LETTERS

Earliest date for milk use in the Near East and southeastern Europe linked to cattle herding

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The domestication of cattle, sheep and goats had already taken place in the Near East by the eighth millennium BC[1–3]. Although there would have been considerable economic and nutritional gains from using these animals for their milk and other products from living animals—that is, traction and wool—the first clear evidence for these appears much later, from the late fifth and fourth millennia BC[4,5]. Hence, the timing and region in which milking was first practised remain unknown. Organic residues preserved in archaeological pottery[6,7] have provided direct evidence for the use of milk in the fourth millennium in Britain[7–9], and in the sixth millennium in eastern Europe[10], based on the δ^{13}C values of the major fatty acids of milk fat[6,7]. Here we apply this approach to more than 2,200 pottery vessels from sites in the Near East and southeastern Europe dating from the fifth to the seventh millennia BC. We show that milk was in use by the seventh millennium; this is the earliest direct evidence to date. Milking was particularly important in northwestern Anatolia, pointing to regional differences linked with conditions more favourable to cattle compared to other regions, where sheep and goats were relatively common and milk use less important. The latter is supported by correlations between the fat type and animal bone evidence.

The use of milk, wool and traction, so-called 'secondary' products, obtained from domestic animals without killing them, marks an important step in the history of domestication[4,5]. But evidence for when and how this first happened is inconclusive. Some researchers have argued that once animals were domesticated the potential benefits of these products would have been exploited rapidly[11]. Others have pointed to the late appearance of unequivocal evidence—that is, representations of milking scenes, carts and ploughs—and to barriers, such as lactose intolerance in humans, suggesting that early domestication was predominantly for meat and hides, postulating a 'secondary products revolution' during the fifth or fourth millennium BC, 2,000–4,000 years after the first domestication of cattle, sheep and goats in the Near East and Europe[5,12]. Evidence provided by figurines and pictures of animals before 4000 BC, and from artefacts (for example, ceramic strainers), has been variously interpreted[13], as has evidence from animal bone assemblages, especially the ages at which animals were killed, taken as reflecting what they were kept for and how they were managed[14–16].

The analysis of lipid residues from pottery, particularly our discovery that ruminant milk fatty acids can be distinguished from those of carcass fats, provided a new tool for detecting early milk use[6,7]. The approach rests upon differences in the δ^{13}C value of the $C_{18:0}$ (in $C_{x:y}$, x is the number of carbon atoms in the fatty acid, and y is the number of double bonds) fatty acid of milk and carcass fats. This arises from a greater proportion of dietary carbohydrate-derived carbon being used in the biosynthesis of carcass fat $C_{18:0}$, compared to milk fat, up to 40% of which derives from biohydrogenated dietary unsaturated C_{18} fatty acids ($C_{18:3}$, $C_{18:2}$ and $C_{18:1}$)[17,18]. Using this approach, we recently provided evidence for widespread milk use at some of the earliest Neolithic sites in southern Britain[7–9]. However, these sites, dating to the early fourth millennium BC, are late in relation to the Neolithic and Chalcolithic of the Near East and southern and central Europe. The same technique has also provided evidence for milk use in Romania before 5000 BC[10].

Reported here are results from analyses of organic residues from sherds of pottery vessels from fifth- to seventh-millennium BC sites in southeastern Europe, Anatolia and the Levant. Vessels most likely to have been used for food preparation were selected to test where milk use started, and whether the use of milk products first began in the region where farming was pioneered, namely within the Fertile Crescent, or whether it was an innovation of other regions. Figure 1 shows the locations of the 23 sites from which the sherds were sampled. The results of the analyses of 2,225 sherds are summarized in Table 1 and Figs 2 and 3; 12% of the sherds (255) yielded sufficient residue for compound-specific stable carbon isotope analysis. Typical gas chromatographic profiles of the residues displayed in Fig. 2 show that the $C_{16:0}$ and $C_{18:0}$ fatty acids predominate, the high abundance of the latter confirming that the residues derive from animal fats. Mean lipid concentrations varied over the range 0.54–1.74 mg per g sherd. The lower concentrations and incidences of lipid residues in these assemblages, compared to pottery from northern European sites, probably relates to differences in vessel use, clay type, the greater age of the pottery and/or degradative factors associated

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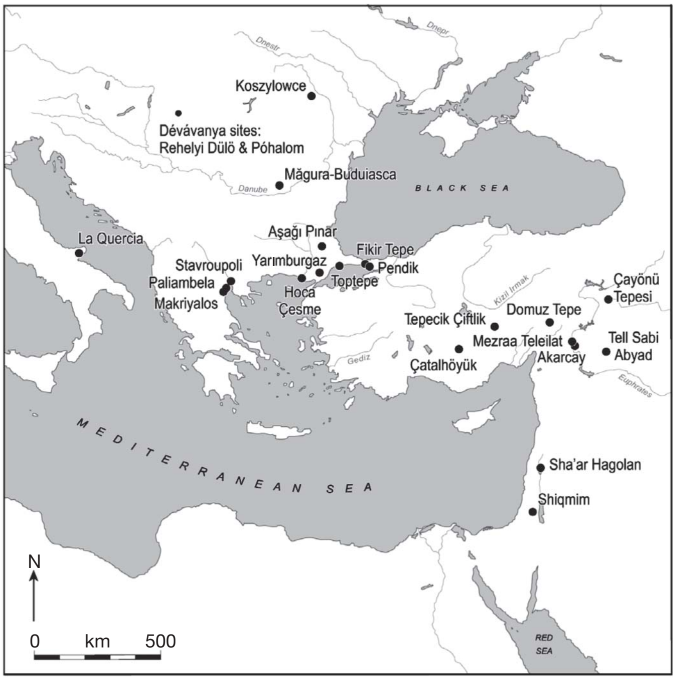

Figure 1 | Map showing the locations of sites providing pottery for organic residue analysis.

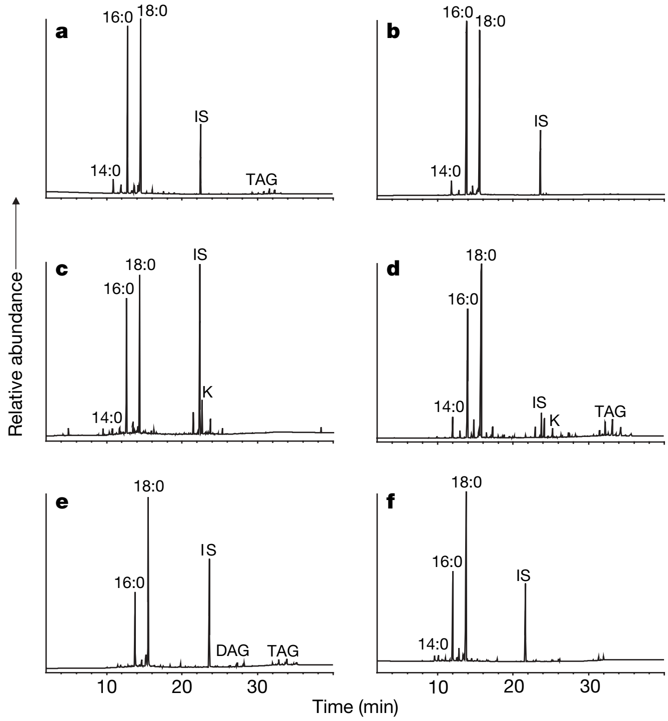

Figure 2 | Partial gas chromatograms of total lipid extracts from pottery. Pottery was from: **a**, Mağura (southeastern Europe); **b**, Makriyalos (northern Greece); **c**, Pendik (northwestern Anatolia); **d**, Çatalhöyük (central Anatolia); **e**, Cayönü Tepesi (southeastern Anatolia); and **f**, Tell Sabi Abyad (Levant). Abbreviations: N:0, fatty acids with N carbon atoms and no double bonds; K, mid-chain ketones with 31, 33 and 35 carbon atoms; DAG, diacylglycerols; TAG, triacylglycerols; and IS, internal standard (*n*-tetratriacontane). Sample reference numbers: **a**, MAG25; **b**, MAK100; **c**, PEN7; **d**, CAT180; **e**, CT53; **f**, SAB21.

with discard and burial. Pyrolytically formed long-chain ketones were present in a number of the vessels, consistent with them having being extensively heated during use[19]. All extracts were investigated for components likely to produce false positives for dairy fats. For example, sherds yielding wax esters diagnostic of beeswax were not included in the stable isotopic analyses[20]. The very low abundance or absence of detectable long-chain *n*-alkanoic (that is, C_{20} and C_{22}), isoprenoid and ω-(*o*-alkylphenyl)-alkanoic acids rules out significant contributions from aquatic resources[21].

Compound-specific stable carbon isotope analyses yielded $\delta^{13}C$ values for $C_{16:0}$ fatty acids in the range −30‰ to −21‰. This range is somewhat wider than that seen for northern European sites (−30‰ to −25‰) as a result of the contribution of C_4 (ref. 22) and water-stressed[23] plants to the diets of domesticated animals in parts of the region. By plotting (Fig. 3) $\Delta^{13}C$ ($= \delta^{13}C_{18:0} - \delta^{13}C_{16:0}$)

Table 1 | Details of sites, dates, sherds, and lipids and their concentrations

Region	Site	Date (kyr BC)	Number of sherds: Total analysed	Number of sherds: With >5 μg g^{-1} lipid	Lipid classes detected	Lipid concentration max/mean (mg g^{-1})
Central/ SE Europe	Koszylowce	4.5–3.5*	339	22	FFA, TAG, WE	0.90/0.08
	Póhalom	4.5–4.0†				
	La Quercia	5.5–4.5*				
	Măgura	5.5–5.2†				
	Rehelyi Dülö	6.0–5.5†				
N Greece	Makriyalos	5.2–4.9*	305	56	FFA, K, WE, TAG	1.74/0.06
	Stavroupoli	5.7–4.2†				
	Paliambela	6.0–4.2*				
NW Anatolia	Aşaği Pınar	5.5–5.0‡	703	102	FFA, K, WE, TAG	0.06/0.06
	Toptepe	5.5–5.0‡				
	Yarımburgaz	6.0–5.5‡				
	Fikir Tepe	6.0–5.5‡				
	Hoca Çesme	6.5–5.5‡				
	Pendik	6.5–6.0‡				
Central Anatolia	Domuztepe	5.9–5.5*	187	34	FFA, K, TAG	0.90/0.08
	Tepecik Çiftlik	5.9–5.6*				
	Çatalhöyük	7.0–6.0†				
SE Anatolia	Akarçay Tepe	7.0–6.2*	236	13	FFA, K, WE, TAG	1.63/0.28
	Çayönü Tepesi	6.5–6.0*				
	Mezraa Teleilat	6.5–6.0*				
Levant	Tell Sabi Abyad	6.5–6.0†	448	28	FFA, K, TAG	0.58/0.06
	Shiqmim	4.8–3.5*				
	Sha'ar Hagolan	6.4–6.0*				

FFA, free fatty acids; K, ketones; WE, wax esters (derived from degraded beeswax); TAG, triacylglycerols.
* Milk fats undetectable.
† < 30% milk fats.
‡ > 30% milk fats.

values, any effects on the classification of animal fats are removed, emphasizing biosynthetic and metabolic characteristics of the fat source[7]. These plots remove selective grazing, browsing or foddering differences between cattle and sheep/goats on fat classifications. Figure 3 presents the carbon isotope plots for the four main regions, showing the classifications of the organic residues to animal fat source.

The most striking feature of the data obtained is the emphatic evidence for extensive processing of dairy products in the pottery from all the sites of northwestern Anatolia (Fig. 3a) dating from about 6500–5000 BC, around the Sea of Marmara. Of the ~700 sherds analysed from the six sites considered in this region—Aşağı Pınar (5500–5000 BC), Toptepe (5500–5000), Yarımburgaz (6000–5500), Fikir Tepe (6000–5500), Hoca Çesme (6500–5500) and Pendik (6500–6000)—about 100 (~15%) yielded appreciable animal fat residues, of which 70% contained predominantly dairy fat residues. Thus, the milking of ruminant animals was clearly practised intensively in the sixth and seventh millennia BC in northwestern Anatolia.

Additional support for the latter interpretations comes from correlations with animal bone evidence. A significant feature of the northwestern Anatolian group of sites is that, where data are available, the proportion of cattle bones in animal bone assemblages is considerably higher than in sites in the other areas, presumably reflecting higher rainfall and greener grazing. A positive correlation ($R^2 = 0.56$) also exists between the proportion of sherds with ruminant milk $\Delta^{13}C$ values from different sites, and the relative importance of cattle in animal bone assemblages from the same sites (Fig. 4a), parallelling findings from British Neolithic sites[9]. Thus, the strong evidence for milk use at the northwestern Anatolian sites can reasonably be related to the importance of cattle in the bone assemblages—although ageing data for cattle and sheep/goats from Fikirtepe and Aşağı Pınar are suggestive of mixed use rather than of specialized milk production[24]. Our results suggest that milk was also used in the other areas studied, but was less important. This accords with the available ageing data: for example, sheep and goats at Çatalhöyük were almost all killed as subadults and young adults, a pattern suggestive of concentration on meat production[25]. Significantly, the proportion of sherds with $\Delta^{13}C$ values characteristic of pig fats from different sites is strongly positively correlated (Fig. 4b; $R^2 = 0.85$) with the estimated relative importance of pig in animal bone assemblages from the same sites, analogous to trends observed in late Neolithic sites in northern Europe[26].

Our results provide new insights into the emergence of dairying as a component of the domestication of animals. The appearance of dairy products at early sites in the region is the earliest evidence so far, by ~1–2 millennia, dating back to the start of ceramics in the region; this indicates an earlier date for the milking of domesticated animals than predicted by reconstructions based on other lines of evidence[3]. Significantly, the high incidence of dairy products in pottery from sites in northwestern Anatolia points to intensification of the milking of ruminant animals, at locations remote from the original region of domestication, namely the Fertile Crescent.

Importantly, the results suggest a pattern of regional variation in the importance of milk use rather than of general change with time. Milk appears to have been particularly important in the sites from northwestern Anatolia, ranging in date from the end of the seventh

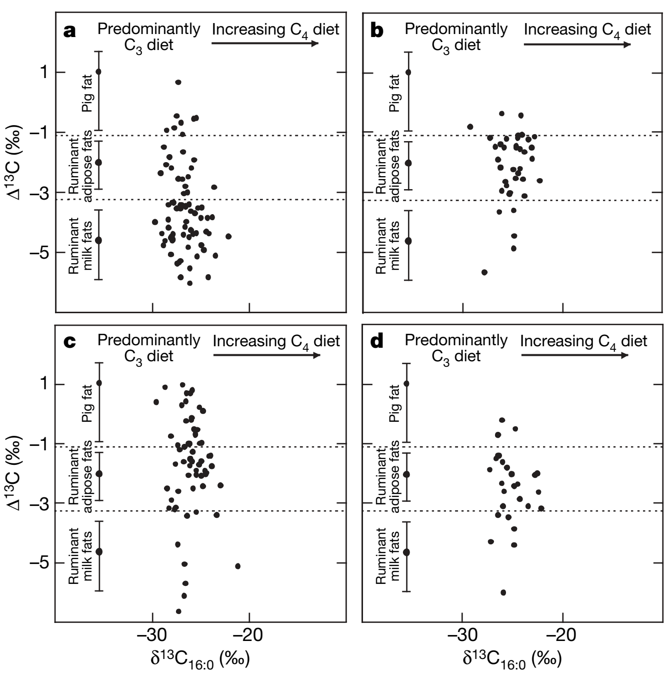

Figure 3 | Plots of the $\Delta^{13}C$ values for archaeological animal fat residues in Neolithic pottery. Pottery was from: **a**, northwestern Anatolia; **b**, central Anatolia; **c**, southeastern Europe/northern Greece; and **d**, eastern Anatolia and the Levant. The $\Delta^{13}C$ values ($= \delta^{13}C_{18:0} - \delta^{13}C_{16:0}$) for the ruminant dairy fats are more depleted than the ruminant adipose fats; the difference in the means is ~2.8‰ which is highly significant (t-test; $P < 0.0005$). Pig fats have positive $\Delta^{13}C$ values which do not exhibit significant variance and the differences in the mean values are also highly significant (ANOVA; $P < 0.0005$ between all three commodity groups; Bonferroni adjustment applied). $\delta^{13}C = [(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard}] - 1$, expressed in per mil. All $\delta^{13}C$ values are relative to Vienna PeeDee Belemnite (VPDB) international standard.

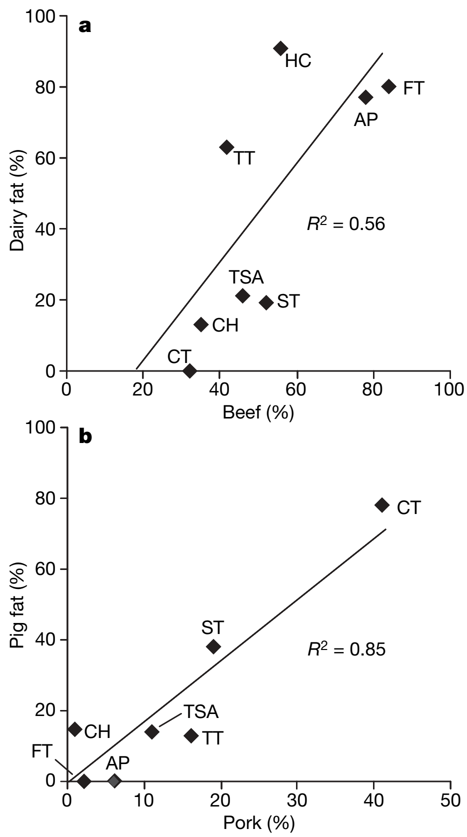

Figure 4 | Percentage animal fat types in pottery versus meat yields based on faunal remains. Both data sets available for: Aşağı Pınar (AP), Çatalhöyük (CH), Çayönü Tepesi (CT), Fikir Tepe (FT), Hoca Çeşme (HC), Tell Sabi Abyad (TSA), Stavroupoli (ST) and Toptepe (TT). Percentage pork and beef is based on ~1,000 to 49,000 identified bones with weightings for pigs (×2) and sheep/goats (×5) to allow for carcass weight and recovery differentials. Percentage dairy and pig fats is based on proportions of 144 residues falling into dairy (**a**) and pig (**b**) fat ranges (Fig. 3). Where more than one bone assemblage is available within periods covered by pottery residues, percentages represent pooled data.

millennium to the fifth millennium BC, contrasting markedly with results from southeastern and central Anatolia at the same time. In neither area is there any strong suggestion of chronological change. In northern Greece, sites dating from the sixth to the fourth millennia BC gave low proportions of sherds with ruminant milk fats; the one site with intermediate results was the sixth millennium Romanian site of Măgura-Buduiasca, which concurs with an earlier study of two sites from this region[10].

Our earlier experiments showed that raw milk lipids absorbed by reproduction ceramics are rapidly destroyed by burial[6,9,27,28], suggesting that the high frequency of ruminant milk lipids from the northwestern Anatolian sites is indicative of milk being processed. Processing milk would have had two important advantages, providing a means of storing surplus milk as products, that is cheese, ghee, and so on, making them available throughout the year, and providing a solution for any problems of lactose intolerance; most lactose intolerant people have fewer problems with consuming processed milk products.

In summary, our findings take the early history of milk use back to the seventh millennium BC, early in the evolution of animal domestication and pottery production and use. The results are significant also for two other reasons: first, they suggest that even at this date (before 6500 BC) milk was processed, making possible the storage of milk products and providing an explanation why, in spite of lactose intolerance, milk use could be adopted quickly, and second, they add to increasing indications of regional differences during the early Neolithic and into the Chalcolithic. Thus, early farming appears not to have been a fixed package; instead, it developed in different ways in different areas, probably in response partly to different environmental conditions and partly to different cultural choices of early farmers.

METHODS SUMMARY

A total of 2,225 well-stratified potsherds were sampled from 23 different archaeological sites across the Near East and southeastern Europe, dated to the Neolithic and Chalcolithic cultural periods. These are grouped within six regions, as follows: central Europe/Ukraine/southeastern Europe (Koszylowce, La Quercia, Măgura, Póhalom and Rehelyi Dülö), northern Greece (Makriyalos, Paliambela and Stavroupoli), northwestern Anatolia (Aşağı Pınar, Fikir Tepe, Hoca Çesme, Pendik, Tepecik Ciftlik, Toptepe and Yarımburgaz), central Anatolia (Çatalhöyük and Domuztepe), southeastern Anatolia (Akarçay, Çayönü Tepesi and Mezraa Teleilat), and the Levant (Tell Sabi Abyad, Sha'ar Hagolan and Shiqmim). Coarsewares and mid-profile sherds were sampled, as these have been found to be the most likely to yield lipid residues[29].

Lipid analyses and interpretations were performed using established protocols described in detail in earlier publications[6,7,9,26,30]. Briefly, ~3 g potsherds were taken and their surfaces cleaned using a modelling drill to remove any exogenous lipids. The sherds were then ground to a powder, an internal standard added and solvent extracted by ultrasonication (chloroform/methanol, 2:1 v/v, 10 ml). The solvent was evaporated under a gentle stream of nitrogen to obtain the total lipid extract (TLE). Aliquots of the TLEs were then trimethylsilylated (*N,O*-bis(trimethylsilyl)trifluoroacetamide 20 μl; 70 °C, 60 min), and submitted to analysis by gas chromatography (GC) and GC/mass spectrometry. Further aliquots of the TLE were treated with $NaOH/H_2O$ (9:1 v/v) in methanol (5% v/v, 70 °C, 1 h). Following neutralization, lipids were extracted into hexane and the excess solvent evaporated under a gentle stream of nitrogen. Fatty acid methyl esters (FAMEs) were prepared by reaction with BF_3-methanol (14% w/v, 70 °C, 1 h). The methyl ester derivatives were extracted with chloroform and the solvent removed under nitrogen. The FAMEs were re-dissolved into hexane for analysis by GC and GC-combustion-isotope ratio MS.

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