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Identification of animal fats via compound specific $\delta^{13}C$ values of individual fatty acids: assessments of results for reference fats and lipid extracts of archaeological pottery vessels

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ABSTRACT – *The possibility of obtaining molecular information from lipid residues associated with archaeological pottery has dramatically increased the potential for deriving new information on the use of ancient vessels and the commodities processed therein. Motivated by the high proportion of the archaeological potsherds that have been shown to contain animal fats, a new approach involving compound specific stable isotope analysis of remnant fats has been developed to retrieve information which will allow new insights into animal exploitation, dietary preferences and vessel use amongst prehistoric peoples. The new approach uses the $\delta^{13}C$ values of the major saturated fatty acid ($C_{16:0}$ and $C_{18:0}$) determined by gas chromatography-combustion-isotope ratio mass spectrometry (GC–C–IRMS) to characterise the origins of animal fat recovered from archaeological pottery.*

IZVLEČEK – *Danes je mogoče dobiti molekularne podatke iz lipidov, ohranjenih na arheološki keramiki, kar je močno povečalo obseg informacij o uporabi starodavnih posod in njihovi vsebini. Ker se je za velik del arheoloških fragmentov izkazalo, da so vsebovali živalske maščobe, so razvili nov način raziskav, ki je vseboval sestavljene analize specifičnih stabilnih izotopov v živalskih ostankih. S tem dobimo podatke, ki nam dajo nov pogled na izrabo živali, način prehranjevanja in uporabo posod pri prazgodovinskih ljudeh. Nov pristop uporablja $\delta^{13}C$ vrednosti najbolj nasičene maščobne kisline ($C_{16:0}$ and $C_{18:0}$), ki jih določimo s posebno masno spektrometrijo (gas chromatography-combustion-isotope ratio mass spectrometry: GC–C–IRMS), da določimo izvor živalskih maščob na arheološki keramiki.*

KEY WORDS – *$\delta^{13}C$ values; animal fats; fatty acids; lipids; archaeological pottery; organic residues*

INTRODUCTION

Natural variation in $\delta^{13}C$ and archaeological research

The use of stable isotopes in archaeological investigations is a relatively recent development which has focused largely on the use of bulk $\delta^{13}C$ and $\delta^{15}N$ measurements, providing information intractable using traditional archaeological techniques. The first applications in archaeology exploited the difference in $\delta^{13}C$ values between C_3 and C_4 plants reflected in bones collagen and apatite in order to assess the relative contributions of these plant types in the diets of ancient peoples (*Vogel and van der Merwe 1977; Jones et al. 1979; Teeri and Schoeller 1979; Tieszen et al. 1979*). Analyses have also enabled isotopic signals from marine and terrestrial sources to be distinguished as components of diet since the heavier isotope (^{13}C) is approximately 5–7‰ more abundant in the tissues of animals that consume marine foods (*Chisholm et al. 1982; Ostrom and Fry 1993*) with applications including the investigation of human migrations, status and social structure (*Sealy and van der Merwe 1986; Murray and Schoeninger 1988*).

Applications of stable carbon isotope analyses in animal nutrition and metabolism studies are numerous (*e.g. Boutton et al. 1988*) since the isotopic compo-

sitions of food and fluids ingested by animals have a strong influence on the isotopic compositions of the tissues they synthesise. However, the precise relationship between the isotopic compositions of ingested materials and a particular tissue or molecular component is complex, responding to changes in nutritional status, biosynthetic pathway and turnover rate of the tissue. Field studies have been conducted in order to elucidate the complexities of how different biochemical fractions translate into consumer tissues in food chains. Attempts have been made to show the offset in $\delta^{13}C$ values between different trophic levels; however, these depend upon the particular biochemical fraction, species type, diet and other environmental factors, and therefore can only be broadly estimated. Lee-Thorpe (*1989*) calculated differences in bulk $\delta^{13}C$ values between the vegetation, herbivore and carnivore trophic levels based upon measurements of meat, collagen and apatite, noting an enrichment in ^{13}C in species further up the food chain. Feeding studies have been carried out to elucidate the relationships between different levels of the food chain and to establish to what extent different fractions in the diet are routed or scrambled to particular tissues in consumers (*e.g. Ambrose and Norr 1993*), with initial findings suggesting some degree of routing of dietary components to specific body tissues.

It is assumed that at a particular location animals raised in antiquity would have consumed relatively restricted diets. Based on this assumption, the dietary contribution of $\delta^{13}C$ values to tissues such as adipose fat would be relatively constant (*DeNiro and Epstein 1978*). However, variation may arise particularly in non-ruminant domesticates due to food supplements (e.g. from domestic waste such as whey left over from cheese production or meat scraps) which would contribute a larger protein component to the diet. In non-ruminant animals, the direct routing of dietary fats to storage organs such as adipose fats means that the isotopic signal of the dietary lipids is retained and will be reflected in the isotopic composition of the tissue, although the situation is complex and varies between species. Tieszen *et al.* (*1983*) showed that fat tissue was 3‰ more depleted in ^{13}C relative to the diet and that the largest departure from dietary ^{13}C relative to other tissues is due to discrimination against ^{13}C during lipid synthesis (*DeNiro and Epstein 1977*). Fat tissue was found to have a relatively short half life of 15.6 days indicating that carbon turnover was relatively rapid compared to other tissues, requiring 208 days for complete recycling of carbon. DeNiro and Epstein (*1978*) showed that the fractionation of ^{13}C from diet to tissue is not identical in animals raised on different diets, possibly because of differential assimilation between the major biochemical fractions, however, the secondary fractionation of carbon isotopes by animal tissues is believed to be relatively small. A recent study of the stable carbon isotope ratios of fatty acids in the body fat of Redhead ducks strongly indicated that fatty acids in the diet are not the sole contributor to adipose tissues and that the ducks also synthesise fatty acids from other fractions in their diet, such as carbohydrates and proteins, which result in higher $\delta^{13}C$ values for the tissue fatty acids (*Hammer et al. 1998*).

Stable isotope analyses of organic residues in archaeological pottery

Morton and Schwarcz (*1985*) first applied stable isotope ($\delta^{13}C$ and $\delta^{15}N$) determinations to the study of residues associated with archaeological ceramics, using bulk measurements to examine carbonised deposits thought to originate from maize. The first application of compound-specific stable carbon isotope approaches to lipids in archaeological materials was that reported by Evershed *et al.* (*1994*). The $\delta^{13}C$ values obtained for individual higher plant leaf wax components in solvent extracts of pottery vessels from the Raunds area project, Northamptonshire, confirmed that the lipids being investigated were of C_3 origin. The distributions of components were consistent with the lipids in the potsherds having deri-

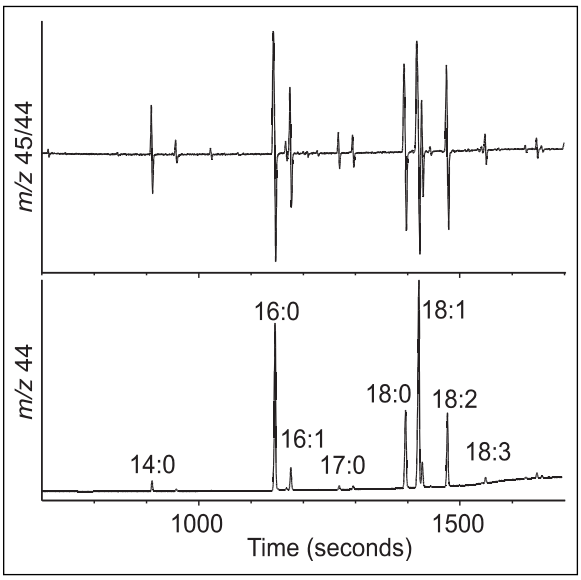


Fig. 1. Partial m/z 44 and m/z 45/44 traces obtained by GC–C–IRMS analysis of fatty acids (as their methyl ester derivatives) in modern pig adipose fat.

ved from *Brassica* species, such as cabbage. The latter study utilised GC–C– IRMS, which allows the isotope ratios of individual compounds within a mixture to be determined (*Santrock et al. 1985*). Compound specific analyses have proven particularly advantageous over bulk analyses in the study of diagenetically altered samples due to the fact that bulk $\delta^{13}C$ values will alter over time as a result of the preferential loss of labile components, e.g. polysaccharides, and the contaminating effects of effects of exogenous components migrating from the burial environment or introduced via the actions of microorganisms (*Evershed et al. 1999*).

Our laboratory was the first to observe the isotopic distinction between preserved fats of ruminant and non-ruminant origin based on the stable carbon isotope composition of fatty acids in sherds from a small assemblage of lamps and 'dripping' dishes from a medieval site at Causeway Lane, Leicestershire (*Evershed et al. 1997; Mottram et al. 1999*). Analyses of fresh cattle lamb and pork fat indicated that fatty acids in ruminant fats are isotopically lighter, by approximately 4‰ and 7‰ for the $C_{16:0}$ and $C_{18:0}$ components, respectively, than the equivalent fatty acids in non-ruminant fats. The variation is believed to result from fundamental differences in metabolic factors and dietary preferences between the species (*Koch et al. 1994*). The $\delta^{13}C$ values obtained clearly distinguished the fats from two different animal origins in the two vessel types, indicating the use of the lamps in burning ruminant tallow and the 'dripping' dishes for the collection of non-ruminant, e.g. porcine fats, perhaps during spit roasting. The isotopic analysis of the extract from a 'cauldron' from the same assemblage gave $\delta^{13}C$ values which were intermediate between those obtained for the lamps and the 'dripping' dishes, indicating that the vessel had once been used to process both ruminant and non-ruminant animal products (*Mottram et al. 1999*). In another study, the potential of compound specific $\delta^{13}C$ values of ancient fats was investigated in an effort to classify the origins of remnant animal fat residues in Late Saxon/ early medieval vessels from West Cotton, Northamptonshire (*Charters 1996*). Extracts of four vessels were studied, including a shelly ware jar (RP78 rim) and three spouted bowls (RP72, 93 spout and 94 rim/body). The mean $\delta^{13}C$ values obtained for the fatty acids in the bowls differed by approximately 4‰ from the values obtained for fatty acids in the jar. The extracts from RP93, 94 and 72 were interpreted as having a ruminant origin, while that from RP78 represented a mixture of fats from different origins, since the $\delta^{13}C$ values were intermediate be-

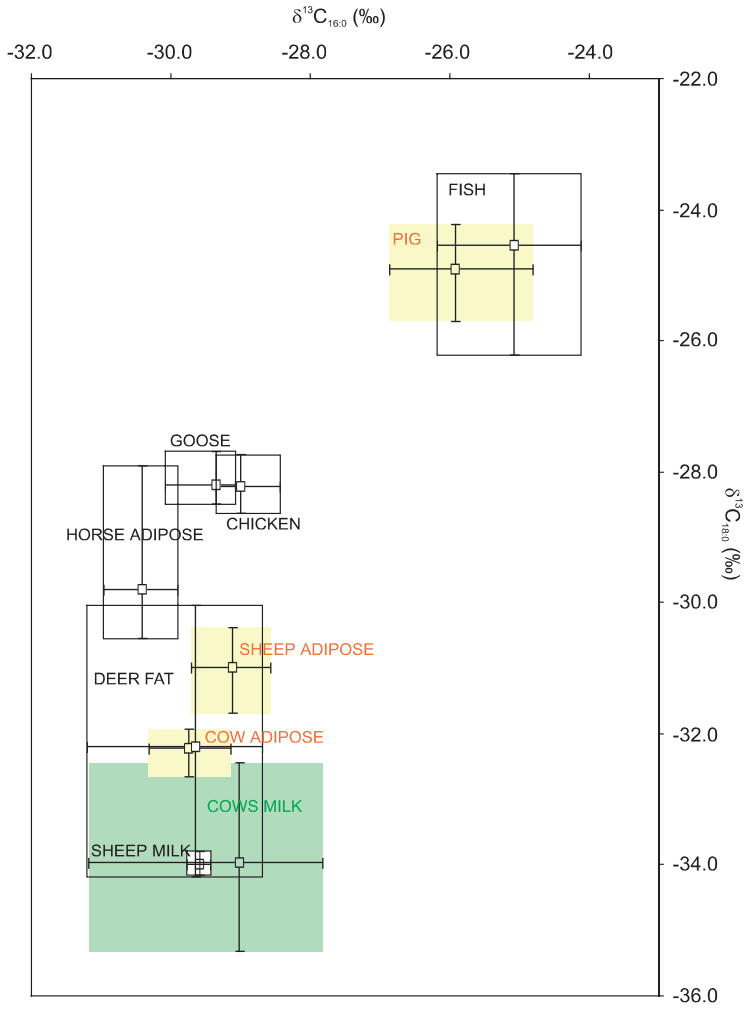


Fig. 2. Plot of the $\delta^{13}C$ values for the major n-alkanoic acid ($C_{16:0}$ and $C_{18:0}$) components of the solvent extracts of modern reference fats. The $\delta^{13}C$ values of the individual fatty acids were determined exactly according to the conditions given in Woodbury et al. (1995) with corrections for the addition of the derivatising methyl carbon. The $\delta^{13}C$ values for the fatty acids in the reference fats have been corrected for the post-Industrial Revolution effects of fossil fuel burning which has decreased the $\delta^{13}C$ value of atmospheric CO_2 by approximately 1.2‰ over the past 130 years (Friedli et al. 1986). The boxed fields encompass the ranges for reference animal fats with the ranges crossing at the arithmetic mean. Instrumental error is ± 0.3‰ and samples were run in triplicate. Instrument operating conditions are described in Dudd and Evershed (1998).

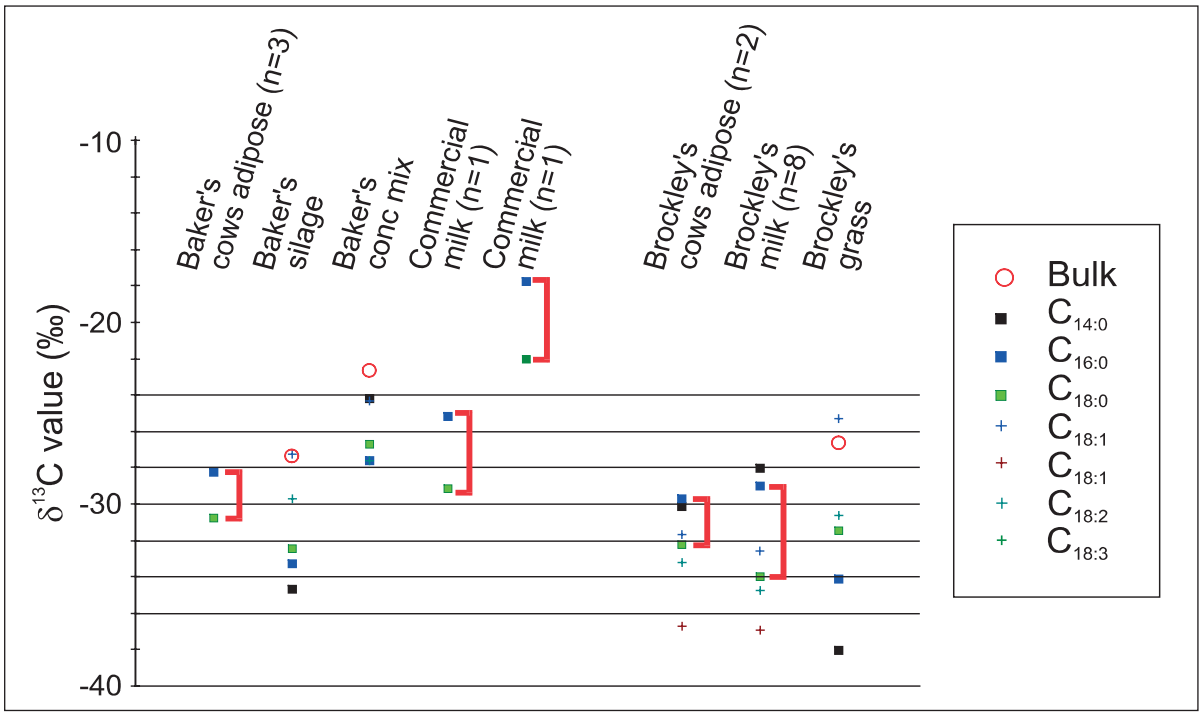


Fig. 3. The relationship between carbon composition of the diet and bovine adipose and milk fatty acids (the variation between the major $C_{16:0}$ and $C_{18:0}$ fatty acids in adipose and milk fats is shown by the red bars).

tween those measured for reference ruminant and non-ruminant adipose fats. The studies by Mottram *et al.* (*1999*) and Charters (*1996*), albeit on a relatively small number of samples, were the first to recognise that differences between the stable carbon isotope compositions of remnant fats could be used to make distinctions between archaeological fats of different animal origins, and provided the stimulus for the work described in this paper.

METHODOLOGICAL CONSIDERATIONS

The selection of modern animal fats as reference materials

The majority of farmed animals available today are unsuitable for comparison with archaeological fats due to the changes which have occurred in the composition of animal fats over the last several hundred years. Reasons for this include: (i) selective breeding, which has resulted in changes in the composition of the fat and milk of the larger domestic animals (*Johansson and Claesson 1957*); (ii) the widespread use of intensive farming methods necessary to maximise yields, which has included the use of nutrient-rich concentrates during the winter when temperatures are low (*Johansson and Claesson 1957, and references therein*); (iii) fossil fuel burning since the Industrial Revolution, and other factors resulting in changes in the isotopic composition of the atmospheric CO_2 which have been reflected in the enrichment of ^{12}C in the tissues of modern animal fats compared to their ancient counterparts, and (iv) C_4 plants (sugar cane) introduced into Europe in the 1500s, which have been incorporated into the diets of farm animals significantly altering the stable carbon isotope composition of their tissues.

In view of the above, reference animal fats for this study have been carefully selected from a number of sources, including:

❶ Fresh fats from modern animals raised on a known diets;
❷ Remnant fats extracted from well-documented ethnographic vessels;
❸ Remnant fats from archaeological pottery assemblages at sites where a preponderance of one species of animal is believed to have been farmed;
❹ Remnant horse fats obtained from a prehistoric permafrost burial.

Determination of compound specific $\delta^{13}C$ values of fatty acids

Compound specific $\delta^{13}C$ values are determined by gas chromatography-combustion-isotope ratio mass spectrometry (GC–C–IRMS). The instrument construction and operating conditions employed to obtain the results described in this paper have been exten-

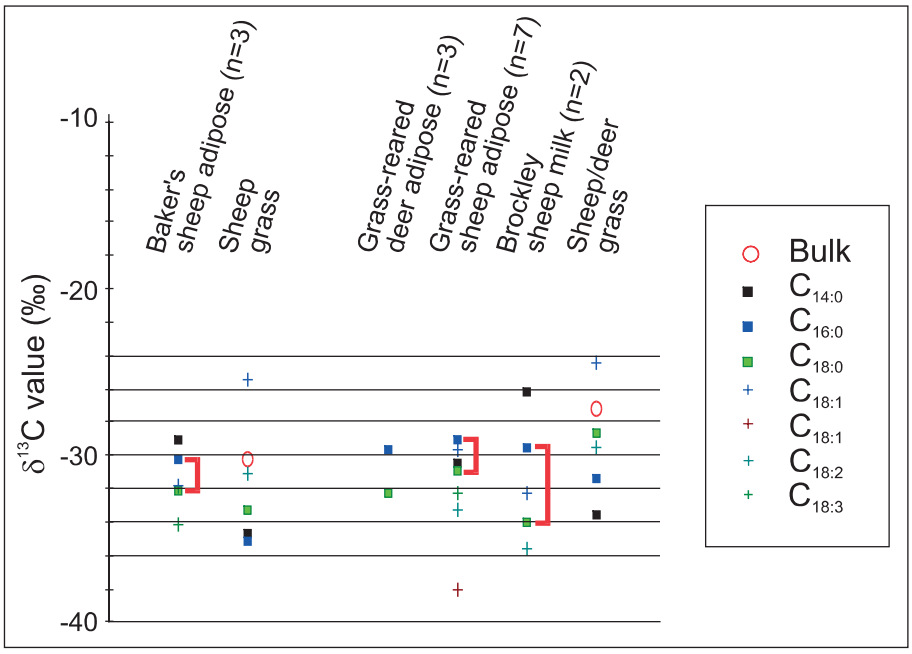


Fig. 4. The relationship between carbon composition of the diet and ovine adipose and milk fatty acids (the variation between the major $C_{16:0}$ and $C_{18:0}$ fatty acids in adipose and milk fats is shown by the red bars).

sively described elsewhere (*Evershed et al. 1994; Dudd and Evershed 1998; Dudd et al. 1999*). The stable carbon isotope ratios are measured as the relative difference between the isotopic ratios of the sample and standard gases, thus adopting the delta (δ) notation (*McKinney et al. 1950*):

$$\delta^{13}C(‰) = \frac{[R_{sample} - R_{standard}]}{R_{standard}} x10^3$$

where the δ13C is the parts per thousand difference between the 13C content of the sample and that of the standard and R is the *m/z* 45/44 ratio of the sample or standard gas. δ13C values are expressed relative to VPDB. This standard has been assigned a δ13C value of 0‰, thus the notation of the δ13C value indicates whether the sample has a higher or lower 13C/12C ratio than VPDB. Samples are run in triplicate and the mean values obtained corrected for the additional carbon of the derivatising agent, BF_3MeOH. Modern fats and oils used as reference samples are corrected for the change in atmospheric CO_2 which has occurred since the Industrial Revolution (*according to Friedli et al. 1986*). Bulk δ13C values reported below for homogenised plant materials and whey were obtained using using an NC 2500 elemental analyser coupled with the Finnigan MAT Delta–S isotope ratio mass spectrometer *via* an open split interface.

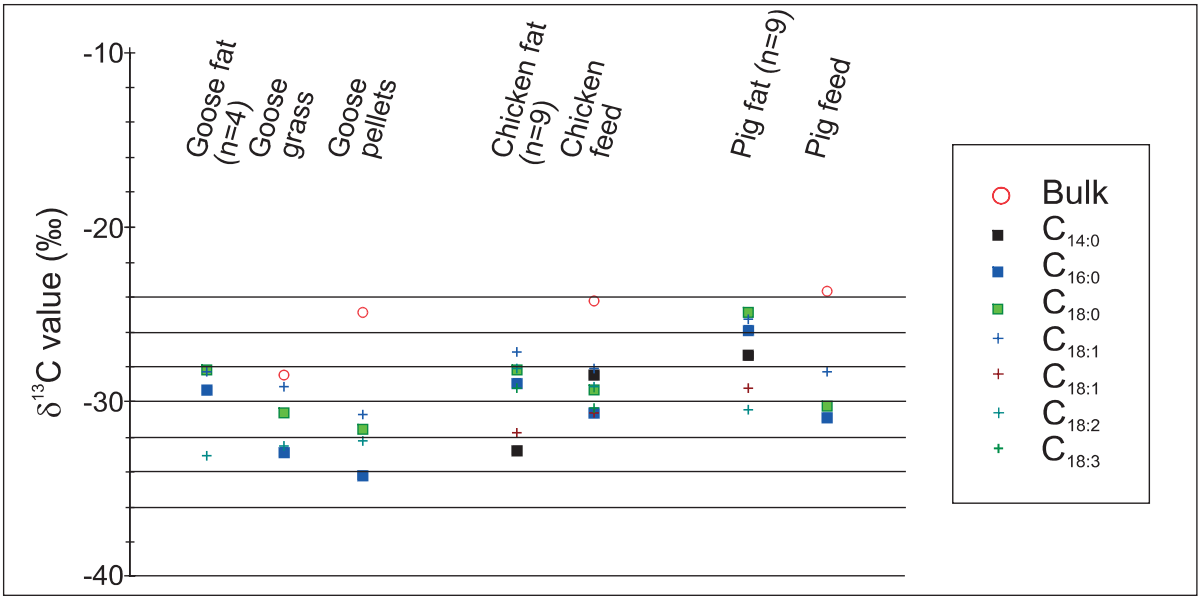


Fig. 5. The relationship between carbon composition of the diet and fatty acids in non-ruminant adipose fats.

Presented in this paper are the ranges of $\delta^{13}C$ values in the body and milk fats of animals known to have been the major domesticated species in antiquity, noting differences between individuals of the same species and between different species. Since the diets of the reference animals are known it is possible to test the relationship between the stable carbon isotope ratios of lipid components in the diet and in the animal fats. The report also constitutes by far the largest study of $\delta^{13}C$ values of individual lipids from archaeological pottery to date, and thus enables an assessment of the usefulness of the chemical information which can be obtained from this type of analysis. The study of prehistoric pot extracts has enabled us to establish whether the distinction in the isotopic signals previously observed between fats of different origin in the medieval pottery from Causeway Lane (*Mottram et al. 1999*) are retained in lipids from early Neolithic vessels.

RESULTS

Compound specific stable carbon isotope analysis of reference fats

An example of the results obtained from a reference animal fat is shown in Figure 1. The lower chromatogram shows the baseline resolution obtained routinely by GC analysis of FAMEs on a 50 m CP WAX 52 CB fused silica capillary column. The upper trace is a ratio of the *m/z* 45/44 ions in the sample detected by the GC–C–IRMS. The $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acids for a range of modern reference fats from different species are shown in Figure 2. The numbers (n) of different reference fats analysed (in triplicate) were: pig adipose fat, n = 9; cow adipose fat, n = 4; sheep adipose fat, n = 7; chicken adipose fat, n = 8; cows' milk fat, n = 8; sheep milk fat, n = 2; horse adipose fat, n = 8; deer adipose fat, n = 7. All the animals were raised on C_3 diets, isotopically representative of the archaeological period, and for this reason numbers of samples suitable for this work were limited.

From the data shown in Figure 2 the following trends have been observed:

❶ Adipose fats from the major ruminant (e.g. ovine and bovine) and non-ruminant (e.g. porcine) domesticates are distinguishable from one another by greater depletion in ^{13}C in the $C_{16:0}$ and $C_{18:0}$ fatty acids in ruminant fats. The mean $\delta^{13}C$ values obtained for adipose fats

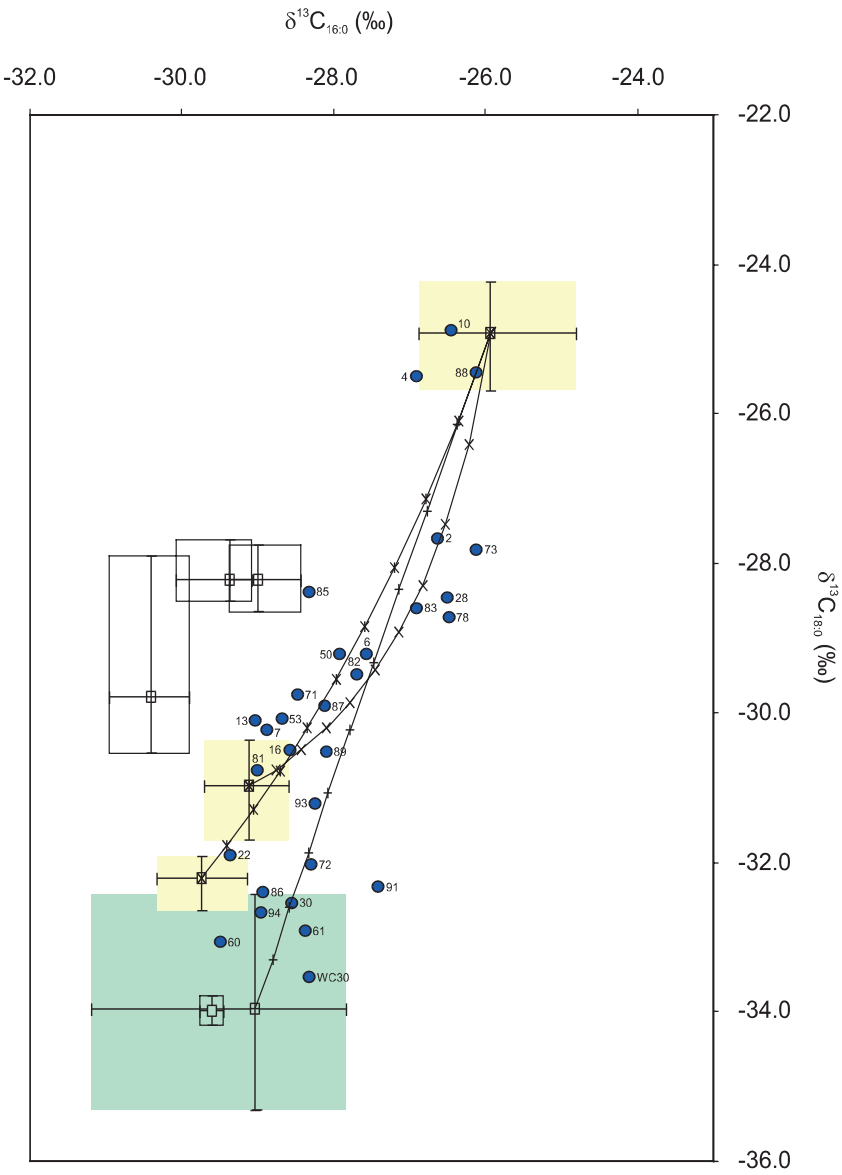


Fig. 6. Plot of the $\delta^{13}C$ values of the major n-alkanoic acid components ($C_{16:0}$ and $C_{18:0}$) from the lipid extracts of potsherds from the Late Saxon/early medieval site of West Cotton, Northamptonshire. The blue-filled circles represent the archaeological fats; sample nos. are labelled. The mixing curves have been calculated to illustrate the $\delta^{13}C$ values which would result from the mixing of ovine and porcine fats (x), bovine and porcine fats (✶) and cow's milk/porcine fats (+) in the vessels.

from the reference pig and sheep differ by 3.2‰ in the $C_{16:0}$ fatty acid and 6.1‰ in the $C_{18:0}$ fatty acid;
❷ The $C_{16:0}$ and $C_{18:0}$ fatty acids in porcine adipose fats and salt-water fish tissues show the least depleted $\delta^{13}C$ values of the reference fats analysed;
❸ The mean $\delta^{13}C$ values obtained for the cattle adipose fats are more depleted than the sheep adipose, by 0.6‰ and 1.2‰ for the $C_{16:0}$ and $C_{18:0}$ fatty acids, respectively;
❹ A distinction can be made between the $\delta^{13}C$ values of fatty acids in ruminant adipose and dairy fats, primarily based on the greater depletion of the $C_{18:0}$ fatty acid in dairy fats (ca. 2–3‰);
❺ The mean $\delta^{13}C$ values obtained for the sheep milk are very similar to the mean values for the cow's milk;
❻ Depot fats from chicken and goose show almost identical $\delta^{13}C$ values for the $C_{18:0}$ fatty acid and a difference of only 0.4‰ between the mean values obtained for the $C_{16:0}$ fatty acid;
❼ The fatty acids in horse adipose fat are slightly more depleted than the other non-ruminant fats, with mean $\delta^{13}C$ values of –30.4‰ and –29.8‰ for the $C_{16:0}$ and $C_{18:0}$ fatty acids, respectively;
❽ The mean $\delta^{13}C$ values of the deer adipose fats are similar to the other ruminant adipose fats, however the range of values obtained is significantly greater. This variation is surprising due to the fact that all these animals were of the same breed and raised on the same pasture;
❾ The $\delta^{13}C$ values for the depot fats of individual animals are closely grouped (with the exception of deer fat), however, in contrast, the range of $\delta^{13}C$ values obtained for the milk fats varied by up to 3.4‰ for the $C_{16:0}$ and 2.9‰ for the $C_{18:0}$ fatty acid.

The relationship between the stable carbon isotope composition of lipid components of adipose fats and diet

The mean $\delta^{13}C$ values for the major saturated and unsaturated fatty acids in the reference ruminant animal fats and their diets are plotted in Figures 3 and 4. Data have been included from cows fed concentrates as a supplement to the diet in order to compare with the data from C_3 grass-fed animals. The fatty acids in adipose tissue from the concentrate-fed and the grass-fed cow differ by ca. 2‰, with the former reflecting the higher $\delta^{13}C$ values of the concentrate supplement. For the silage and fresh grasses analysed, the bulk $\delta^{13}C$ value is significantly less depleted in ^{13}C, by ca. 6‰, than the individual $C_{16:0}$ and $C_{18:0}$ fatty acids. Since the majority of higher plant tissue is composed of carbohydrate, with only ca. 7% lipid, the

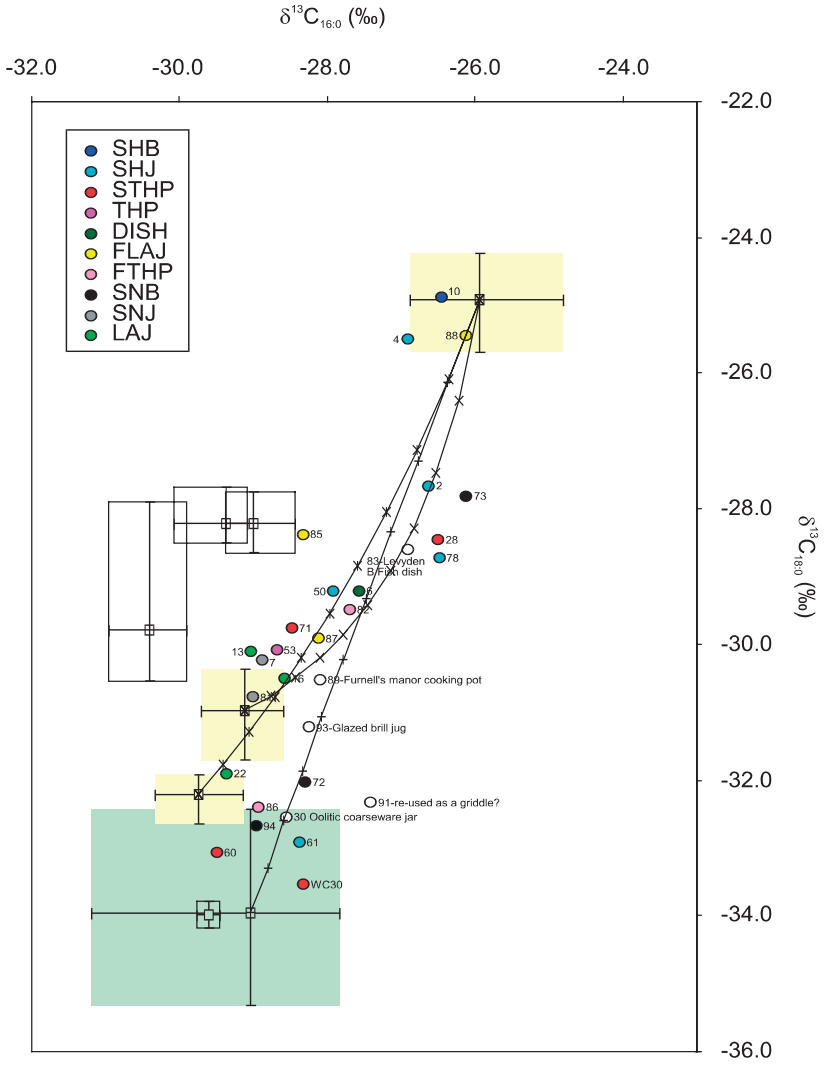


Fig. 7. Plot of the $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acid components from the lipid extracts of potsherds from the Late Saxon/early medieval site of West Cotton, Northamptonshire, correlated with vessel form. The abbreviations denoting vessel form are as follows: SHB, shelly ware bowl; SHJ, shelly ware jar; STHP, shelly ware 'top hat' pot; THP, 'top hat' pot; DISH, dish; FLAJ, Furnell's Manor Lyveden A ware jar; FTHP, Furnell's Manor 'top hat' pot; SNB, St. Neots bowl; SNJ, St. Neot's jar, and LAJ, Lyveden A ware jar.

bulk value obtained reflects the isotopically heavier carbohydrate. $\delta^{13}C$ values for individual fatty acids in the grasses have shown that the $C_{14:0}$ is most depleted isotopically, followed by the $C_{16:0}$ and the $C_{18:0}$ fatty acids.

The variations in $\delta^{13}C$ values obtained for the individual fatty acids in the grasses and those from cow adipose tissue illustrate that the relationship between diet and tissue is extremely difficult to interpret. This is due to the complexity of the metabolic and physiological processes determining adipose fat formation in different animal species, as mentioned above. The $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$ in cow adipose tissue are generally less depleted than the same fatty acids in the diet indicating that a proportion of these components are synthesised *de novo* and reflect a contribution from other sources of carbon in the diet, e.g. carbohydrate and protein. The relationship between diet and fat cannot be explored fully without examining the routing of different sources of dietary carbon and utilisation of stored carbon in the whole animal (*viz DeNiro and Epstein 1978*). The $\delta^{13}C$ values for the sheep from Baker's farm fed some supplements to their diet are very similar to those for the grass-reared sheep from Brockley since the bulk of their diet was grass.

In ruminant animals there is a large (ca. 3‰) difference between the $\delta^{13}C$ values for the $C_{16:0}$ and $C_{18:0}$ fatty acids in adipose and an even larger difference of up to 6‰ between the same fatty acids in milk fat. This relatively large difference in the $\delta^{13}C$ values of the fatty acids indicates different sources for these components, i.e. the direct routing of dietary fatty acids and the synthesis of components in different organs of the body, e.g. liver, adipose, mammary gland, etc. from different precursors which may result in differing degrees of isotopic discrimination in fat synthesis. The direct routing of fatty acids in the formation of adipose fats is thought to be minor since the fat content of the diets of the major domesticated animals is relatively low (<5%) and thus the major portion of the fat deposited as adipose fat will be biosynthesised by the animal itself (*Emery 1980*).

In the cow adipose and milk samples, including those from concentrate-fed animals, the $C_{16:0}$ is less depleted in ^{13}C than the $C_{18:0}$ fatty acid. The $\delta^{13}C$ values of the $C_{18:0}$ fatty acids in cow's milk are depleted by approximately 2‰ relative to the $C_{18:0}$ fatty acid in adipose fat and may reflect a direct contribution from the more depleted fatty acids in the grass/forage (Fig. 3). The relative importance of these different contributions are discussed further below. The $\delta^{13}C$ values of fatty

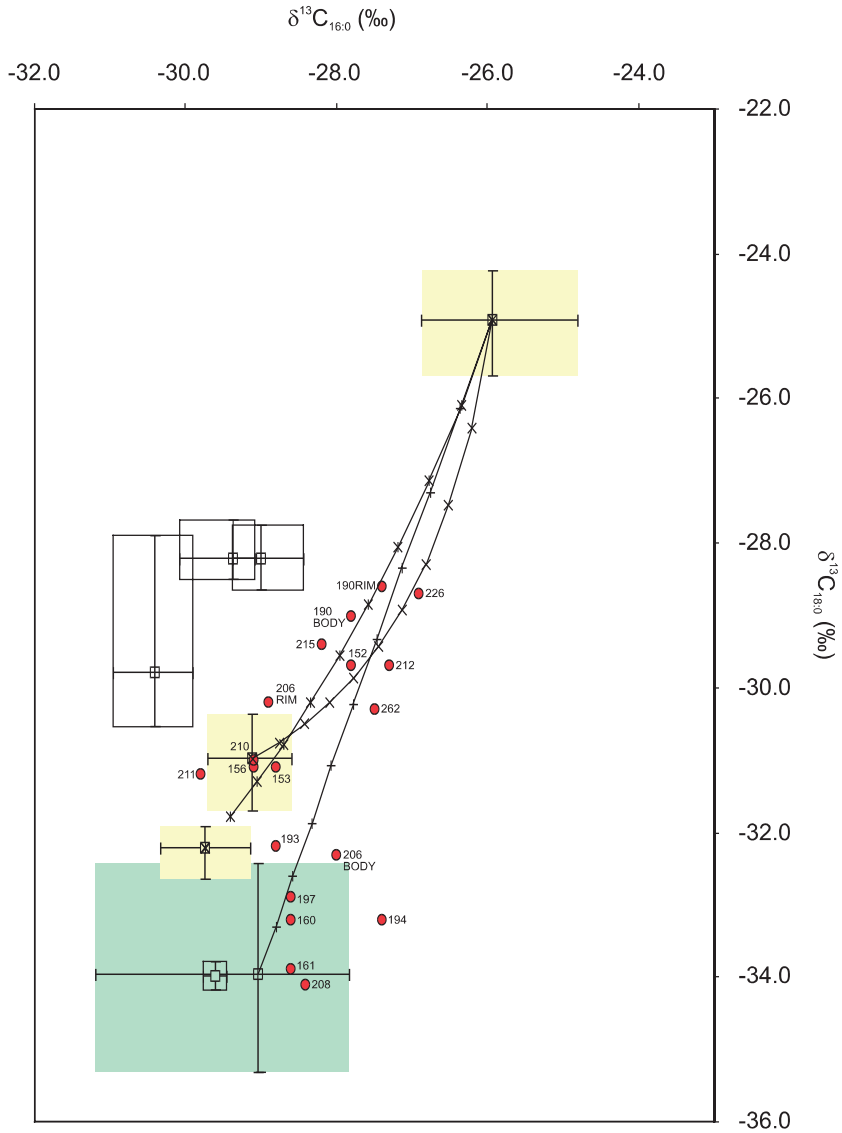


Fig. 8. Plot of the $\delta^{13}C$ values of the major n-alkanoic acid components ($C_{16:0}$ and $C_{18:0}$) from the lipid extracts of potsherds from the Iron Age/Romano-British site of Stanwick, Northamptonshire, compared with data from modern reference fats.

acids in milk from concentrate-fed animals are less depleted than milk from grass fed animals due to the influence of the supplements in their diet, however, the $C_{18:0}$ is still significantly more depleted in ^{13}C than the $C_{16:0}$. The $\delta^{13}C$ value of the $C_{18:0}$ fatty acid is also depleted relative to the $C_{16:0}$ by ca. 4‰ in sheep milk (Fig. 4). The difference between the $C_{16:0}$ and $C_{18:0}$ fatty acids in sheep adipose is of lower magnitude (ca. 2‰). The effect of diet on the composition of milk fats is clearly shown by the relatively higher $\delta^{13}C$ values (up to –18‰) of the fatty acids in dairy fats from cows fed concentrate supplements. Thus, there appears to be a distinction between the $\delta^{13}C$ values of milk and adipose fats from ruminant animals probably reflecting differences in the physiological and metabolic processes involved in their production.

Non-ruminant fats comprise fatty acids have higher $\delta^{13}C$ values in the order $C_{14:0}<C_{16:0}<C_{18:0}$ (Fig. 5). However, in ruminant milk and adipose fats the opposite is the case, with the $C_{14:0}$ displaying the higher $\delta^{13}C$ value (Fig. 4). In non-ruminant body fats there is less variation (ca. 0.5–1‰) between the $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acids compared with ruminant fats. The fatty acids in the tissues display significantly higher $\delta^{13}C$ value than the fatty acids in the diet and more closely reflect the bulk $\delta^{13}C$ value obtained for the diet. The ~2–4‰ depletion in ^{13}C relative to the bulk diet is possibly due to discrimination against ^{13}C during *de novo* fat biosynthesis in non-ruminants.

The lipid components of the porcine fats are ca. 5‰ more enriched than that of the herbivores, probably reflecting several factors, including: i) the isotopic composition of the diet; ii) the proportion of protein components in the diet (e.g. meat protein); iii) the degree to which different animals rely on different fractions of the diet for energy metabolism [e.g. carnivores depend mainly on protein for energy metabolism, whereas herbivores and omnivores may use excess protein for energy (*Krueger and Sullivan 1984*)]; iv) the presence of the rumen in herbivores which facilitates breakdown and absorption of complex organic materials, and v) the differences in metabolism by which different fractions in the diet are routed or scrambled into the production of body fats. Studies have suggested that carbon in dietary proteins is routed to collagen in rats (*Chisholm et al. 1982*) indicating that some routing occurs rather than simple scrambling of all the different components in the diet (*Schwarcz et al. 1985; Spielmann et al. 1990*). However, at present, factors controlling the isotopic composition of tissues of

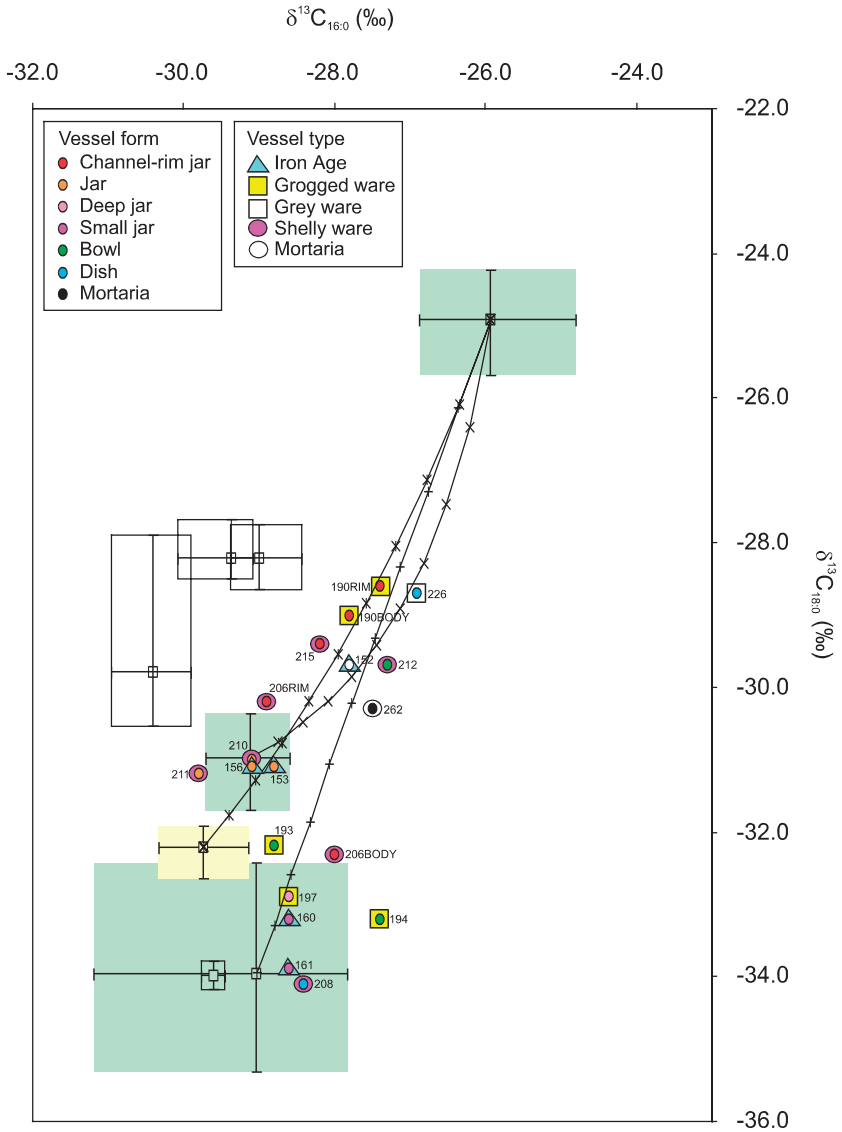


Fig. 9. Plot of the $\delta^{13}C$ values of the major n-alkanoic acid components ($C_{16:0}$ and $C_{18:0}$) from the lipid extracts of potsherds from the Iron Age/Romano-British site of Stanwick, Northamptonshire, compared with vessel type and form.

different herbivorous and omnivorous animals are poorly understood.

Figure 5 shows that a distinct pattern exists in the relationship between the diet and adipose tissue of non-ruminant animals, with both the $C_{16:0}$ and $C_{18:0}$ fatty acids equally enriched in ^{13}C relative to the diet. The degree of enrichment is variable between species, with differences of ca. 1‰ between fatty acids in chicken feed and adipose fat, and up to 5‰ between fatty acids in pig feed and pig adipose fat.

Compound specific $\delta^{13}C$ values of animal fats preserved in archaeological pottery

Archaeological samples were selected for stable carbon isotope analysis on the basis that the overall distribution of lipid components resembled a degraded animal fat, and that they contained sufficient quantities of $C_{16:0}$ and $C_{18:0}$ fatty acids for analysis by GC–C–IRMS. Extracts in which leaf wax components were also identified were generally avoided in order to obtain pure animal fat signals (*Charters et al. 1993*). In order to assess the effect of mixtures of fats from different reference animals on the isotopic signal, theoretical mixing lines have been constructed according to Woodbury *et al.* (*1995*); such mixing curves take into account both the relative proportions of the major *n*-alkanoic acids and the $\delta^{13}C$ values of the acids present in the pure fats. The $\delta^{13}C$ values for mixtures of fats in varying proportions are plotted for comparison with the archaeological data.

Sites with well-documented faunal assemblages

West Cotton (Late Saxon/early medieval) – $\delta^{13}C$ values were obtained for the $C_{16:0}$ and $C_{18:0}$ fatty acids in the selected remnant fats from West Cotton. The data are plotted in Figure 6 together with $\delta^{13}C$ values obtained for the fats of modern equivalents of the domesticated animals represented in the faunal assemblage at West Cotton. Three archaeological fats, sample nos. RP4, 10 and 88, correspond closely with the data obtained for the reference pig fats. The majority of the remainder contain fatty acids with $\delta^{13}C$ values which plot along the mixing curves between the reference ruminant and non-ruminant adipose fats and in the region of the reference ruminant fats. Several of the archaeological fats from West Cotton were found to correspond to the data obtained for the reference ruminant milk fats, including RP30, 60, 61, 86, 94 and WC30, distinguished by a lighter isotopic signal (mean –33‰) for the $C_{18:0}$ fatty acid. Two other archaeological fats, sample nos. RP72 and 91, cluster around the mixing curve between the reference milk and non-rumi-

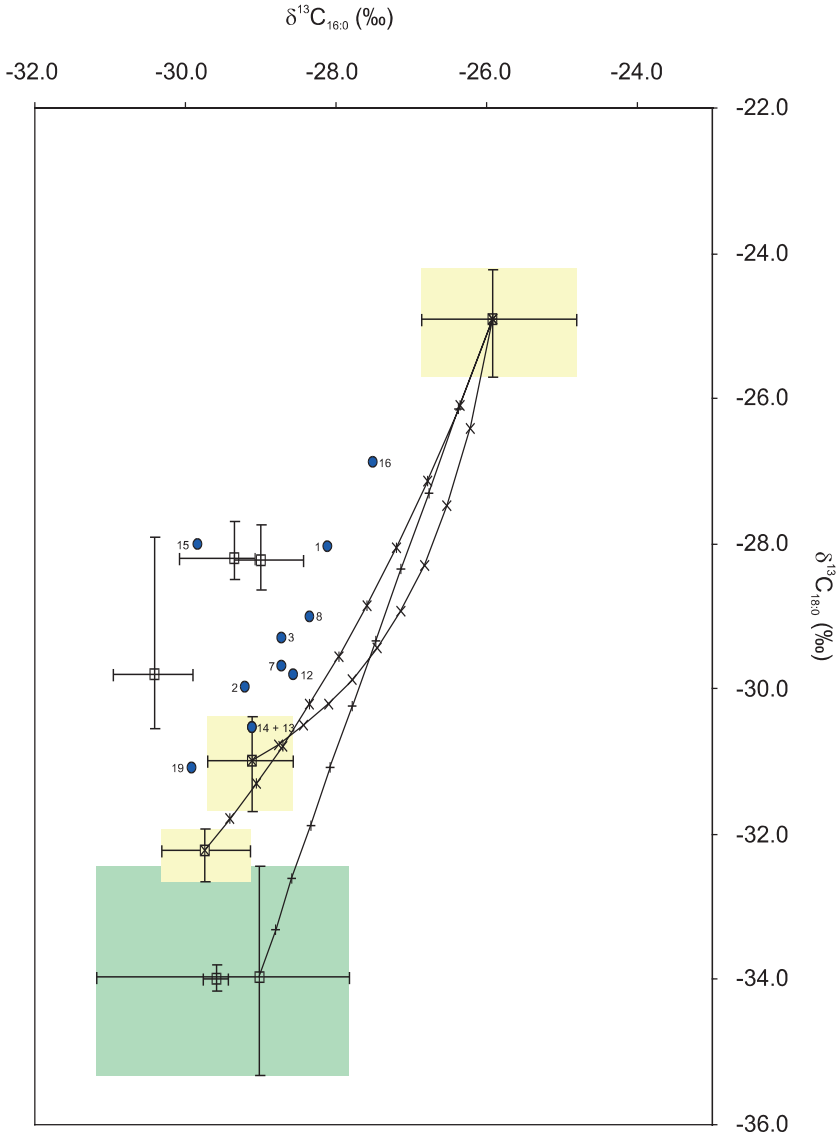
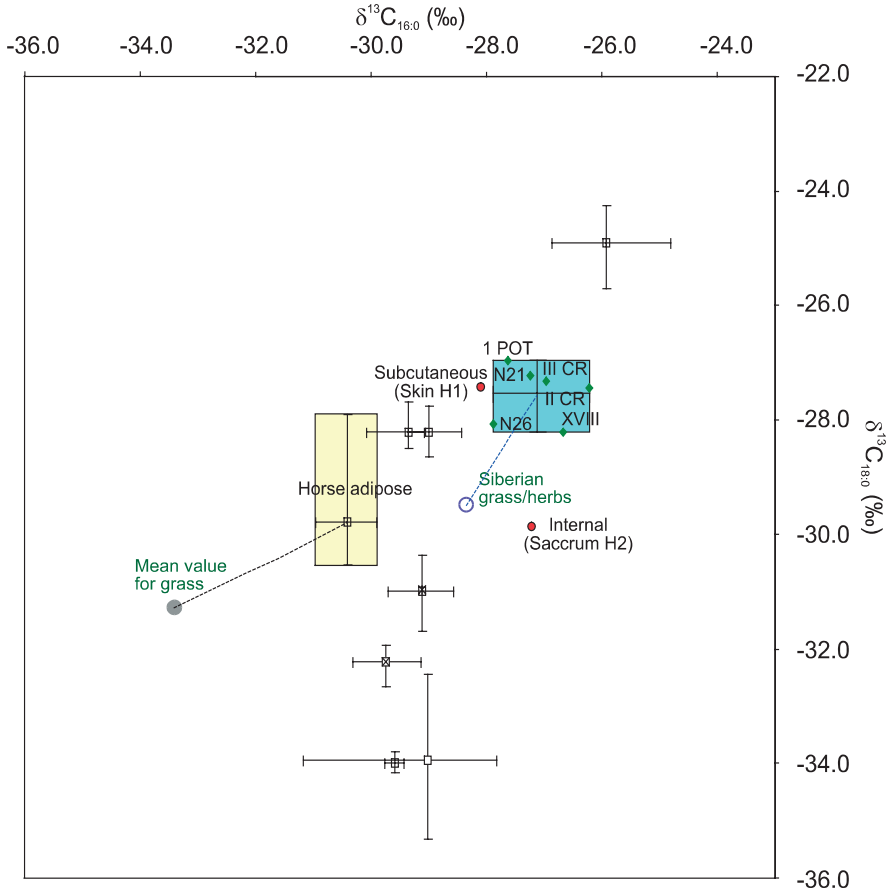


Fig. 10. Plot of the $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acids in solvent extracts of Wicken Bonhunt potsherds compared with data from modern reference fats.

nant fats. Based on the distributions of lipid components, it had previously been assumed that the majority of remnant fats from West Cotton derived from degraded adipose fats (*Charters 1996*), probably of an ovine origin due to the high abundance of sheep bones recovered from the site. However, in the light of these new data, at least six remnant fats appear to have a dairy origin based on their $\delta^{13}C$ values. WC30, the medieval 'top hat' vessel from West Cotton contained an appreciable abundance of short-chain fatty acids which are diagnostic of milk fats. It is notable that the $\delta^{13}C$ values for the $C_{16:0}$ and $C_{18:0}$ fatty acids from this residue plot in the range for the reference milk fats since this unusually well-preserved residue helps to validate the methods described herein for the detection of acid fat. None of the other remnant fats from West Cotton contained such a high abundance of short-chain fatty acid components which could identify them as dairy fats, the only evidence indicating their dairy origin has been obtained through determination of the $\delta^{13}C$ values of their $C_{16:0}$ and $C_{18:0}$ fatty acid components.

The $\delta^{13}C$ values obtained in the re-analysis of the West Cotton vessels previously studied by Charters (*1996*) show that the original interpretations still stand, with vessel RP78 derived from either a ruminant adipose origin or from a mixture of ruminant and non-ruminant fats. The results of the re-analysis also indicate that the fats from the spouted bowls all derive from ruminant animals, with $\delta^{13}C$ values correlating with those obtained for the reference dairy fats. The $\delta^{13}C$ values indicate a common function for these spouted bowls. Since sub-samples of the same potsherds were re-extracted prior to stable carbon isotope analysis as part of this study, the close similarity of the results obtained in analyses by Charters (*1996*) and the new data provide further validation of the analytical procedures employed and illustrate the reproducibility of the compound-specific $\delta^{13}C$ analyses.

Figure 7 shows a plot of the $\delta^{13}C$ values compared with vessel form. Vessel function does not correlate well with form, although some observations can be made, e.g. both of the St Neots Jars [refer to Charters (*1996*) for a detailed description] have given $\delta^{13}C$ values which indicate ruminant adipose fats are present. Shelly ware jars were apparently used for a range of culinary functions since various sherds from these vessels have isotope values corresponding with ruminant adipose and dairy fats and non-ruminant fats; $\delta^{13}C$ values from the Shelly ware bowl indicate the presence of non-ruminant fat.

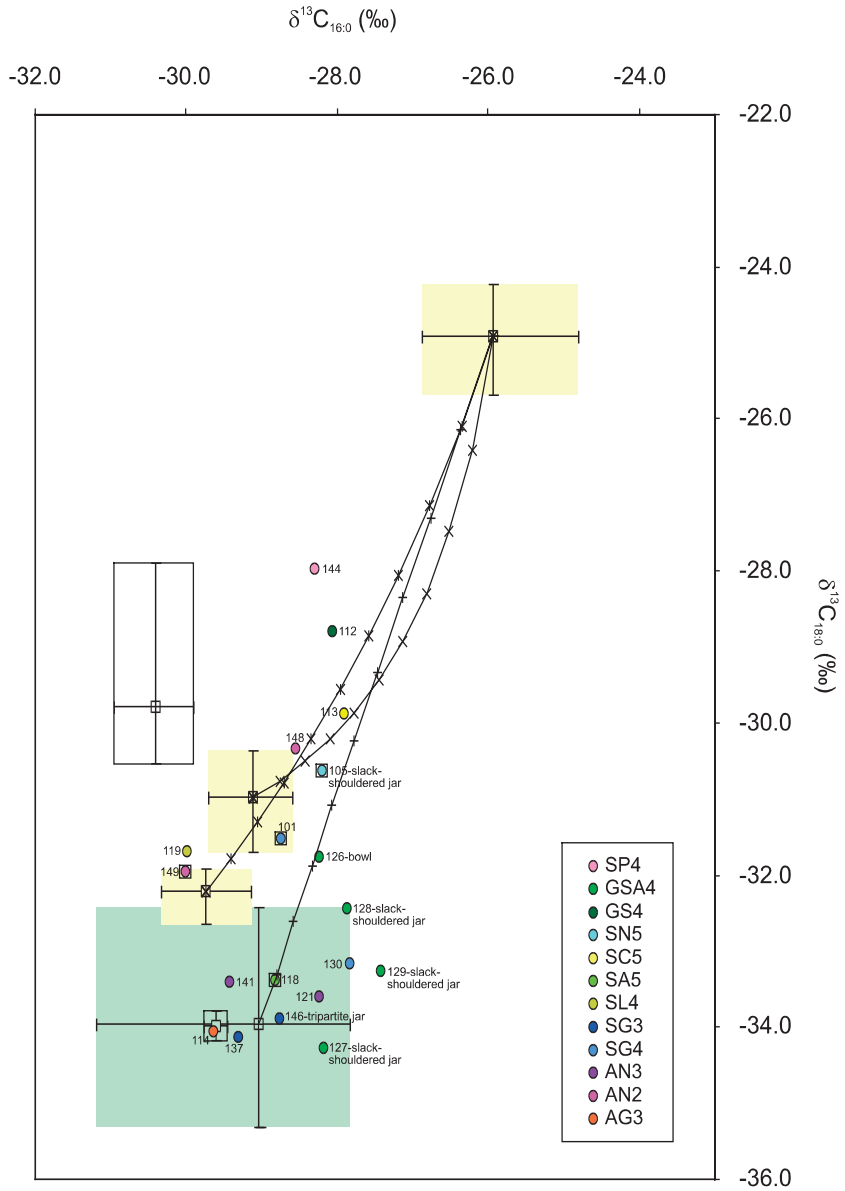


Some correlation can be observed between $\delta^{13}C$ values and date, including: i) the Late Saxon residues plot in the region of the ruminant adipose and dairy fats; ii) the early medieval pots, ca. 1100 to 1150 AD (site of an early medieval settlement and manor) plot in the region of the ruminant adipose and in line with the mixing curve indicating mixtures of ruminant and non-ruminant fats, however, there is no isotopic evidence for dairy

Fig. 11. Plot of the $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acids in extracts of archaeological potsherds from Botai, Kazakhstan, compared with values for reference animal fats and the fatty acid components in their diet.

or pure non-ruminant fats; iii) data from four sherds dating ca. 1150–1225 AD include two which plot closely together quite high up the mixing curve towards the reference porcine fats and two which plot together in the region of the dairy fats, and iv) all of the sherds dating between 1225 and 1300 AD (site of medieval manor and hamlet) plot in the region of the ruminant adipose fats. Unfortunately, dates are not known for all of the sherds analysed; however, it appears that residues from both ruminant adipose and dairy fats are associated with all periods at West Cotton. A larger data set for the main periods would provide a clearer picture of changes or trends in vessel and commodity use.

Stanwick (Iron Age/Romano-British) – Figure 8 is a plot of the stable carbon isotope data obtained for the $C_{16:0}$ and $C_{18:0}$ fatty acids from archaeological fats from the Stanwick assemblage compared with the same reference fats and mixing curves as previously described. The clustering of archaeological fats clearly illustrates the predominance of ruminant adipose and dairy fats in these Romano-British and Iron Age sherds. In contrast to West Cotton, none of the remnant fats from Stanwick plot with the non-ruminant (e.g. porcine) reference fats; however, a number of the archaeological fats plot along the mixing curve between the ruminant and non-ruminant fats. Several of the fats appear to have a dairy origin due to their close correlation with data obtained for the modern reference milk fats, including ST193, 206 body, 197, 160, 194, 161 and 208. The reliability and wider application of the stable isotope approach is re-enforced by the data obtained from these analyses since the spread of $\delta^{13}C$ values from Stanwick, seen in Figure 8, mirrors that seen in Figure 6 for the West Cotton extracts, except for the notable absence of non-ruminant (e.g. porcine) fats amongst the Stanwick assemblage.



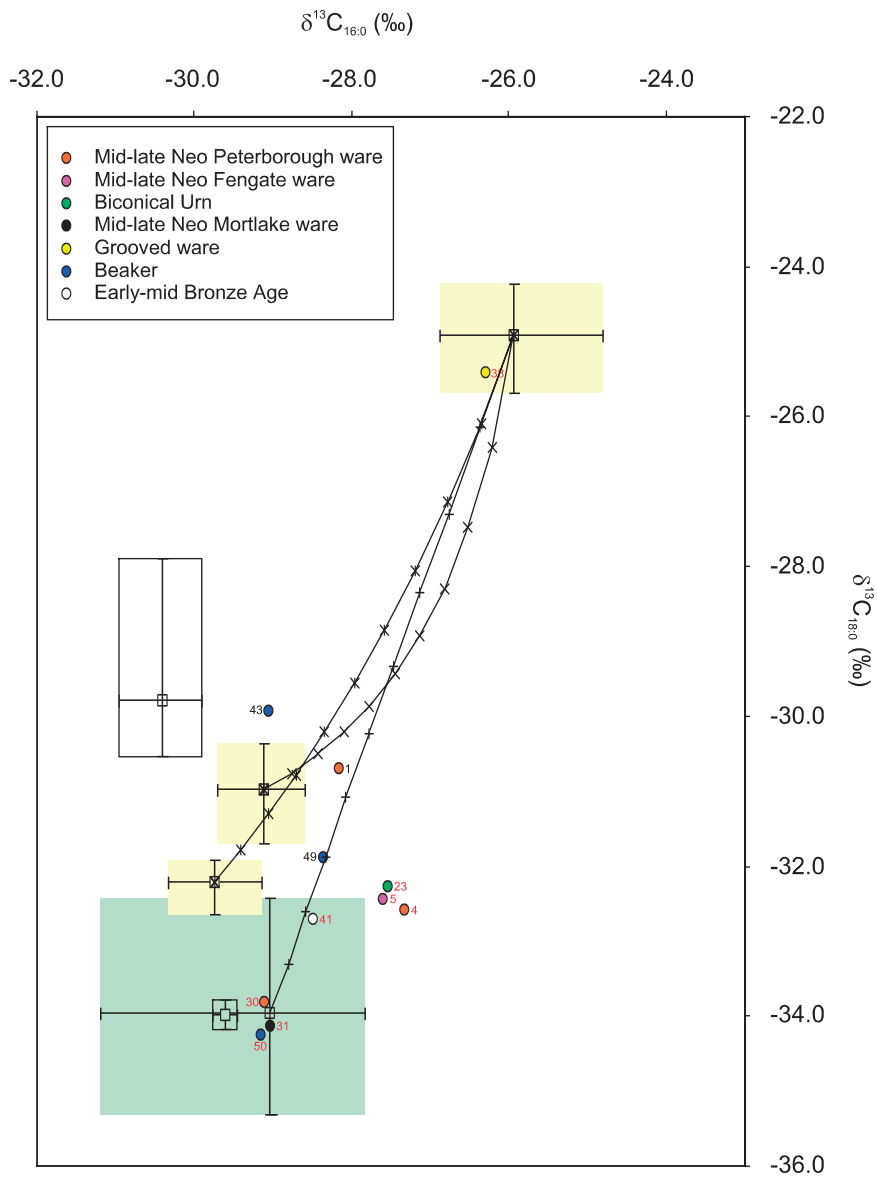
The correlation of vessel form/fabric type and stable carbon isotope ratios of the fatty acids in the Stanwick vessels shown in Figure 9 illustrates that both Grogged ware bowls contain residues which plot with the reference dairy fats, and both sherds from the Grogged Channel-rim jar plot closely together on the mixing curve between the reference ruminant and non-ruminant adipose fats. The two Iron Age Channel-rim jars correlate with the reference dairy fats and both of the Iron Age jars plot in the region of the reference ovine adipose fats.

Fig. 12. Plot of the $\delta^{13}C$ values of the fatty acids from lipid extracts of the Yarnton Cresswell field assemblage correlated with vessel fabric and form (where known) and compared with the values obtained for the modern reference fats. The fabric type abbreviations are explained fully in Table 7, Appendix 1 of Dudd (1999).

Sites with an unusually strong bias in the faunal record

Wicken Bonhunt (Romano-British/Middle Saxon) – The aim of these analyses was to investigate whether the high proportion of pig bone present at the site was reflected in residues preserved in the potsherds. The $\delta^{13}C$ values obtained for the potsherd extracts from the Middle Saxon site plot in a broad distribution between the modern ruminant and non-ruminant reference fats (Fig. 10). The fatty acids from the archaeological extracts are more depleted than those in the modern reference pig fats by up to 6‰. Several of the remnant fats plot close to the reference sheep adipose, although the majority of archaeological fats plot along the line of the mixing curve, indicating that these data may represent mixtures of fats processed in the same vessel or multiple usage of vessels. There is no clear correlation between the archaeological data and the porcine reference fats, nor is there any indication from the stable carbon isotope data that any of the archaeological fats from Wicken Bonhunt derive from a dairy origin.

Botai (early Neolithic) – Potsherds from Botai were sampled in anticipation of retrieving data from degraded horse fats due to the strongly attested association of this site with horse breeding. The $\delta^{13}C$ values obtained for fatty acids in the potsherd extracts are shown in Figure 11. The data points group together with mean $\delta^{13}C$ values of –27.1‰ for the $C_{16:0}$ and –27.5‰ for the $C_{18:0}$ fatty acids, but are distinct from the modern reference fats. The grouping of the data for the archaeological fats is relatively tight, indicating that these remnant fats all derive from the same animal origin. The remnant fats are less depleted by ca. 2–3‰ (in both the $C_{16:0}$ and $C_{18:0}$ fatty acids) than the modern reference horse fats from the UK. However, this difference can be attributed to differences in the isotopic composition of the diet of the horses raised in Kazakhstan from that of modern horses raised on forage in the UK. The data indicate that comparison of the stable isotope data from the fats of animals raised in different geographic locations are not directly comparable.



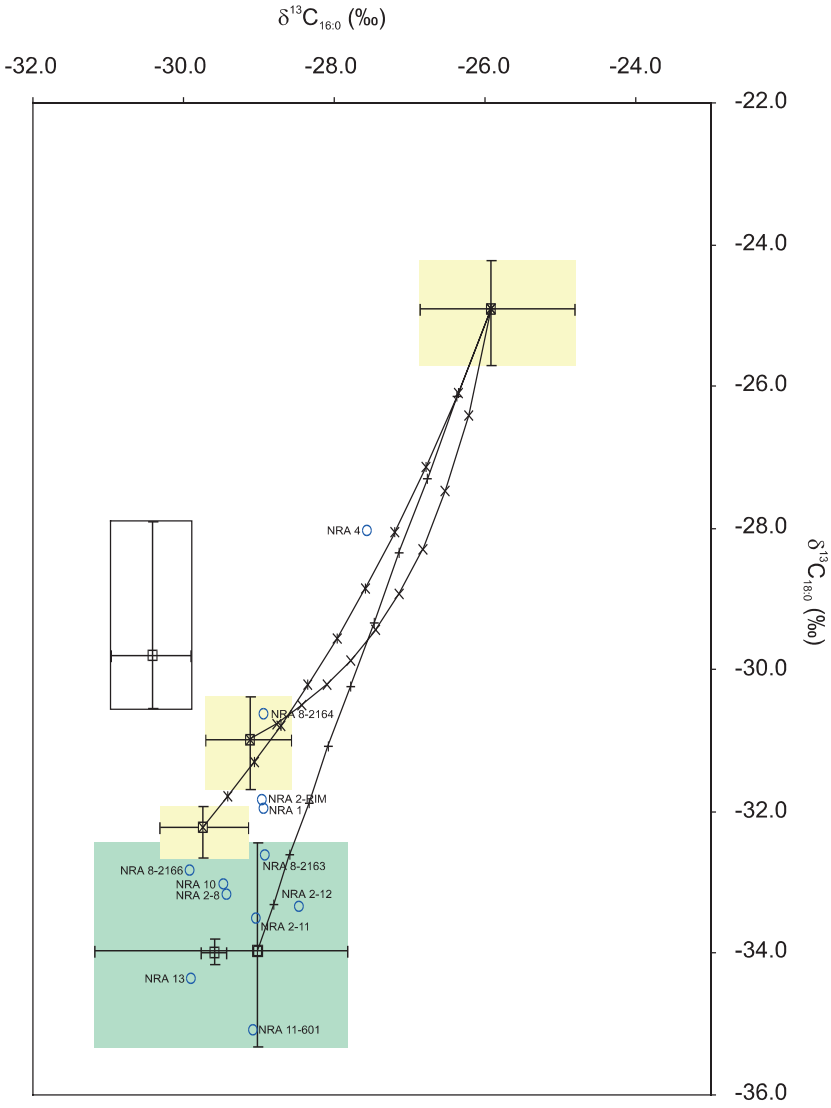
Prehistoric British archaeological sites

Yarnton Cresswell field (early-middle Iron Age) – The $\delta^{13}C$ values for the majority of the Yarnton Cresswell field extracts are relatively depleted in ^{13}C and plot in the region of the ruminant adi-

Fig. 13. Plot of the δ13C values of the fatty acids from lipid extracts of the Yarnton flood plain assemblage correlated with vessel type and compared with the values obtained for the modern reference fats typical of the archaeological period.

pose and dairy fats (Fig. 12). Approximately half of the archaeological extracts plot within the range of the modern reference cows' milk. Several extracts plot close to the mixing curves between the ruminant and non-ruminant reference fats. None of the remnant fats correlate with the reference porcine fats. There is some correlation between fabric types from Cresswell field and the $\delta^{13}C$ values of the extracts (Fig. 12), with the majority of the GSA4 (grog, shell and quartz sand; coarse textured) and SG3 (shell and grog; medium coarse textured) types plotting within the range for reference dairy fats as does sample 114 [fabric type AG3 (quartz sand and grog; medium-coarse textured)]. Sample 144 [fabric type SP4 (shell and clay pellets; coarse textured)] contains the remnant fat exhibiting the least depleted $\delta^{13}C$ values.

Yarnton flood plain (Neolithic-Bronze Age) – The stable carbon isotope data for fatty acids from the Yarnton flood plain extracts are plotted in Figure 13. The majority of the archaeological data points cluster in the region of the ruminant fats, with 4 vessels plotting well within the range for the reference cows' milk fats. Only one data point (sample 38) out of 11 analysed falls within the range of the non-ruminant (e.g. porcine) reference fats. Several vessels, including sample nos. 49, 23, 5 and 4 plot around the mixing curve between the milk and non-ruminant reference fats. In general, the archaeological vessels comprising higher abundances (>100 µg g^{-1}) of absorbed lipid also exhibited more depleted $\delta^{13}C$ values, resembling dairy fats. This is possibly a reflection of the ease with which certain fats are absorbed within the porous pottery, or the different ways in which vessels were used to process commodities, i.e. boiling or roasting. During the dosing of sherds for laboratory decay experiments we noted that substantially larger quantities of fat are absorbed when soaked in butter fat than in milk (*Dudd, Aillaud and Evershed, unpublished data*), suggesting that archaeological vessels containing substantial quantities of remnant dairy fats may have derived from butter rather than milk fats.



Correlation of vessel type with $\delta^{13}C$ values reveals a distinction between the residues from the Peterborough ware and the Grooved ware vessels, with the former yielding $\delta^{13}C$ values comparable to the reference ruminant fats and the latter consistent with reference porcine fats. This result is significant since we have recognised the same distinction in residues from Peterborough and Grooved ware vessels from the Neolithic settlement at Upper Ninepence, Walton (*Dudd et al. 1999*). Figure 13 also indicates that a range of different vessel types, including Peterborough and Mortlake wares from the mid-late Neolithic, a beaker and an Early-Mid-

Fig. 14. Plot of the $\delta^{13}C$ values of the fatty acids from lipid extracts of the Eton Lake End Road assemblage compared with the data obtained for the modern reference fats.

dle Bronze Age vessel, appear to have been associated with the processing of dairy fats.

Eton Lake End Road (late Neolithic-Early Bronze Age) – Similar to the Eton Rowing Lake assemblage, the majority of the extracts from Eton Lake End Road plot within the region of the reference ruminant dairy fats, with $\delta^{13}C$ values of <–28‰ for the $C_{16:0}$ fatty acid and <–32‰ for the $C_{18:0}$ fatty acid (Fig. 14). Three of the extracts, NRA 8–2164, NRA 2–rim and NRA 1 are slightly less depleted in ^{13}C, particularly in the $C_{18:0}$ fatty acid, and cluster with the reference ruminant adipose fats. Only one sample, NRA 4 is less depleted still and falls along the mixing curve between the ranges of the ruminant adipose and non-ruminant fats, possibly representing a mixture of ruminant and non-ruminant fats.

Eton Rowing Lake (early Neolithic) – All of the $\delta^{13}C$ values for the Eton Rowing Lake samples cluster within the ranges of the reference ruminant adipose and dairy fats (Fig. 15), indicating that all of the remnant fats are derived from a ruminant source. Samples 12 and 20 are less depleted than the other samples and correlate with the ranges for the reference adipose fats, while the remainder correlate well with the reference dairy fats. None of the extracts have $\delta^{13}C$ values suggesting a significant non-ruminant fat contribution.

Upper Ninepence (early-late Neolithic) – The $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acids in three absorbed residues from the Peterborough ware (P1, P3 and P5), two absorbed residues from the Grooved ware (P66 and P68) and three carbonised (interior) surface residues from the Grooved ware (P33, P38 and P39) are plotted in Figure 16. Clearly, there is a distinction between the absorbed residues from the Peterborough ware and the Grooved ware and between the absorbed and carbonised residues from the Grooved ware, based on differences in the $\delta^{13}C$ values of both the $C_{16:0}$ and $C_{18:0}$ fatty acids. The absorbed archaeological fats from the Grooved ware (both from site context 133) plot together near to the non-ruminant (e.g. porcine) reference fats, whilst the three archaeological fats from the Peterborough ware plot in the region of the ruminant fats, within the range of the reference dairy fats. The carbonised residues adhering to three other Grooved ware vessels, all excavated from the same pit, plot with the reference dairy fats, with the exception of sample P39, which is more depleted in ^{13}C. The absorbed residues from these same vessels were poorly preserved, all comprising <13 µg g^{-1} of lipid. The Grooved ware vessels associated with ruminant fat residues were excavated from a different archaeological feature

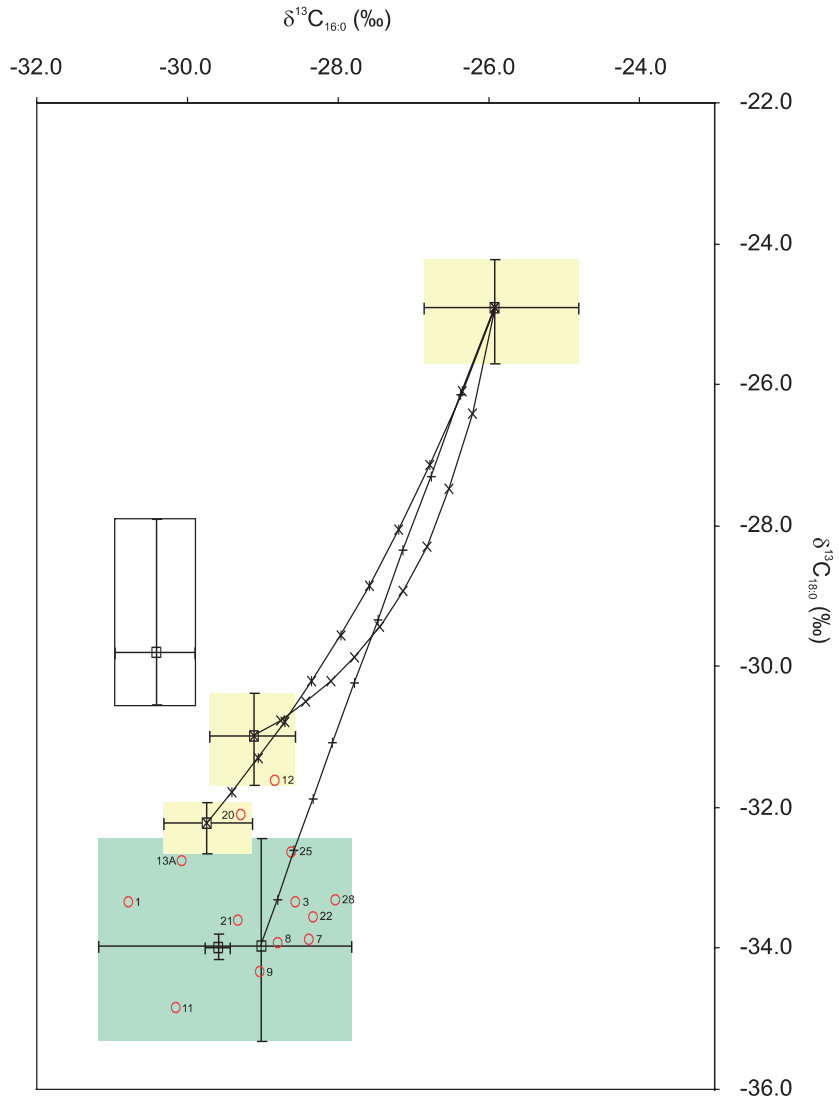


Fig. 15. Plot of the $\delta^{13}C$ values of the fatty acids from lipid extracts of the Eton Rowing Lake assemblage compared with the values obtained for the modern reference fats typical of the archaeological period.

than those of the same period corresponding with the non-ruminant reference fats.

Archaeological horse fats and tissues – Since the remnant fats from Botai were not directly comparable with UK reference horse fats, we obtained samples of Siberian horse fats for use as reference data. The sample of stomach lining from Horse 1 comprised free fatty acids and highly abundant hydroxy acids. The degraded fat contained some intact triacylglycerols, however these were present in very low abundance. The lipid components of the stomach contents were also analysed, with the saponified, methylated extract comprising a range of long-chain, saturated and unsaturated free fatty acids. The subcutaneous fat (skin; Horse 1) comprised free fatty acids, hydroxyoctadecanoic acid, cholesterol and a greater abundance of intact triacylglycerols than in the sample of stomach lining from the same horse (Fig. 17). The Sacrum meat and crumbled flesh associated with the coccygeal vertebra from Horse 2 yielded an abundance of free fatty acids and also di- and triacylglycerols in low abundance. The lipid components are similar in distribution to the subcutaneous fat from Horse 1. The distributions of free fatty acids, mono-, di- and triacylglycerols in this sample are consistent with other degraded animal fats described and are remarkably well preserved due to the permafrost burial conditions.

The $\delta^{13}C$ values obtained for the $C_{16:0}$ and $C_{18:0}$ fatty acids in the archaeological horse fats from the Siberian tomb are less depleted than the modern reference horse fats, particularly with respect to the $C_{18:0}$ fatty acid (Fig. 11). The $\delta^{13}C$ values of the fatty acids from the internal (sacrum) and subcutaneous (skin) fat samples from the Siberian horses vary by ca. 1‰ and 2.4‰ for the $C_{16:0}$ and $C_{18:0}$, respectively. This may reflect the fact that the fat sample from the sacrum resembled adipocere, consisting predominantly of free fatty acids, which may have been contaminated by fatty acids from micro-organisms. There may also be some natural variation between tissues from different parts of the body.

The bulk $\delta^{13}C$ values obtained for the grass in the stomach of the Siberian horse were more depleted by approximately 2‰ than modern UK grasses (25.9‰ compared with 27.9‰); this was also reflected in the $\delta^{13}C$ values of the individual fatty acids in the grass (Fig. 11). The stomachs of these horses were found to contain a wide range of herbs and grasses typical of a rich upland pasture, whereas the diets of our modern reference horses was dominated by one or two grass species, from heavily grazed fields. Since non-ruminants and pseudo-ruminants are believed to be more directly

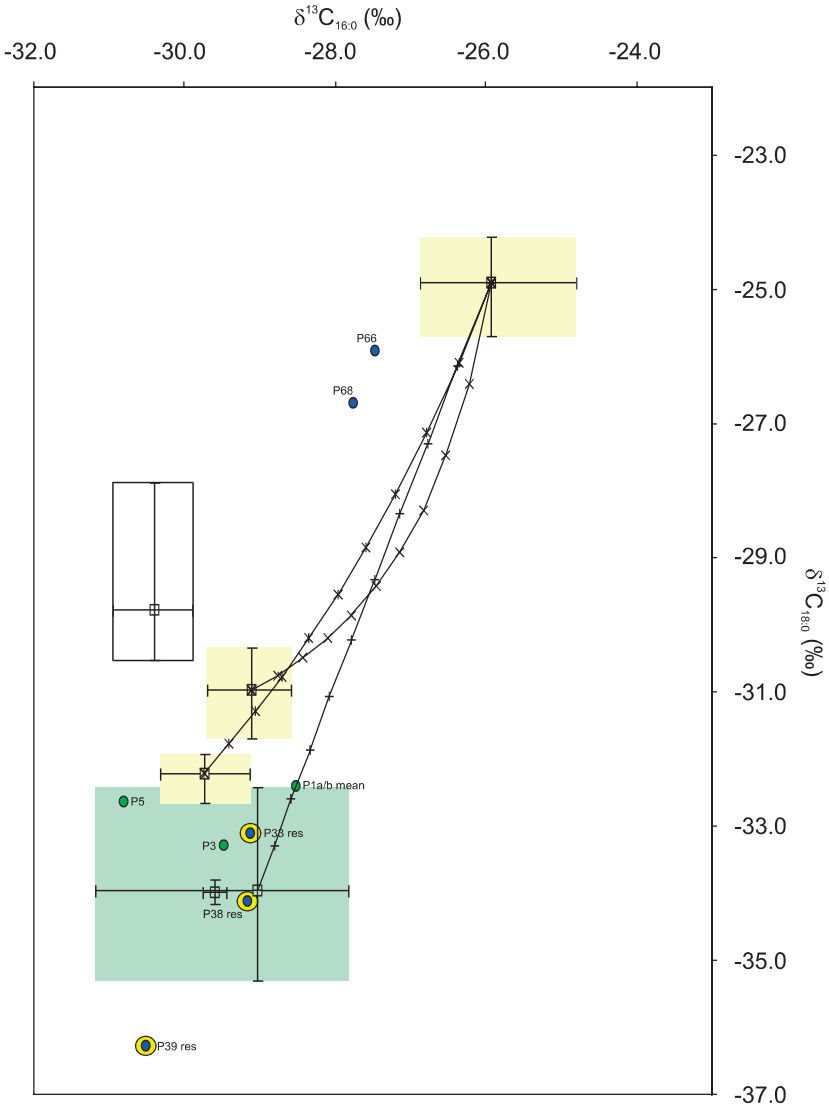


Fig. 16. Plot of the $\delta^{13}C$ values for the major n-alkanoic acid ($C_{16:0}$ and $C_{18:0}$) components of the lipid extracts of potsherds from the Walton assemblage: Grooved Ware = blue-filled circles; Peterborough Ware = green-filled circles; Carbonised surface residues = yellow ring around the data point.

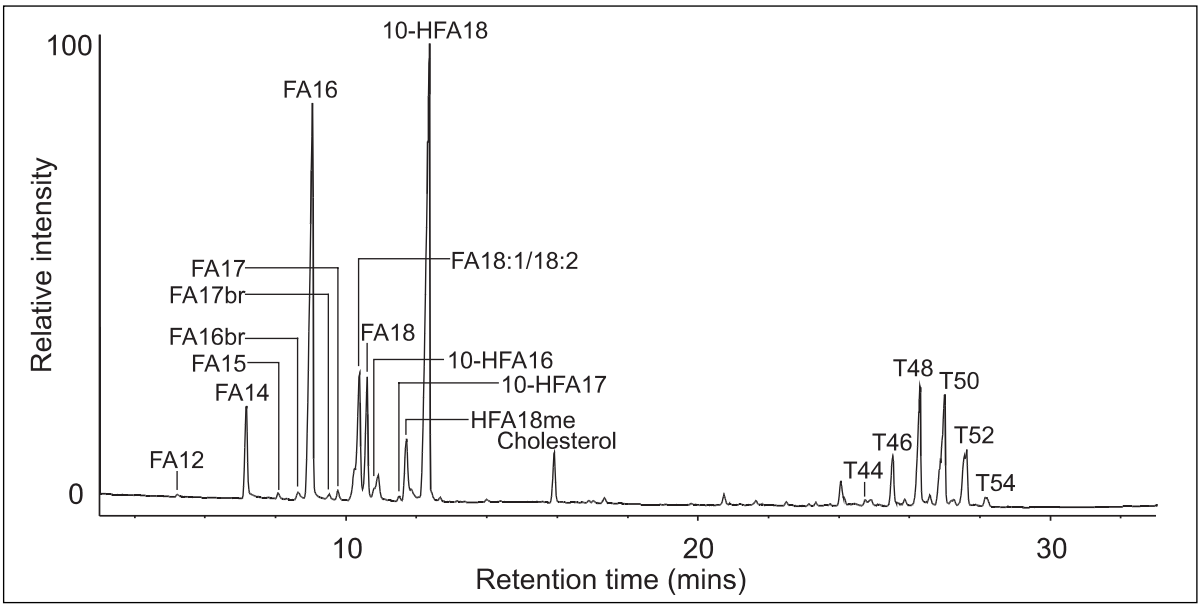


Fig. 17. Partial HTGC profile of the trimethylsilylated total lipid extract of the remnant fat associated with the horse skin/hide from the 'Ice Princess' burial in the Altay mountains. Peak identities are: FA12, FA14, FA15, etc. correspond to n-alkanoic acids with 12, 14 and 15 carbon atoms, etc., respectively; FA17br refers to a branched-chain alkanoic acid with 17 carbon atoms; FA16:1 and FA18:1 refer to monounsaturated n-alkanoic acids containing 16 and 18 carbon atoms, respectively; T44, T46, T48, etc. correspond to triacylglycerols bearing 44, 46, 48, etc. acyl carbon atoms, respectively; IS = internal standard (n-tetratriacontane) added at the extraction stage to enable quantification of lipid. All peak assignments have been confirmed by GC/MS analysis. In addition, 10-HFA 16, 17 and 18 refer to 10-hydroxy fatty acids with 16, 17 and 18 carbon atoms, respectively; HFA 18 me refers to methyl ester of the C18 10-hydroxy fatty acid.

influenced by their diet, the differences in the $\delta^{13}C$ values of their tissues can be readily related to differences in the composition of the diet. Clearly, the heavier dietary carbon consumed by the Siberian horses had led to less depleted values for the fatty acids in their depot fats compared with our modern reference horses.

The data from the Siberian horse fats are clearly more comparable with the data obtained for the remnant fats from the Botai potsherd extracts than the modern horse fats. This positive correlation obtained for remnant fats from the more similar geographical region indicates that the absorbed residues in the Botai potsherds are, indeed, derived from horse fats processed in the vessels in antiquity.

DISCUSSION

Reference fats

The compound specific $\delta^{13}C$ values recorded for individual fatty acids have enabled clear distinctions to be drawn between adipose fats from the major species of domesticated ruminants, non-ruminants and poultry, and furthermore, has shown that significant differences exist between the composition of adipose and milk fats from dairy animals based on $\delta^{13}C$ values of the major saturated fatty acids.

Inter-species variation – The $\delta^{13}C$ values obtained for the reference ruminant adipose fats are relatively similar between different individuals of the same animal species. The sheep adipose gave mean $\delta^{13}C$ values of –29.1‰ ± 0.6 and –31‰ ± 0.7 and the cow adipose gave mean values of –29.7‰ ± 0.6 and –32.2‰ ± 0.4 for the $C_{16:0}$ and $C_{18:0}$ fatty acids, respectively. The variation can be attributed to the way in which ruminants can break down and reassimilate components from various sources of carbon in the diet and also to the fact that body fats represent an average value for carbon accumulated over several months. Conversely, the $\delta^{13}C$ values measured for the bovine dairy fats cover a broader range (mean values of –29.0‰ ± 2.2 and –34‰ ± 1.5 for the $C_{16:0}$ and $C_{18:0}$ fatty acids, respectively), which is thought to be partly a reflection of the recent diet of the animal, due to the turnover of carbon in milk production being significantly faster than that of body fats (*Tieszen et al. 1983*). Thus, the composition of the dairy fats may vary significantly in isotopic composition, e.g. according to the availability of particular forage materials or seasonal variations in $\delta^{13}C$ values of the plant tissues. No correlation could

be found between the stable isotope composition of the reference milk samples and the time of year or stage of lactation during which they were collected.

The range of $\delta^{13}C$ values measured for fatty acids in the porcine fats is similar but, slightly greater than for the ruminant adipose fats, and is probably representative of the range of foodstuffs a pig will consume and the direct routing of dietary fats to body fats. It is well established that in non-ruminants such as pigs, little modification of the fats occurs unless utilised for energy (*Christie et al. 1972*). The range of $\delta^{13}C$ values for fatty acids in tissues of goose and chicken fats were comparable with the ruminant reference fats, however, the values for the same fatty acids in deer adipose fats varied significantly, by up to 2‰ and 4‰ in the $C_{16:0}$ and $C_{18:0}$ fatty acids, respectively. Since the deer fats were all taken from animals of the same herd raised on the same unimproved pasture, the $\delta^{13}C$ values are surprisingly variable. To some extent this may be a reflection of 'ecological variability', referred to as the 0.2–2‰ standard deviation found for animals of the same species raised in similar environments on the same diets (*DeNiro and Epstein 1978; Teeri and Schoeller 1979; Tieszen et al. 1983*).

Contribution of dietary fat to milk – It has been recognised that the $\delta^{13}C$ value of the $C_{18:0}$ component of milk obtained from cows grazing on C_3 pastures differs isotopically from the same compound in subcutaneous adipose fat of cattle grazing on the same pasture. This distinction reflects the well-established pathways involved in the formation of milk and adipose fats in ruminant animals (*Church 1988; McDonald et al. 1988*), demonstrated by Tove and

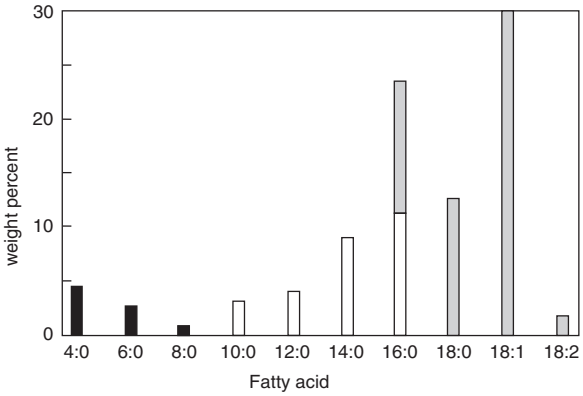


Fig. 18. Probable origin of fatty acids in ruminant milk (from* Dimick et al. 1970*): 4-carbon unit ■; malonyl-CoA pathway □ and circulating blood lipids ▨.

Mochrie (*1963*) in an investigation of tissue and milk fats sampled simultaneously from cows fed whole ground soybeans. They observed that the percentage of both $C_{18:0}$ and $C_{18:1}$ increased markedly in the milk fat. This was compensated for by a decrease in the percentage of $C_{14:0}$ and $C_{16:0}$, and confirmed the contribution of dietary long- chain fatty acids to milk fat in ruminant animals.

Figure 18 illustrates the probable contribution of carbon from different sources to milk lipids according to Dimick *et al.* (*1970*). The substantial proportions of short- and medium-chain fatty acids in milk lipids are a result of a very active *de novo* synthesis from the simple metabolites acetate and β-hydroxybutyrate, which are supplied to the mammary gland (*Dils 1983*). It is well-established that a proportion of the $C_{16:0}$ and essentially all the C_{18} acids are derived *via* the circulating blood lipids. $C_{16:0}$ is known to be derived from two sources; pre-formed from the

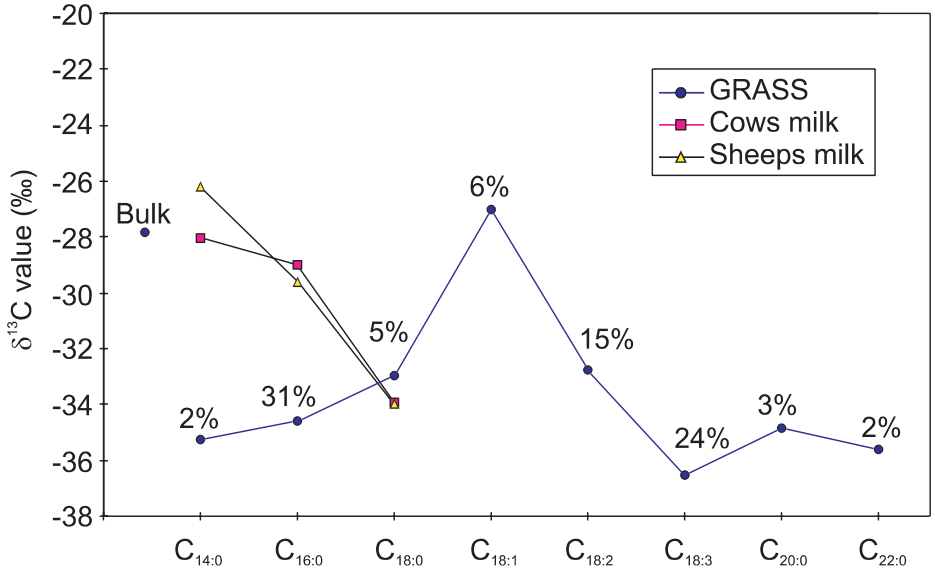


Fig. 19. Stable carbon isotope data obtained for the major saturated fatty acids in cows (mean of 8 individuals) and sheep (mean of 2 individuals) milk compared with $\delta^{13}C$ values for the bulk diet (grass) and individual fatty acids in the diet. The relative abundances (mean %) of the different fatty acids in grass are shown.

blood, and synthesised within the mammary gland from 2 carbon units (*Dimick et al. 1970*). Triacylglycerols in the blood may arise either directly from absorbed (exogenous) fat or from endogenous fat *via* liver synthesis of very low density lipoproteins (VLDL; *Dils 1983*).

Various studies have been carried out to determine the proportions of dietary fat which contribute directly to fatty acids used in milk production. Banks *et al.* (*1976a*) showed that a low fat ration limited milk production, and tracer studies have indicated that 54% of dietary C_{18} fatty acids (*Banks et al. 1976b*) and 76% of dietary $C_{18:2}$ (*Palmquist and Mattos 1978*) are transferred directly to milk fat. However, estimates are dependant upon the physiological state of the animal and will also reflect changes in the contribution of endogenous (adipose) fatty acids to milk secretion as fatty acid intake varies, and contributions from rumen-synthesised fatty acids. Other isotopic labelling studies have estimated that 44% of milk fat is of direct dietary origin with approximately 6% of long-chain fatty acids from endogenous sources (*Garton 1963*). Plowman *et al.* (*1972*) noted a rapid change in milk fatty acid composition when protected fat was fed to lactating ruminants; milk fat with increased polyunsaturated fatty acids was produced by feeding cows a diet containing a H_2CO-treated safflower oil-casein particle. The treatment protected the $C_{18:2}$ acid in safflower oil from biohydrogenation in the rumen and $C_{18:2}$ acid content in the milk increased from 3 to 35% of the total fatty acids.

It has been suggested that in the mammary gland, long-chain fatty acids are produced by chain elongation. This has been demonstrated in ruminant adipose tissues by the formation of labelled $C_{18:0}$ and $C_{18:1}$ from [1-^{14}C]-acetate *in vitro*, where between 45–55% and 60–70% of the fatty acids synthesised in bovine (*Pothoven et al. 1974*) and ovine (*Deeth and Christie 1979*) adipose tissue slices, respectively, were elongated to C_{18} fatty acids. The $C_{18:0}$ in milk fat could therefore be partially derived from the $C_{14:0}$ and $C_{16:0}$ fatty acids in the diet following chain elongation, and would thus incorporate the relatively depleted carbon from these fatty acids. It should also be remembered that many other forage materials, including herbs and shrub vegetation, will also contribute long-chain fatty acids to the diet. As previously mentioned, these fatty acids are believed to be significantly more depleted than those in grass and probably contribute to the more depleted values for the $C_{18:0}$ in milk fat. Figure 19 shows the relative abundances of the individual long-chain fatty acids in a typical ruminant diet (mainly grass). The $C_{18:0}$ component comprises only 5% of the total, while together the unsaturated C18 fatty acids with $\delta^{13}C$ values of less than –34‰ constitute a total of 29%.

The stable isotope data obtained for the $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$ fatty acids in the reference ruminant milk fats are also compared in Figure 19. There are large differences between the $\delta^{13}C$ values of the major fatty acid components of the milk fats, amounting to ca. 6‰ and 8‰ between the $C_{14:0}$ and $C_{18:0}$ in cow's milk and sheep milk, respectively. The $\delta^{13}C$ value of the $C_{14:0}$ in milk is clearly not consistent with a direct dietary origin, due to the difference in $\delta^{13}C$ value from the $C_{14:0}$ in the grass, but reflects the value for the bulk diet (i.e. mainly carbohydrate). The $\delta^{13}C$ value of the $C_{18:0}$ fatty acid in the milk fats does not reflect the bulk value for milk which indicates that this component is derived, at least partially, from a source other than the carbohydrate in the diet. The relatively depleted $\delta^{13}C$ values recorded for the C_{16} and C_{18} fatty acids in the diet (ca. –27 to –37‰) indicate that these components could be contributing to the depleted values of the C_{16} and C_{18} fatty acids in the milk fat.

The almost linear relationship between the $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$ fatty acids shown in Figure 19 supports the work by Dimick *et al.* (*1970.Fig. 17*) who have suggested that the $C_{14:0}$ fatty acid is derived predominantly from the malonyl-CoA pathway while the $C_{16:0}$ fatty acid forms from approximately equal contributions from both the circulating blood lipids (including dietary fatty acids) and *de novo* synthesis. The majority of the C_{18} is reported to be derived from circulating blood lipids which include up to 50% of long-chain dietary fatty acids ($C_{18:0}$ and unsaturated C_{18} after biohydrogenation).

Stable carbon isotope analyses of the different biochemical fractions which comprise grasses and herbs from unimproved pastures are currently under investigation in our laboratory in order to investigate the various sources of carbon available to animals which consume them. Results obtained to date have shown C_{18} fatty acids with highly depleted $\delta^{13}C$ values in the range of –36‰, e.g. in herbs such as clover (*Docherty and Evershed, unpublished data*). The consumption of these highly depleted fatty acids would explain the more depleted $\delta^{13}C$ values for the $C_{18:0}$ components in milk fat since herbivorous grazers will consume a variety of foliage and herbage as well as grass. The regulation of milk production and adipose fat formation is far from simple

and as yet not fully understood but since fatty acid output in the milk of lactating cows usually exceeds daily intake of fatty acid, lipid metabolism must play an important, if not central, role in the energy economy of the lactating cow.

In addition to dietary fat content and metabolic variations, the range of stable carbon isotope values obtained for the reference and archaeological milk samples probably reflect variation in the proportion of fibre in the animals diets, the effects of a range of environmental stresses on the animals and also the stage of lactation. Palmquist and Mattos (*1978*) suggest that their estimates of fatty acid transfer from the diet may not be valid during the non-steady state when the cow is rapidly losing adipose stores during early lactation.

Changes in plant carbon isotope ratios may occur due to environmental heterogeneity which are most likely associated with either large differences in soil moisture content (affecting plant water status) or light intensity (*Fogel and Cifuentes 1993; Lockheart et al. 1997*). Lowdon and Dyck (*1974*) have shown that the $\delta^{13}C$ values of maple leaves and a grass species collected at a single location may vary more than 5‰ during the growing season. A recent study of the $\delta^{13}C$ values of individual fatty acids in vegetable oils has shown sources of variability relating to geographical origin of the oil, year of harvest and the particular variety of the oil (*Woodbury et al. 1998*).

Archaeological pottery

All of the archaeological fat extracts prepared as FAME and analysed by GC-C-IRMS have yielded $\delta^{13}C$ values which correlate closely with the range of data obtained for the modern reference fats. The data from each site appear to correspond either with the ruminant dairy, ruminant adipose, non-ruminant adipose or lie along the line of the theoretical mixing curve between ruminant and non-ruminant adipose fats. None of the values appear to be erroneous or affected adversely by decay so that they lie far from the reference fat data points. Coupled with the distributional data, which show that the fats comprise an abundance of saturated $C_{16:0}$ and $C_{18:0}$ fatty acids characteristic of animal fats, the $\delta^{13}C$ values have also indicated that all the archaeological fats studied derive from a terrestrial source rather than a marine source.

The study of assemblages from the well-documented sites of West Cotton and Stanwick has provided an excellent starting point for the exploratory use of compound specific $\delta^{13}C$ values in the identification of remnant fats, since the major domesticated animals at these sites are known to be predominantly of ovine, bovine or porcine origin, enabling assumptions to be made about the remnant fats processed in the vessels. The data from West Cotton have shown how compound specific $\delta^{13}C$ values can be used to distinguish between remnant fats which derive from non-ruminant, ruminant adipose and ruminant dairy fat origins. A number of remnant fats plotted along the mixing lines between the reference ruminant and the reference non-ruminant fats. These are thought to represent adipose fats, the intermediate $\delta^{13}C$ values derived from the mixing of different fats, the non-specific use of individual vessels in processing animal products or possibly shifted isotopic values due to a different dietary regime in antiquity. The data for the Stanwick extracts exhibit a comparable spread of $\delta^{13}C$ values for the ruminant fats, however, no non-ruminant (e.g. porcine) fats are present.

The $\delta^{13}C$ analysis of the Saxon pottery residues from Wicken Bonhunt has not provided clear evidence for the dedicated processing of pigs in these vessels, however, based on comparison with the theoretical mixing curves we have tentatively identified mixtures of appreciable amounts of porcine and ruminant fats. The apparent lack of pure remnant porcine fats may be a reflection of cooking methods, since pigs are traditionally thought to have been cooked by spit-roasting, perhaps with processing in pottery vessels of secondary importance. Furthermore, since faunal evidence of other animal species have also been recovered from the site, it is likely that these species are also represented in domestic wares with the more depleted $\delta^{13}C$ values representing fats from the ruminant species identified amongst the faunal remains. The emphasis at this site clearly appears to be on the production and processing of animal meat/fat in preference to dairy products.

The ranges of $\delta^{13}C$ values for the prehistoric assemblages from Yarnton flood plain and Cresswell field assemblages are comparable, with the majority of residues from both sites corresponding to the reference ruminant fats. At both sites there are examples of archaeological samples which plot just outside the range for the reference cows' milk fat. These residues would appear to represent degraded dairy fats or mixtures of dairy fats and non-ruminant fats, as would arise in multiple uses of vessels (*discussed by Charters 1996*). In the earlier, Neolithic assemblage, one residue corresponds to the reference

non-ruminant fats. In the later period, there are a number of residues which would appear to represent mixtures of ruminant and non-ruminant fats. Similarly, at Eton Rowing Lake, the early Neolithic assemblage contains residues which cluster within the region of the ruminant fats, whilst the Neolithic/ Bronze Age sherds from the nearby Lake End Road site also contain residues which are less depleted isotopically and may represent more varied vessel use.

The analysis of materials from Siberia and Kazakhstan have illustrated that caution needs to be taken when comparing isotopic data from samples originating from different geographical locations, probably largely due to the variation in the $\delta^{13}C$ values of dietary components, e.g. grass and forage, rather than differences in metabolism or physiology of different breeds of horse. Nonetheless, the stable carbon isotope data have clearly indicated that the Botai potsherd residues derive from horse fats due to the close correlation between the Siberian horse fats and the archaeological pot residues. These data provide direct evidence for the exploitation of horses for their meat as well as for work animals by the Kazakhstan peoples.

CONCLUSIONS

Based on the stable carbon isotope analyses carried out on the modern reference animal fats and archaeological fats, the following conclusions can be drawn:

❶ Stable carbon isotopic analysis has enabled distinctions to be drawn between modern fats from the major domesticated ruminant and non-ruminant animal species. The less depleted $\delta^{13}C$ values seen for the fatty acids in non-ruminant fats compared to the fatty acids in ruminant fats reflect differences in the complex metabolic and biochemical processes involved in the formation of body fats between the different species and to a lesser extent reflect differences in diet. These distinctions are clearly reflected in the archaeological fats from West Cotton.

❷ Fats from a number of archaeological sites have been identified as deriving from a ruminant dairy origin based upon the greater depletion in the $\delta^{13}C$ values of $C_{18:0}$ fatty acids in dairy fats compared with adipose fats. It is proposed that the difference in the isotopic signal of the $C_{18:0}$ fatty acid in milk and adipose derives largely from known metabolic pathways involved in lactation, the physiological demands of which result in a shift in the energy balance such that a greater proportion of the $C_{18:0}$ fatty acid present in milk is derived directly from the long-chain fatty acids in the diet. The $C_{18:0}$ fatty acid is produced partially through biohydrogenation in the rumen, therefore reflecting the depleted $\delta^{13}C$ values of the $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ fatty acids which predominate in grass and forage materials, and partially through chain elongation of the $C_{14:0}$ and $C_{16:0}$ fatty acids in the diet. The more negative $\delta^{13}C$ values (ca. –32.5 to –34.0‰) seen for $C_{18:0}$ in milk compares favourably with the depleted values recorded for C_{18} fatty acids in pastures and fodders, i.e. up to –36.5‰. These distinctions are clearly reflected in the archaeological fats from West Cotton and Stanwick.

❸ It is a well recognised fact that $\delta^{13}C$ values of fatty acids of plants will always be more depleted, by approximately 5‰, than those of carbohydrates from the same source (*Deines 1980*). Thus, notwithstanding the proportion of carbon routed from stored fat and dietary carbohydrate, milk and adipose fats from animals raised on similar diets are separable since the isotopic relationships between the major biochemical fractions, in this case milk and adipose fats, will always be qualitatively preserved, thus establishing a secure basis for detecting dairying at different geographical locations and during different periods in prehistory (*Dudd and Evershed 1998*).

❹ The isotopic data have provided the first direct evidence for the processing of dairy fats at prehistoric sites, and has indicated that a large number of vessels from both Yarnton and Eton were probably used for the storage or processing of milk or milk products. The fatty acids recovered from the vessels exhibited highly depleted $\delta^{13}C$ values for the $C_{18:0}$ components which correlates closely with the dairy fats from modern animals raised on C_3 pastures.

If the trends in the $\delta^{13}C$ values are supported by a range of other chemical criteria being considered, including fatty acid and triacylglycerol compositions (*Evershed et al. 1997; Dudd 1999; Mottram et al. 1999*), then the close correlation between the $\delta^{13}C$ values obtained for the remnant fats and the modern fats is remarkable considering the great age of some of the assemblages and thus the potential for alteration of the original isotopic signal. Clearly, based on the data presented herein, the measurement of $\delta^{13}C$ values has proven the single most effective criterion of those considered in distinguishing between degraded fats of ruminant, non-ruminant and dairy origin.

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