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Ruminant animal milk in ceramic baby bottles from European prehistoric child graves

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Abstract – 200 words

The study of childhood diet, including breastfeeding and weaning, has important implications for infant mortality, early and later life health and fertility in past societies[1]. Nitrogen stable isotopic analyses of infant bone collagen and dentine have provided information on the timing and duration of weaning[2], yet little is known of what foods were consumed by infants in prehistory. Possible infant feeding vessels, made from clay, first appear in Europe in the Neolithic, becoming more commonplace throughout the Bronze and Iron Ages. However, these vessels, complete with a spout, through which liquid could be poured, have also been suggested to be feeding vessels for the sick or infirmed[3,4]. Here, we report the first unequivocal evidence for the foods contained in such vessels based on lipid 'fingerprints' and the compound-specific $\delta^{13}C$ and $\Delta^{13}C$ values of the major fatty acids (FAs) from three small, spouted vessels found in Bronze and Iron Age infant graves in Bavaria. The results confirm the vessels were used for feeding ruminant milk products to infants, possibly mixed with small amounts of meat broth. This first direct evidence of the type of foodstuffs used either to feed or wean prehistoric infants confirms the importance of animal milk from domesticates for these early communities and provides the first direct information on infant feeding behaviours practised by prehistoric human groups.

The study of past infancy, including infant care, breastfeeding and weaning practices, provides valuable information on population demographics and health, reproduction rates, mortality patterns and fertility in past societies. Today, feeding practices for babies and young infants can be attributed to various ecological and socioeconomic constraints and cultural factors, such as health beliefs and food taboos[1,5]. Prehistoric humans likely practised a range of infant feeding behaviours[2,3,4], with profound consequences for biological and social wellbeing. Ethnographic, historical and social studies show differences across the breastfeeding phase, the nature of the addition of supplementary foods (during weaning) and the timing of complete cessation of breastfeeding[1,5,6].

Breastfeeding is integral to infant care of all human (mammal) groups and fundamental to the mother-infant relationship[4]. Breast milk provides an infant with all the macro- and micronutrients required to sustain growth for the first six months of life[7], together with bioactive components, which protect the infant from pathogenic organisms and facilitate immune system development and maturation[8]. The introduction of energy and nutrient-rich, easily digestible, supplementary foods in infant feeding (known as weaning) is unique to humans[9,10]. This is an evolutionarily derived (i.e. apomorphic) species characteristic that coevolved with changes in life history and physiology, reducing the maternal costs of lactation[1,10]. Supplemental foods are generally introduced at around six months, when the metabolic requirements of an infant exceed what the mother can provide through milk energy yield, contributing to the infant diet as chewing, tasting and digestive competencies develop[1,10,11].

Considerable variation exists in the practice and duration of breastfeeding and the subsequent addition of supplementary/weaning foodstuffs between human groups. Hunter-gatherers typically breastfeed for several years, whereas adoption of a sedentary lifestyle in early farming communities led to shortening of the breastfeeding period[12], likely due to the introduction of agriculture, when new foods became available for weaning infants, e.g. animal milk and cereal products. The widespread use of animal milk, either to feed babies or as a supplementary weaning food, became possible with the domestication of dairy animals during the European Neolithic[12], leading to improved nutrition and contributing to increased birth rate, with shorter inter-birth intervals, resulting in significant growth in human population: the so-called Neolithic Demographic Transition[13]. Broad trends identified from the Neolithic to Iron Age in

Central Europe suggest supplementary foods were given to babies at around 6 months, with weaning complete by 2-3 years[3].

Possible infant feeding vessels, made from clay, first appear in Neolithic Europe. One of the earliest finds is a Linear Pottery Culture (LBK) feeding vessel from Steigra, Germany, dating to *c*. 5500-4800 BC[14]. These unique vessels, with a small spout through which liquid could be poured or suckled, come in many forms and sizes and, occasionally, of zoomorphic design (Fig. 1). They become more common in Central Europe during the Late Bronze and Early Iron Age[4], being found in settlements, as stray finds, and in graves, particularly those of children, strongly suggesting they were feeding or weaning vessels for infants.

The precious nature and, often small, openings of these vessels makes their sampling for organic residue analysis extremely challenging. However, infant feeding vessels of open, bowl form, found in graves in cemeteries of Dietfurt-Tankstelle and Dietfurt-Tennisplatz, recently became available for chemical analysis. The graves are part of a large Early Iron Age cemetery complex, dating to *c*. 800–450 BC, in the lower Altmühl valley in Bavaria, the former encompassing 99 burials in 72 graves[20] and the latter 126 burials[21]. Child grave 80 at Dietfurt-Tennisplatz contained an east-west oriented inhumation of a young child (0-6 years old), of which only parts of the cranium and long bones were preserved[21]. Feeding vessel 1 (Fig. 2a), was placed at the child's feet, with a small bronze bracelet found where the left arm of the child would have been. Grave 65 at Dietfurt-Tankstelle comprised the inhumation of a *c*. one-year-old child, placed extended on the back with the head oriented to the south and the child's arms folded over the upper body. Feeding vessel no. 2 (Fig. 2b), shaped like a small pipe, was found within a bowl deposited at the right hip[20]. Both vessels were of similar size, *c*. 50 mm diameter, although vessel 1 has a much shorter spout. A further broken vessel (3), cremation burial of a 1-2 year old child at Augsburg-Haunstetten 1, Bavaria[22], a Late Bronze Age necropolis (*c*. 1200-800 BC), was also analysed.

Organic residue analyses were performed largely as described in earlier publications[23,24], except that a modified sampling procedure was adopted for vessels 1 and 2 to minimise damage to these precious artefacts. Part of the internal surface layer of vessel was removed by abrasion, to remove contamination, then the underlying fabric taken as a powder for analysis of absorbed organic residues (ca. 0.84 and 0.33 g for vessels 1 and 2, respectively). A small fragment (ca. 0.95 g) of vessel 3 was destructively sampled after surface cleaning by abrasion and grinding

to powder. Appreciable lipid (29.7, 1.5 and 0.9 mg g^{-1}) was recovered from vessels 1, 2 and 3, respectively (Table 1), using acidified methanol extraction, suggesting the vessels had been used in sustained processing/consumption of high lipid-containing commodities. All extracts were dominated by palmitic (C_{16}) and stearic (C_{18}) fatty acids (FAs), typical of degraded animal fat[24] (Fig. 3a,b,c) with vessels 1 and 3 also comprising long-chain fatty acids (in low abundance), containing C_{20} to C_{28} carbon atoms (Fig. 3a,c), likely originating directly from the ruminant animal's plant diet[26]. Shorter-chain FAs (C_{12} and C_{14} in vessel 1 and C_{14} in vessels 2 and 3, Fig. 3a,b,c), rarely detected in archaeological pottery, were also present. The latter are likely remnants of C_4 to C_{14} FAs only seen in fresh milk fats[25,27], as the shorter chain homologues $\leq C_{10}$ have been shown to be lost in degradation experiments via leaching or volatilisation[23].

Further characterisation of the fats was achieved through stable carbon isotope ($\delta^{13}C$) values of the $C_{16:0}$ and $C_{18:0}$ FAs[23,25,28]. Vessels 2 and 3 plot just outside the reference range for dairy products (Fig. 3d), suggesting these were primarily used to process ruminant dairy products, with vessel 1 (Fig. 3d) plotting between the dairy and non-ruminant adipose ranges, indicating minor mixing of non-ruminant (pig or, possibly, human milk) and dairy products. $\Delta^{13}C$ values obtained for the lipid residues from vessels 1, 2 and 3, at -3.4, -3.7 and -3.6 ‰, respectively, plot in the ruminant dairy region, consistent with the processing/feeding predominantly of dairy products in these vessels[28] (Fig. 3e). We interpret the results from vessel 3 with caution due to the presence of minor, possibly contaminating compounds, however, as this was a cremation these could be pyrolytically-derived if the baby bottle was included in the funeral pyre.

Since the $\Delta^{13}C$ values lie at the top of the range for dairy fats the vessels were also analysed by solvent extraction[23] using high-temperature gas chromatography (HTGC) and high-temperature gas chromatography-mass spectrometry (HTGC-MS) for diagnostic intact acyl lipids[25]. Figure 3f clearly shows triacylglycerols (TAGs) and their degradation products, di- and monoacylglycerols (DAGs and MAGs) were present in vessel 2, with TAGs comprising C_{40} to C_{54} carbon atoms, C_{48} being the most abundant. The latter were not detectable in vessel 1 and 3 indicating complete diagenetic hydrolysis of the acyl lipids. Fresh adipose fats are characterised by TAGs containing 48 to 54 carbon atoms whereas dairy fats are distinguished by TAGs containing 24 to 54 carbon atoms[27]. While shorter chain TAGs (24 to 38) are rarely seen in degraded archaeological fats due to diagenetic loss (also demonstrated experimentally[23]), C_{40} to C_{46} TAGs are highly diagnostic of dairy fats[23,25]. In summary, our

findings provide unequivocal evidence that all three vessels were used predominantly to process dairy fats.

A wide variety of methods for artificially feeding infants in the past have been documented, including using bottles or feeding vessels made from ceramic (e.g. Figs 1 and 4), glass, wood, silver and pewter, animal horns, suckling bags and feeding tubes, as well as feeding directly from the teat of an animal[17,29]. What went into the vessels likely varied. Historical writers, including Aristotle and Galen, discuss various foodstuffs as additives to, or substitutes for, breastfeeding, including animal milk, milk products, honey, wine, bread and eggs[19,29]. Recipes for pap, gruel and panada[19], semi-solid foods comprising combinations of cereals, bread or flour, cooked in water, milk or broth, first appear in the medieval and renaissance literature. The low nutritional value of these mother's milk replacements would have had potential health consequences possibly resulting in high morbidity and mortality rates[4,19,29].

The finding of these three obviously specialised vessels in child graves combined with our new chemical evidence points strongly to these vessels having been used to feed animal milk to babies (in place of human milk) and/or during weaning onto supplementary foods. Interestingly, lipid evidence of feeding other products to infants, such as honey (beeswax residues often taken to indicate honey use) or plants, as suggested by historical writers, is lacking, although the carbon isotope data suggest that the vessels may have occasionally held ruminant carcass products. Given the narrowness of the spout this must have been a very thin solids-free gruel or broth, to provide valuable extra nutrients.

Although ruminant animal milk may have provided a valuable extra source of nutrition, it is important to note its potential negative effect on infant health[12]. Milks are species-specific and there are key differences in the composition of human and ruminant milk. Animal milk could have been used as a supplementary food but it would not have been a full replacement for human milk, which contains similar amounts of lipid, more carbohydrate (in the form of lactose) but considerably less protein. These differences might affect an infant in various ways. For instance, cow's milk is harder for an infant to absorb as it contains higher quantities of saturated FAs and much larger fat globules than human milk[30], causing reduced energetic input for the infant. The processing of animal milk and the incorporation of meat-based gruel may have served to balance out nutritional deficiencies. However, introduction of inappropriate supplementary foods would have provided an opening for infectious agents and pathogens,

causing diarrhoeal and other diseases, putting the infant at greater risk of iron-deficiency anaemia[12]. They may also have been nutritionally inadequate, leading to malnutrition and detriment to future development. Furthermore, feeding unpasteurised animal milk comes with a risk of contamination and transmission of zoonoses[4], with bacterial contamination from the vessel itself also possible. Notwithstanding the latter, our discovery of ruminant milk-based foods in these prehistoric baby bottles offers a rare glimpse into the ways prehistoric families were attempting to deal with the challenges of infant nutrition and weaning at this inherently risky phase of the human lifecycle.

Method summary

Lipid analyses were performed largely as described in earlier publications[22,23], except that a sampling procedure was adopted to minimise damage to these precious artefacts. Part of the internal surface layer of vessel was removed by abrasion, to remove contamination, then smaller samples than usual of the underlying fabric taken as a powder for analysis of absorbed organic residues (e.g. [22,23]). All solvents used were HPLC grade (Rathburn) and the reagents were analytical grade (typically > 98% of purity). An internal standard, typically 40 µL, was added to enable quantification of the lipid extract (*n*-tetratriacontane; Sigma Aldrich Company Ltd). Following the addition of 5 mL of H_2SO_4/MeOH 2 - 4% ($\delta^{13}C$ measured), the culture tubes were placed on a heating block for 1 hour at 70 °C, mixing every 10 min. Once cooled, the methanolic acid was transferred to test tubes and centrifuged at 2500 rpm for 10 min. The supernatant was then decanted into another furnace culture tube (II) and 2 mL of DCM extracted double distilled water was added. In order to recover any lipids not fully solubilised by the methanol solution, 2 x 3 mL of hexane was added to the extracted potsherds contained in the original culture tubes, mixed well and transferred to culture tube II. The extraction was transferred to a clean, furnaced 3.5 mL vial and blown down to dryness. Following this, 2 x 2 mL hexane was added directly to the H_2SO_4/ MeOH solution in culture tube II and whirlimixed to extract the remaining residues. This was transferred to the 3.5 mL vials and blown down under a gentle stream of nitrogen until a full vial of hexane remained. Aliquots of the fatty acid methyl esters (FAME's) were derivatised using *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1 % *v/v* trimethylchlorosilane (TMCS; Sigma Aldrich Company Ltd.; 20 µL; 70°C, 1 h). Excess BSTFA was removed under nitrogen and the derivatised FAME was dissolved in hexane prior to analysis by gas chromatography (GC), gas chromatography–mass

spectrometry (GC–MS) and gas chromatography–combustion–isotope ratio mass spectrometry (GC–C–IRMS).

Further analysis was carried out using the solvent extraction method. An internal standard was added to the sherd powder and they were solvent extracted by ultrasonication (chloroform/methanol 2:1 *v/v*, 30 min, 2 x 10ml). The solvent was evaporated under a gentle stream of nitrogen to obtain the total lipid extract (TLE). Aliquots of the TLE were trimethylsilylated (*N,O*-bis(trimethylsilyl)trifluoroacetamide 20 µL, 70° C, 1 h), and submitted to analysis by HTGC and HTGC-MS.

All FAMEs initially underwent high-temperature gas chromatography using a gas chromatograph (GC) fitted with a high temperature non-polar column (DB1-HT; 100% dimethylpolysiloxane, 15 m x 0·32 mm i.d., 0.1 µm film thickness). The carrier gas was helium and the temperature programme comprised a 50°C isothermal hold followed by an increase to 350°C at a rate of 10°C min^{-1} followed by a 10 min isothermal hold. A procedural blank (no sample) was prepared and analysed alongside every batch of samples. Further compound identification was accomplished using gas chromatography-mass spectrometry (GC-MS). FAMEs were then introduced by autosampler onto a GC-MS fitted with a non-polar column (100% dimethyl polysiloxane stationary phase; 60 m x 0.25 mm i.d., 0·1 µm film thickness). The instrument was a ThermoFinnigan single quadrupole TraceMS run in EI mode (electron energy 70 eV, scan time of 0·6 s). Samples were run in full scan mode (*m/z* 50–650) and the temperature programme comprised an isothermal hold at 50°C for 2 min, ramping to 300°C at 10° min^{-1}, followed by an isothermal hold at 300°C (15 min). Data acquisition and processing were carried out using the HP Chemstation software (Rev. C.01.07 (27), Agilent Technologies) and Xcalibur software (version 3.0). Peaks were identified on the basis of their mass spectra and gas chromatography (GC) retention times, by comparison with the NIST mass spectral library (version 2.0).

Carbon isotope analyses by GC-C-IRMS were also carried out using a GC Agilent Technologies 7890A coupled to an Isoprime 100 (EI, 70eV, three faraday cup collectors *m/z* 44, 45 and 46) via an IsoprimeGC5 combustion interface with a CuO and silver wool reactor maintained at 850°C. Instrument accuracy was determined using an external FAME standard mixture (C_{11}, C_{13}, C_{16}, C_{21} and C_{23}) of known isotopic composition. Samples were run in duplicate and an average taken. The $\delta^{13}C$ values are the ratios $^{13}C/^{12}C$ and expressed relative to the Vienna

Pee Dee Belemnite, calibrated against a CO_2 reference gas of known isotopic composition. Instrument error was ±0.3‰. Data processing was carried out using Ion Vantage software (version 1.6.1.0, IsoPrime).

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Author contributions

The project was designed by JD and KRS, and the paper was written by JD, KRS, RS and RPE. JD and CWD performed analytical work and data analysis. AF provided vessels and helped with sampling.

The authors declare there are no competing interests

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Figures

Figure. 1 Selection of Late Bronze/Early Iron Age feeding vessels, from Vienna, Oberleis, Vösendorf and Statzendorf, *c*. 1200-600 BC (photos: KRS)

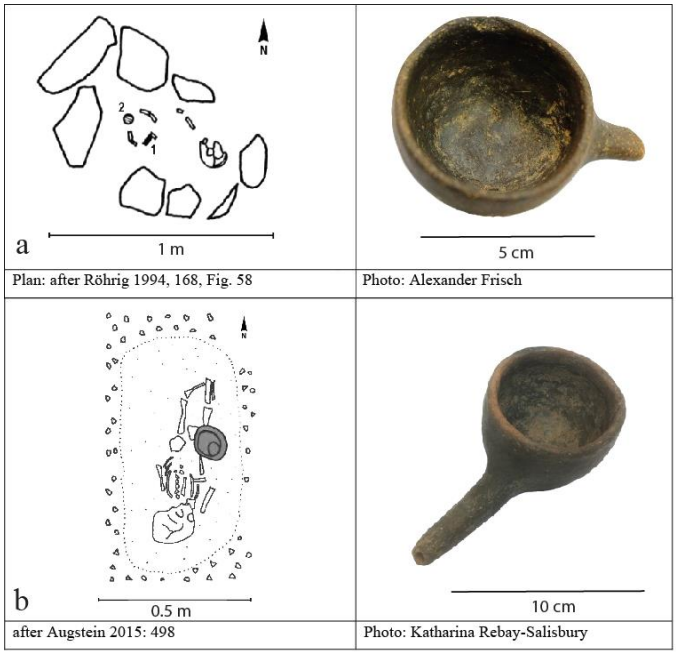

Figure 2. Drawings of child graves from Dietfurt cemetery with images of the feeding vessels found in each grave

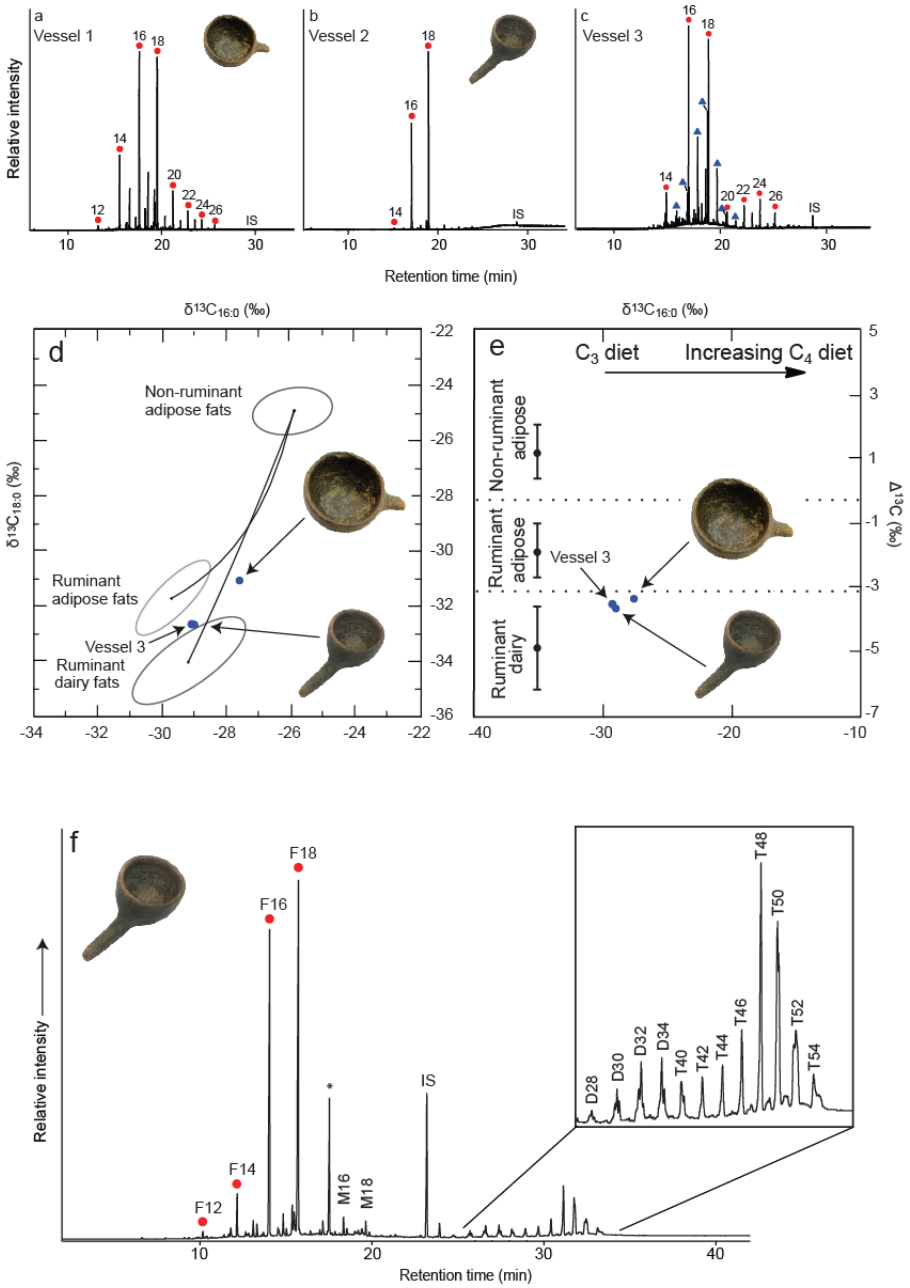

Figure. 3 Partial gas chromatograms and plots of $\delta^{13}C$ and $\Delta^{13}C$ values of alkanoic acids in infant feeding vessels from Dietfurt and Augsburg cemeteries, Bavaria (*n*=3), respectively. a, b, partial gas chromatograms of trimethylsilylated FAMEs from infant feeding vessels 1 and 2. Red circles, *n*-alkanoic acids (fatty acids, FA); blue triangles, *n*-alkanes; IS, internal standard, C_{34} *n*-tetratriacontane. c, $\delta^{13}C$ values for the $C_{16:0}$ and $C_{18:0}$ fatty acids for archaeological fats extracted from infant feeding vessels 1, 2 and 3. The three fields correspond to the P = 0.684 confidence ellipses for animals raised on a strict C_3 diet in Britain[22]. Each data point represents an individual vessel. d, shows the $\Delta^{13}C$ ($\delta^{13}C_{18:0} - \delta^{13}C_{16:0}$) values from the same vessels. The ranges shown here represent the mean ± 1 s.d. of the $\Delta^{13}C$ values for a global database comprising modern reference animal fats as detailed previously[27], published elsewhere. e. partial high-temperature chromatogram of trimethylsilylated total lipid extract of infant feeding vessel 2, showing degraded animal fat. Red circles indicate short- and long-chain fatty acids with x carbon atoms, monoacylglycerols (M) containing 16 and 18 acyl carbon atoms, diacylglycerols (D) containing 28, 30, 32 and 34 carbon atoms, triacylglycerols (T),

containing 40, 42, 44, 46, 48, 50, 52 and 54 carbon atoms, * denotes plasticiser and IS, internal standard *n*-tetratriacontane (*n*-C_{34}). Note: replication was not possible due to the unique and irreplaceable nature of the archaeological artefacts sampled, although the objects were analysed using two different extraction methods.

Figure 4. Modern-day baby feeding from reconstructed infant feeding vessel of the type investigated here (photo: Helena Seidl da Fonseca)