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## Dynamics of blood chylomicron fatty acids in a marine carnivore: implications for lipid metabolism and quantitative estimation of predator diets

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**Abstract** Blubber fatty acid(s) (FA) signatures can provide accurate estimates of predator diets using quantitative FA signature analysis, provided that aspects of predator FA metabolism are taken into account. Because the intestinal absorption of dietary FA and their incorporation into chylomicrons (the primary transport lipoproteins for dietary FA in the blood) may influence the relationship between FA composition in the diet and adipose tissue, we investigated the metabolism of individual FA at these early stages of assimilation. We also investigated the capacity of chylomicron signatures to provide quantitative estimates of prey composition of an experimental meal. Six captive juvenile grey seals (*Halichoerus grypus*) were fed either 2.3 kg ( $n=3$ ) or 4.6 kg ( $n=3$ ) of Atlantic herring (*Clupea harengus*). Although chylomicron FA signatures resembled diet signatures at all samplings, absolute differences were smallest at 3-h post-feeding, when chylomicrons were likely largest and had the greatest ratio of triacylglycerol to phospholipid FA. Specific FA that differed significantly between diet and chylomicron signatures reflected either input from endogenous sources or loss through peroxisomal  $\beta$ -oxidation. When these aspects of metabolism were accounted for, the quantitative predictions of diet composition generated using chylomicron signatures were extremely accurate, even when tested against 28 other prey items.

**Keywords** Fatty acids · Chylomicrons · Pinnipeds · Diet

**Abbreviations** FA: Fatty acid(s) · FAME: Fatty acid methyl ester(s) · K-L: Kulback-Leibler · MUFA: Monounsaturated fatty acid(s) · PL: Phospholipid(s) · PUFA: Polyunsaturated fatty acid(s) · QFASA: Quantitative fatty acid signature analysis · SFA: Saturated fatty acid(s) · TAG: Triacylglycerol(s) · VLDL: Very low-density lipoproteins

### Introduction

Fatty acid (FA) signature analysis can be used to study the diets of free-ranging marine predators (Iverson 1988, 1993). Until recently, FA have only been used qualitatively, to infer the dominant prey species of several marine mammals and birds (e.g. Iverson et al. 1997a, b; Raclot et al. 1998; Dahl et al. 2000) and to determine spatial and temporal shifts in diets, both within and between marine mammal species (e.g. Iverson 1993; Käkälä et al. 1993; Smith et al. 1996; Iverson et al. 1997a, b; Smith et al. 1997). Although these studies clearly demonstrate the value of FA in elucidating foraging patterns, they do not do so quantitatively. Using FA signatures to quantitatively estimate the species composition of diets is not straightforward and requires a careful evaluation of the effects of FA metabolism within the predator. Because of this metabolism, the composition of adipose tissue triacylglycerol (TAG) will never be identical to that of the diet (Charnock et al. 1985; Roshanai and Sanders 1985; Lhuillery et al. 1988; Schwalm 1992; Kirsch et al. 1998; Kirsch et al. 2000; Summers et al. 2000). Recent work has demonstrated that when the differential metabolism of individual FA within the predator is accounted for, adipose tissue FA signatures can provide quantitative estimates of predator diets (Iverson et al. 2004).

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The metabolism of dietary FA can be separated into early and late stages. The early stages include the hydrolysis of FA from the ingested TAG, absorption of FA by the small intestine, and incorporation of FA into blood chylomicrons. Chylomicrons are TAG-rich lipoproteins synthesised in the small intestine, which act as the primary transport lipoproteins for dietary FA in the aqueous environment of the blood. They consist of a core of TAG and cholesterol esters surrounded by a phospholipid (PL) monolayer with associated cholesterol and apolipoproteins (Zilversmit 1965). Although chylomicron TAG are not exclusively dietary in origin (Karmen et al. 1963; Sheehe et al. 1980; Mansbach and Parthasarathy 1982; Shiau et al. 1985) it is well documented that the FA composition of TAG in chylomicrons reflects that of the diet (Ockner et al. 1969; Griffiths et al. 1994; Lambert et al. 1996; Fielding et al. 1999; Summers et al. 2000). The FA composition of the other chylomicron lipids (PL and cholesterol esters), however, does not (Kayden et al. 1963; Whyte et al. 1963; Redgrave and Dunne 1975). Because TAG is the main class of FA-containing lipid in mammalian chylomicrons (80–95%) (Redgrave 1983; Brindley 1991), the overall chylomicron FA composition is reflective of the diet as well.

Differential metabolism of individual FA throughout digestion, absorption, and incorporation into chylomicrons will influence the relationship between the FA composition of the diet and that in the adipose tissue. For example, in terrestrial species, the in vivo partitioning of absorbed FA between newly synthesized PL and TAG has been shown to vary for individual FA (Nilsson et al. 1987a; Nilsson and Melin 1988; Perez et al. 1999). Because FA esterified in chylomicron TAG will be more readily incorporated into adipose TAG, if such partitioning of dietary FA between PL and TAG occurs in marine carnivores such as pinnipeds, it will affect the relationship between FA in dietary and adipose tissue. Summers et al. (2000) suggest that this early metabolic processing of dietary FA is the main determinant of their deposition in adipose tissue. Given the distinct characteristics of marine lipids and the expected adaptation of marine carnivores to consuming them, it is reasonable to predict that differences may exist between marine carnivores and terrestrial species in the relationship between dietary and chylomicron FA compositions. Few studies have looked at this relationship in marine carnivores. Our first aim was, therefore, to investigate the metabolism of individual dietary FA at these early stages of assimilation by characterizing the FA composition of chylomicrons over the complete digestion period in grey seals (*Halichoerus grypus*) and comparing these with that of the meal consumed.

Our second aim was to investigate the capacity of chylomicron FA signatures to provide quantitative estimates of diet composition using quantitative fatty acid signature analysis (QFASA) (Iverson et al. 2004). Common methods used in diet studies (stomach content analyses and the recovery of hard parts from faecal

samples) are associated with several well-understood but difficult to overcome biases (Bigg and Fawcett 1985; da Silva and Neilson 1985; Jobling and Breiby 1986; Jobling 1987; Dellinger and Trillmich 1988; Bowen 2000). One of the primary advantages of QFASA over these methods is that it provides a relatively long-term (weeks to months) integration of an animal's diet, as opposed to just its most recent intakes. Situations exist, however, when the prey composition of a recent meal is of interest. If the QFASA of chylomicron signatures is viable, it will provide another tool, which could be used in conjunction with longer-term QFASA diet estimates from the adipose tissue of the same individual.

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## Materials and methods

### Animal maintenance and sampling

Six grey seals (age 2–3 years), originally captured as weaned pups from the Scotian Shelf population off eastern Canada, were housed in a seawater tank at the Dalhousie University Aquatron Facility. Seals were maintained throughout on diets of various lots of Atlantic finfish species, but mostly Atlantic herring (*Clupea hargenus*). Prior to the start of our experiment, seals were fasted for approximately 24 h. Early the next morning, the seawater tank was drained and an initial blood sample ( $\leq 10$  ml) was taken from the hind flipper of each seal into vacutainers containing anticoagulant. Each animal was then fed a meal from a single lot of Atlantic herring. Three seals received 2.3 kg of herring while the other three received 4.6 kg. Ten whole herring were randomly sampled from the same lot and stored at  $-20^{\circ}\text{C}$  until analysis. Seals were captured in a net at 1, 3, 6, 9, 12, and 24 h after feeding and a blood sample ( $\leq 10$  ml) was taken at these times as above.

### Chylomicron isolation

The lipoprotein classes of blood are generally defined and separated based on their densities. There is, however, some overlap in the sizes of the different classes. Chylomicrons are typically 35–250 nm in diameter, very low-density lipoproteins (VLDL) range from 30–110 nm, and the remaining lipoprotein classes are much smaller (Davis 1991). When a separation density of 1.006 g/ml is used, lipoproteins with diameters greater than 100 nm are isolated. We used this density to isolate the majority of blood chylomicrons while minimizing contamination from VLDL. For all postprandial blood samples, we refer to this density fraction ( $\rho < 1.006$  g/ml) as chylomicrons, despite some VLDL contamination. For the post-absorptive (e.g., 0 h and 24 h) plasma samples, VLDL will likely constitute a large proportion of the very small quantity of lipoproteins expected to be recovered. We, therefore, designate the isolated fraction here simply as post-absorptive lipoproteins.

Fresh blood samples were centrifuged and then visually inspected for the presence and relative amount of chylomicrons, as evidenced by the cloudy white layer floating at the top of the sample. Due to constraints placed on the captive sampling situation, which resulted in reduced volumes of blood being obtained as sequential blood sampling progressed, we were often unable to obtain sufficient samples for both accurate measurement of total lipid concentration and isolation and quantification of chylomicron FA. Our primary interest was in accurately measuring chylomicron FA composition and linking this to the visual appearance of their presence in blood samples, as this would be the situation faced by most investigators in the field when sampling free-ranging animals. Thus, blood samples were visually ranked as being clear or having low, moderate, high, or very high concentrations of chylomicrons. Plasma (1–4 ml) was collected from these samples and overlaid with a solution of 0.196 M NaCl ( $\rho$  1.006 g/ml), at pH 7.4 containing EDTA (0.01% w/v) and  $\text{NaN}_3$  (2 mM). The ratio of sample to saline was 1:1 v/v. The saline solution was carefully layered with a Pasteur pipette with the tip bent at a right angle and placed against the wall of the tube at the meniscus to avoid mixing. Tubes were then centrifuged at 26,000  $g$  for 20 min at 15°C in a Spinco-Beckman L65 ultracentrifuge (Beckman, Palo Alto, CA, USA). The top layer, containing all lipoproteins with  $\rho < 1.006$  g/ml, was carefully withdrawn, the centrifuge tube was refilled with saline and centrifuged again as described above. The lipoprotein fractions collected from the two centrifugations were pooled into a new centrifuge tube and 30–40 mg of polyethylene glycol (26,000 MW, BDH) was added. The same volume of saline as polyethylene glycol was added on top and the tubes were again centrifuged. The top layer, containing the purified chylomicrons or post-absorptive lipoproteins, was collected and frozen at –35°C in a  $\text{N}_2$  atmosphere until further analysis.

### Lipid analysis

Lipids were extracted from the purified chylomicron samples into chloroform according to the method of Folch et al. (1957) as modified by Iverson (1988), using the ratios of 18 parts 2:1 chloroform-methanol (v/v) to one part sample and three parts solvent to one part aqueous salt (see also Iverson et al. 2001). Fatty acid methyl esters (FAME) were prepared from 100 mg of the pure extracted lipid (filtered and dried over anhydrous sodium sulfate), using 1.5 ml of 8% boron trifluoride in methanol (v/v) and 1.5 ml of hexane, capped under nitrogen, and heated at 100°C for 1 h (Morrison and Smith 1964). FAME were extracted into hexane, concentrated, and brought up to volume (50 mg/ml) with high-purity hexane.

Duplicate analyses of FAME were performed using temperature-programmed gas-liquid chromatography on a Perkin Elmer Autosystem II Capillary FID gas

chromatograph fitted with a 30-m column (0.25-mm inside diameter) coated with 50% cyanopropyl polysiloxane (0.25- $\mu\text{m}$  film thickness; J&W DB-23; Folsom, CA, USA). Identifications of FA were determined from a number of sources, including known standard mixtures (Nu Check Prep., Elysian, MN, USA), silver-nitrate chromatography, and GC-mass spectrometry (Iverson et al. 1992, 2002). Individual FA are expressed as mass percentage of total FA and designated by A:Bn-x where A is carbon chain length, B is the number of double bonds, and n-x designates the location of the double bond nearest the terminal methyl group.

### Data analysis

In order to assess the degree to which chylomicron FA composition at the various sampling times resembled that of the test meal, the average Kulback-Leibler (K-L) distance (Kotz and Johnson 1983) between chylomicron and herring FA signatures were calculated. The average K-L distance represents a measure of the distance between two profiles of proportional data and is calculated as follows:  $\sum_j (C_j - D_j) \log(C_j/D_j)$  where  $j$  = individual FA,  $C$  = one profile (e.g., chylomicron signature), and  $D$  = a second profile (e.g., test meal signature). The smaller the K-L distance the more similar are the two sets of proportional data. Significant differences in the levels of individual FA between the meal ( $n = 10$ ) and chylomicron signatures sampled at the time corresponding to the lowest K-L distance (3-h post-feeding,  $n = 6$ ) were then determined by a MANOVA that used the five (i.e.,  $n - 1$ ) FA with the largest coefficient of variation.

Iverson et al. (2004) have developed a statistical model (QFASA) that provides quantitative estimates of the proportions of prey species in the diets of individual predators, using FA signatures. We tested the model's ability to accurately identify the experimental herring as the only prey item in the meal, using the chylomicron FA signatures sampled at the various times post-feeding. All prey FA signatures used in this study, except that of the experimental meal, were taken from a FA database of prey collected from the Scotian Shelf, which consists of 954 individuals of 28 fish and invertebrate species (Budge et al. 2002). An important component of the QFASA model is the use of calibration coefficients for individual FA to account for lipid metabolism within the predator and thus that the FA signature of the predator will never exactly match that of its prey. Calibration coefficients can be calculated when the complete diet FA signature that produced the predator FA signature is known. According to Iverson et al. (2004), the calibration coefficient,  $c_j$ , of a particular FA ( $j$ ) is computed as the 10% trimmed mean of the following  $r_{li}^j$ 's

$$r_{li}^j = \frac{\text{seal}_{ij}}{\text{diet}_{lj}}$$

for all  $l$  and  $i$  where  $l$  represents an individual diet signature and  $i$  represents an individual seal.

We used two sets of calibration coefficients to model the test meal of seals from their chylomicron signatures: (1) existing blubber calibration coefficients developed from a separate captive feeding study of grey seals (Iverson et al. 2004) and (2) chylomicron calibration coefficients calculated using the FA signatures of chylomicrons sampled at 3-h post-feeding (i.e., the lowest K-L distance from diet signature). Within each treatment group (2.3-kg meal and 4.6-kg meal), the chylomicron signatures of individual seals were modelled using the average calibration coefficients calculated from the other two seals in that group. We then modelled meals using two sets of prey inputs. The first set used only five potential prey options: argentine (*Argentina silus*), capelin (*Mallotus villosus*), northern sandlance (*Ammodytes dubius*), redfish (*Sebastes* sp.), and the herring actually fed to the seals in our study. These prey types were chosen based on hierarchical cluster analysis (Iverson et al. 2004), which showed that these prey FA signatures were more similar to each other than they were to all other species in the prey database, and thus represented a potentially difficult estimation scenario. The second prey set used the entire Scotian Shelf prey database and the experimental herring labelled separately from the other herring in the database. Here we wished to determine whether an increased number of prey options introduced significant noise in the model predictions.

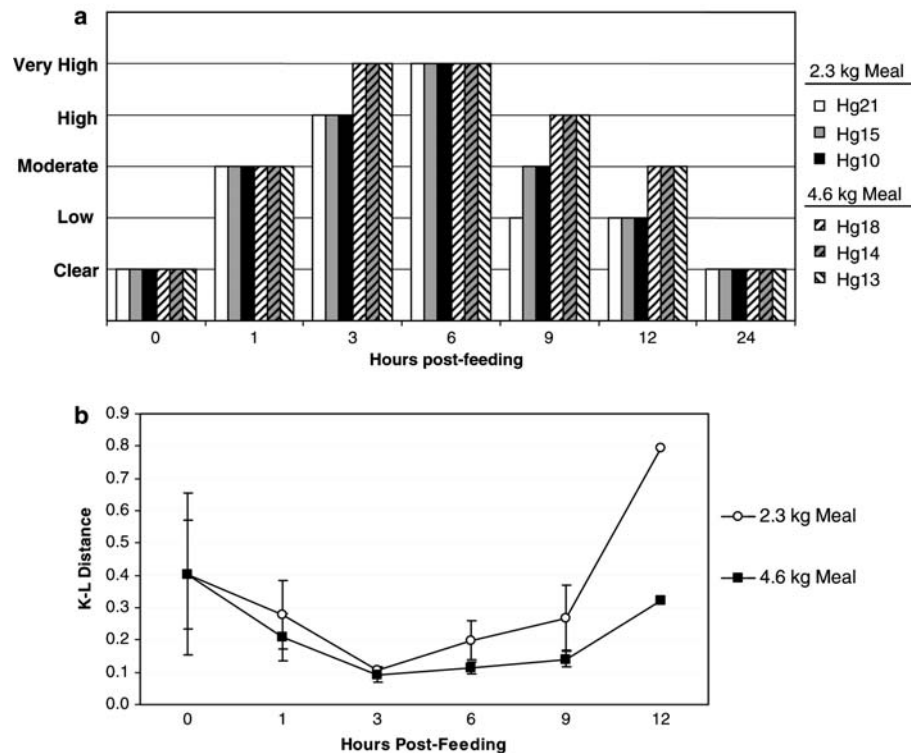
## Results

We obtained sufficient lipid for chylomicron FA analyses in all 1-, 3-, 6- and 9-h postprandial samples. We

were unable to obtain sufficient chylomicron lipid for reliable FA analysis in one 0-h post-absorptive sample, in four of the six 12-h samples and in all 24-h samples. This was in part due to very low lipid levels at these times, particularly 0 h and 24 h (see Fig. 1a), but primarily due to the reduced volumes of blood obtained as sequential sampling progressed (especially at 12 h and 24 h, see **Materials and methods**). The qualitative visual assessment of serum samples for the presence and relative amount of chylomicrons (cloudy white layer) indicated that chylomicrons began to appear in the blood by 1-h post-feeding (Fig. 1a). The visual density of chylomicrons in the blood peaked between 3- and 6-h post-feeding and then slowly declined until chylomicrons were no longer evident at some point between 12- and 24-h post-feeding. All study animals exhibited this pattern; however, the visual density of chylomicrons in seals fed the 4.6-kg meal appeared to peak earlier, was generally greater, and persisted longer than that of the seals fed the 2.3-kg meal.

The lowest K-L distances calculated between the test meal and chylomicron FA signatures occurred at 3-h post-feeding, indicating that the FA signatures of chylomicrons and the meal were most similar at this time. Values did not differ between meal sizes at this time but all other distances were lower for seals fed the 4.6-kg meal than for those fed the 2.3-kg meal (Fig. 1b), indicating greater similarity with increased meal size (i.e., fat intake). Both groups exhibited similar temporal patterns in K-L distances over time, with the greatest distances measured in the essentially post-absorptive lipoprotein samples (0 h and 12 h).

**Fig. 1** **a** Appearance and visual assessment of chylomicron concentration in serum samples taken at various times post-feeding. *Solid bars* Seals fed 2.3 kg of experimental meal. *Hatched bars* Seals fed 4.6 kg of experimental meal. **b** Average Kulback-Leibler distance ( $\pm 1$  SD) between the FA signature of the meal and those of the chylomicrons sampled at various times post-feeding; sample sizes for each group are  $n=3$  except at 0 h ( $n=2$ , for the 2.3-kg group) and 12 h ( $n=1$  for each group) samplings





The FA composition of the herring meal, in comparison to that of the post-absorptive lipoproteins (0 h) and postprandial chylomicrons (3 h), is presented in Table 1. The herring diet contained 19% saturated fatty acid (SFA), 62% monounsaturated fatty acid (MUFA), and 19% polyunsaturated fatty acid (PUFA). The majority of each of these classes was accounted for by only two FA each: 90% of the SFA were 14:0 and 16:0, 69% of the MUFA were 20:1n-9 and 22:1n-11, and 60% of the PUFA present were 20:5n-3 and 22:6n-3. The dominant FA found in the seals' post-absorptive lipoproteins were generally those that were dominant in the test herring, which also represented the habitual diet. However, the relative proportions of total SFA, MUFA, and PUFA (28, 42, and 31%, respectively) in post-absorptive lipoproteins differed considerably from those in herring, as did the relative contributions to these classes of individual FA (Table 1). For example, these lipoproteins were extremely enriched in 18:0, 18:2n-6, 20:4n-6, and 20:5n-3, and reduced in 20:1n-9 and 22:1n-11 relative to the diet. In contrast to these post-

absorptive lipids, the FA composition of the 3-h postprandial chylomicrons was similar to that of the meal, with SFA, MUFA, and PUFA comprising about 20, 54, and 26% of total FA, respectively (Table 1). Nevertheless, some differences remained between the absolute levels of individual FA in chylomicrons and those in the diet. For instance, levels of 18:0, 20:4n-6, and 20:5n-3 were higher while 20:1n-9 and 22:1n-11 were lower in chylomicrons compared to the diet.

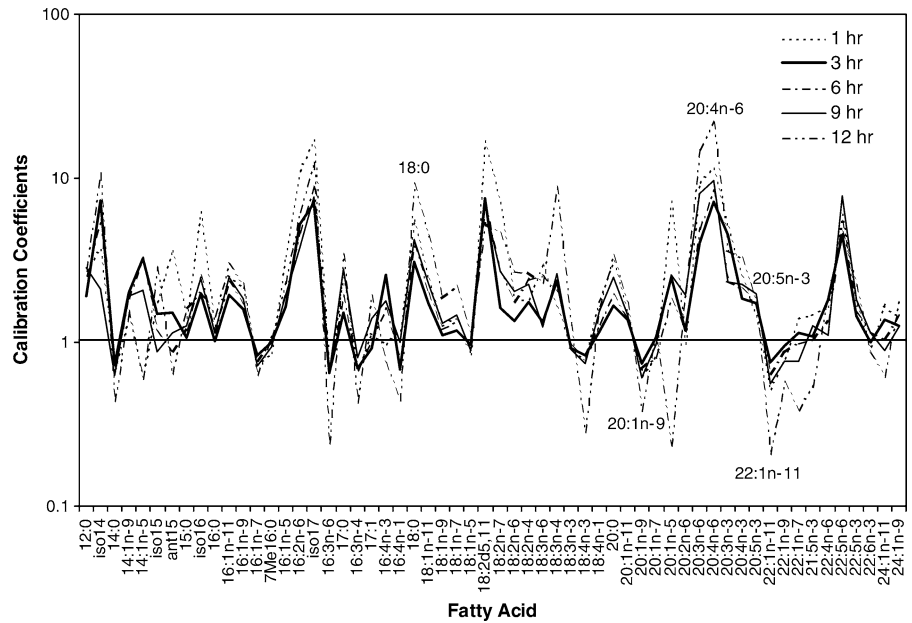
Although we used the calibration coefficients calculated from the 3-h chylomicrons for the actual diet modelling, we calculated coefficients from all post-feeding chylomicron signatures to assess the degree to which these differed from one another (Fig. 2). The majority of FA whose ratio deviated greatly from 1.0 were present in very small amounts (<0.5%, e.g. 12:0, 18:2Δ5,11, 22:5n-6). The exceptions to this were 20:1n-9 and 22:1n-11 which had ratios significantly less than 1.0, and 18:0, 20:4n-6, and 20:5n-3 which had ratios significantly greater than 1.0 (MANOVA,  $P < 0.001$ ). The ratios between chylomicron and diet FA changed over time.

**Table 1** Fatty acid composition of the herring diet in comparison to post-absorptive (0 h) lipoproteins and postprandial (3 h) chylomicrons from seals fed either 2.3 kg or 4.6 kg of the diet

Fatty acid	Herring diet $n = 10$	0 h $n = 5$	Chylomicrons	
			3 h 2.3 kg $n = 3$	3 h 4.6 kg $n = 3$
14:0	6.4±0.39	3.6±1.19	4.9±0.20	4.6±0.14
14:1n-9	0.2±0.01	0.4±0.15	0.5±0.01	0.5±0.06
15:0	0.3±0.01	0.4±0.06	0.3±0.02	0.3±0.03
16:0	10.6±0.31	13.8±1.68	10.8±0.58	10.7±0.55
16:1n-11	0.3±0.02	0.7±0.27	0.5±0.06	0.5±0.02
16:1n-9	0.1±0.01	0.3±0.15	0.2±0.06	0.2±0.03
16:1n-7	5.3±0.39	3.1±1.10	4.3±0.29	4.4±0.13
16:2n-6	0.1±0.01	0.5±0.11	0.3±0.02	0.5±0.09
16:3n-6	0.7±0.03	0.4±0.23	0.4±0.01	0.4±0.00
16:3n-4	0.4±0.04	0.4±0.18	0.4±0.06	0.3±0.02
16:4n-1	0.8±0.09	0.6±0.20	0.7±0.06	0.4±0.17
18:0	0.9±0.05	6.7±3.01	2.7±0.16	2.8±0.60
18:1n-11	0.6±0.04	2.0±0.69	1.0±0.09	1.1±0.08
18:1n-9	5.7±0.91	9.3±2.82	6.4±0.03	6.4±0.25
18:1n-7	1.6±0.13	2.6±0.58	1.9±0.10	1.9±0.13
18:1n-5	0.4±0.02	0.3±0.07	0.4±0.06	0.4±0.02
18:2n-6	0.8±0.05	2.8±1.70	1.0±0.09	1.2±0.14
18:3n-3	0.5±0.08	0.4±0.12	0.5±0.05	0.5±0.04
18:4n-3	1.6±0.33	1.0±0.48	1.3±0.12	1.4±0.05
20:0	0.2±0.01	0.6±0.18	0.3±0.02	0.3±0.01
20:1n-11	1.0±0.08	1.4±0.16	1.4±0.03	1.3±0.03
20:1n-9	16.7±0.36	7.9±3.56	12.7±0.71	12.0±0.44
20:1n-7	0.5±0.12	0.3±0.07	0.6±0.04	0.5±0.03
20:4n-6	0.2±0.01	3.3±1.36	1.6±0.11	1.5±0.18
20:4n-3	0.3±0.03	0.8±0.16	0.5±0.04	0.5±0.06
20:5n-3	6.1±0.42	10.1±2.09	10.2±0.54	10.9±1.07
22:1n-11	25.7±0.60	8.9±6.07	20.0±1.58	18.5±1.23
22:1n-9	1.7±0.24	1.9±0.68	1.5±0.17	1.6±0.17
22:1n-7	0.3±0.05	0.2±0.06	0.3±0.03	0.4±0.11
21:5n-3	0.2±0.03	0.2±0.08	0.3±0.01	0.3±0.03
22:5n-6	0.1±0.00	0.3±0.26	0.3±0.13	0.4±0.14
22:5n-3	0.7±0.03	1.5±0.39	1.1±0.03	1.0±0.06
22:6n-3	5.5±0.45	5.0±1.27	5.5±0.50	5.5±0.39
24:1n-11	0.3±0.02	0.3±0.22	0.3±0.09	0.5±0.15
24:1n-9	0.7±0.04	1.0±0.26	0.9±0.06	0.9±0.07
Saturated	19.2±0.71	27.5±4.43	20.3±0.81	20.5±1.55
Monounsaturated	61.6±1.83	41.7±6.36	53.6±2.27	52.2±1.11
Polyunsaturated	19.2±1.33	30.8±3.18	26.1±1.46	27.3±0.45

Values are mean mass percent ± SD of fatty acids (35 out of 69) present at levels ≥0.2%. Sufficient lipid for analysis was only obtained for five of the six seals at 0 h

**Fig. 2** Log plot of calibration coefficients for individual FA calculated from the average chylomicron FA signatures (2.3- and 4.6-kg meals combined) measured at the various times post-feeding. A value >1.0 indicates that the FA is present at a higher concentration in the chylomicrons than in the meal and vice versa for a value <1.0



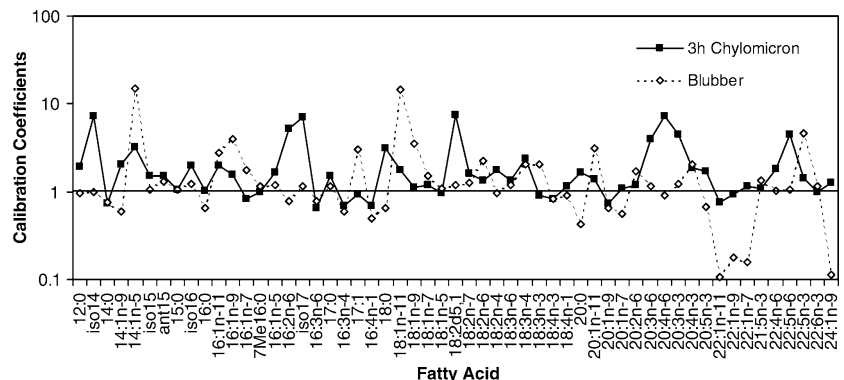
Consistent with the findings from K-L distances, ratios generally had the smallest deviations from 1.0 at 3-h post-feeding and subsequently increased in the later postprandial samples, with the greatest differences observed in 12-h chylomicrons (Fig. 2). We compared the 3-h chylomicron coefficients with those previously calculated from grey seal blubber signatures (Iverson et al. 2004; Fig. 3). The calibration coefficients of the FA that were greater in chylomicrons than blubber included 20:4n-6, all *iso* FA, 18:2Δ5,11, the SFA 18:0, and 20:0, and the isomers of 22:1. The FA with higher calibration coefficients in blubber compared to chylomicrons were 22:5n-3 and the MUFA 14:1n-5, 17:1, 20:1n-11, and all isomers of 16:1 and 18:1.

The QFASA model accurately identified the experimental herring as the main prey item contributing to the chylomicron signatures (and thus meal), using the reduced prey set of similar signatures and either the blubber or chylomicron calibration coefficients (Fig. 4a, b). At 3- and 6-h post-feeding, when chylomicrons appeared most abundant in all animals

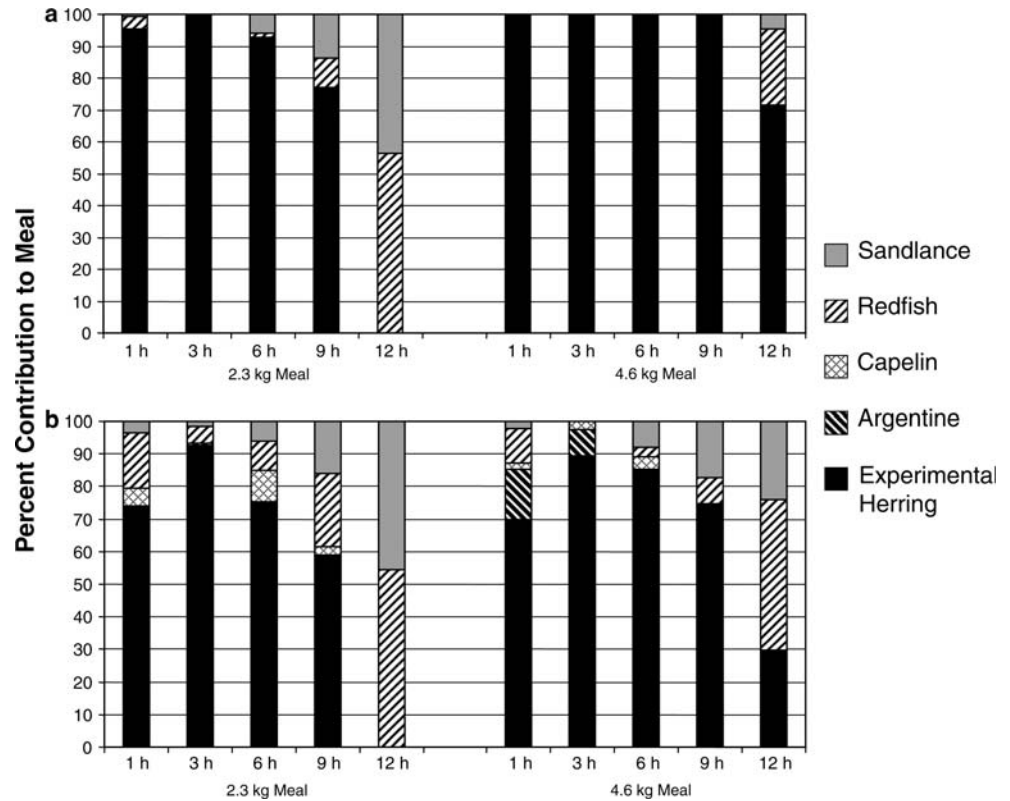
(Fig. 1a), the model estimated the experimental herring to comprise 90–100% and 75–100% of the meal, respectively. Estimates were generally less accurate using either the 1- or 9-h chylomicrons and degraded entirely using the 12-h chylomicrons, especially with the animals fed 2.3-kg herring. The meal was generally best estimated using the blubber coefficients and in the animals fed the 4.6-kg meal. Sandlance and redfish were the main other prey species estimated a contributing to the chylomicron signatures, particularly at 12-h post-feeding.

When chylomicron signatures were modelled using the same calibration coefficients but with the entire Scotian Shelf prey database, the diet was again extremely well estimated (Fig. 5a, b). The experimental herring was estimated to comprise about 100% of the meal at the 3-h sampling with either meal size, and >90% of the meal at both 1- and 6-h post-feeding. Again, the estimates degraded in the 9-h and especially 12-h samples, although generally less so than for the 5-species model (Fig. 4 vs. Fig. 5). At all times, the

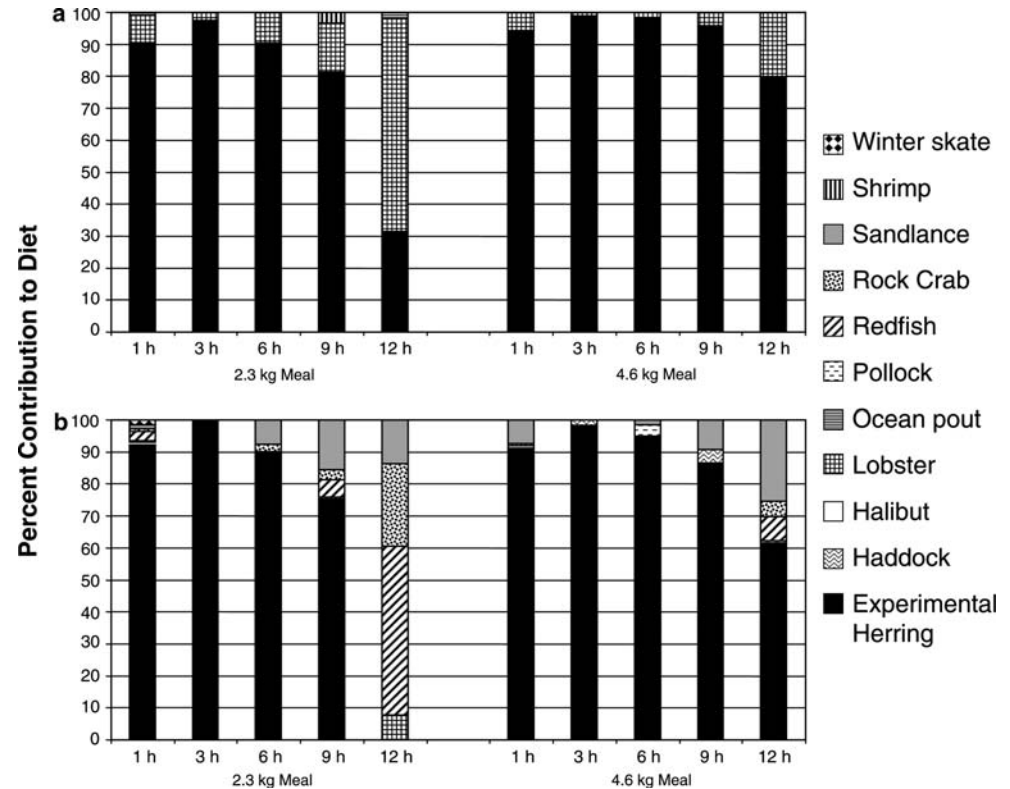
**Fig. 3** Log plot of the calibration coefficients calculated from chylomicron FA signatures measured at 3-h post-feeding (2.3- and 4.6-kg meals combined) in comparison to those previously reported (Iverson et al. 2004) from grey seal blubber FA signatures. A value >1.0 indicates that the FA is present at a higher concentration in the chylomicrons or blubber than in the meal and vice versa for a value <1.0



**Fig. 4** Model estimates of the percent contribution of prey items to the meal using chylomicron FA signatures at the various times post-feeding and **a** calibration coefficients derived from blubber FA signatures (Iverson et al. 2004) or **b** calibration coefficients derived from 3-h chylomicron signatures. Meals were modelled with a prey input of the experimental herring and four species from the Scotian Shelf prey database (Budge et al. 2002), with relatively similar FA signatures to that of herring to assess a potentially difficult estimation scenario



**Fig. 5** Model estimates of the percent contribution of prey to the meal using chylomicron FA signatures at the various times post-feeding and **a** calibration coefficients derived from blubber FA signatures (Iverson et al. 2004) or **b** calibration coefficients derived from 3-h chylomicron signatures. Meals were modelled with a prey input of the experimental herring and the entire Scotian Shelf prey database of 28 species, including a different lot of herring (Budge et al. 2002). Only the prey species that appeared in any of the meal estimates are listed on the figure



experimental herring was identified as the herring source rather than the other samples of herring contained in the Scotian Shelf prey database. Sandlance, redfish, lobster,

and rock crab were the main prey items erroneously estimated as contributors to the meal, especially at 12-h post-feeding.

## Discussion

The results of our study demonstrate that chylomicron FA signatures resemble those of the diet, but more importantly can be used to provide accurate, quantitative predictions of the prey composition of a recent meal in a marine carnivore. Nevertheless, we have also shown that individual dietary FA likely experience differential metabolism leading up to their incorporation into chylomicrons. These differences should ultimately have an effect on the incorporation of different FA into predator adipose tissue. Therefore, studying the metabolism of individual FA at these early stages is important for understanding the relationship between dietary and adipose tissue FA compositions. When the differences between the FA composition of whole chylomicrons and that of the diet are accounted for by using calibration coefficients for individual FA, the QFASA model of Iverson et al. (2004) accurately predicts the meal when postprandial chylomicrons are present. The model identified the specific lot of herring fed over all other lots of herring, as well as other prey contained in the broader Scotian Shelf prey database. Although further work should examine mixed-species diets and different feeding regimes as well as the effect of isolating only the TAG chylomicron FA for application of the QFASA model, this is the first time that diet has been studied, in a quantitative fashion, using the FA composition of chylomicrons.

### FA metabolism within the predator

In this study, we were not able to accurately measure total lipid concentration of serum samples and, therefore, used the visual assessment of serum samples as an index for the presence and relative concentration of chylomicrons. Chylomicron concentration is not entirely visually based. As their TAG core is metabolized, chylomicrons become smaller and their visual appearance in the serum can change. However, because we isolated and analysed the <1.006 density fraction, we were likely only working with the larger chylomicrons that would be responsible for producing the milky appearance of the serum. Thus, our FA data corresponded well with the visual assessment of the serum (i.e. lowest K-L distances were measured at peak visual density of chylomicrons). Since TAG account for the majority of FA in large chylomicrons (Redgrave 1983; Brindley 1991), we did not separate chylomicron TAG and PL prior to measuring chylomicron FA composition. However, the degree to which PL FA contribute to the overall chylomicron FA composition clearly depends on both the time since feeding and the level of fat consumed, and is discussed below.

The temporal appearance of chylomicrons in grey seals (Fig. 1a) agrees well with studies performed on humans and rats in which chylomicrons typically ap-

peared at some point before 2-h post-feeding and chylomicron TAG concentration peaked between 4- and 6-h post-feeding (Harris et al. 1988; Gibney and Daly 1994; Griffiths et al. 1994; Sakr et al. 1997; Lai and Ney 1998; Summers et al. 2000). Our finding that the FA profiles of the chylomicrons sampled at 3-h post-feeding were most similar to that of the diet (i.e. smallest K-L distance, Fig. 1b) is also in keeping with this. Chylomicrons were still visible in all study animals at 12-h post-feeding (Fig. 1a). Lai and Ney (1998) found that, in rats, chylomicron TAG concentration remained above fasting values for at least 12-h post-feeding. In humans, however, Sakr et al. (1997) found that by 8-h post-feeding the concentration of the chylomicron fraction was at or below fasting values.

While our density gradient used for isolating chylomicrons was aimed at minimizing contamination from VLDL, it is possible that as the amount of chylomicrons decreased with increased time post-feeding, the relative contribution of VLDL FA to the overall signature increased. This could, in turn, increase the K-L distance between the chylomicron and diet signatures. In several species studied, chylomicrons and VLDL contain different forms of apoB (Davis 1991). Nothing is known about the types of apoB found in seals but if different forms are produced in the small intestine and liver, it would allow direct determination of the relative proportions of chylomicrons and VLDL in a given density fraction.

The earlier apparent peak and generally greater visual density of chylomicrons in the seals fed 4.6 kg of herring relative to those fed 2.3 kg (Fig. 1a) was expected. The former seals consumed twice as much fat and would, therefore, have more to transport in the blood. Chylomicron size is primarily dependent on the amount of TAG absorbed and transported (Fraser et al. 1968; Boquillon et al. 1977; Hayashi et al. 1990). As the fat load increases, the size but not number of chylomicrons also increases. Larger chylomicrons have higher TAG:PL ratios and as a result their FA composition should be more reflective of diet. Our finding that the K-L distances were consistently lower for seals fed the larger meal (Fig. 1b) is in keeping with their greater fat intake and suggests that these seals may have been transporting relatively larger chylomicrons, with a higher TAG:PL ratio.

The FA that were present in greater levels in the seal chylomicrons relative to their experimental diet (18:0, 20:4n-6 and 20:5n-3; Table 1, Fig. 2) have to have arisen from endogenous sources (Christensen and Høy 1996; Lambert et al. 1996; Becker et al. 2001). The primary endogenous sources of mucosal FA are plasma non-esterified FA and lipoproteins (Gangl and Ockner 1975; Gangl and Renner 1978; Mansbach and Dowell 1992), chylomicron remnants (Mansbach and Dowell 1994, 1995), and bile (Noma 1964; Shrivastava et al. 1967; Melin et al. 1996). The differential handling of individual FA, both in terms of chylomicron formation as well as during subsequent lipoprotein metabolism, determines



the FA composition of these endogenous sources. Because chylomicrons preferentially incorporate nascent TAG and preformed PL (Mansbach and Parthasarathy 1982; Mansbach and Nevin 1998; Luchoomun and Hussain 1999), it is likely that a proportionately greater amount of the endogenous FA found in the whole chylomicrons are associated with the PL fraction.

The partitioning of dietary FA between PL and TAG within the enterocyte differs for individual FA. In previous studies, 18:0, 20:4n-6, and 20:5n-3 all show greater incorporation into PL than do other FA (Whyte et al. 1963; Chen et al. 1985; Nilsson et al. 1987a; Nilsson and Melin 1988; Nilsson et al. 1992; Emken et al. 1993; Perez et al. 1999). Subsequent lipoprotein metabolism then contributes to the ultimate return of these FA to the intestine via endogenous sources, where they are again likely to become associated with the PL portion of the chylomicrons. Chylomicron and VLDL TAG-associated 20:4n-6 and 20:5n-3 have been shown to be relatively resistant to hydrolysis by lipoprotein lipase (Ridgway and Dolphin 1984; Nilsson et al. 1987b; Ekström et al. 1989; Melin et al. 1991; Levy and Herzberg 1999). Wang and Koo (1993) showed that 18:0 is also released slowly from chylomicrons. For this reason, chylomicron remnants are generally enriched in these three FA relative to their parent chylomicrons (Nilsson and Landin 1988; Melin et al. 1991; Nilsson et al. 1992; Mansbach and Dowell 1995; Lambert et al. 1996; Hansen et al. 1998). Finally, rat bile PL can contain 15–20% 20:4n-6 (Kawamoto et al. 1980; Patton et al. 1984). Both 20:4n-6 and 20:5n-3 have high affinities for pathways that catalyze their incorporation into tissue PL (Pelech and Vance 1984; Leyton et al. 1987; MacDonald and Sprecher 1991), suggesting that when the habitual diet contains significant quantities of 20:5n-3, this FA would also be predominant in bile. However, Levy and Herzberg (1996) studied the FA composition of bile PL in rats adapted to fish oil diets and found that even when diets containing 13% 20:5n-3 were consumed, this FA comprised less than 2% of the bile PL FA. Thus, bile PL may be an endogenous source of 20:4n-6 in grey seals but this may not be the case for 20:5n-3.

The experimental diet consisted primarily of MUFA, as did the 3-h chylomicrons but to a somewhat lesser degree (Table 1). This was mainly the result of lower levels of 20:1n-9 and 22:1n-11 in the chylomicrons relative to the diet (Table 1, Fig. 2). These lower concentrations could in part result from dilution of dietary FA with the endogenous FA of the PL fraction. However, it is likely that they are primarily due to the partial chain shortening of these FA, via peroxisomal  $\beta$ -oxidation, within the enterocytes (Novikoff and Novikoff 1972; Thomassen et al. 1985). The peroxisomal  $\beta$ -oxidation system is quite active with 20:1 and 22:1 fatty acyl-CoA esters as substrate (Osmundsen et al. 1979) and is induced by the intake of 22:1 FA-containing diets (Christiansen et al. 1979; Neat et al. 1980, 1981; Thomassen et al. 1985; Veerkamp and Zevenbergen 1986; Rouvinen and Kiiskinen 1989). Considering that

our study animals had been maintained on a diet of Atlantic herring, which contains a high concentration of 22:1n-11 (26%, Table 1), their peroxisomal  $\beta$ -oxidation systems were expected to be highly active. This assumption was supported by the finding that the levels of 20:1n-9 and 22:1n-11 in chylomicrons were at most only 74% and 75%, respectively, of that in the diet (Table 1). Further support for this conclusion comes from 20:1n-11 and 18:1n-11 (the primary products of chain shortening), being enriched in chylomicrons relative to diet (1.3-fold and 1.8-fold respectively; Fig. 2). These results are consistent with in vivo studies of seals using radio-labelled 22:1n-11 (Cooper 2004).

The differences between calibration coefficients calculated using chylomicron signatures versus blubber signatures are extremely informative. Because the dietary FA concentration is always in the denominator of the calculation, these differences in calibration coefficients are indicative of differences between the levels of individual FA in the two fat sources, one immediately after absorption and one after final deposition in tissue. This, in turn, provides information regarding the origin of certain FA as well as the predominant location of various modification processes. For example, the calibration coefficient for 22:1n-11 was much lower for blubber than for chylomicrons, whereas those for 20:1n-11 and 18:1n-11 were much higher. This suggests that despite the fact that some chain-shortening in the mucosa was implied by the chylomicron composition (Fig. 2), the primary site of peroxisomal  $\beta$ -oxidation is downstream of chylomicron formation and most likely resides in the liver as has been suggested previously (Ong et al. 1977; Bremer and Norum 1982).

Differences between chylomicron and blubber calibration coefficients of several other FA are also in keeping with the fact that the majority of FA modification occurs post chylomicron formation (Cook 1991). For instance, the calibration coefficient of 22:5n-3, which is a modification product of 20:5n-3 (Terano et al. 1983; von Schacky and Weber 1985), was greater in blubber than in the chylomicrons, implying that the primary site of this inter-conversion reaction may reside in either the liver or the blubber itself. Secondly, the calibration coefficients of SFA were typically higher in the chylomicrons than in the blubber, whereas the calibration coefficients of their immediate desaturation products (MUFA) were higher in the blubber. In a comparison between FA concentrations of chylomicrons and depot fat, Becker et al. (2001) found identical patterns in rats. Finally, the seal chylomicron calibration coefficients for the branched-chain FA (*i*14:0, *i*15:0, *i*16:0 and *i*17:0) were > 1.0 and relatively larger for chylomicrons than for blubber (Figs. 2, 3). This is in keeping with both their proposed origin and site of metabolism. High proportions of branched-chain FA have been linked to several categories of gut bacteria including Gram positive micro-organisms and certain Gram negative anaerobes (Moore et al. 1994; Hopkins et al. 2001), which accounts for their enrichment in chylomicrons.

The relatively higher levels of these FA in chylomicrons than in blubber, is likely due to their subsequent metabolism within the predator. Branched-chain FA are primarily oxidized within the peroxisomes (Poulos et al. 1988; Singh et al. 1992). As discussed above, peroxisomal  $\beta$ -oxidation in our study animals was likely very active. As a result, the branched-chain FA present in chylomicrons would be effectively oxidized, primarily in the liver, and thus not available for deposition in the blubber.

#### Quantitative diet estimation from chylomicron FA

The QFASA model performed well in estimating diets from postprandial chylomicron signatures both when using a select group of prey items of similar FA composition (Fig. 4) and when using the entire Scotian Shelf prey base (Fig. 5). In fact, when the chylomicron calibration coefficients were used, the model produced more accurate predictions when the entire prey base was included as opposed to just the select group of similar prey items (Figs. 4b, 5b). In the former case, the model identified the experimental herring as the primary diet item over all other prey (28 species) in the database, including other lots of herring. Nevertheless, some prey species were falsely identified as contributing minor amounts to the meal. The appearance of redfish and sand lance with both prey sets, especially at 9- and 12-h post-feeding (Figs. 4, 5), was likely due to the similarity of their FA signatures to that of herring and the increased “noise” introduced in chylomicron signatures as the PL:TAG ratios increased with time since feeding. The appearance of crustaceans in the model predictions using the full prey base (Fig. 5), again especially at 9- and 12-h post-feeding, was due to the very high levels of n-6 FA, particularly 20:4n-6, in these prey. The model included these prey in the estimates to account for the level of 20:4n-6 being much higher in the chylomicron signatures of seals than in the diet.

Although the QFASA model provided accurate estimations of diet in postprandial whole chylomicrons, the time since feeding and the size of the meal influenced the accuracy of the results. The predictions were best when 3-h chylomicron signatures were used. When chylomicrons sampled within 9 h of feeding were used, predictions were still generally better than the 75% and 85% accuracy for seals fed 2.3 kg or 4.6 kg of herring, respectively. However, estimates clearly degrade as the time post-feeding increases. The processes involved in the metabolism of chylomicrons that increase the ratio of PL to TAG with time undoubtedly contribute to this finding. Isolation of TAG FA from PL FA in the samples taken within 9 h of feeding would, therefore, have improved our diet estimations. A potentially increasing relative contribution from VLDL FA with increased time post-feeding may also play a role. Thus, whether predictions would be similarly improved in the largely post-absorptive lipoproteins at 12 h is not known.

It is clear from this work that in order to use chylomicron FA signatures to estimate diet, chylomicrons must be visibly present and the <1.006 density fraction must be isolated from the rest of the blood sample prior to FA analysis. If whole blood, plasma, or serum were analyzed, the increased input of endogenous FA, carried in the other lipoprotein classes, would lead to highly erroneous results in the modelling of diets. Where moderate levels of visible chylomicrons are present, one can be relatively confident in obtaining useful model predictions of diet, even in free-ranging animals when time since feeding is unknown. We believe that since endogenous FA associated with chylomicron PL introduce noise in diet estimates, even when chylomicrons are largest and most abundant, isolating the FA in TAG from those in PL will further improve results and provide the most reliable estimates of diets from chylomicron FA. In addition, it is possible that if only the FA of chylomicron TAG were analyzed, diet estimates would remain accurate for longer durations post-feeding but this requires further investigation. Future studies should also examine mixed-species diets and feeding regimes that might be more representative of the foraging behaviour of free-ranging animals, to assess their effect on the accuracy of model predictions.

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