TECHNICAL COMMENT

Metabolism of Dietary Cetoleic Acid (22:1n-11) in Mink (*Mustela vison*) and Gray Seals (*Halichoerus grypus*) Studied Using Radiolabeled Fatty Acids

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ABSTRACT

Cetoleic acid (22:1n-11) is a good indicator of diet in marine predators and has proven to be an important fatty acid (FA) when using adipose tissue FA composition to study diet in marine mammals and seabirds. Feeding studies have shown that 22:1 isomers are predictably underrepresented in adipose tissue relative to diet, implying that metabolism within the predator strongly influences the relationship between the level of these FAs in diet and adipose tissue. Fully understanding such metabolic processes for individual FAs is important for the quantitative estimation of predator diets. We employed a dual-label radioisotope tracer technique to investigate the potential modification of 22:1n-11 and its recovery in the blubber of gray seals (*Halichoerus grypus*) and in the adipose tissue and liver of mink (*Mustela vison*), a smaller model carnivore also accustomed to fish-based diets. In both seals and mink, ^3H radioactivity was found in the chain-shortened products of 22:1n-11, with 18:1 being the dominant product. We also found ^3H radioactivity in saturated FAs. The distribution patterns of ^3H radioactivity across the FAs isolated from seal blubber and mink subcutaneous adipose tissue were comparable, indicating that mink are a good model for the investigation of lipid metabolism in marine carnivores.

Introduction

Cetoleic acid (22:1n-11) has proved to be an important fatty acid (FA) when using adipose tissue FA composition to study diet in marine mammals and seabirds (Iverson 1993; Käkelä et al. 1993; Smith et al. 1996; Iverson et al. 1997*a*, 1997*b*; Raclot et al. 1998; Brown et al. 1999; Dahl et al. 2000; Iverson and Springer 2002). Although theoretically vertebrates can synthesize 22:1n-11, this FA primarily originates from the fatty alcohols (wax esters) of certain copepod species (Lee et al. 1971; Pascal and Ackman 1976; Ackman et al. 1980; Falk-Petersen et al. 1990). The concentration of this FA also varies widely among different fish and invertebrate species (Ackman 1980; Iverson 1993; Dahl et al. 2000; Budge et al. 2002; Iverson et al. 2002), making 22:1n-11 a good indicator of diet when found in the predator. Feeding studies have shown, however, that the isomers of 22:1 (n-11, n-9, and n-7) are generally underrepresented in adipose tissue relative to the diet (Holland et al. 1990; Lin and Connor 1990; Lin et al. 1993; Kirsch et al. 1998, 2000; Cooper et al. 2001; Iverson et al. 2004). Nevertheless, this underrepresentation is both predictable and highly consistent among both pinnipeds and seabirds (Iverson et al. 2004). This implies that metabolism within the predator has a strong and predictable influence on the relationship between the levels of these FAs in the diet and the adipose tissue. A better understanding of this metabolism will allow more accurate use of 22:1n-11 in quantitatively estimating diets of marine predators using quantitative FA signature analysis (Iverson et al. 2004).

In this study, we investigated the modification and deposition of 22:1n-11 using radiolabeled FA to provide more direct insight into the relationship between ingestion and deposition of 22:1 FAs in predators and to better understand the origin and fates of potential modification products. Radioisotope tracers are commonly used to study the in vivo metabolism of individual FAs (Owen et al. 1975; Thomassen et al. 1985; Hjelte et al. 1990; Linares and Henderson 1991; Green and Yavin 1993; Rabinowitz and Myerson 1994; Nilsson et al. 1996). Pinnipeds present a significant problem in tracking ingested labeled FA. They have a large body size, and blubber constitutes a high percentage of body mass (approximately 10%–45%; Worthy and Lavigne 1987; Ryg et al. 1990; Iverson et al. 1995; Arnould et al. 1996; Aarseth et al. 1999; Kirsch et al. 2000), both of

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which result in a large dilution of the labeled FA. Thus, the cost of feeding sufficient amounts of such labeled compounds can be quite high. One way to circumvent this logistical problem is to use a much smaller model animal. A second approach, developed by Budge et al. (2004), is to employ a more sensitive method of analysis that is capable of identifying labeled FA in the blubber of pinnipeds when small doses (<1 mCi) of labeled lipid are ingested. In this study, we employed both approaches to determine the metabolic fate of dietary ^{3}H-labeled 22:1n-11 in pinnipeds. Mink (*Mustela vison*) were used as a model for marine mammals because they too are carnivores accustomed to fish-based diets (Linscombe et al. 1982; Tolonen 1982), but their much smaller size avoids the problem of extreme signal dilution.

In addition to tracing ^{3}H-labeled 22:1n-11, simultaneously administering a differently labeled control FA allows for comparison between the levels of deposition of the two FAs, giving a quantitative measure of relative recovery. We chose ^{14}C-labeled 18:1n-9 as a control FA because it was expected to experience little modification between ingestion and final deposition in adipose tissue (Cook 1991; Budge et al. 2004), and its metabolism is generally representative of the dietary FA pool as a whole (Hagenfeldt et al. 1972; Jones et al. 1985; Wang and Koo 1993). Thus, this measure of relative recovery will indicate the degree to which the underrepresentation of 22:1n-11 in the adipose tissue relative to the diet is a direct consequence of its metabolism.

Material and Methods

Isotopically Labeled Fatty Acids

Because ^{3}H-labeled 22:1n-11 is not commercially available, it was necessary to first isolate 22:1n-11 from a natural source and then label it. We isolated 22:1 from surplus FA methyl ester (FAME) samples from marine lipids using a combination of argentation thin-layer chromatography and reverse-phase high-performance liquid chromatography (HPLC), according to the methods detailed in Budge et al. (2004). A sample of 50 mg was isolated to ensure an appropriate yield of labeled product. The isolated 22:1n-11 was sent to Perkin Elmer Laboratories for tritium labeling. The [1-^{14}C]-oleic acid (18:1n-9) was purchased from DuPont NEN (Boston). All radioisotopes were purchased under a license and permit held by Sara Iverson at Dalhousie University. The use of radioisotopes at the Nova Scotia Agricultural College (NSAC) Canadian Centre for Fur Animal Research was approved under an NSAC radioisotope use permit.

Mink Experiment

Five adult male mink housed at the NSAC Canadian Centre for Fur Animal Research were maintained on a herring-based diet from the time of weaning up to the time of the experiment. Thus, all the animals were accustomed to consuming marine lipids, including 22:1n-11. All animals were housed in identical conditions and led relatively sedentary lives. For the experiment, the mink were fed 1 mCi ^{3}H-labeled 22:1n-11 as FAME and 0.01 mCi ^{14}C-labeled 18:1n-9 as free FA using an eyedropper in combination with a 100-g meal of fish. After a 6- or 9-h incubation period, the mink were anesthetized by intramuscular injection of ketamine hydrochloride at 25 mg kg^{-1} body weight. The animals were then euthanized by intracardiac injection of sodium pentobarbital at 0.44 mL kg^{-1} body weight. Tissue samples weighing approximately 1 g were taken from the liver and from the mesenteric, omental, perirenal, inguinal, and subcutaneous adipose depots. For simplicity of presentation, the data for the inguinal, omental, and perirenal adipose depots were averaged to form the visceral category. Samples were placed in glass vials (with Teflon-lined caps) with chloroform and 0.01% BHT and then frozen until further lipid analysis was possible. Experiments using mink were approved by the NSAC Animal Care and Use Committee.

Seal Experiment

Two free-ranging juvenile gray seals were captured on Sable Island, Nova Scotia, placed in a fenced enclosure on the beach, and fasted for approximately 12 h. Each animal was then fed 1.5 mCi ^{3}H-labeled 22:1n-11 as FAME and 0.1 mCi ^{14}C-labeled 18:1n-9 as free FA by gastric intubation. Budge et al. (2004) found that administering 0.5 mCi of labeled FA was sufficient to produce a detectable signal in the blubber. However, because 22:1n-11 typically experiences reduced deposition relative to other FAs, we chose to use three times as much radioactivity in this experiment. On the other hand, 18:1n-9 is expected to experience a relatively direct deposition, so only 0.1 mCi of ^{14}C-labeled 18:1n-9 was used. A 24-h incubation period was chosen in light of the very low level of absolute deposition (<2%) of ingested ^{3}H-labeled triolein found by Budge et al. (2004), using a 12-h incubation period. Blubber biopsies totaling approximately 0.5 g per animal were taken from both the right and left flanks of each animal according to Kirsch et al. (2000). The animals were then released. Samples were placed in glass vials (with Teflon-lined caps) with chloroform and 0.01% BHT and frozen until further lipid analysis was possible. Experiments using seals were approved by the Dalhousie University Committee on Laboratory Animals.

Lipid Analysis

Lipids were extracted from adipose and blubber samples using 2 : 1 chloroform : methanol according to a modified Folch et al. (1957) procedure described in detail in Iverson et al. (2001). FAMEs were formed by reaction of approximately 100 mg of lipid with 1.5 mL of fresh anhydrous boron trifluoride in methanol (8% v/v) and 1.5 mL of hexane. The mixture was heated at 100°C for 1 h in a nitrogen atmosphere and FAMEs were extracted with hexane. FAMEs were then separated by degree of unsaturation using argentation thin-layer chromatography according to Budge et al. (2004). The FAMEs of each fraction were subjected to reverse-phase HPLC, and individual FAMEs were manually collected in glass test tubes. The purity of each isolate was assessed using temperature-programmed gas liquid chromatography according to Iverson et al. (1997*b*) on a Perkin Elmer Autosystem II Capillary FID GC equipped with a flexible fused silica column (30 m × 0.25 mm i.d.) coated with 50% cyanopropyl polysiloxane (0.25-μm film thickness; J & W/Agilent DB-23, Folsom, CA) and linked to a computerized integration system (Turbochrom, ver. 4.1, Perkin Elmer Nelson). FAMEs were identified by comparison of retention times with known standards (Nu-Check Prep, Elysian, MN), as well as by gas chromatography mass spectrometry. Each FAME fraction was then mixed with a scintillation cocktail (ScintiVerse II) and counted in a Beckman scintillation counter (LS3801).

Results

The relative recovery of 22:1n-11 was calculated as the ratio of ^{3}H-labeled 22:1n-11 to ^{14}C-labeled 18:1n-9 recovered in a depot divided by the ratio of ^{3}H-labeled 22:1n-11 to ^{14}C-labeled 18:1n-9 ingested. In the mink that had a 6-h incubation period, there was a large degree of individual variation in the relative recovery of 22:1n-11 (Fig. 1). In general, mink 1 had the greatest relative recovery, with its highest value being 0.60 in the mesenteric adipose tissue, while mink 3 had the lowest relative recoveries, <0.11 in all tissues. In the mink with a 9-h incubation period, the relative recovery was similar for both animals and was similar across all tissues sampled. In all tissues, the relative recovery of 22:1n-11 was less at 9 h than it was after a 6-h incubation period. In seals 1 and 2, the relative recovery of 22:1n-11 in blubber was 0.67 and 0.84, respectively.

The concentrations of ^{3}H radioactivity present in the various FAs isolated from the different mink tissues indicate considerable individual variation in the incorporation of the ^{3}H label (Table 1). This variation could have been caused by differences in mink body size and composition, both of which would affect the dilution space of the label. Body mass of the mink showed little variation (2.3 ± 0.1 kg), but body composition was not measured in this study. In a different study, however, body composition was measured in adult male mink and found to show relatively little variation (total body fat: 28.7% ± 3.2%; total body protein: 21.3% ± 1.1%; Boudreau 2005). For this reason, it is unlikely that variations in body mass or body composition contributed greatly to the large variation seen in the incorporation of the ^{3}H label. Generally speaking, the concentration of ^{3}H radioactivity in the mink incubated for 6 h was greater than that in mink incubated for 9 h. At both 6 h and 9 h, the concentration of ^{3}H-labeled 22:1 was greater in the liver than in any of the adipose depots (linear mixed-effects model, $P < 0.044$).

To account for the large amount of individual variation in the absolute concentrations of radioactivity and to make the data comparable across individuals, the radioactivity in each FA was expressed as a percent of the total ^{3}H present in a specific tissue. The distribution pattern of ^{3}H radioactivity among the various FAs provided insight into the extent of FA chain shortening and recycling (Figs. 2, 3). Even after the radioactivity data were standardized, individual differences in the metabolic processing of the ingested ^{3}H-labeled 22:1n-11 were apparent in some depots after a 6-h incubation period. For example, in mesenteric adipose, mink 3 contained the majority of its radioactivity in 18:1 (63.7%) with relatively little remaining in 22:1 (10.3%), whereas mink 2 had a relatively large amount of its radioactivity remaining in 22:1 (38.1%), with a smaller amount found in 18:1 (18.6%; Fig. 2).

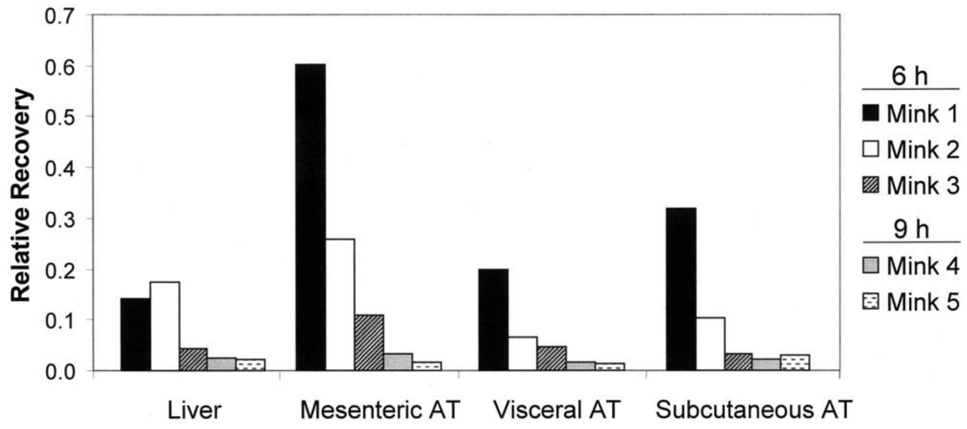

Figure 1. Relative recovery ([^{3}H-labeled 22:1$_{\text{recovery}}$/^{14}C-labeled 18:1$_{\text{recovery}}$]/[^{3}H-labeled 22:1$_{\text{ingested}}$/^{14}C-labeled 18:1$_{\text{ingested}}$]) of labeled fatty acids in liver and adipose tissue (*AT*) depots of adult male mink fed 1 mCi ^{3}H-labeled 22:1n-11 and 0.01 mCi ^{14}C-labeled 18:1n-9 after a 6- or 9-h incubation period.

Table 1: ^{3}H radioactivity recovered in fatty acids isolated from various tissue samples of mink (M1–M5)

	Mesenteric AT					Liver				
	6 h			9 h		6 h			9 h	
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
14:0	1.0	1.7	13.2	.3	.1	20.4	363.0	318.3	34.8	15.1
16:0	4.9	5.7	20.8	2.1	.2	51.6	452.2	422.4	590.6	113.7
18:0	2.9	6.2	19.1	1.8	.2	59.8	517.4	643.0	618.3	102.8
16:1	2.6	1.5	3.0	1.0	.1	13.3	224.7	54.2	29.2	9.8
18:1	5.7	7.1	146.5	6.2	.5	11.0	121.2	119.4	95.4	41.7
20:1	1.0	1.5	3.7	.7	.1	5.3	44.8	59.7	34.6	10.5
22:1	5.9	14.5	23.7	1.2	.1	17.3	70.9	109.3	26.4	17.4
Total	24.1	38.1	230.1	13.5	1.4	178.6	1,794.3	1,726.2	1,429.3	311.1
	Visceral AT					Subcutaneous AT				
	6 h			9 h		6 h			9 h	
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
14:0	6.7	1.5	4.3	.2	.1	.9	.9	5.7	.1	.0
16:0	7.2	11.7	12.0	.9	.2	3.6	6.5	8.6	.7	.1
18:0	11.5	11.9	10.1	.8	.4	2.2	10.9	13.3	.5	.2
16:1	2.6	3.3	6.4	.8	.1	.6	1.5	12.7	.7	.1
18:1	5.7	6.2	11.9	3.4	.4	1.4	5.4	35.8	2.3	.2
20:1	2.9	2.0	4.1	.3	.1	.8	.9	4.6	.2	.1
22:1	1.4	2.0	1.8	.3	.1	.9	2.0	2.2	.3	.1
Total	38.1	38.5	50.6	6.7	1.3	10.3	27.9	82.7	4.7	.9

Note. AT = adipose tissue. Values are 1,000 × dpm g^{-1} lipid.

The distribution pattern of radioactivity among the FAs in the liver was quite different from that of the adipose depots in that the vast majority of the ^{3}H recovered was located in the saturated FAs (SFAs; average of 76.1%, Fig. 2). The liver tissue sampled after a 9-h incubation period maintained this distribution pattern distinct from the adipose depots, with an average of 80.7% of radioactivity in the SFAs. The distribution of radioactivity among the FAs in the visceral and subcutaneous adipose depots of the 6-h minks showed somewhat different patterns from those of the mesenteric adipose tissue of the same animals (Fig. 2). In both the visceral and subcutaneous adipose depots, little of the radioactivity in any of the mink was remaining in 22:1 (<7%). Somewhat less radioactivity is found in the monounsaturated FAs (MUFAs) of the visceral (34.4%) and subcutaneous (39.5%) depots compared with the mesenteric depot (44.0%), whereas much more of it was found in the SFAs (average of 61.4%, 54.3%, and 31.7%, respectively). At 9 h, the distribution patterns of radioactivity in all adipose depots were similar, with SFAs accounting for an average of 35.2%, 39.6%, and 30.7%, and MUFAs accounting for an average of 56.1%, 54.5%, and 58.4% for the mesenteric, visceral, and subcutaneous depots, respectively (Fig. 3).

Comparison of these data from 6- and 9-h incubated animals revealed interesting findings (Figs. 2, 3). The distribution pattern of radioactivity in the FA of the liver was virtually identical in the 6- and 9-h mink. Also, in both the visceral and subcutaneous adipose tissue, the proportion of ^{3}H radioactivity in 18:1 was greater in the 9-h mink (average of 38.8% and 37.3%, respectively) than in the 6-h mink (average of 18.2% and 25.3%, respectively). This was coupled with a general reduction in the amount of ^{3}H radioactivity found in the SFAs at 9 h relative to 6 h for both the visceral (average of 39.6% vs. 61.4%) and subcutaneous depots (average of 30.7% vs. 54.3%).

In seals, significant amounts of ^{3}H were found in each of the SFAs and MUFAs isolated from blubber samples (Table 2). Tritium recovery, on a per gram blubber basis, was comparable in the two seals, with seal 1, the smaller of the two (42.0 vs. 50.5 kg), showing a 1.4-fold greater concentration. However, body composition measurements were not taken from these animals, so the absolute dilution of the ingested radioactivity was not known. In seals 1 and 2, the FA with the largest proportion of total recovered radioactivity was 18:1 (37.8% and 43.1%, respectively; Fig. 4). Seal 2 had a larger proportion of its total radioactivity remaining in 22:1 (23.0% vs. 12.6%) accompanied by a smaller proportion of its total radioactivity in the various SFAs (19.3% vs. 32.6%).

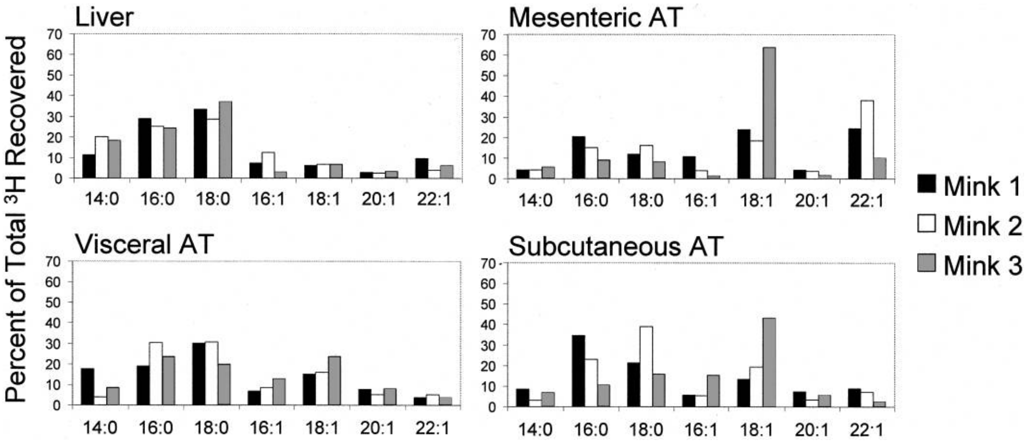

Figure 2. Percent of total recovered ^{3}H radioactivity found in each fatty acid isolated from liver and adipose tissue (*AT*) depots of adult male mink fed 1 mCi ^{3}H-labeled 22:1n-11 and sampled after a 6-h incubation period.

To assess the extent to which mink were useful animal models for the investigation of the metabolism of marine lipids by a pinniped, we compared the distribution of ^{3}H radioactivity among the FAs in the subcutaneous adipose depot of mink and the blubber of seals (Fig. 5). The small sample size and variation within each of the treatment groups prevented firm conclusions; however, the proportion of radioactivity recovered from each FA in the seals was quite similar to the proportions found in the 6- and 9-h mink.

Discussion

The 22:1 FAs are generally underrepresented in adipose tissue relative to the diet (Holland et al. 1990; Lin and Connor 1990; Lin et al. 1993; Kirsch et al. 1998, 2000; Cooper et al. 2001; Iverson et al. 2004). It has been suggested that the lower levels of 22:1 in depot triacylglycerol (TAG) are a result of poor digestibility and lower esterification rates of the 22:1 FAs (Thomasson 1956; Caselli et al. 1979). The most important factor governing the observed levels of these FAs in depot TAG, however, is more likely the peroxisomal chain shortening of 22:1 FAs (Bremer and Norum 1982). Animals that are unaccustomed to consuming large amounts of 22:1 FAs have a limited capacity for their metabolism, ultimately resulting in an intracellular cardiac lipidosis which causes a deterioration of myocardial function (see Bremer and Norum 1982 for a review). This cardiac lipidosis is, however, temporary because peroxisomal β-oxidation is induced by the intake of 22:1 FA–containing diets (Christiansen et al. 1979*a*, 1979*b*; Thomassen et al. 1979, 1985; Neat et al. 1980, 1981). As might be expected, animals accustomed to diets high in 22:1 FAs are more able to chain-shorten these FAs, thus avoiding any potentially harmful cardiac lipidosis and perhaps leading to an even greater un-

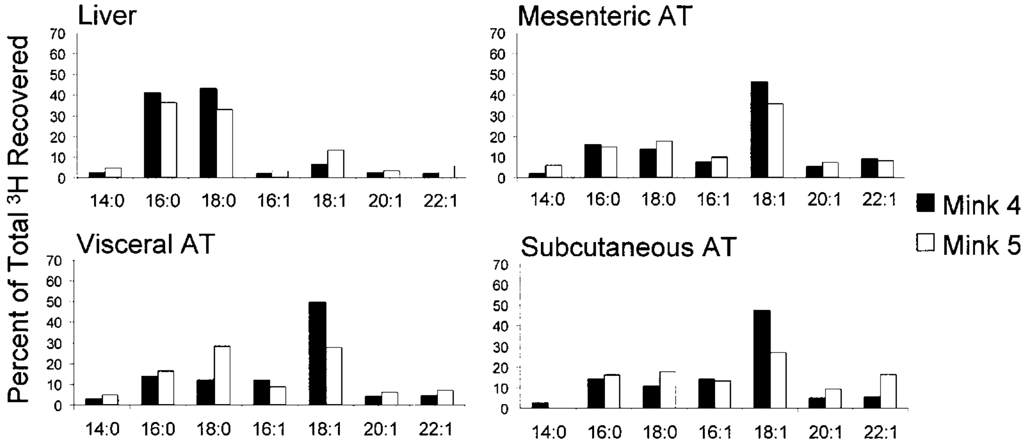

Figure 3. Percent of total recovered ^{3}H radioactivity found in each fatty acid isolated from liver and adipose tissue (*AT*) depots of adult male mink fed 1 mCi ^{3}H-labeled 22:1n-11 and sampled after a 9-h incubation period.

Table 2: ^{3}H radioactivity recovered in fatty acids isolated from blubber samples

	^{3}H dpm g^{-1} Blubber	
Fatty Acid	Seal 1	Seal 2
14:0	7.7	3.8
16:0	3.2	2.0
18:0	11.0	1.2
16:1	6.4	2.2
18:1	30.0	31.0
20:1	6.1	2.8
22:1	8.8	9.0
Total	73.2	52.0

Note. Values are 1,000 × dpm g^{-1} blubber.

derrepresentation of these FAs in adipose depots, relative to the diet. For example, Rouvinen and Kiiskinen (1989) showed that mink, whose wild diet is predominantly fish based (Linscombe et al. 1982; Tolonen 1982), accumulated 22:1 FAs to a lesser degree than did blue foxes (*Alopex lagopus*), which consume fish only occasionally (Samuel and Nelson 1982), when both species were fed diets high in 22:1 FAs.

The mink and seals studied here exhibited a strong capacity for the metabolism of 22:1n-11, as evidenced by the consistently lower recovery of ^{3}H-labeled 22:1n-11 relative to ^{14}C-labeled 18:1n-9. However, a caveat regarding the form in which the two FAs were administered must be made. The ^{3}H-labeled 22:1n-11 was fed as a FAME, whereas the ^{14}C-labeled 18:1n-9 was fed as a free FA. TAG is the form in which FAs are naturally consumed. Although there is some controversy in the literature (Nørdoy et al. 1991; Krokan et al. 1993), it appears that FAs consumed as TAG are more biologically available than those consumed as alkyl esters (Ikeda et al. 1995; Hong et al. 2003). In addition, free FAs are more slowly but ultimately equally well absorbed as FAs from TAG (Ikeda et al. 1995). This implies that the ^{14}C-labeled 18:1n-9 may have been more readily absorbed and, therefore, more biologically available for incorporation into tissue lipids than was the ^{3}H-labeled 22:1n-11. If so, the values calculated for the relative recovery of ^{3}H-labeled 22:1n-11 versus ^{14}C-labeled 18:1n-9 may be due in part to lower availability in addition to peroxisomal β-oxidation of the ^{3}H-labeled 22:1n-11.

In the 6-h mink, both the relative recovery of ^{3}H-labeled 22:1 and the distribution pattern of ^{3}H radioactivity across the various FAs indicate that there is considerable individual variation in the ability to metabolize 22:1n-11. For example, mink 3 appeared to have a much higher capacity than either mink 1 or mink 2 (Figs. 1, 2). This variation may be due to differences in the activity of their peroxisomal β-oxidation systems. It could also be caused by differences in food passage rate among the mink. The average food passage rate in mink is approximately 2–5 h (Jørgensen 1985; Szymeczko and Skrede 1990; Atkinson 1996). After only a 6-h incubation period, each mink could have been at a different point in the processing of the meal. Consistent with this interpretation, there was less individual variation in the same data from the 9-h mink. After a 9-h incubation period, the recovery of ^{3}H-labeled 22:1n-11 relative to ^{14}C-labeled 18:1n-9 is lower in all mink tissues studied, and there is very little difference in the relative recovery of ^{3}H-labeled 22:1n-11 across depots (Fig. 1). There is also a generally lower concentration of ^{3}H radioactivity in all tissues at the later sampling period (Table 1). The uniformity of the relative recovery data and the lower ^{3}H concentrations and relative recoveries at 9 h suggest that the extra time provided by the 9-h incubation period allowed the mink to more fully metabolize the ^{3}H-labeled 22:1n-11.

We anticipated the presence of ^{3}H in the chain-shortened

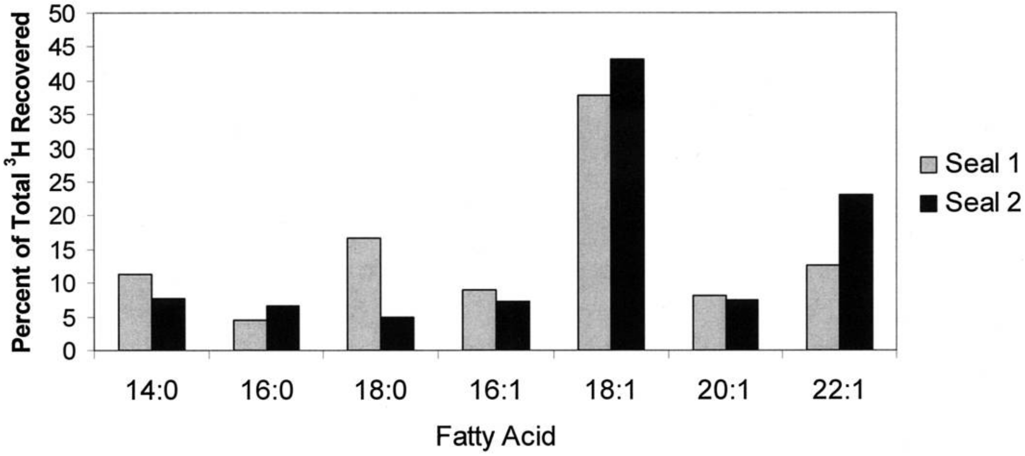

Figure 4. Percent of total recovered ^{3}H radioactivity found in each fatty acid isolated from the blubber of gray seals fed 1.5 mCi ^{3}H-labeled 22:1n-11 and sampled after a 24-h incubation period.

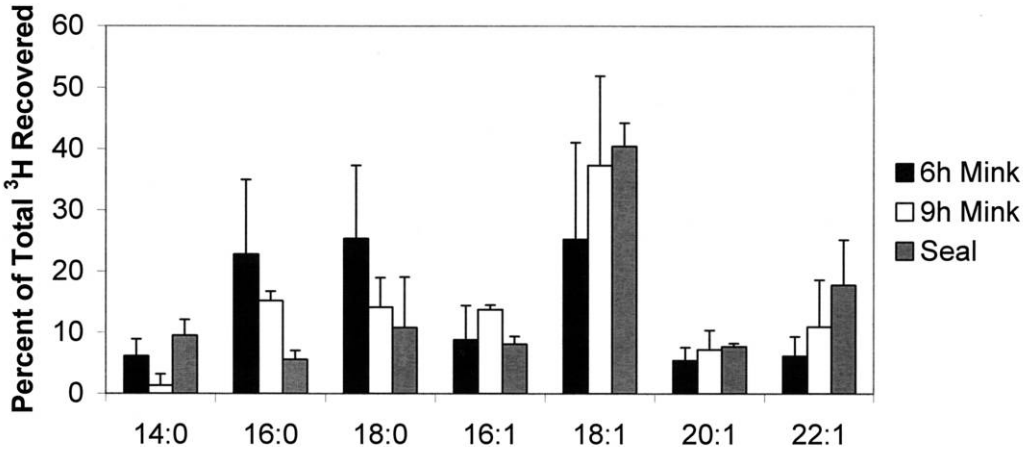

Figure 5. Percent of total recovered ^{3}H radioactivity found in each fatty acid isolated from the subcutaneous adipose tissue of adult male mink fed 1 mCi ^{3}H-labeled 22:1n-11 and sampled after a 6- or 9-h incubation period and the blubber of gray seals fed 1.5 mCi ^{3}H-labeled 22:1n-11 and sampled after a 24-h incubation period. Values are averages ± 1 SD.

products of 22:1, namely 20:1, 18:1, and 16:1, isolated from adipose samples of animals fed ^{3}H-labeled 22:1n-11 (Figs. 2–4). In peroxisomal chain shortening, only one or a few β-oxidation cycles take place (Osmundsen et al. 1979), making 20:1, 18:1, and 16:1 the expected products. Norseth and Christophersen (1978) found that the main product of the chain shortening of 22:1n-9 was 18:1n-9, with some 20:1n-9 and 16:1n-9 also being formed. Our results with 22:1n-11 are similar in that the proportion of total radioactivity found in 18:1 of all mink adipose depots, as well as seal blubber, was generally more than twice that found in either 16:1 or 20:1 (Figs. 2–4).

The extent of ^{3}H radioactivity found in the SFAs was somewhat surprising (Figs. 2–4). Radioactivity can appear in these FAs if the chain-shortened products of peroxisomal β-oxidation are transported to the mitochondria for complete breakdown and the resultant acetyl groups are then utilized in de novo FA synthesis. The main product of de novo FA synthesis is 16:0 (Volpe and Vagelos 1973), but some 14:0 and 12:0 may also be formed, as well as traces of 18:0 (Wakil et al. 1983). The presence of the vast majority of the ^{3}H radioactivity of the liver in SFA is consistent with this process of recycling the ^{3}H-labeled acetyl units into de novo synthesized SFA being important in this organ. Whether the ^{3}H-labeled SFAs present in the adipose depots originated in the depots themselves or were transported there from the liver is not known.

In both the visceral and subcutaneous adipose depots, the proportion of total radioactivity present in 18:1 was greater in the 9-h mink than it was in the 6-h mink (Figs. 2, 3). The small sample sizes and the cross-sectional nature of the data prevent firm conclusions, but we suggest that some of the de novo synthesized SFAs may have been elongated and/or desaturated to form 18:1 FAs. Isomers of individual FAs cannot be isolated using reverse-phase HPLC, so we cannot assess the contribution of the different isomers to the total ^{3}H radioactivity associated with individual FAs at the two sampling times. The larger proportion of ^{3}H radioactivity associated with 18:1 at 9 h may simply be due to an increased amount of ^{3}H-labeled 18:1n-11 from continued chain shortening of the ingested ^{3}H-labeled 22:1n-11. If, however, it is caused by an increased contribution from the 18:1n-7 or 18:1n-9 isomers, this would further indicate a progression in the metabolism of the ingested radioactivity at this time.

Pinnipeds accustomed to consuming diets high in 22:1 FAs are expected to have efficient peroxisomal chain-shortening systems. This expectation is supported by Iverson et al. (2004), who showed that the concentration of 22:1n-11 in the blubber of gray and harp seals is much lower than its concentration in the diet (proportional recoveries of 0.20 and 0.34, respectively). We also found a reduced recovery of ^{3}H-labeled 22:1n-11, in this case relative to ^{14}C-labeled 18:1n-9, in the two seals studied here (0.66 and 0.84). The reduced deposition of 22:1n-11 calculated by Iverson et al. (2004) is measured in relation to all other FAs present in the diet and blubber, including those that can be synthesized de novo in the seal. This ratio reflects total FA metabolism in the animal, and therefore, the ratio is lower than can be accounted for by the direct metabolism of 22:1n-11. Because our calculation of the recovery of 22:1n-11 is relative to only a single FA, 18:1n-9, our measure of reduced recovery more directly reflects the role of peroxisomal β-oxidation in determining the relationship between dietary and blubber levels of 22:1n-11. Because the two FAs were fed in different forms (free FA vs. FAME), however, our measure of relative recovery is still not an ideal reflection of peroxisomal β-oxidation in these animals. Future work should aim to synthesize and administer labeled TAG for each FA studied. In addition, while our sample size has allowed us to only begin to characterize metabolic patterns, it will be important to include a greater sample size to examine individual variability in relation to factors such as body size and metabolic rate.

In the past, mink have been used as models for nonruminant animals in general (Urlings et al. 1993) and carnivores specifically (Tauson et al. 1994). Because they are naturally carnivorous aquatic animals, mink seem to be reasonable models for studying marine mammals (Donnelly et al. 2000). Insofar as both mink and seals metabolized the tritium ingested as 22:1n-11 into chain-shortened MUFAs and de novo synthesized SFAs, the distribution patterns of ^{3}H radioactivity across the FAs isolated from seal blubber and mink subcutaneous adipose tissue were comparable (Fig. 5). Thus, our results indicate that mink are a suitable model to investigate the metabolism of marine lipids by a carnivore accustomed to their consumption. Mink have faster rates of passage of ingesta (2–5 h; Jørgensen 1985; Szymeczko and Skrede 1990; Atkinson 1996) relative to seals (5–13 h; Helm 1984; Krockenberger and Bryden 1994), as well as higher mass-specific metabolic rates (Kleiber 1975), as a result of their smaller body size. This may explain the apparently greater progression of the metabolism of 22:1n-11 in the mink compared with the seals (particularly at the 9-h sampling time), but the 24-h incubation period used in the seal experiments appears to be roughly equivalent to the shorter incubation periods used in the mink experiments. Therefore, given their smaller body size and demonstrated suitability as a model, the use of mink will allow more comprehensive studies to be conducted than would be possible using marine mammals.

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