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# The carbon isotopic composition of individual fatty acids as indicators of dietary history in arctic foxes on Svalbard

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## SUMMARY

We have measured the carbon isotopic compositions of individual fatty acids isolated from the adipose tissue of arctic foxes (*Alopex lagopus*) from the island of Svalbard on the Spitsbergen archipelago to examine whether dietary sources can be distinguished in the fatty acid isotopic signature. The diets of Svalbard arctic foxes are unusual in that some members of the population are believed to feed largely in the terrestrial food chain while others feed mainly from the marine food chain. Isotopic data were obtained for the five major fatty acids present (16:0, 16:1, 18:0, 18:1, and 20:1). A wide range in $\delta^{13}C$ values for specific fatty acids in Arctic foxes is observed and the mixing lines produced by the correlation in $\delta^{13}C$ of the 16- and 18-carbon fatty acids indicate that both marine and terrestrial dietary sources determine fatty acid isotope composition. The differences in $\delta^{13}C$ values between marine and terrestrial organisms appear to be passed on to individual fatty acids. The abundance and isotopic composition of 16:1 indicates that the foxes derive this acid from marine sources, whereas, 16:0 and 18:0 appear to have predominantly terrestrial sources.

## 1. INTRODUCTION

Stable isotope analysis has become an increasingly widely used tool in tracing the flow of organic matter in both modern and prehistoric food webs (DeNiro & Epstein 1978; Chisholm *et al.* 1982; Schoeninger *et al.* 1983; Fry & Sherr 1984; Ostrom & Fry 1993). The procedure is based on the observation that the carbon and nitrogen isotopic compositions of a consumer organism are similar to, or vary predictably from, that of its food. Isotopic methods of food-web analysis offer several advantages over conventional methods such as the comparison of stomach content data which identify ingested material. Stable isotope composition reflects the time-integrated average of the assimilated diet thereby enabling the nutritional dependence of an animal on a particular food source to be assessed. Tissue-specific studies, in particular of bone collagen (Schoeninger *et al.* 1983; Tuross *et al.* 1988; Ostrom *et al.* 1990), have yielded information on an animal's lifetime average diet and provide information on the relative sources of animal and vegetable protein (Ambrose & DeNiro 1986) or marine and terrestrial foods (Chisholm *et al.* 1982; Schoeninger *et al.* 1983; Ambrose & DeNiro 1986; Cormie & Schwarcz 1994). However, with isotopic analysis of whole-tissue or whole-organism samples an estimate of the trophic fractionation has to be taken into account when calculating the contribution of a particular food source to the diet in mixing models (Harrigan *et al.* 1989). Recent studies have extended stable isotope analysis of diet to individual compounds, namely alkanes and amino acids (Des Marais *et al.* 1980; Tuross *et al.* 1988; Hare *et al.* 1991). Des Marais *et al.* (1980) found that the isotopic composition of individual alkanes in bat guano reflected the differences in $\delta^{13}C$ between $C_3$ and $C_4$ plants in the diets of insects, the bat's primary food source. Similarly, Hare *et al.* (1991) found that the carbon isotope compositions of individual amino acids isolated from consumers fed $C_3$ and $C_4$ diets reflected differences in the isotopic composition of the carbon source. Most isotopic studies have focused on aquatic ecosystems or plant–herbivore relations, relatively few attempts have been made to look at carnivore relations (McConnaughey & McRoy 1979; Ambrose & DeNiro 1986; Ramsey & Hobson 1991).

Information about the dietary history of an animal can also be obtained from the relative compositions of storage lipids. The simplest lipids, the fatty acids, are also the constituents of many more complex lipids such as adipose tissue triacylglycerols (the principal form in which fatty acids are stored in animals). Their composition reflects that of the diet in monogastric animals and a direct connection between the fatty acid


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compositions of dietary and storage lipids has been demonstrated experimentally (Valero-Garido *et al.* 1990). This has led to the use of fatty acid abundances in adipocytes as a tool in dietary analysis, as there are consistent differences in the fatty acid composition of lipids in marine and terrestrial organisms (James & Nichols 1966; Erwin 1973).

The isotopic composition of individual fatty acids is controlled by the nature and availability of carbon sources and biosynthetic fractionations associated with the animal's metabolism. The ability to measure the isotopic compositions of individual compounds in a complex mixture (termed compound specific isotope analysis (CSIA)), has been a long-held goal of stable isotope mass spectrometry (Abelson & Hoering 1961; Colombo *et al.* 1965; Sano *et al.* 1976; Matthews & Hayes 1978; Gilmour *et al.* 1984; Engel & Macko 1984). However, it is the advent of combined gas chromatography-isotope ratio mass spectrometry (GC-IRMS) (Freeman *et al.* 1990; Hayes *et al.* 1990) that has enabled the isotopic compositions of individual compounds to be determined, to high levels of precision, in complex mixtures and seen the application of CSIA to an increasing range of geochemical and biological problems (Guo *et al.* 1993; Beylot *et al.* 1994; Gilmour & Pillinger 1994; Schoell *et al.* 1994). A complicating requirement for the study of fatty acids is that they need to be derivatized to their more volatile methyl esters for gas chromatography, which introduces an additional step in their isotopic analysis as an extra carbon atom is introduced into the molecule. In the case of amino acids and carbohydrates derivatization is known to be accompanied by isotopic fractionations, requiring time consuming individual corrections for each compound studied (Silfer *et al.* 1991; Moers *et al.* 1993). As part of this investigation we considered whether any isotopic fractionations were associated with the esterification reaction.

Our aim in this study was to examine whether dietary sources can be distinguished in the fatty acid isotopic signature of adipose tissue triacylglycerols of arctic foxes from Svalbard. Because Svalbard has only two native terrestrial mammals, the Svalbard reindeer (*Ragnifer tarandus*) and the arctic fox (*Alopex lagopus*), the diet of the latter is unusual in that some members of the population are believed to feed largely or entirely in the terrestrial food chain (i.e. eating reindeer carrion, ptarmigan and herbivorous ducks and geese), whereas others apparently feed mainly from the marine food chain on fish, seabirds and seal carrion (Frafjord 1993).

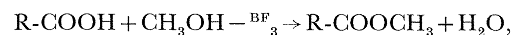
## 2. SAMPLES AND EXPERIMENTAL METHODS

Details of the sample locations and specimens are described elsewhere (Pond *et al.* 1995*a*, *b*). The foxes came from four locations on the island of Spitsbergen in the Svalbard archipelago: Gråhuken near to the north coast of Spitsbergen, Austfjordneset near the southern ends of the Austfjorden and Wijdefjorden fjords on the north coast, Tempelfjorden on the western side of Spitsbergen, and Adventdalen near the town of Longyearbyen. The fatty acid compositions of triacylglycerols from perirenal and inguinal adipose tissue samples were studied.

A set of fatty acid standards (odd numbered from $C_{15}$ to $C_{23}$) were obtained from Alltech Associates together with a polyunsaturated fatty acid methyl ester standard (PUFA-2, Matreya) which was used as a chromatographic calibration standard. A single lot of 14% (by volume) boron Trifluoride/Methanol solution (Sigma Chemical Co.) was used for all esterifications. Details of the isolation of adipose tissue and sample preparation are given elsewhere (Pond *et al.* 1995*a*, *b*). Reagents and standards were stored below 5 °C. All solvents used were of high performance liquid chromatography (HPLC) grade and all glassware was scrupulously cleaned using ultrasonication in Decon 90 (Decon Laboratories Ltd) and roasting at 450 °C for at least 2 h. Conventional isotopic analysis of fatty acid standards followed the procedures outlined in Grady *et al.* (1982).

The fatty acid methyl esters were analysed for their carbon isotopic compositions by using a Finnigan MAT Delta s/GC GC-IRMS. In this study, the gas chromatograph was equipped with one of two GC columns. For the initial analyses a 50 m × 0.32 mm i.d. fused silica capillary column coated with a 0.5 μm film of 5% phenyl-methyl silicone (BPX-5, Scientific Glass Engineering (S.G.E.), Milton Keynes) was temperature programmed as follows: after a 2 min hold at 50 °C, the oven temperature was raised from 50 °C to 140 °C at 10 °C min$^{-1}$, then from 140 °C to 220 °C at 2 °C min$^{-1}$ with a final hold time of 7 min. However, this column proved incapable of resolving polyunsaturated fatty acid methyl esters (FAMES). For this a 50 m × 0.32 mm i.d. coated with a 0.25 μm film of polar stationary phase (BPX-70, S.G.E., Milton Keynes, U.K.) was used. This column was programmed after a 2 min hold from 40 °C to 220 °C at 5 °C min$^{-1}$, with a final hold time of 22 min. Splitless injection was used in both cases with the injector being purged after 2 min. GC-IRMS data reduction was performed using Finnigan MAT Isodat V 5.1 and V 5.2 software. The accuracy attainable in GC-IRMS analysis is determined by two main factors: (i) amount of sample relative to background (Merritt & Hayes 1994); and (ii) the presence of coeluting peaks. The samples used in this study were exceptionally clean consisting entirely of methyl esters, as confirmed by gas chromatography mass spectroscopy (GCMS) (Pond *et al.* 1995*a*), and backgrounds were found to be extremely low for both of the columns used so that the corrections required were minimal. Multiple sample injections were made, both to determine reproducibility, and to bring the ion-beam signals within the optimal linear range of the mass spectrometer (m/z 44 signals of 0.1–2 V) for samples that had a wide range in concentrations of individual fatty acids. Routine precision for well resolved peaks was better than 0.3‰ for a sample size of 1 nmol; the lowest precision accepted for poorly resolved peaks was 0.6‰ ($\pm 1\sigma$).

Provided that no isotopic fractionations accompany the derivatization reaction, the fatty acid methyl esters should have $\delta^{13}C$ values that reflect the different proportions of carbon from the fatty acid and methanol and their respective carbon isotope compositions. For the derivatization reaction:



$$\text{R-COOH} + \text{CH}_3\text{OH} \xrightarrow{BF_3} \text{R-COOCH}_3 + \text{H}_2\text{O},$$

the stoichiometric mass balance for fatty acid methyl esters can be written as

$$\delta^{13}C_{FAME} = X \cdot \delta^{13}C_{FA} + (1 - X) \cdot \delta^{13}C_{ME},$$

where $\delta^{13}C_{FAME}$, $\delta^{13}C_{FAME}$, and $\delta^{13}C_{ME}$ are the carbon isotopic compositions of the methyl ester, the underivatized fatty acid, and the methanol respectively, while $X$ and $1 - X$ are the mole fractions of carbon from each source. Comparison of



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Table 1. *Mean fatty acid abundances and carbon isotope compositions for adipose tissue samples from arctic foxes*

(nd = not detected, nm = not measurable). Localities: G = Gråhuken, Au = Austfjordneset, T = Tempelfjorden, Ad = Adventdalen.)

| | | % total fatty acids | | | | | | | | | | | $\delta^{13}C_{PDB}$ (‰) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| fox | locality | 14:0 | 14:1 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:1 | 20:4 | 20:5 | 16:1 | 16:0 | 18:1 | 18:0 | 20:1 |
| 1 | Ad | 5.9 | 0.5 | 13.6 | 4.6 | 10.2 | 34.6 | 0.8 | 0.2 | 29.5 | 0.1 | 0.1 | −27.6 | nm | −28.5 | nm | −26.8 |
| 11 | Ad | 2.2 | 0.1 | 31.2 | 3.0 | 28.1 | 26.8 | nd | nd | 8.6 | 0.1 | nd | −27.6 | nm | −28.5 | nm | nm |
| 12 | Ad | 4.6 | 0.8 | 17.0 | 8.4 | 9.5 | 48.8 | nd | nd | 10.5 | 0.1 | 0.2 | −24.2 | −24.9 | −25.3 | −25.7 | −25.7 |
| 14 | Ad | 4.7 | 1.3 | 19.4 | 20.6 | 8.6 | 33.2 | 0.7 | 0.4 | 9.5 | 1.7 | 0.7 | −22.6 | 24.3 | −25.7 | −26.2 | −25.4 |
| 15 | Ad | 1.9 | 0.3 | 20.8 | 5.6 | 14.4 | 46.5 | 2.3 | nd | 9.1 | 0.2 | 0.2 | −25.4 | nm | −26.6 | nm | nm |
| 23 | Ad | 3.4 | nd | 20.4 | 6.4 | 8.7 | 42.0 | 3.0 | 0.3 | 17.1 | 0.3 | 0.1 | −25.0 | −28.2 | −27.3 | −28.3 | −27 |
| 33 | Ad | 4.9 | 0.5 | 27.6 | 4.5 | 20.6 | 34.4 | 1.5 | nd | 6.5 | 0.3 | 0.1 | −25.1 | −28.2 | −30.0 | −33.0 | −26.8 |
| 4 | Au | 4.7 | 4.7 | 23.9 | 3.6 | 13.8 | 36.3 | nd | nd | nd | 0.1 | 12.9 | nm | −26.4 | −28.8 | −31.3 | nm |
| 24 | Au | 5.0 | 2.3 | 18.8 | 6.5 | 10.1 | 40.2 | nd | nd | 0.2 | 0.2 | 16.8 | −26.8 | −26.6 | −26.8 | −28.0 | −27.6 |
| 32 | Au | 4.9 | 0.4 | 26.0 | 3.2 | 20.2 | 38.3 | 1.8 | 0.3 | 5.8 | 0.1 | nd | nm | −28.2 | −28.9 | −31.5 | −27.6 |
| 3 | G | 4.8 | 0.9 | 13.0 | 10.5 | 5.1 | 41.0 | 2.2 | 0.6 | 22.0 | 1.0 | 0.3 | −26.4 | −25.5 | −25 | −27.2 | −25.1 |
| 5 | G | 4.5 | 0.4 | 20.7 | 6.6 | 13.5 | 38.4 | 0.7 | nd | 14.8 | 0.6 | 0.1 | −25.7 | −25.7 | −26.5 | −28.0 | −25.4 |
| 6 | G | 2.5 | 0.1 | 20.8 | 7.2 | 10.9 | 39.7 | 2.9 | 0.7 | 14.6 | 0.6 | nd | −25.2 | −25.5 | −25.4 | −26.9 | −26.0 |
| 7 | G | 4.7 | 0.2 | 23.3 | 7.5 | 13.3 | 33.8 | 1.2 | 0.2 | 15.1 | 0.7 | 0.1 | −27.1 | −27.8 | −26.8 | −28.6 | −25.8 |
| 8 | G | 4.0 | 0.2 | 20.1 | 6.5 | 13.6 | 37.4 | nd | nd | 17.4 | 0.6 | 0.2 | −26.7 | −26.9 | −27.2 | −28.8 | −25.9 |
| 9 | G | 4.7 | 0.2 | 18.2 | 5.5 | 10.4 | 34.4 | 0.5 | nd | 25.8 | 0.4 | 0.2 | −25.4 | −25.1 | −26.6 | −27.0 | −26.6 |
| 17 | G | 5.1 | 1.9 | 21.3 | 15.6 | 11.3 | 33.5 | 1.2 | 0.4 | 6.8 | 1.6 | 2.6 | −22.8 | −25.0 | −26.5 | −28.5 | −25.6 |
| 19 | G | 5.0 | 0.4 | 26.9 | 3.2 | 20.8 | 37.2 | 1.2 | nd | 6.0 | 0.2 | nd | −26.5 | −27.3 | −28.2 | −30.0 | −25.2 |
| 10 | T | 4.0 | 0.7 | 17.1 | 8.1 | 9.6 | 46.8 | 2.9 | nd | 10.6 | 0.2 | 0.1 | −24.5 | nm | −25.5 | nm | nm |
| 13 | T | 6.1 | 2.0 | 18.3 | 22.1 | 7.1 | 32.2 | 1.1 | 0.4 | 8.7 | 1.8 | 1.0 | −22.1 | −24.9 | −24.0 | −23.8 | −27.1 |
| 16 | T | 4.2 | 1.5 | 21.3 | 9.6 | 13.0 | 42.2 | 1.4 | 0.4 | 6.3 | 0.9 | 1.6 | −22.2 | −24.3 | −25.1 | −26.9 | −26.0 |
| 28 | T | 4.9 | 0.4 | 25.6 | 3.0 | 19.6 | 40.5 | 1.8 | nd | 5.5 | 0.1 | nd | −27.1 | −27.7 | −28.9 | −30.2 | −25.8 |
| 31 | T | 5.2 | 0.4 | 24.7 | 3.1 | 18.1 | 42.1 | 1.9 | 0.4 | 5.5 | nd | nd | −25.8 | −26.6 | −28.8 | −28.4 | −28.8 |
| 35 | T | 4.4 | 0.4 | 26.8 | 3.2 | 20.3 | 37.9 | 1.4 | 0.3 | 6.1 | 0.1 | nd | −29.0 | −28.2 | −29.2 | −30.1 | −26.1 |

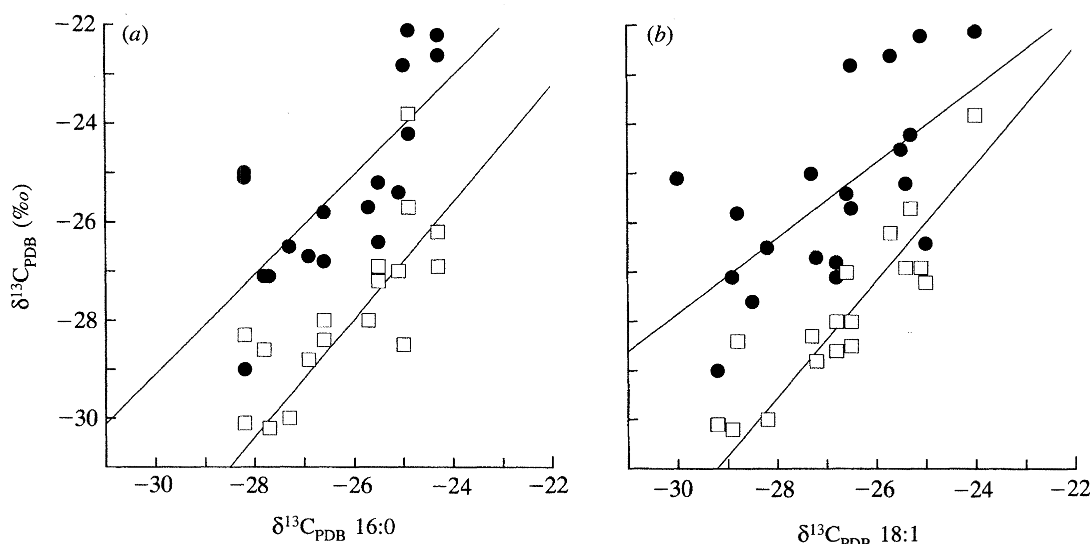


Figure 1. Correlations between isotopic compositions of individual fatty acids. (*a*) Comparison of 16:0 with 16:1 (filled circles; $R = 0.74$, $p < 0.01$) and 18:0 (open squares; $R = 0.77$, $p < 0.01$). (*b*) Comparison of 18:0 with 16:1 (filled circles; $R = 0.66$, $p < 0.01$) and 18:0 (open squares; $R = 0.92$, $p < 0.01$).

the $\delta^{13}C$ values measured on fatty acids using conventional isotopic analysis with those predicted by mass balance calculations from the GC-IRMS FAME $\delta^{13}C$ values gave good agreement. Replicate analyses were within 0.4‰ of the true values, indicating that there is apparently no isotopic fractionation associated with the methylation reaction. This is in agreement with the conclusions reached by Abrajano *et al.* (1994) who undertook a similar investigation. Thus, a simple mass balance calculation can be used to determine the correct isotopic composition for each fatty acid. It is not the case with other groups of compounds such as amino acids (Silfer *et al.* 1991), and highlights the need for careful study of the isotope effects associated with analytical derivatization reactions before they can be used routinely for CSIA studies.

## 3. RESULTS

The mean percentage abundance and $\delta^{13}C$-values of individual fatty acids in samples of perirenal and inguinal adipose tissue from each fox are summarized in table 1. No differences in fatty acid abundance or isotopic composition have been observed between these

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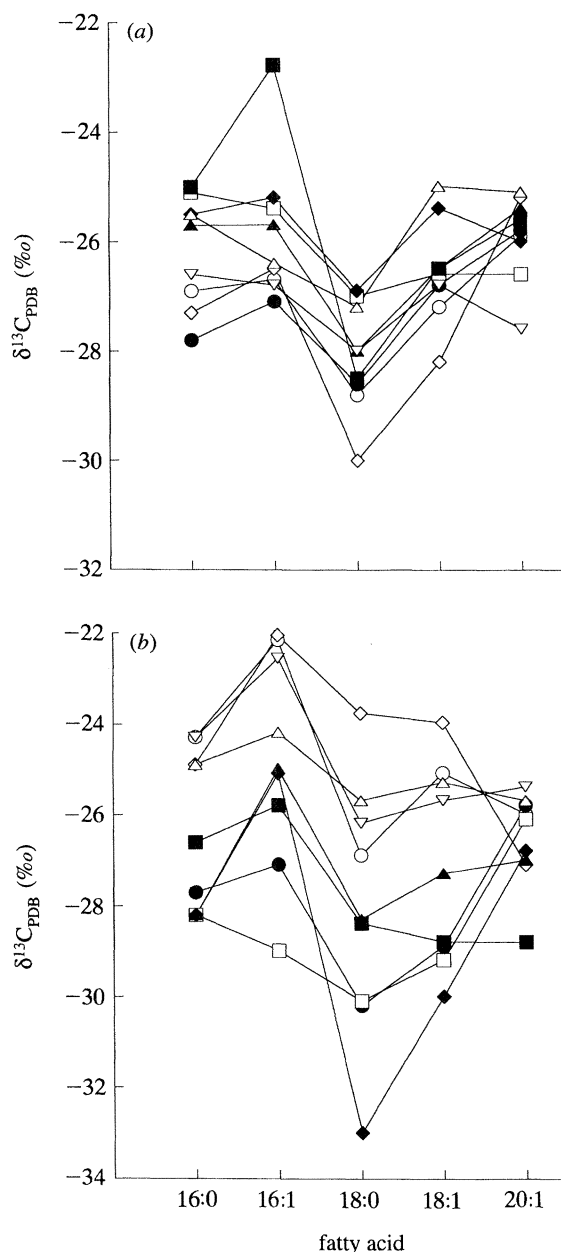


Figure 2. Intermolecular isotope distribution of the fatty acids 16:0, 16:1, 18:0, 18:1 and 20:1 from adipose tissue triacylglcerols in Svalbard arctic foxes. (*a*) Foxes from Gråhuken (open symbols; circles, individual 8; squares, 9; diamonds, 19; upright triangles, 3; inverted triangles, 24) and Austfjordneset (filled symbols; circles, individual 7; squares, 17; diamonds, 6; upright triangles, 5). (*b*) Foxes from Tempelfjorden (open symbols; circles, individual 16; squares, 35; diamonds, 13; upright triangles, 12; inverted triangles, 14) and Adventdalen (filled symbols; circles, individual 28; squares, 31; diamonds, 33; upright triangles, 23).

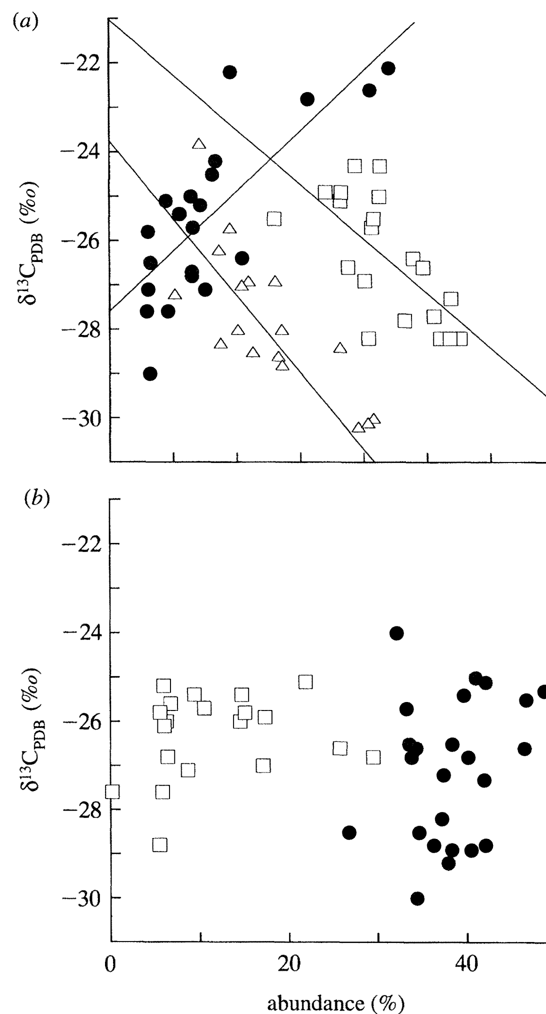


Figure 3. Correlation between the isotopic compositions of individual fatty acids and their abundance. (*a*) 16:1 (filled circles) shows a strong positive trend ($R = 0.77$, $p < 0.01$), whereas 16:0 (open squares) and 18:0 (open triangles) show strong negative trends ($R = -0.68$, $p < 0.01$; $R = -0.79$, $p < 0.01$). (*b*) Neither 18:1 (filled circles) or 20:1 (open squares) shows any significant correlation with abundance ($R = 0.25$, $p > 0.2$).

two depots (Pond *et al.* 1995*a*). Isotopic data are given for the five major fatty acids (16:0, 16:1, 18:0, 18:1, and 20:1); the abundances of the 14-carbon and polyunsaturated fatty acids were too low in the majority of samples for reliable isotopic measurements to be made. The isotopic compositions of fatty acids vary between −22 and −33‰, however, ranges for individual acids differ. For palmitic acid (16:0), $\delta^{13}C$ values encompass 3.9‰ compared with 6.9‰ for palmitoleic acid (16:1), 9.2‰ for stearic acid (18:0), 6‰ for oleic acid (18:1), and 3.7‰ for 20:1. There are significant correlations between the isotopic compositions of individual acids. The two 18-carbon acids and the two 16-carbon acids correlate, as do the saturated acids, 16:0 and 18:0 and to a lesser degree the unsaturated acids, 16:1 and 18:1 (see figure 1). No significant correlation was found between the isotopic composition of 20:1 and any other acid.

Isotopic compositions of fatty acids also vary within a single animal. For foxes 13, 17, and 33, $\delta^{13}C$ values have a range of more than 5‰, whereas a range of less than 2‰ is observed for foxes 6, 9, 12 and 24. Figure 2 shows the intermolecular isotope distribution for the 18 animals from which a complete dataset was obtained. A similar pattern between the fatty acids is observed for the majority of animals, although those with a low overall level of unsaturated acids (Pond *et al.* 1995*a*) show marked differences (i.e. foxes 19, 28, 31, 33, 35). This pattern is reflected in the mean $\delta^{13}C$-values for each acid from all foxes, −26.4‰ for 16:0, −25.5‰ for 16:1, −28.4‰ for 18:0, −27.1‰ for

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18:1, and −26.3‰ for 20:1. There is a strong positive correlation (see figure 3) of fatty acid abundance and $\delta^{13}C$ for 16:1 and a strong negative correlation for 16:0 and 18:0. Neither the $\delta^{13}C$ values of 18:1 nor 20:1 correlated with fatty acid abundance.

## 4. DISCUSSION

Equilibrium fractionation in aquatic systems between $CO_2$ and dissolved $HCO_3^-$ results in a difference in the $\delta^{13}C$ values of atmospheric $CO_2$ (−8‰) and marine carbonate (0‰), the inorganic carbon reservoirs from which autotrophs derive their carbon. Isotopic fractionation during the fixation and diffusion of $CO_2$ into the cells of plants produces a depletion in $^{13}C$ of the plant, relative to the inorganic carbon source (O'Leary 1981). In comparison to terrestrial plants, marine phytoplankton are more commonly diffusion-limited so that a larger proportion of the $CO_2$ within the cell is fixed, thereby reducing the isotopic fractionation associated with enzymatic incorporation (O'Leary 1981). The resulting differences in $\delta^{13}C$ values between marine and terrestrial organisms are maintained through successive trophic levels in both marine and terrestrial food webs (Chisholm *et al.* 1982). Differences in the primary carbon-fixing enzymes and associated fractionations also account for the 15‰ difference between the $C_3$ and $C_4$ photosynthetic products (Smith & Epstein 1971). However, the Arctic is predominantly a $C_3$ biome (Tieszen & Boutton 1988) and no $C_4$ vegetation occurs on Svalbard (Bliss & Malveyeva 1992).

Arctic foxes are circumpolar in distribution, and since they can travel long distances over ice, have colonised many arctic islands. They are opportunistic feeders, and prosper on a wide variety of foods. On mainland Europe, Asia, North America and large islands such as Greenland, their diet is mostly rodents, but in the absence of small mammals on Svalbard, foxes eat substantial quantities of birds, fish, and (in summer) mammalian carrion (Frafjord 1993). Fox adipose tissue occurs in distinct depots that can be identified by site-specific anatomical and biochemical properties (Pond *et al.* 1995*b*). On Svalbard, foxes have few natural predators and starvation seems to be the main cause of mortality. Perhaps as an adaptation to these ecological circumstances adult foxes are often obese, as evidenced by the high adipose tissue contents of these animals (Pond *et al.* 1995*b*). Observations of various wild and captive animals have shown that diet is important in determining the fatty acid composition of adipose tissue triacylglycerols, e.g. mink and arctic foxes (Rouvinen & Kiiskinen 1989), mongeese, (Reidinger *et al.* 1985) polar bears (Colby *et al.* 1993), and American badgers and coyote (Harlow & Varnell 1990). Examination of the isotopic composition of fatty acids from arctic fox adipose tissue triacylglycerols may indicate the extent to which isotopic signatures of diet are maintained at a molecular level.

The ranges in $\delta^{13}C$ values for individual fox fatty acids fall within that observed for $C_3$ plants, however, lipids in both plants and animals are generally $^{13}C$-depleted with respect to either the bulk plant (Park & Epstein 1961; DeNiro & Epstein 1977) or other tissue samples (Tieszen *et al.* 1983). Individual plant lipids, for instance leaf wax n-alkanes, can be even more depleted in $^{13}C$ than the bulk lipid fraction (Rieley *et al.* 1993). In polar bears and ringed seals fat from adipose tissue was found to be 9–10‰ lighter than bone collagen (McConnaughey & McRoy 1979; Ramsey & Hobson 1991). There are only limited data available on the isotopic composition of individual fatty acids, as most previous food web studies have used protein or protein-rich tissues. Results from metabolic and clinical tracer studies, most likely representing 'terrestrial' diets, give values of −28 to −31‰ for 16- and 18-carbon fatty acids (Metges *et al.* 1994). Fatty acids from marine algae in a symbiotic relationship with giant clams can have $\delta^{13}C$ values of −10 to −15‰ compared to −14 to −27‰ for fatty acids from the clams (Johnston *et al.* 1995). We have analysed 16- and 18-carbon fatty acids isolated from Indo-Pacific molluscs and found them to have $\delta^{13}C$ values of between −19 to −22‰. Thus differences are apparent in the carbon isotopic composition of marine and terrestrial lipids that mirror those seen in whole organisms. However, the dataset is still extremely limited.

The origin of the variations in $\delta^{13}C$ of individual fatty acids can be attributed to two possible effects or a combination thereof: (i) isotopically distinct dietary sources for each acid; and (ii) isotopic fractionations associated with modifications due to desaturation or chain elongation (Monson & Hayes 1982). The wide range in $\delta^{13}C$ values for specific fox fatty acids and the mixing lines produced by the correlations in $\delta^{13}C$ of the 16- and 18-carbon fatty acids (see figure 1), suggest that marine and terrestrial dietary sources exert an overall control on fatty acid isotope composition. Animals feeding predominantly on the marine food chain (i.e. those with heavier $\delta^{13}C$ values) apparently obtain some of each acid from marine sources. However, it is also possible that fatty acids are produced by the modification of dietary derived acids. Two possible mechanisms exist: desaturation, and elongation. It has been postulated, though not confirmed by experiment, that desaturation and elongation would have an associated kinetic isotope effect (Fang *et al.* 1993). Nevertheless, isotopic depletion between potential precursor and product fatty acids does not, by itself, indicate that metabolic processes are exerting the greatest influence on the intermolecular isotope distribution, as terrestrial dietary sources are also more $^{13}C$ depleted with respect to marine sources. However, examining the intermolecular isotope distribution for all foxes, the mean difference between 16:0 and 16:1 is +0.94‰ ($t_{17} = 3.1$, $p = .006$) and between 18:0 and 18:1 it is +1.37‰ ($t_{19} = 6.7$, $p < .001$) which is the reverse of what would be expected if desaturation was taking place. It therefore appears that diet is the predominant control on the isotopic composition of these fatty acids, rather than metabolic processes such as *de novo* desaturation. Though lipid metabolism has not been studied in Arctic foxes, the capacity to elongate and desaturate lipids is generally weak in carnivores: cats lack Δ6 and Δ5 desaturases



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and the activities of these enzymes are very low in microsomes isolated from dogs' livers (Brenner 1989). Arctic foxes are phylogenetically more closely related to dogs than cats, but, especially on Svalbard, their diet is almost entirely vertebrate tissues, and is thus similar to that of cats. It is unlikely that arctic foxes are capable of more extensive transformations than dogs and cats.

It is also apparent from figure 2*a* that animals from Gråhuken display closer similarities in the pattern of their intermolecular isotope distribution than do animals from the other two areas with similar numbers of samples (see figure 2*b*). In a study of amino acids in modern and fossil proteins, Hare *et al.* (1991) observed consistent similarities in the $\delta^{13}C$ values of individual amino acids between consumers and their diets, and interpreted the differences in $\delta^{13}C$ values between individual amino acids as a result of metabolic fractionation. However, the variations in intermolecular $\delta^{13}C$ values observed between foxes from Gråhuken and those from Tempelfjorden and Adventdalen are more likely to reflect the differing availability of food sources between these areas. For instance, both Tempelfjorden and Adventdalen are areas with many reindeer when compared with Gråhuken (Pond *et al.* 1995*a*).

There are also significant correlations between individual fatty acids and their abundance. These correlations provide strong evidence of the dominant dietary sources for each acid. Foxes with a higher abundance of 16:1 appear to obtain it from marine sources (as indicated by the concomitant increase in $\delta^{13}C$ values), suggesting that most animals may have had a significant marine component to their diet at some point in the lives. The reverse is apparently the case for 16:0 and 18:0 which both become isotopically lighter with increasing abundance, indicating that these acids are more readily available from terrestrial food sources. The two other fatty acids show no correlation with abundance. For 18:1 this probably reflects the widespread availability of this acid in the majority of the foxes' food sources, as it is a common and highly abundant component of ruminant adipose tissue. The narrow range in $\delta^{13}C$ for 20:1 of 3.7‰ may indicate that this acid has relatively few sources, most likely marine. However, the range for 16:0 is not that dissimilar (3.9‰), yet it is clearly correlated with both its abundance and the isotopic composition of other acids.

## 5. CONCLUSIONS

The $\delta^{13}C$ values of individual fatty acids in adipose tissue triacylglycerols from arctic foxes reflect the isotopic composition of their diet. The differences in $\delta^{13}C$ values between marine and terrestrial organisms appear to be passed on to individual fatty acids. The abundance and isotopic composition of 16:1 indicates that it is apparently more readily available in marine sources, in contrast, 16:0 and 18:0 appear to have predominantly terrestrial sources. The origins of other acids (18:1 and 20:1) are less clearly defined or are seemingly readily available from a wide variety of food sources. In contrast to field observations or stomach content analysis (Frafjord 1993), the results of this study suggest that all of the foxes studied have had a significant marine component to their diet at some stage in their lives. Despite variations in dietary $\delta^{13}C$, a similar pattern of isotopic composition of individual fatty acids exists among the majority of foxes. Further studies will be required to determine if this pattern of isotopic compositions reflects only dietary sources or is partly a consequence of metabolic processes.

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