results from this sample were therefore removed from all further analyses.

All male and female individuals are only referred to by their gender hereafter. $\delta^{15}N$ values in this population ranged from 8.75 ‰ to 11.50 ‰, while $\delta^{13}C$ values ranged from -23.63 ‰ to -21.74 ‰ (Figure 4, Table 5). Although most teeth used in the analysis had formed during the same period of life, adult and non-adult values were tested for significant differences between survivors (adults) and non-survivors (children). There were no significant differences between the groups, either for $\delta^{15}N$ values (for males, U=29, z=-0.58, p=0.5619; for females, U=18.5, z=0.94, p=0.3472) or $\delta^{13}C$ values (U=22.5, z=0.13, p=0.8966 for males, and U=26, z=0.06, p=0.9522 for females) therefore they were treated as a single group for all subsequent statistical tests, except for testing adult and non-adult groups with differential social status as expressed by grave goods.

Table 5 Summary of minimum and maximum $\delta^{15}N$ and $\delta^{13}C$ values in males and females, by gender

Gender	**$\delta^{15}N$ values ‰ AIR**				**$\delta^{13}C$ values ‰ PDB**			
	Min (burial)	Max (burial)	Mean	SD	Min (burial)	Max (burial)	Mean	SD
F	8.75 (254)	10.04 (344)	9.39	0.37	-23.63 (254)	-21.74 (223)	-22.76	0.54
M	9.32 (221)	11.50 (232)	10.44	0.64	-23.20 (346)	-21.97 (332)	-22.48	0.36

Overall, $\delta^{15}N$ values were significantly higher in males than females (U=192, z=-3.78, p=0.0002, Figure 4). When analysed by period, $\delta^{15}N$ values were significantly higher in males than females from the chronologically later period (Period 2; U=64, z=-3.31, p=0.0009), but not the earlier period (Period 1; U=33, z=-1.64, p=0.101). Between Period 1 and Period 2 males, $\delta^{15}N$ values appeared higher in Period 2, but the difference was not statistically significant (U=15, z=1.1, p= 0.2713). Likewise, for females of both chronological periods, the differences in $\delta^{15}N$ values were not statistically significant (U=34, z=-0.64, p= 0.5222). The $\delta^{15}N$ values of two adult male individuals with

low social status were overlapping with female $\delta^{15}N$ values (burial 221, aged 45-50 (9.32 ‰) and burial 114, older than 30 years (9.55 ‰)). Both of these individuals were from Period 1.

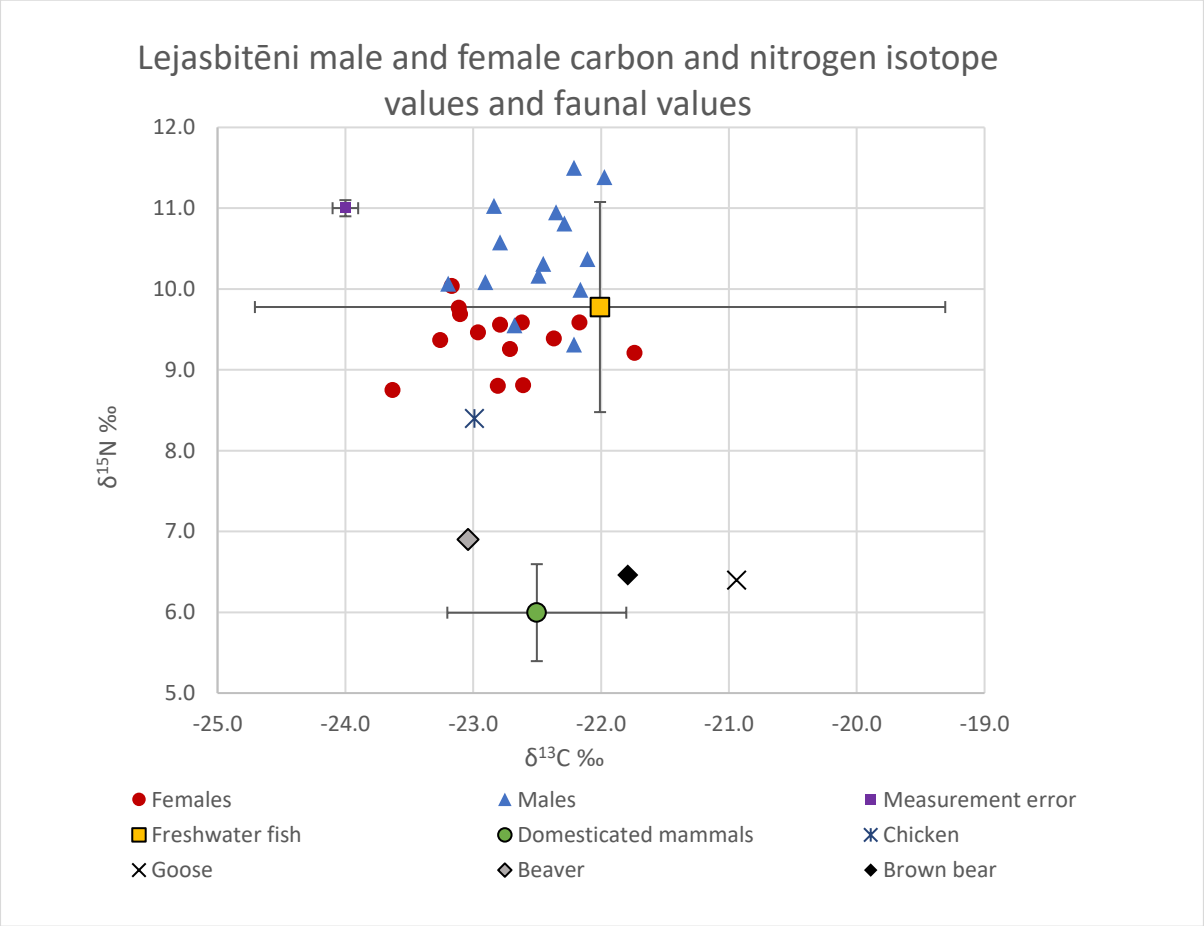

Figure 4 $\delta^{13}C$ and $\delta^{15}N$ values of male and female gender individuals in the Lejasbitēni cemetery. Measurement error is shown on the small square point at the bottom right. Data from Lejasbitēni is shown together with reference values of faunal remains from the Daugmale and Aizkraukle hillforts (11^{th}-12^{th} centuries CE), from Gunnarsone et al., 2020. Only mean values and standard deviation (error bars) are shown for freshwater fish and domesticated mammals

There were also significant differences in $\delta^{13}C$ values between males and females from both periods together (U=153.5, z=-2.09, p=0.0366), but not within Period 1 and 2 (for Period 1 males and females, U=32, z=-1.5, p=0.1336; for Period 2 males and females, U=44.5, z=-1.26, p=0.2077). The $\delta^{13}C$ values were not significantly different between individuals from both periods in either gender group (U=34, z=-0.64, p=0.5222 for females and U=27, Z=-0.32, p=0.749 for males).

Due to the small sample size, it was not possible to investigate if social status in children as expressed by grave goods was linked to differential diet (Figure 5A). With regard to investigating if childhood diet was linked to adult social status, no significant differences were observed either between high and low status males (N=8, U=2, Critical Value=0 for $\delta^{15}N$ values, and U=6, Critical Value=0 for $\delta^{13}C$ values) or females (N=8, U=7, Critical Value=0 for $\delta^{15}N$ values, and U=10, Critical Value=0 for $\delta^{13}C$ values) (Figure 5B).

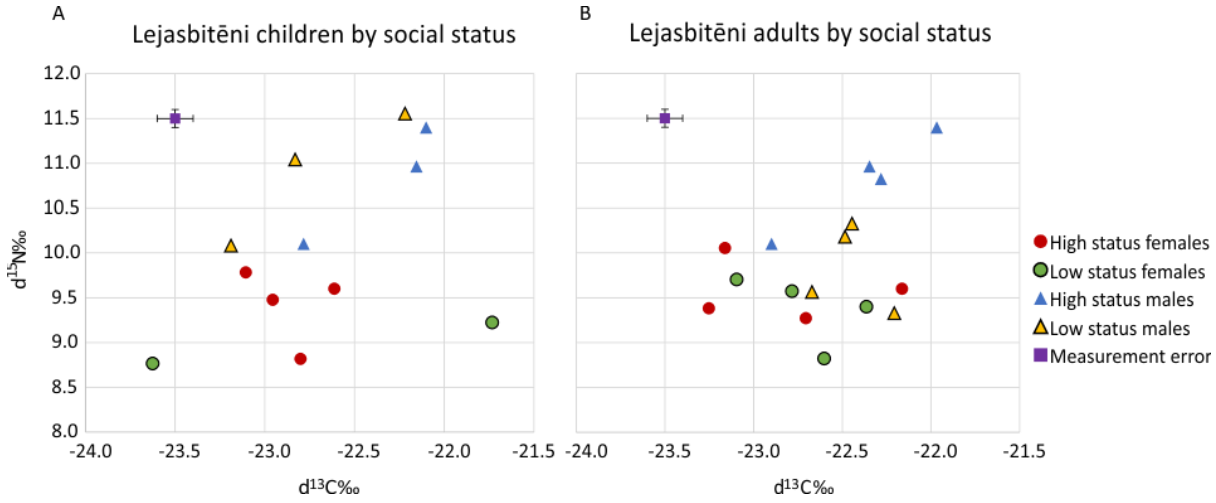

Figure 5 $\delta^{13}C$ and $\delta^{15}N$ values of male and female gender non-adults (A) and adults (B), according to social status as expressed by grave goods. Measurement error is shown on the square point at the top left

5. DISCUSSION

5.1 Diet of the Lejasbitēni population

To put the $\delta^{13}C$ and $\delta^{15}N$ values of the Lejasbitēni population in context, they were compared with data from faunal remains from two slightly more recent hillforts, Daugmale and Aizkraukle (in use between 11th – 12th centuries CE, Gunnarsone et al. 2020, Figure 1). The hillfort of Daugmale is

located approximately 60 km downstream from Lejasbitēni, and recently obtained bone collagen carbon and nitrogen stable isotope values were available for freshwater fish (N=13) of various species (bream, pike, zander, perch, chub, ide), and two domestic pigs (Gunnarsone et al., 2020). A few reference carbon and nitrogen isotope values were also available from the Aizkraukle hillfort near the Lejasbitēni cemetery for wild and domesticated mammals and domesticated birds (one each of beaver, brown bear, cow, horse, chicken, and goose) (ibid., Figure 4).

The difference between the mean human (9.9 ‰) and faunal (6.0 ‰) $\delta^{15}N$ values was 3.9 ‰, which is consistent with one trophic level (2 - 5 ‰, Schoeninger and DeNiro, 1984). This suggests that the main protein in the diet of the Lejasbitēni population probably came from domesticated mammals and/or their products, such as milk. The mean human and domestic faunal $\delta^{13}C$ values, however, were very similar (-22.5 ‰ and -22.6 ‰, respectively), thus not showing the expected rise of 1 ‰ in humans, if domestic animals were their only dietary source (Schoeninger and DeNiro, 1984). The relatively low $\delta^{13}C$ values in Lejasbitēni humans could partly be explained by the consumption of freshwater fish, especially given the variation seen in both $\delta^{13}C$ and $\delta^{15}N$ values of fish from Daugava (-26.01 ‰ to -17.5 ‰ and 6.62 ‰ to 11.22 ‰, respectively, Gunnarsone et al., 2020). Likewise, the abundance of fishbones from the two late Iron Age habitation sites (ibid.) strongly suggests that this aquatic resource was a substantial part of the diet of these populations.

Another plausible cause for the low $\delta^{13}C$ values in the Lejasbitēni human population could be the consumption of freshwater molluscs, which would have been readily available from the river Daugava, but with expected $\delta^{13}C$ and $\delta^{15}N$ values lower than freshwater fish (due to their low trophic level, and as demonstrated elsewhere by Fischer et al., 2007).

No evidence for the consumption of millet was observed in this population, although it was widely grown in Eastern Europe since the late Bronze Age, with $\delta^{13}C$ values between –11 ‰ and -9 ‰

(Antanaitis and Ogrinc, 2000; Mueller-Bieniek et al., 2019). The oldest site with evidence for millet in Latvia is the Late Bronze Age - early Iron Age Ķivutkalns settlement (650 cal. BC – 2nd century CE, Vasks and Zariņa, 2014). Most archaeological evidence for millet from Latvian sites was found in the late Iron Age layers in the Tērvete and Koknese hillforts (Rasiņš and Tauriņa, 1983: 154).

A striking difference between male and female $\delta^{15}N$ values was observed, with mean male values significantly (1.05 ‰) higher than mean female values. The difference in $\delta^{13}C$ values was also significant but small (0.28 ‰). As mentioned above, $\delta^{13}C$ and $\delta^{15}N$ values in dentine can hold information about various dietary and physiological changes over the time of the tooth formation. Although the sample size is small, it is unlikely that the significant differences between male and female children were due to random individual variation. Instead, it appears that the childhood diet for boys and girls was distinctly differential, with a lower proportion of animal protein in the girls' diet, and/or more of their protein coming from a source with a lower trophic level. The dietary data for all but two individuals in this population reflects the period from the age of three years onwards, which is beyond the age where breastmilk could supply a sufficient proportion of an individual's protein requirement to be detectable using isotope analysis (Halcrow et al., 2017). For this reason, differential periods of breastfeeding for male and female children can be ruled out as a possible cause.

Although the sample sizes are small for many of the comparisons made here, the fact that the significant differences in diet only occurred in individuals living in the chronologically later period are intriguing. These, along with changes in burial traditions, might suggest an external cultural influence, which will be discussed in more detail in the next section. This was also the case in several sites from the Orkneys, where a general trend towards increased hierarchy, including gender-based dietary differences, was observed with the advance of Viking Age (Barrett and Richards, 2004; Richards et al., 2006).

5.2 The link between possible external cultural influence and social differentiation in the Lejasbitēni population

The external cultural influences which were possibly responsible for significant differences in childhood diet in Period 2 burials in the Lejasbitēni population can also be traced in grave inventories, potentially pointing to increased social differentiation. For example, while most males in the earlier period were buried with weapons and relatively few personal jewellery items (for example, burials 114 and 221 only contained two spearheads and a knife, and an axe and a spearhead, respectively), in the later period male and female burials included numerous personal jewellery items (Figure 6).

Figure 6 Artefacts from a female grave No 344 at Lejasbitēni cemetery. The artefacts are stored in the National History Museum of Latvia (LNVM A 11847: 49-63). Photograph by A. Vilcāne

In fact, the most richly furnished graves of all people are from the chronologically later period, with some adult female gender and non-adult burials including very rare items, such as axes with a

406 decorative bronze strip around the handle (Figure 7). These items are considered very rare, with a
407 total of 25 found in Latgalian cemeteries so far. Axes with handles decorated in this way have
408 traditionally been regarded as a part of rich male "warrior" grave inventory, only associated with
409 male gender individuals of a very high social status, especially in the earier Iron Age periods (Atgāzis,
410 2019: 132). The rare occasions when axes with decorated handles appear in female gender graves
411 from the 8th – 9th centuries CE have been interpreted as a sign of a particular, probably new, social
412 role for these individuals (Atgāzis, 1964: 118; Urtāns, 1970: 73). Five out of nine such axes at
413 Lejasbitēni were placed in adult male gender graves, just one in an adult female gender grave, and
414 three in non-adult male gender graves, including burial 416, which was one of the most richly
415 furnished graves in the cemetery (Figure 7). Six of these axes are dating from Period 2. The
416 appearance of richly furnished non-adult graves in Period 2, alongside those of other children of a
417 similar age, whose graves only contained a few simple items, mimics the trend towards increased
418 social differentiation through the distribution of grave goods in the chronologically later period seen
419 in adult burials.
420
421
422
423

Figure 7 Artefacts from a non – adult grave No 416 at Lejasbitēni cemetery. The artefacts are stored in the National History Museum of Latvia (LNVM A 11847: 454-464). Photograph by A. Vilcāne

A new burial tradition also appears in Period 2, whereby the flat grave is covered with stones. In total, 66 graves in Lejasbitēni were covered with stones, all relating to the chronologically later period. Adult males had been buried in 53 of these graves, while only seven individuals were adult females, four were non-adult males, and two were non-adult females. Some of these stone burials proved to be among the most elaborately furnished in the Lejasbitēni cemetery, including 346, 349, 379, and 416. Stones were commonly placed on top of inhumation burials in other Viking Age Scandinavian cemeteries, for example, Gotland, Sweden (Kosiba et al., 2007). Their presence in the

Lejasbitēni cemetery during the Viking period, compared to the earlier period, suggests possible cultural influence in this population, and strontium analysis is under way to investigate the geographic origin of the interred individuals.

While burial traditions in the Lejasbitēni population were consistent with increased hierarchy during the Viking Age, it is currently unclear if social status was linked to differential access to resources. No significant differences in $\delta^{13}C$ and $\delta^{15}N$ values were found between adult individuals with and without rich grave inventories, suggesting that in the tested individuals there was no relation between childhood diet and social status in adulthood, although the sample size was very small. It was not possible to investigate this link in children. It is also not currently clear if the distinct dietary differences between boys and girls continued into adulthood, and if there were dietary differences between adults with differential grave inventories. This will be addressed by further dietary isotope analysis in a forthcoming study about Iron Age populations in Latvia, including Lejasbitēni.

6. CONCLUSIONS

The pilot study of ancient DNA analysis in this population confirmed that in the few tested individuals the estimated gender was consistent with their biological sex.

The significantly different $\delta^{13}C$ and $\delta^{15}N$ values between male and female gendered individuals suggested distinct dietary differences between boys and girls. Because the dietary differences were only significant in the later period, it is possible that this was a part of cultural changes also reflected in burial traditions in this population, which were consistent with the development of the Viking Age in the region. A larger and more diverse sample size would be necessary to further explore dietary changes and differences between people living at Lejasbitēni during different periods.

No relation was found between childhood diet and social status in adulthood. A larger sample size which also includes bone from adult individuals, is needed to explore the link between social status and diet in this population further.

The most significant differences in social status observed in this study were expressed in burial traditions and were apparent in adults and children of both genders. In Period 2 burials, a shift towards increased social differentiation was observed, expressed in more richly furnished graves for particular adult and non-adult individuals of both genders, the inclusion of rare grave goods for more individuals, and a new burial tradition of covering the grave with stones, compared to the earlier period. The changes in burial traditions in the chronologically later period were interpreted as a result of possible cultural influence, similar to changes observed in other Northern European populations during the Viking Age.

This study has achieved its main aim and provided more understanding about how social status and diet in the Lejasbitēni population changed over time, also revealing rich potential for future research not only in the Lejasbitēni population, but also other Iron Age populations in the region. The childhood dietary isotope data obtained is an important addition to Iron Age studies in the Baltic States.

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638

Supplementary Material A

Table 1S List of ^{14}C dates of human bone samples from Lejasbitēni cemetery. All dates were obtained during the current research. The dates were calibrated by OxCal v4.4.2 (Bronk Ramsey, 2009) using IntCal20 (Reimer et al. 2020) and are reported with ranges rounded out to the next 5 years (Millard 2014)

No.	Burial*	Sample	Lab no.**	14C age (BP)	Cal CE (68.3%)	Cal CE (95.4%)
1	17	Bone	Poz-136767	1385 ± 30	610 (7.3%) 620 CE 640 (61.0%) 665 CE	600 (95.4%) 680 CE
2	82	Bone	Poz-136853	1270 ± 30	680 (59.7%) 750 CE 755 (8.6%) 770 CE	660 (84.8%) 780 CE 785 (10.7%) 830 CE
3	113	Bone	Pozs-117605	1270 ± 30	680 (59.7%) 750 CE 755 (8.6%) 770 CE	660 (84.8%) 780 CE 785 (10.7%) 830 CE
4	119	Bone	Poz-136855	1370 ± 30	660 (33.4%) 685 CE 740 (34.9%) 775 CE	650 (51.9%) 710 CE 720 (43.6%) 775 CE
5	264	Bone	Poz-136905	1235 ± 30	700 (24.2%) 740 CE 785 (34.1%) 830 CE 850 (9.9%) 875 CE	680 (33.4%) 750 CE 755 (62.0%) 885 CE
6	265	Bone	Poz-136907	1205 ± 30	780 (68.3%) 880CE	700 (7.2%) 740 CE 770 (87.4%) 895 CE 930 (0.9%) 945 CE
7	259	Bone	Poz-136908	1225 ± 30	705 (9.1%) 725 CE 785 (40.8%) 835 CE 845 (18.4%) 880CE	685 (22.4%) 745 CE 770 (73.1%) 890 CE
8	275	Bone	Poz-136909	1190 ± 30	775 (11.4%) 795CE 805 (1.6%) 810 CE 820 (55.3%) 890 CE	705 (1.6%) 725 CE 770 (88.0%) 900 CE 920(5.8%) 955 CE
9	349	Bone	Poz-136911	1125 ± 30	890 (11.5%) 905 CE 910 (56.8%) 980CE	770 (2.3%) 785 CE 830(2.5%) 850 CE 875 (90.6%) 995 CE
10	393	Bone	Poz-117606	1265 ± 30	6795(60.1%) 750 CE 755 (8.2%) 770 CE	665 (79.7%) 780 CE 785 (13.8%) 830 CE 855 (1.9%) 875 CE
11	416	Bone	Poz-117607	1130 ± 30	885 (12.0%) 905 CE 911 (56.3%) 980 CE	770 (3.0%) 790 CE 830 (3.5%) 855 CE 870 (88.9%) 995 CE
12	435	Bone	Poz-136855	1210 ± 30	780 (41.4%) 835 CE 840 (26.9%) 880CE	700 (9.7%) 745 CE 770 (85.7%) 895 CE

*Archaeological context information given as it appears in primary documentation and/or publications

**Laboratory code “Poz” = Poznan Radiocarbon Laboratory, Poland

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Supplementary Material B

Methods and results of ancient DNA analysis for human bone samples

DNA extraction was based on the protocol by Keyser-Tracqui & Ludes 2005. Bone or tooth samples were washed with 10% sodium hypochlorite, 70% ethanol and deionized water to remove surface contaminants. The samples were then UV irradiated for 30 minutes from each side, covered and left to air dry overnight. When dry, the samples were ground up in a Retsch Cryomill using one 30 second cycle of grinding. 0.5 grams of the powder were transferred to a 5 ml Eppendorf tube together with 2 ml of digestion buffer containing 5mM EDTA, 2% SDS, 10 mM pH 8.0 Tris-HCl, 0.3M NaOAc and 1 mg/ml proteinase K. One tube containing only the incubation buffer was added as a blank control, and all further operations were also carried out on the blank. The tubes were incubated for 36 hours at 50 °C with constant agitation to keep the powder suspended.

After digestion the tubes were centrifuged at 1000g for 10 minutes. The supernatant was transferred to a new 5 ml tube and 2 ml of phenol-chloroform-isoamyl alcohol (25:24:1) were added, the mix was vortexed and centrifuged at 1000g for 10 minutes. The supernatant was transferred to a new 5 ml tube, 2 ml of chloroform were added and the tube was vortexed and centrifuged at 1000g for 10 minutes. One 1 ml of the supernatant containing the aDNA was transferred to a new tube for further purification, the rest of the supernatant was stored at -20 °C.

aDNA was purified using the Zymo Research Genomic DNA Clean & Concentrator-10 kit. 4 ml of ChIP DNA Binding Buffer were added to the 1 ml of supernatant and mixed by pipetting. 1 ml of the mix was put into the spin column and the column was centrifuged in a tabletop centrifuge for 30 seconds. The filtrate was discarded, and the rest of the aDNA mix was centrifuged through the column. The column was then washed by adding 200 µl of Wash Buffer and centrifuging for 1 minute. The wash was repeated for a total of three times. The column was transferred to a new LoBind 1.5 ml tube and aDNA was eluted by adding 30 µl of nuclease-free water, incubating at room temperature for 1 minute and centrifuging for 1 minute.

Libraries were prepared using Qiagen Ultralow Input Library Kit for Illumina according to the manufacturer's protocol. The quality of the libraries was checked using Agilent 2100 Bioanalyzer with High Sensitivity DNS Kit. When necessary, library reads were size selected using Nucleomag NGS

Clean-up and Size Select Kit. The libraries were sequenced on a Illumina NextSeq 550 Series machine.

The sequenced reads were quality checked using FastQC (Andrews 2010), trimmed using Trimmomatic (Bolger *et al.* 2014) with the trimming steps ILLUMINACLIP:TruSeq3-SE.fa:2:30:10 SLIDINGWINDOW:4:20, aligned to human reference sequence hg38 using bwa mem (Li 2013) with default settings, duplicates were removed using samtools markdup (Li *et al.* 2009) and the aligned reads were filtered for softclipped reads using samclip (Seeman 2018). The authenticity of the aDNA was checked using mapDamage (Jónsson *et al*. 2013) and sex was determined by the ratio of reads aligning to X and Y chromosomes, using the script described in Skoglund *et al.* 2013.

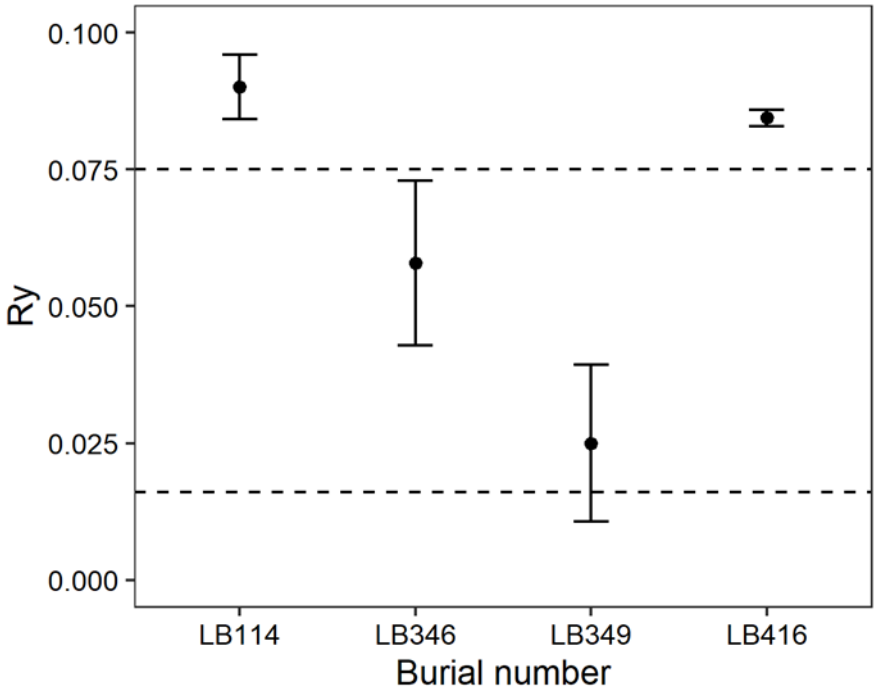

Figure 1S Ry values with standard error for burials 114, 346, 349, 416. Dashed lines represent the limit for assignment of male (top line, 0.075) and female (bottom line, 0.016) sex

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Supplementary Material C

Carbon and nitrogen stable isotope sample preparation and analysis

Initial sample preparation and collagen extraction was carried out in the Archaeological Isotope and Peptide Research Laboratory (AIPRL), Durham University where all samples were cleaned and abraded using a tungsten carbide dental drill to remove surface contamination. Collagen extraction was carried out following the procedure outlined in O'Connell and Hedges (1999). Approximately 300mg of cleaned bone was demineralised in 0.5M HCl at 4°C for several days then rinsed thoroughly in ultra-pure water (18.2MΩ·cm). Following this, samples were gelatinised in a pH 3 solution of HCl at 70°C for 48 hours, after which insoluble residues were removed using Ezee® filters. The filtered samples were then frozen at -18°C and freeze dried for 48 hours, the resultant collagen was then weighed into tin capsules. Stable isotope analysis was carried out in the Stable Isotope Biogeochemistry Laboratory (SIBL), Durham University using a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer. Calibration using internal reference samples (e.g., Glutamic Acid, Glycine, SPAR and Urea) and international reference standards (e.g., USGS 24, USGS 40, IAEA 600, IAEA N1, IAEA N2) determined a standard deviation of ±0.1‰ (1σ) for collagen carbon and nitrogen isotopes.

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