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Short Article

Deuterium depletion in the fatty acids from beef

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Abstract

We examined stable hydrogen (δD, ‰ relative to V-SMOW) and carbon isotopic compositions (δ^{13}C, ‰ relative to V-PDB) of fatty acids extracted from beef, and compared them with those from feeds (mixtures of plant materials) for the cattle. The δD value of stearic acid in beef is similar to that in the feeds, whereas the palmitic and oleic acids in beef are both significantly depleted in D (by ca. 60‰) as compared with the corresponding acids in the feeds. On the other hand, these fatty acids in beef are enriched in ^{13}C (by up to 5.7‰) relative to the corresponding acids in the feeds. Thus, the isotopic compositions of fatty acids in beef are not identical to those in the feeds. These results suggest that fatty acids in beef are an admixture of fatty acids derived from *de novo* biosynthesis in the cattle and from the feeds, and may imply that a considerable D-depletion process (e.g., hydrogenation of feed-derived fatty acids and/or dehydrogenation–hydrogenation cycling) occur in the fatty acids of the cattle.

Key words: fatty acids, hydrogen isotopic composition, heterotroph

1. Introduction

The stable hydrogen isotopic composition of lipid biomarkers has been employed as potential hydrologic proxies in the study of paleoenvironments and paleoecosystems. This is possible because of the empirical correlation observed between the isotopic composition of algal- and plant-derived lipid biomarkers found in surface soils and sediments, and that of ambient water (see the review by Sachse et al., 2012). However, most lipid biomarkers, particularly short-chain fatty acids such as palmitic (16:0) and stearic acids (18:0) and their unsaturated homologues, are potentially derived from multiple sources, including heterotrophic animals and bacteria. The isotope records of lipid biomarkers in geological and ecological samples therefore potentially reflect not only algal and plant signals but also heterotrophic activities occurring during burial and ecological processes. An understanding of the isotopic composition of lipids and related fractionation mechanisms in heterotrophs are therefore an important topic and worthy of investigation (e.g., Chikaraishi, 2006; Zhang et al., 2009).

Early studies involving heterotrophic cultures of algae (Estep and Hoering, 1980) and bacteria (Zhang et al., 2009) reported that the D content of heterotrophically produced lipids is significantly greater (>50‰) than that of photoautotrophically produced lipids. It has been proposed that heterotrophs mainly utilize relatively D-enriched NADPH (nicotinamide adenine

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dinucleotide phosphate reduced) derived from sugar metabolism (e.g., *via* the oxidative pentose phosphate pathway), whereas photoautotrophs utilize strongly D-depleted NADPH produced by photosynthesis (Zhang et al., 2009). However, the above findings are not always consistent with the isotopic composition of lipids found in natural heterotrophic organisms; for example, long-chain *n*-alkanes in insects are depleted in D by 30–40‰ as compared with the values in plants in the same environment (Chikaraishi et al., 2012).

In this study, we investigated the hydrogen and carbon isotopic compositions of fatty acids, including those of palmitic and stearic acids and their unsaturated homologues, extracted from beef, and compared them with the corresponding acids in feeds used for the cattle. We focused on short-chain fatty acids because they are typical lipids in heterotrophs, and because they may be recycled and employed as precursors of other lipids in heterotrophic organisms and ecosystems.

2. Samples and methods

Ten pieces of rump beef were purchased from markets in Matsuzaka (six samples) and Hida (four samples), Japan. Feeds (admixture of barley, soybean, corn, etc.) used for raising the cattle yielding these meats were also collected from farmers; we were able to identify the feeds by using the beef traceability system. These samples were stored at −20°C prior to analysis.

Lipids were extracted using methanol/chloroform (1/2, v/v) and subsequently saponified using 0.5 M KOH in methanol/water (95/5, w/w) by refluxing for 1 hour. The carbon isotopic composition ($\delta^{13}C$, ‰ relative to Vienna-Peedee Belemnite; V-PDB) of the residue was determined using a Flash Elemental Analyzer (1112HT) coupled to a Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS) *via* a ConFlo III interface (Thermo Fisher Scientific). Neutral lipids, such as sterols, were removed from the alkaline solution using *n*-hexane/dichloromethane (2/1, v/v); then, fatty acids were extracted using *n*-hexane/dichloromethane (1/1, v/v) after addition of 12 M HCl to establish a pH < 1. The fatty acids were esterified using methylchloroformate/acetonitrile/pyridine/methanol (1/22/2/1, v/v) at room temperature for 5 min using the procedures of Aoyagi et al. (2012). The esterified fatty acids were extracted using *n*-hexane after addition of saturated $NaHCO_3$ solution.

The isotopic compositions of hydrogen (δD, ‰ relative to Vienna-Standard Mean Ocean Water; V-SMOW) and carbon ($\delta^{13}C$, ‰ relative to V-PDB) in the esterified fatty acids were determined by a Delta V Advantage IRMS attached to a Trace Ultra Gas Chromatograph (GC) through a GC-C III interface (GC/IRMS, Thermo Fisher Scientific). The GC was equipped with an HP-5ms capillary column (30 m length × 0.25 mm i.d. × 0.10 μm film thickness; Agilent Technologies). The isotopic compositions of the fatty acids were obtained *via* an isotopic mass balance calculation (Goto et al., 2011). Standard deviation (1σ) of the isotope measurements was 5‰ for hydrogen and 0.5‰ for carbon.

3. Results and discussion

3.1. Hydrogen and carbon isotopic compositions

The isotopic compositions of the fatty acids analyzed in this study are summarized in Fig. 1. Palmitic, stearic, and oleic acids are the major fatty acids in beef. On the other hand, palmitic and stearic acids and their unsaturated homologues with 18 carbon atoms (18:1, 18:2, 18:3) are the major short-chain fatty acids in the feeds. Because the unsaturated homologues in the feeds were not sufficiently resolved on the baseline of the GC/IRMS chromatogram, a single composite value is reported as 18:x.

The δD values of palmitic, stearic, and oleic acids in beef are −272±4‰, −223±7‰, and −277±4‰, respectively, for the Matsuzaka samples (N=6), and −276±5‰, −224±8‰, and −267±4‰, respectively, for the Hida samples (N=4) (Fig. 1). No substantial difference is found between the δD values of the fatty acids in the two locations. However, although the δD value of stearic acid in beef is similar to that in the feeds (−234‰ in Matsuzaka and −222‰ in Hida), the palmitic and oleic acids in beef are significantly

depleted in D by approximately 60‰ as compared with these acids in the feeds (−222‰ and −231‰, respectively, in the Matsuzaka samples, and −198‰ and −195‰, respectively, in the Hida samples).

Figure 1 also shows that $\delta^{13}C$ values of palmitic, stearic, and oleic acids in Matsuzaka beef (−23.4±0.4‰, −23.2±0.5‰, and −23.7±0.6‰, respectively; N=6), are smaller by approximately 2.8−3.4‰ than those in Hida beef (−20.5±0.8‰, −19.8±1.0‰, and −20.3±1.0‰, respectively; N=4). This difference in the $\delta^{13}C$ value of fatty acids in the Matsuzaka and Hida samples probably reflects the isotopic composition of the bulk feeds in these two locations, which are −22.5‰ in Matsuzaka and −17.9‰ in Hida, and thereby that of the bulk beef in Matsuzaka (−21.4±0.3‰) and Hida (−17.1±0.8‰). On the other hand, the $\delta^{13}C$ values of the fatty acids in the feeds in both locations are much smaller (by up to 5.7‰ in Matsuzaka and 5.3‰ in Hida) than those of the fatty acids in beef.

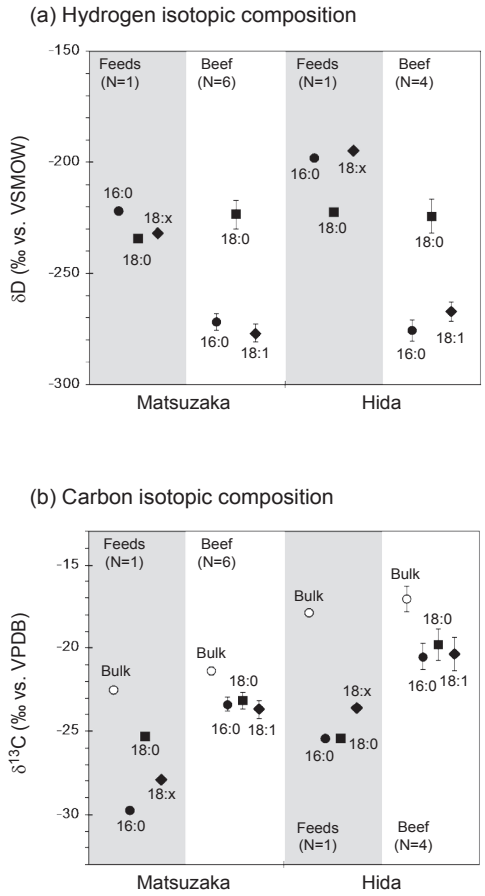

Fig. 1. (a) Hydrogen isotopic composition of fatty acids in beef and their feeds, and (b) carbon isotopic composition of bulk and fatty acids in beef and their feeds.

3.2. The origin of beef fatty acids

The $\delta^{13}C$ values of bulk beef are thus slightly larger than those of bulk feeds (Fig. 1), which is consistent with the general trend of trophic enrichment in ^{13}C observed during grazing processes (e.g., +0.4±0.1‰; McCutchan et al., 2003). In contrast, except for stearic acid, fatty acids in beef are significantly depleted in D and enriched in ^{13}C as compared with corresponding fatty acids in the feeds (Fig. 1). Because few data are available for the isotopic fractionation of hydrogen and carbon during lipid biosynthesis in heterotrophs, we are currently unable to identify specific mechanisms explaining the observed isotopic signatures in beef.

However, the difference in the $\delta^{13}C$ value of fatty acids in beef vs. feeds implies that all of the cattle fatty acids do not come from the feed fatty acids. Moreover, it is likely that the isotopic discrimination in ^{13}C in bulk beef vs. fatty acids (1.4−2.7‰ in Matsuzaka and 2.4−3.7‰ in Hida) can be attributed to isotopic fractionation during *de novo* biosynthesis, as similar magnitudes of fractionation have been found in the biosynthesis of fatty acids in plants (e.g., 2.4−9.9‰; Chikaraishi et al., 2004). Thus, the $\delta^{13}C$ values of fatty acids may be explained by a scenario in which the fatty acids in beef are mainly produced by *de novo* biosynthesis in the cattle but partially derived from the fatty acids in the feeds.

On the other hand, the considerable depletion in D for palmitic and oleic acids in beef might not be simply explained by this scenario, as previous experiments with cultures of heterotrophic bacteria have demonstrated an opposite trend: namely, that heterotrophically produced fatty acids are less depleted in D as compared with those produced by photoautotrophically (Valentine, 2009; Zhang et al., 2009). Moreover, a less depletion in D has been reported in a typical lipid (i.e., cholesterol) in natural gastropods (Chikaraishi, 2006). However, Chikaraishi et al. (2012) observed that

long-chain *n*-alkanes in insects are depleted in D by 30−40‰ as compared with values in plants in the same environment. Thus, the results of this and previous studies suggest the co-occurrence of two processes in the production of fatty acids in heterotrophs: *de novo* biosynthesis that is characterized by less depletion in D; and an alternative process characterized by considerable depletion in D.

In general, based on the kinetic isotopic fractionation, strong depletion in D is observed in biosynthetic products of hydrogenation, whereas strong enrichment and minor change in D are observed in respective biosynthetic precursors and products of dehydrogenation (Chikaraishi et al., 2004, 2009). According to this knowledge, significant enrichment in D of stearic acid relative to palmitic and oleic acids in beef is probably explained by the isotopic fractionation during dehydrogenation of stearic acid to form oleic acid in the cattle. Moreover, the considerable depletion in D for palmitic and oleic acids in beef may be explained by hydrogenation of feed-derived fatty acids and/or dehydrogenation–hydrogenation cycling with respect to the recycling of fatty acids in the cattle.

We predict that these hydrogenation of feed-derived fatty acids and dehydrogenation–hydrogenation cycling in heterotrophic organisms are likely candidates as the alternative process, which potentially leads to considerable depletion in D for fatty acids and their secondary alkyl derivatives (e.g., *n*-alkanes) in geological and ecological samples. Further investigation is obviously needed particularly for identifying the specific mechanisms and evaluating the universality of the considerable depletion in D in heterotrophs, but these studies would allow us better understanding of the isotope record on lipid biomarkers in geological and ecological samples.

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