Effect of a Low-Fat Diet on Body Composition and Blubber Fatty Acids of Captive Juvenile Harp Seals (*Phoca groenlandica*)

Penny E. Kirsch[1]
Sara J. Iverson[1,*]
W. Don Bowen[1,2]
[1]Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada; [2]Marine Fish Division, Bedford Institute of Oceanography, Dartmouth, Nova Scotia B2Y 4A2, Canada

ABSTRACT

We investigated the effects of a change from a high-fat diet to a low-fat diet of differing fatty acid (FA) composition on the body composition and blubber FA of five captive juvenile harp seals. Seals that had been maintained for 1 yr on a diet of Atlantic herring (≥9% fat) were switched to a diet of Atlantic pollock (1.7% fat) for 30 d. On days 0, 14, and 30, mass and body composition (using isotope dilution) were measured, and blubber biopsies (5 cm × 6 mm) were taken for FA analysis. Fat accounted for 38%–49% of body mass at the start of the experiment. When switched to the pollock diet, and despite food intakes averaging 6.5 kg/d (32.3 MJ/d), body fat declined by an average of 6.4 kg or by 32% over the 30-d experiment. In contrast, body protein increased in direct relation to protein intake ($r^2 = 0.836$, $P = 0.030$). Despite substantial loss of body fat, blubber FA signature changed significantly to reflect the changes in dietary intake of FA, and the deposition of FA was quantifiably predictable. Our results suggest that young growing phocids are unable to maintain body fat stores on low-fat diets even when protein intakes are high. This may have significant implications for juvenile pinniped survival in the wild. In addition, turnover and deposition of dietary FA in blubber takes place in nonfattening seals.

Introduction

Pinnipeds (seals, sea lions, and fur seals) are top predators in marine ecosystems and, as such, may have significant effects on or be affected by prey populations (Bowen 1997). The diets of pinnipeds may serve as an indicator of relative abundance and distribution of prey species (see, e.g., Sinclair et al. 1994). Also, there has been considerable interest in understanding the diets of free-ranging pinnipeds and the effects of variation in prey abundance and prey quality on pinniped population dynamics (see, e.g., Alaska Sea Grant College Program 1993; NRC 1996).

Traditional methods of diet analysis include recovery of prey hard parts from stomach contents and fecal samples. Although these methods provide important information about the diets of pinnipeds, they involve biases that are in some cases well understood yet often unavoidable. For instance, prey species that lack hard parts or whose hard parts are easily digested may be underestimated (Jobling 1987; Dellinger and Trillmich 1988; Pierce et al. 1991; Bowen and Harrison 1994). In contrast, accumulation in the stomach of hard parts such as squid beaks may overestimate the importance of these prey in the diet (Bigg and Fawcett 1985). Finally, and perhaps most important, only the last meal consumed, and usually only that consumed near haul-out locations and breeding sites, can be studied using these methods, and thus diets from other parts of the species' foraging range may be missed.

The limitations of these traditional methods have led to the development of other methods, including serology, stable isotopes of C and N, and fatty acid signatures, for understanding the diet of free-ranging pinnipeds (Pierce et al. 1990; Rau et al. 1992; Iverson 1993). Fatty acids are the predominant constituent of most lipids. A relatively limited number of fatty acids are able to be biosynthesized by animals (Cook 1985), and during fattening, those of carbon chain length of 14 or greater can be deposited in animal tissue directly as consumed in diet. In marine ecosystems, fatty acids are remarkably complex and diverse, often arising from specific taxa or habitats. Fatty acid analysis integrates dietary intake over periods of weeks or months and thus can provide information on more than just the last meal. Also, because the use of fatty acids does not depend on the recovery of hard parts, all major components of the diet could in principle be identified.

Milk and blubber fatty acids in free-ranging pinnipeds have been used as indicators of or changes in diet (Iverson 1993; Iverson et al. 1997*a*, 1997*b*; Smith et al. 1997). However, to use fatty acids with confidence and, eventually, in a quantitative manner, it is important to understand the direct effects of diet on fatty acid composition and turnover in predator lipid stores and to assess the relative effects of intake level (e.g., degree of

*To whom correspondence should be addressed; e-mail: siverson@is.dal.ca.

fattening) on these patterns. Unfortunately, controlled experiments are expensive, time consuming, and logistically difficult to carry out. To date, studies have demonstrated the direct influence of diet on the fatty acid composition of planktivorous and piscivorous fish (Yu et al. 1977; Sargent 1989; Dos Santos et al. 1993; Kirsch et al. 1998). Similarly, the influence of fish fatty acids in the diet has been demonstrated in various tissues of commercially important fur-bearing carnivores, including mink (*Mustela vison*), silver fox (*Vulpes vulpes*), arctic fox (*Alopex lagopus*), polecats (*Mustela putorius*), and raccoon dogs (*Nyctereutes procyonoides*; Rouvinen and Kiiskinen 1989; Rouvinen 1992; Rouvinen et al. 1992; Pond et al. 1995).

In pinnipeds, the predominant site of fatty acid storage and release is the superficial layer of adipose tissue known as blubber. The mass and depth of this tissue is most notably altered during periods of fasting or fattening, and hence, it is the tissue most strongly influenced by diet (see, e.g., Iverson et al. 1995). It is evident that during rapid fattening on a high-fat diet, the blubber fatty acid composition of seals may mirror that of diet (Iverson et al. 1995). However, the effects of a low-fat diet and little or no net fat deposition on blubber fatty acid composition are not known. In this study, we investigated the effects of a change from a high-fat diet to a low-fat diet on the body composition and blubber fatty acid patterns of captive juvenile harp seals (*Phoca groenlandica*). Our objectives were to determine whether the fatty acid composition of harp seal blubber changed when diet was changed and, if changes in blubber did occur, if these reflected the relative differences in diet. Our other aim was to determine the extent to which changes in blubber fatty acid composition were dependent on mass gain and fattening.

Material and Methods

Animal Maintenance and Sampling

Five juvenile harp seals (four females aged 1–3 yr, one male aged 1 yr) were housed in an outdoor seawater pool for approximately 1 yr on a diet of Atlantic herring (*Clupea hargenus*). Before the beginning of the diet trial (April 13, 1994; day 0), seals were fasted overnight. On day 0, each seal was caught, restrained by a net, and weighed to the nearest 0.5 kg on a 100-kg Salter scale. Total body water and food intake were measured by isotope dilution (see, e.g., Speakman 1997). A precisely weighed quantity (at 1 g/kg body mass) of deuterium oxide (D_2O, 99.8 atom%, Sigma Aldrich) was administered to each seal from a 60-cm^3 syringe by gastric intubation using a 12 French stomach tube. The syringe and stomach tube were rinsed with two 5-cm^3 aliquots of distilled water, and air was blown through the tube as it was withdrawn to ensure complete isotope delivery. A blubber biopsy was then taken from the posterior flank of the animal. To do this, an area was first shaved and cleaned, and a local anesthetic was given. A small incision (≤ 1 cm) in the skin was made with a scalpel, through which

a sterile 6-mm biopsy punch was inserted. A core was taken through the full depth of the blubber layer (about 5 cm), excluding the skin, and the incision was cleaned and sutured. Seals did not have access to water or food for 3 h to allow the isotope to equilibrate. Blood samples (>2 mL) were taken from the hind flipper at approximately 2.5 and 3 h after isotope administration to determine equilibration concentration.

After the final blood sampling, animals were returned to the pool and fed a diet consisting solely of the same lot of Atlantic pollock (*Pollachius virens*) for 30 d. The seals were fed, ad lib., twice daily during the week and once daily on the weekends. Seals were also given vitamin (thiamin, B/C complex, and vitamin E) and salt supplements. Because of the large size of the pollock, the heads were removed and the remainder cut into 2-in-thick pieces (cut dorsal-ventrally and containing all parts of the fish, including organs, muscle, and fat) for feeding. Seals were fed by throwing pieces of fish around the tank, as much as possible to each individual, such that each seal generally received all parts of the pollock minus the head (i.e., all organs as well as muscle and fat tissue). Seals were fed until they lost interest in the food. Although all seals were fed in the same tank, we attempted to visually estimate the amount that each individual consumed based on the known total mass of pollock pieces fed at each feeding and the estimated number of pieces that each seal consumed. These visual estimates were used only to compare with the food consumption rates measured by isotope dilution. Throughout the 30-d experiment, 24 whole pollock were randomly sampled and stored in plastic bags at $-20°$C until they could be processed for proximate and fatty acid composition. Unfortunately, samples of the Atlantic herring fed to the harp seals during the year before our experiment were not available for analysis. However, as we knew these herring were caught on the Scotian Shelf, we collected other samples of Scotian Shelf Atlantic herring ($n = 17$) from trawl surveys to use as an estimate of the original diet composition and to compare with the pollock diet. These were stored in the same manner as described for pollock.

Each seal was weighed weekly. On days 14 and 30 of the experiment, all of the above procedures were repeated: animals were fasted overnight, an initial bleed was taken to measure residual isotope, another dose of isotope was administered and allowed to equilibrate for the measurement of body composition, a blubber biopsy was taken, and equilibration blood samples were taken at 2.5 and 3 h.

The blubber biopsies were chilled (30 min) in tinfoil until returning to the lab. They were then weighed and transferred to glass tubes (with Teflon-lined caps) containing 2 mL of chloroform with 0.005% 2,6-di-*tert*-butyl-4-methyl-phenol (BHT) and stored frozen ($-20°$C) until lipid extractions were performed. After collection, blood samples were centrifuged, and the serum was stored frozen ($-20°$C) in cryovials until analysis.

Laboratory Procedures

Lipid was extracted from the harp seal blubber samples using a modification of the method of Folch et al. (1957). Fatty acid methyl esters were prepared from the lipid extracts using 8% boron trifluoride in methanol as described previously and tested against 0.5 N H_2SO_4 in methanol (Iverson et al. 1997b; Kirsch et al. 1998). After we recorded length and weight, each whole pollock ($n = 24$), minus the head (i.e., precisely as fed to the seals), and whole herring were individually ground and analyzed in duplicate for dry matter (by forced convection drying to constant mass), protein (by Kjeldahl method), fat (using a quantitative Folch extraction), and fatty acid composition (below).

Duplicate analyses of fatty acid methyl esters were performed using temperature-programmed gas liquid chromatography, according to Iverson (1988) and Iverson et al. (1992), on a Perkin Elmer Autosystem II Capillary FID gas chromatograph fitted with a 30 m × 0.25 mm i.d. column coated with 50% cyano-propyl polysiloxane (0.25-μ film thickness; J&W DB-23; Folsom, Calif.) and linked to a computerized integration system (Turbochrom 4 software, PE Nelson). Identifications of fatty acids and isomers and response factors were determined as described previously (Iverson et al. 1997b). Fatty acids are expressed as mass percent of total fatty acids and are designated by shorthand IUPAC (International Union of Pure and Applied Chemistry) nomenclature of carbon chain length:number of double bonds and location (n-x) of the double bond nearest the terminal methyl group.

Total free water was collected from the blood serum by heat distillation (Oftedal and Iverson 1987). The concentration of D_2O was determined by quantitative infrared spectrophotometry on a Perkin-Elmer 283B infrared spectrophotometer as described by Oftedal and Iverson (1987).

Isotope Calculations

Isotope equilibration was considered to have occurred if the isotope levels of the two sequential blood samples were within 0.01 percentage points of each other. According to this criterion all animals at each administration day were considered to have equilibrated by 2.5 h. Isotope dilution space on days 0, 14, and 30 was calculated as described by Iverson et al. (1993). Dilution space at each sampling time was converted to total body water (TBW, kg) using the equation (approximately 3% correction) developed by Bowen and Iverson (1998).

Body composition was calculated from total body mass (BM) and TBW using regression equations derived for harp seals (Gales et al. 1994). Thus, lean body mass (LBM), total body protein (TBP), and total body fat (TBF) were calculated using the following equations:

$$LBM \ (kg) = (1.45 \times TBW) - 1.182,$$

$$\%TBF = (-1.25 \times \%TBW) + 92.40,$$

$$\%TBP = (0.26 \times \%TBW) + 4.54.$$

Total water intake (TWI; kg/d) and food intake (FI; kg/d) over the periods of 0–14 and 14–30 d were calculated for each animal according to procedures of Oftedal and Iverson (1987). Serum isotope concentrations at days 14 and 30 were corrected for changes in TBW from time 0 according to Iverson et al. (1993). The fractional turnover rate (k) of isotope was then calculated from the semi-log (ln) linear decline in isotope over time. TWI was then determined as the sum of total water loss (k × average TBW) and total water gain or loss over the time period (change in TBW). TWI was then converted to FI using the equation

$$FI = 100 \times \frac{[TWI + (1.07 \times F_D) + (0.42 \times P_D)]}{[\%W_P + (0.9095 \times \%F_P) + (0.357 \times \%P_P)]},$$

where F_D and P_D represent daily fat and protein deposition (kg/d), respectively, of individual seals during each time period, and $\%W_P$, $\%F_P$, and $\%P_P$ represent the water, fat, and protein content of pollock, respectively. This equation was derived from Oftedal et al. (1987) and modified assuming 85% metabolizable energy of pollock, as has been found for herring fed to grey seals (*Halichoerus grypus*) and harp seals (Keiver et al. 1984; Ronald et al. 1984; Worthy 1990). FI was converted to energy intake using the factors of 39.3 MJ/kg fat and 23.6 MJ/kg protein (Schmidt-Nielsen 1979).

Statistical Analyses

Data were analyzed using a combination of regression, univariate, and multivariate analysis of variance (ANOVA and MANOVA) models. In graphs of food intakes versus mass and body nutrient changes, although data points for the two time periods (0–14 and 14–30 d) are presented, regression analyses were performed on only the average for each individual over the full 30-d feeding trial (i.e., $n = 5$) to avoid problems of serial correlation. Because of constraints of sample size, a subset of 14 fatty acids (arcsine transformed) were used in ANOVAs and MANOVAs. The 14 fatty acids were a combination of the most abundant components and those that tend to be good indicators of diet (see, e.g., Iverson 1993). Fatty acid data were also analyzed using classification and regression trees (CART) in S-Plus, as described in Iverson et al. (1997a) and Smith et al. (1997), because CART allows the use of all fatty acids without requiring a subjective or restricted selection of variables for analysis. All data are presented as mean ± SE unless otherwise indicated.

Table 1: Mass and body composition of harp seals during experimental period

	Seal					Mean	P^a
	74	70	73	71	Sid		
Mass (kg):							
0 d	61.3	59.5	39.3	27.3	19.8	41.4 ± 8.35	
14 d	59.3	66.0	39.8	28.5	23.5	43.4 ± 8.35	
30 d	51.8	64.0	38.8	28.8	23.8	41.4 ± 7.40	NS
TBW (kg):							
0 d	24.3	20.8	14.6	11.5	8.6	16.0 ± 2.91	
14 d	27.0	27.1	16.9	14.5	11.2	19.3 ± 3.27	
30 d	26.2	31.2	20.8	14.6	12.6	21.1 ± 3.48	.006
TBW (%):							
0 d	39.7	35.0	37.2	42.4	43.5	39.6 ± 1.59	
14 d	45.5	41.0	42.4	50.8	47.6	45.5 ± 1.77	
30 d	50.5	48.8	53.7	50.9	53.0	51.4 ± .88	<.001
LBM (kg):							
0 d	34.1	29.0	20.0	15.6	11.3	22.0 ± 4.22	
14 d	38.0	38.1	23.3	19.8	15.0	26.8 ± 4.75	
30 d	36.7	44.1	29.0	20.1	17.1	29.4 ± 5.05	.006
LBM (%):							
0 d	55.7	48.7	50.9	57.1	57.1	53.9 ± 1.73	
14 d	64.1	57.7	58.5	69.5	64.0	62.8 ± 2.16	
30 d	71.0	68.9	74.8	69.7	71.9	71.3 ± 1.02	<.001
TBF (kg):							
0 d	26.2	29.0	18.0	10.7	7.5	18.3 ± 4.18	
14 d	21.0	27.1	15.7	8.2	7.7	16.0 ± 3.73	
30 d	15.1	20.1	9.8	8.3	6.2	11.9 ± 2.53	.007
TBF (%):							
0 d	42.7	48.7	45.9	39.4	38.0	42.9 ± 1.98	
14 d	35.5	41.1	39.4	28.9	32.9	35.6 ± 2.21	
30 d	29.2	31.4	25.3	28.7	26.1	28.2 ± 1.10	<.001
TBP (kg):							
0 d	9.1	8.1	5.6	4.2	3.1	6.0 ± 1.13	
14 d	9.7	10.0	6.2	5.1	4.0	7.0 ± 1.23	
30 d	9.1	11.0	7.2	5.1	4.4	7.4 ± 1.24	.014
TBP (%):							
0 d	14.9	13.6	14.2	15.6	15.9	14.8 ± .41	
14 d	16.4	15.2	15.6	17.8	16.9	16.4 ± .46	
30 d	17.7	17.2	18.5	17.8	18.3	17.9 ± .23	<.001

Note. Animals ordered by initial body mass from largest to smallest.

[a] Differences over the course of the experiment tested by repeated-measures ANOVA.

Results

Harp Seal Diet and Body Composition

Before the start of our experiment, the five harp seals had been maintained on a diet consisting solely of Atlantic herring (approximately 9% fat). The seals were then switched to a diet consisting solely of pollock (length: 46.0 ± 0.52 cm; mass: 685.4 ± 29.52 g), which averaged 1.7% ± 0.21% fat and 18.2% ± 0.15% protein ($n = 24$). The seals generally took well to the new diet, consuming a combined total of about 30 kg/d of pollock, despite having begun to molt.

At the start of the experiment, the body mass of seals ranged from 19.8 to 61.3 kg. After switching to the pollock diet, the seals as a group tended to maintain their body mass, averaging 41.4 kg at day 0 and 41.4 kg at day 30 (Table 1). However, there was variation among individuals in that some gained mass

slightly, especially between days 0 and 14, while others lost mass during some intervals (Fig. 1). Total mass changes over the 30-d experiment for seals 74, 70, 73, 71, and Sid (in order from initially largest to smallest) were −9.5, +4.5, −0.5, +1.5, and +4.0 kg, respectively (Table 1).

Although body mass changed little during the course of the experiment, body composition changed significantly ($P < 0.01$; Table 1). On day 0, body mass of seals was composed of 49%–57% LBM, 38%–49% fat, and 14%–16% protein. By day 30, seal body mass was composed of 69%–75% LBM, 25%–31% fat, and 17%–19% protein. Individuals lost an average of 2.3 kg fat (range: −0.2 to 5.2 kg) during the first 14 d and lost an additional average of 4.2 kg fat (range: 0.1–7.0 kg) during the subsequent 16 d. In contrast to a decline from 42.9% to 28.2% TBF, all animals gained LBM and protein both absolutely and as a percentage of body mass (Table 1). Individuals gained an average of 1.0 kg (range: 0.6–1.9 kg) and 0.4 kg (range: −0.6 to 1.0 kg) protein during the first and second periods, respectively. Average TBP increased from 14.8% to 17.9% over the 30 d.

Figure 1. Detailed body mass changes of the five harp seals during the course of the 30-d experiment.

Harp Seal Water Turnover, Food Intake, and Nutrient Deposition

Daily water intake, calculated from fractional water turnover rates, averaged about 5.9 kg/d but ranged widely among individuals from 3.1 to 11.0 kg/d (Table 2). Hence measures of food intake among individuals derived from isotope dilution also ranged widely from 3.6 to 12.6 kg pollock per day during the 30-d period. Although it was not possible for us to obtain accurate visual measures of food intake by each individual because of the feeding method, we attempted to estimate meal size during each feeding for comparison. These visual estimates compared well with food intake derived by isotope dilution ($r = 0.857$, $P = 0.002$). There was no significant difference between overall food intakes estimated visually (5.9 ± 0.34 kg/d) and those measured from isotope dilution (6.5 ± 0.94 kg/d, $P = 0.517$, paired t). With the removal of seal 70, which had the highest calculated intakes (but also the greatest mass gains), visual estimates and isotope measures of food intake averaged 5.5 and 5.3 kg/d, respectively. However, given their greater reliability, we used only measures of food intake from isotope dilution in further analyses.

Despite average food intakes of 6–7 kg/d, these corresponded to mean fat intakes of only 120 g/d and 102 g/d during the first and second periods, respectively (Table 2). There was no direct relationship between levels of food intake (kg/d) and daily mass change ($r^2 = 0.180$, $P > 0.4$, $n = 5$). Seals lost 1.3–11.1 kg fat over the course of the experiment, representing a loss of 31.7% ± 5.49% of initial fat stores. Daily rates of fat depletion were not related to either daily total food intake or daily fat intake ($P > 0.4$; Fig. 2a, 2b). In contrast, daily protein intake was an order of magnitude higher than fat intake (Table 2),
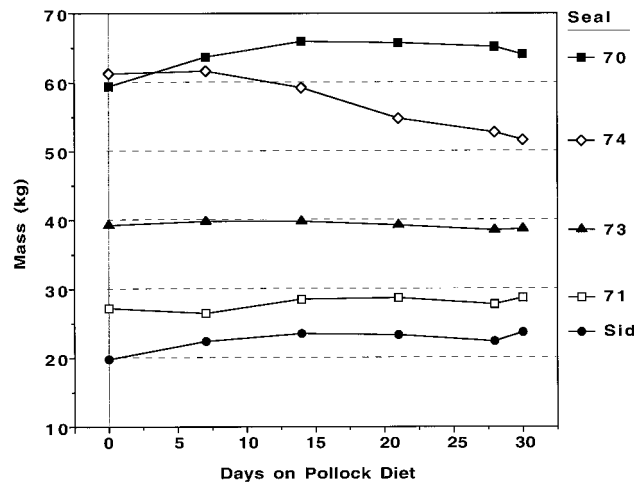
and almost all seals gained body protein (1.3 ± 0.47 kg total). Daily protein deposition was directly related to total daily food and protein intakes ($r^2 = 0.836$, $P = 0.030$; Fig. 2c, 2d). Similarly, deposition of LBM (kg/d) was also highly correlated to food and protein intakes ($r^2 = 0.896$, $P = 0.015$).

Gross energy intake of seals averaged 35.1 and 29.9 MJ/d during the first and second periods, respectively, or 32.3 MJ/d overall (Table 2). Expressed on a metabolic body size basis ($kg^{0.75}$), energy intakes were 2.2 and 1.8 MJ/$kg^{0.75}$/d during the first and second periods, respectively. The overall intake per metabolic body size averaged 2.0 MJ/$kg^{0.75}$/d ($n = 5$). Although gross energy intake was not correlated with rates of mass change ($r^2 = 0.180$, $P > 0.4$), energy intake per metabolic body size explained 89% of the variation in mass change among individuals ($P = 0.016$; Fig. 3).

Harp Seal Diet and Fatty Acid Composition

Sixty-six fatty acids were identified in all harp seal, herring, and pollock samples. Based on a subset of fatty acids, Scotian Shelf Atlantic herring (9.1% ± 0.89% fat, $n = 17$) differed significantly from the experimental pollock diet (Fig. 4a; $P < 0.05$, MANOVA). Scotian Shelf herring were characterized by high levels of 20:1n-9 and 22:1n-11, whereas the pollock fed to seals contained much lower levels of these components. Pollock was also lower in 14:0 and 20:1n-11 than was herring. Pollock was further characterized by high levels of 18:0, 18:1n-9, and 20:5n-3 and particularly high levels of 20:4n-6 and 22:6n-3 in comparison to herring (Fig. 4a). These two prey species were also readily separated using classification trees. Using all 66 fatty acids, CART analysis correctly classified all (100%) 41

Table 2: Water and food intake and nutrient deposition in harp seals during first and second parts of experimental period

	Seal 74		Seal 70		Seal 73		Seal 71		Sid		Mean ± SEM	
	0–14 d	14–30 d	0–14 d	14–30 d	0–14 d	14–30 d	0–14 d	14–30 d	0–14 d	14–30 d	0–14 d	14–30 d
Initial mass (kg)	61.3	59.3	59.5	66.0	39.3	39.8	27.3	28.5	19.8	23.5	41.4 ± 8.35	43.4 ± 8.35
Water turnover rate (k)	.126	.165	.197	.180	.192	.178	.159	.200	.159	.222	.167 ± .013	.189 ± .010
Intake:												
Total water intake (kg/d)	5.88	3.55	10.99	9.41	5.27	7.30	5.01	3.07	4.17	4.04	6.26 ± 1.213	5.47 ± 1.232
Food intake (kg/d)	6.36	3.63	12.61	10.36	5.90	8.01	5.59	3.55	4.86	4.57	7.07 ± 1.407	6.02 ± 1.355
Fat intake (kg/d)	.11	.06	.21	.18	.10	.14	.10	.06	.08	.08	.12 ± .024	.10 ± .023
Protein intake (kg/d)	1.16	.66	2.30	1.89	1.08	1.46	1.02	.65	.89	.83	1.29 ± .256	1.10 ± .247
Energy intake (MJ/d)	31.6	18.0	62.6	51.4	29.3	39.8	27.8	17.6	24.2	22.7	35.1 ± 6.98	29.9 ± 6.73
Energy intake ($MJ/kg^{-0.75}$)	1.44	.84	2.92	2.22	1.87	2.51	2.33	1.43	2.58	2.12	2.23 ± .261	1.83 ± .303
Deposition:												
Mass gain (g/d)	−143	−469	464	−125	36	−63	89	16	268	16	143 ± 103.6	−125 ± 90.0
Fat deposition (g/d)	−368	−368	−131	−440	−168	−366	−180	2	16	−95	−166 ± 61.5	−253 ± 86.9
Protein deposition (g/d)	43	−35	137	62	43	61	59	3	60	23	69 ± 17.6	23 ± 18.3

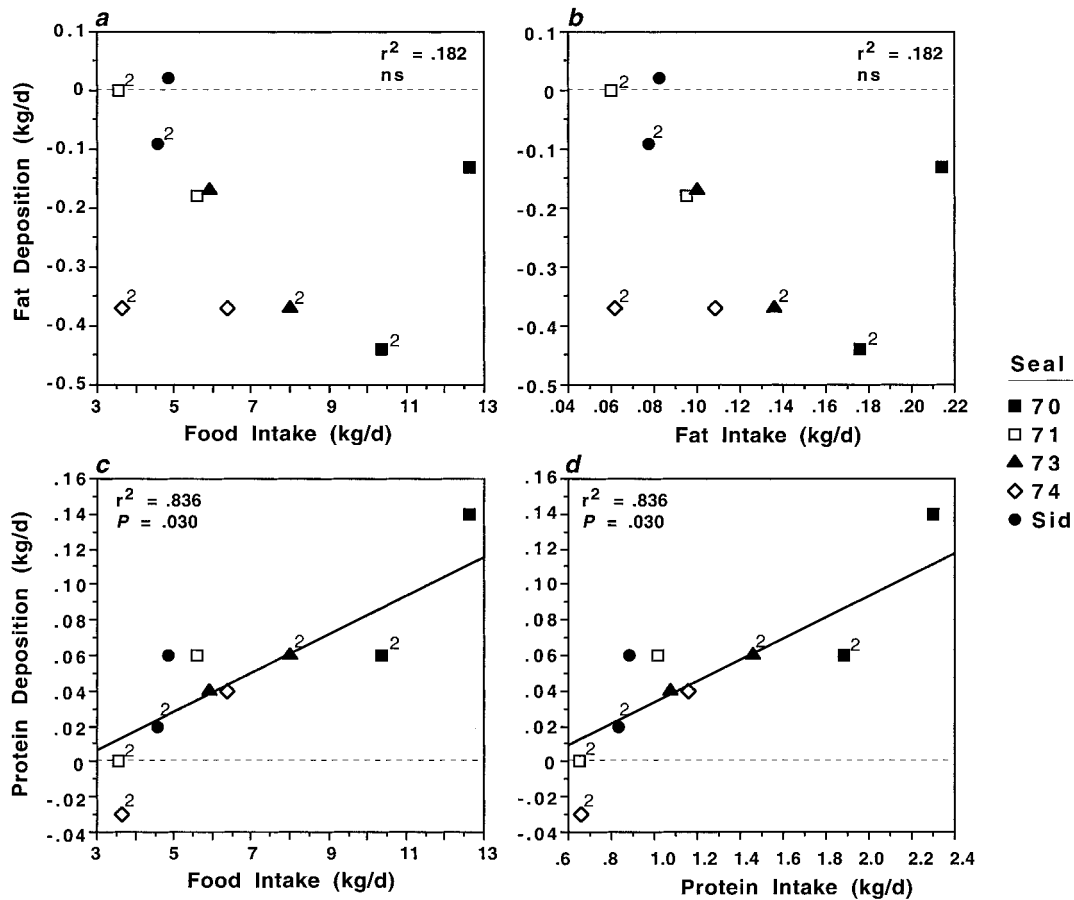Note. Animals ordered by initial body mass from largest to smallest.

Figure 2. Daily fat deposition in harp seals as a function of (*a*) daily food intake and (*b*) daily fat intake, and daily protein deposition in seals as a function of (*c*) daily food intake ($y = -0.027 + 0.11x$) and (*d*) daily protein intake ($y = -0.027 + 0.06x$). Food intake and deposition rates were measured using isotope dilution. Data points represent two time periods of measurement for each individual: 0–14 d (*plain symbols*) and 14–30 d (*symbols with superscript 2*); however, regression relationships and lines plotted were determined from one overall average (0–30 d) for each individual (i.e., $n = 5$).

herring and pollock using only three fatty acids: 22:1n-7, 18:0, and 16:1n-9.

The fatty acid composition of harp seal blubber at 0, 14, and 30 d is presented in Table 3. Fatty acid composition of blubber changed significantly during the 30-d period, as illustrated by the same 14 fatty acids used to illustrate differences between the two prey (Fig. 4*b*). Changes in levels of most fatty acids occurred gradually, with the greatest changes having occurred by day 30 (Table 3; Fig. 4*b*). The levels of most major fatty acids that were higher in pollock than in herring (Fig. 4*a*) increased in harp seal blubber after the switch to a pollock diet (Fig. 4*b*), while those that were lower in pollock than in herring decreased in harp seal blubber. CART analysis using all 66 fatty acids also indicated differences in blubber fatty acid composition of harp seals over the course of the experiment. Although the sample size was small for this analysis, day 0 separated from days 14 and 30 based on a decline in either 22:1n-11 or

20:1n-9; days 14 and 30 tended to be separated further from one another but with two errors.

Given the variation in initial blubber fatty acid composition among seals and in the daily rates of pollock intake and mass deposition, we also examined changes in blubber fatty acids of each individual seal. Four dietary fatty acids illustrate the changes that took place (Fig. 5). Despite small changes in body mass and significant rates of fat loss in seals over the course of the experiment, the fatty acid composition of the blubber changed in the direction of changes in dietary fatty acid intake. The proportions of the fatty acids 20:1n-9 and 22:1n-11, which are typically high in herring and low in pollock (Fig. 4*a*), decreased significantly when seals were switched from herring to a diet of pollock. Conversely, the proportions of the fatty acids 20:4n-6 and 22:6n-3, which are typically relatively low in herring and high in pollock, increased significantly (Fig. 5).

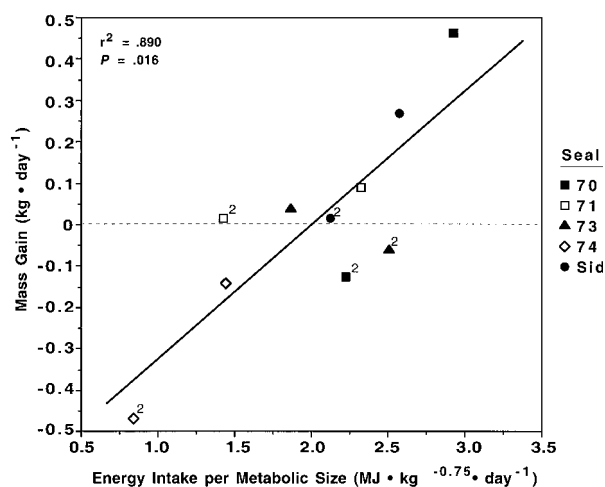These changes occurred despite no net deposition of body

Figure 3. Daily mass gain in harp seals as a function of energy intake per metabolic body size ($y = -0.647 + 0.324x$). Data points represent two time periods of measurement for each individual: 0–14 d (*plain symbols*) and 14–30 d (*symbols with superscript 2*); however, regression relationship and line plotted were determined from one overall average (0–30 d) for each individual (i.e., $n = 5$).

fat. For example, seal 73 maintained its body mass but lost 2.3 and 5.9 kg of fat during the first and second periods, respectively (Table 1). Despite this, changes in the proportions of fatty acids reflected changes in dietary intake: proportions of 20:1n-9 and 22:1n-11 decreased in blubber from 13.7% to 9.7% and 11.2% to 4.6%, respectively, while proportions of 20:4n-6 and 22:6n-3 increased in blubber from 0.38% to 0.53% and from 8.4% to 10.7%, respectively (Fig. 5).

Because one of our aims was to assess how predictable the changes in blubber fatty acids would be with a change in diet, we attempted to model the proportion of dietary fatty acids that might be deposited in blubber. We estimated each seal's expected fatty acid composition at day 30 using its initial quantity (kg) of body fat at day 0, the fatty acid composition of that fat, its total fat intake over the 30-d period measured by isotope dilution (assuming 85% metabolizable energy of pollock), and the fatty acid composition of the pollock consumed. Given that seals were at or below maintenance energy intakes, and hence likely using much of their fat intake directly for energy, we used three scenarios: deposition of 100%, 50%, or 25% of the available dietary fatty acids consumed over the 30-d period. We then compared these estimated levels of deposition with the actual observed fatty acid composition of harp seal blubber measured at 30 d (Fig. 6). In all three cases, our estimated final blubber fatty acid signature was more similar to the observed final (day 30) signature than to the initial (day 0) signature. Differences between the observed and estimated final signatures were measured using the forward Kullback-Liebler distance, which represents a measure of the distance

between two distributions of proportions. Based on this statistic, both the 50% and 25% deposition scenarios were significantly closer to the observed final signature than was the 100% deposition estimate ($P < 0.020$). The estimate based on 25% deposition was the closest to the observed final composition in all seals, but only marginally so over the estimate based on 50% deposition ($P = 0.075$). Final estimates based on <25% deposition resulted both in larger differences in more components and in greater distance measures. Several individual fatty acids, 16:1n-7 and 18:1n-9, were not well predicted by direct dietary fat intake (Fig. 5). These were consistently higher in seal blubber than predicted, although not significantly for 16:1n-7.

Discussion

Potential Sources of Error

Hydrogen isotopes have been widely used to measure TBW in animals, and a number of studies have validated their use in marine mammals (reviewed in Bowen and Iverson 1998). Providing that the isotope equilibrates with body water, the calculation of TBW can then be used to accurately predict body composition from species-specific equations (Reilly and Fedak 1990; Farley and Robbins 1994; Speakman 1997; Hilderbrand et al. 1998). As stated previously, equilibration was confirmed at all administrations of D_2O, and we used the relationship between body composition and TBW derived from chemical analysis of 26 harp seals (ages juvenile and adults) derived by Gales et al. (1994). Thus, our estimates of body composition and changes in composition should be accurate.

A more significant source of error in our use of isotopes might have arisen in calculations of food intake. Here we assumed that all water intake came from preformed food water or production of metabolic water. Several studies have demonstrated that seals meet all of their water requirements through food water and metabolism and that consumption of sea water is rare or minimal (see, e.g., Depocas et al. 1971; Keiver et al. 1984). Although direct consumption of water was never observed in our captive animals, if some had occurred this would have caused us to overestimate food consumption. However, estimated total food intake by all seals during the 30-d trial (976 kg) was similar to that known to have been available for feeding (approximately 950 kg); thus the effect of any direct water intake appears to be minimal.

Effects of Low Fat Intake on Body Composition of Harp Seals

Atlantic herring is an energy-dense fish (approximately 7.8 MJ/kg) usually averaging 8%–10% fat, but fat may reach 21%, or 12 MJ/kg (Hodder et al. 1973). In general, seals readily fatten on high-fat diets. Thus, although all five harp seals were young, growing animals, they were quite fat (38%–49% fat) at the start of the experiment, having been fed on herring for some time.
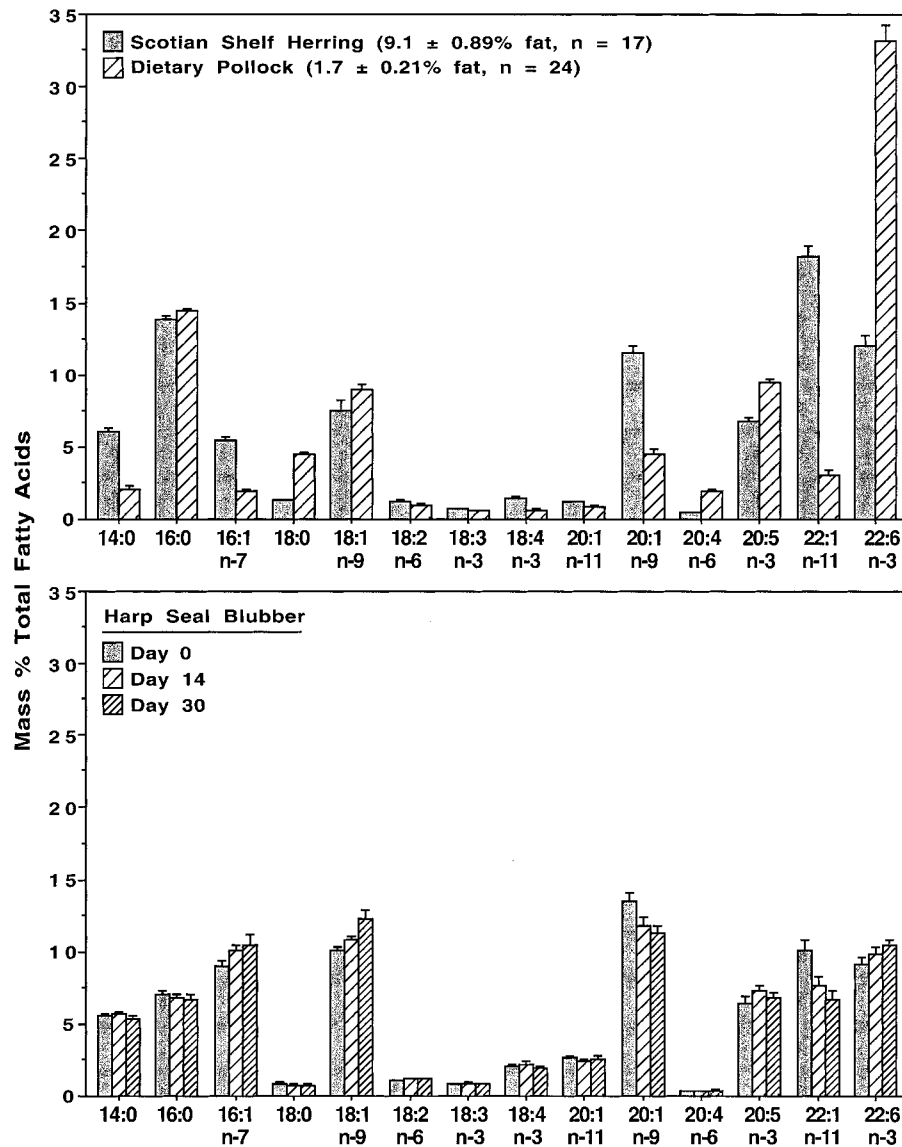
Figure 4. Selected fatty acids in (*top*) a sample of Scotian shelf herring and the pollock fed to harp seals and (*bottom*) in harp seal blubber sampled on days 0, 14, and 30 after the start of the pollock diet. Bars are means, and vertical lines are 1 SEM. Pollock differed significantly from herring (MANOVA, $P < 0.05$); levels of all fatty acids differed between species except 16:0 and 18:4n-3 ($P < 0.05$, ANOVA on arcsin transformed data). Significant differences in blubber over time were found for 18:1n-9, 18:2n-6, 18:3n-3, 18:4n-3, 20:1n-9, 20:4n-6, 22:1n-11, and 22:6n-3 ($P < 0.05$, repeated-measures ANOVA on arcsine-transformed data).

In contrast, when switched to a low-fat (1.7%), high-protein diet containing about 5.0 MJ/kg, seals gained little or lost body mass. More important, they also lost a substantial amount of body fat. Despite food intakes averaging 6.5 kg/d, corresponding to about 27.5 MJ/d of metabolizable energy, body fat declined by an average of 6.4 kg, or by 32%, over the 30-d experiment (Tables 1, 2). These results suggest that young, growing phocids are unable to maintain body fat stores on low-fat diets, even when protein intakes are high.

On average, while harp seals lost TBF, they gained TBW and LBM (Table 1). The rates of LBM and protein deposition (kg/d) were directly proportional to protein intake (e.g., Fig. 2d). In young elephant seals (*Mirounga angustirostris*), when total food intake was less than that of their metabolic requirements, seals maintained their weight and stored protein despite losses of adipose tissue (Condit and Ortiz 1987). Although these patterns of deposition and loss appear comparable to those of the harp seals in our study, the cause could not be attributed to

Table 3: Fatty acid composition of harp seal blubber

Fatty Acid	Day 0	Day 14	Day 30
14:0	5.62 ± .14	5.69 ± .20	5.36 ± .28
14:1n-9	.25 ± .01	.26 ± .02	.23 ± .01
14:1n-5	.67 ± .03	.84 ± .05	.88 ± .08
15:0	.29 ± .01	.31 ± .02	.31 ± .01
16:0	7.06 ± .17	6.79 ± .21	6.64 ± .41
16:1n-11	.54 ± .02	.60 ± .03	.62 ± .04
16:1n-9	.29 ± .01	.32 ± .01	.34 ± .02
16:1n-7	8.96 ± .46	10.13 ± .38	10.46 ± .69
7Me16:0	.26 ± .00	.29 ± .01	.29 ± .02
16:2n-4	.24 ± .02	.24 ± .02	.25 ± .02
16:3n-6	.62 ± .02	.69 ± .03	.63 ± .02
17:1	.66 ± .02	.63 ± .09	.63 ± .07
16:4n-1	.72 ± .08	.73 ± .19	.77 ± .06
18:0	.85 ± .06	.78 ± .06	.78 ± .11
18:1n-11	4.08 ± .21	4.13 ± .24	4.32 ± .33
18:1n-9	10.04 ± .29	10.79 ± .25	12.24 ± .66
18:1n-7	2.42 ± .15	2.55 ± .14	2.70 ± .22
18:1n-5	.41 ± .02	.41 ± .01	.46 ± .04
18:2n-6	1.10 ± .02	1.16 ± .03	1.20 ± .03
18:3n-3	.80 ± .03	.88 ± .05	.84 ± .05
18:4n-3	2.03 ± .11	2.24 ± .14	1.91 ± .17
18:4n-1	.24 ± .01	.32 ± .03	.28 ± .02
20:1n-11	2.68 ± .11	2.41 ± .20	2.50 ± .26
20:1n-9	13.53 ± .60	11.77 ± .64	11.27 ± .51
20:1n-7	.38 ± .03	.36 ± .03	.34 ± .03
20:4n-6	.35 ± .02	.37 ± .01	.41 ± .03
20:4n-3	.54 ± .03	.57 ± .04	.55 ± .03
20:5n-3	6.50 ± .37	7.28 ± .34	6.83 ± .30
22:1n-11	10.14 ± .65	7.61 ± .63	6.64 ± .63
22:1n-9	.70 ± .02	.57 ± .04	.53 ± .04
21:5n-3	.46 ± .02	.48 ± .02	.46 ± .02
22:5n-3	4.16 ± .10	4.39 ± .12	4.61 ± .09
22:6n-3	9.17 ± .40	9.90 ± .46	10.43 ± .43

Note. Values are mean mass percent ± SEM of fatty acids (33 out of 66) present at levels ≥ 0.25%. $n = 5$. The full listing of all fatty acids identified can be found in Kirsch 1997.

reduced energy intakes. Harp seals in our study consumed about 0.82 MJ/kg/d or 2.0 MJ/kg$^{0.75}$/d. These rates are approximately 2.5 times those previously reported as maintenance requirements for captive juvenile harp and grey seals fed herring (Keiver et al. 1984; Ronald et al. 1984). However, Keiver et al. (1984) found that when harp seals were fed relatively low-fat herring (4.2% fat), they had greater fecal energy losses and were less efficient at assimilating energy than when fed diets of higher-fat herring (9.5% and 18.4% fat). As a consequence, they consumed more low-fat food (6%–13% body mass/d) than higher-fat food (2.5%–3.5% of body mass). When fed shrimp, seals were even less efficient at assimilating energy but did not

necessarily consume more food to meet energy requirements (Keiver et al. 1984).

In our study, the diet was even lower in fat than that fed by Keiver et al. (1984), suggesting that the harp seals may have been even less efficient at assimilating the gross energy consumed. This was apparently the case because intakes corresponded to about 15% of body mass per day. Thus, it appears that on a low-fat, high-protein diet, young harp seals may experience a reduction in the rate of energy assimilation. They deposit body protein but are unable to maintain body fat stores. These results suggest that young, growing phocids faced with access to only a low-fat, high-protein diet might have difficulty in storing lipids and therefore could be more vulnerable to periods of food shortages. However, the ecological implications of these results are difficult to evaluate, as it is unlikely that pinnipeds in the wild would consume only one type of prey.

Effects of Diet on Blubber Fatty Acid Composition of Harp Seals

Lipids have been used as biological markers and indicators of diet in a number of studies on fish and copepods and in terrestrial, aquatic, and marine carnivores (Lee et al. 1971; Sargent et al. 1988; Fraser et al. 1989; Klungsoyr et al. 1989; Iverson and Oftedal 1992; Graeve et al. 1994; Pond et al. 1995; Smith et al. 1996; St. John and Lund 1996; Iverson et al. 1997*a*, 1997*b*; Kirsch et al. 1998). Iverson et al. (1995) also demonstrated the direct and quantitative incorporation of dietary fatty acids into blubber of suckling hooded seal (*Cystophora cristata*) pups, the composition of which subsequently looked almost identical to that of the milk consumed. However, controlled studies of animals consuming fish prey are required to understand the processes of dietary fatty acid incorporation into pinniped fat stores. Our experiment shows that a change in dietary fatty acid intake resulted in a significant change in fatty acid signatures in harp seal blubber (Figs. 4, 5). But in assessing changes in fatty acids, some of the underlying biochemistry must be considered. For instance, 16:0, 16:1n-7, 18:0, and 18:1n-9 can be produced by mammalian fatty acid synthetase and thus could be synthesized by the seal in addition to that consumed in the diet. In contrast, other abundant fatty acids (e.g., Figs. 4–6) arise almost entirely from diet (Cook 1985; Nelson 1992) since fatty acids with double bonds in the n-6 and n-3 position cannot be synthesized by mammals. In addition, large amounts of 20:1n-9 and 22:1n-11 are known to arise from marine copepods, to be transferred to planktivores such as herring, and on to marine mammals (Iverson 1993). In fact, it was in most of these "indicator" fatty acids that changes were most apparent with changes in diet. Changes in the levels of these fatty acids in blubber corresponded to the increases or decreases in their intakes with increases in diet.

Although we could not directly compare the initial blubber fatty acid composition of our harp seals with that of their
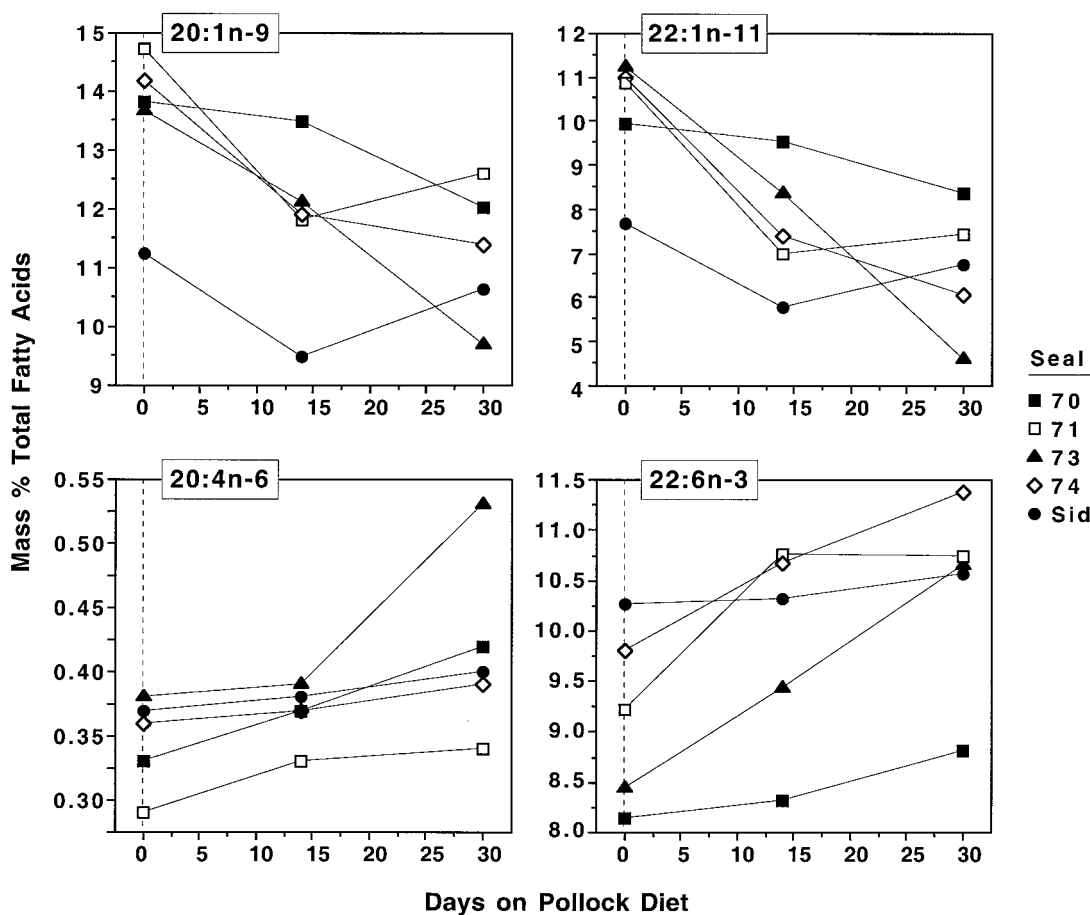
Figure 5. Individual changes in four selected fatty acids in the blubber of harp seals over the course of the experiment when seals were switched to a diet of pollock.

previous herring diet because samples of this were unavailable, fatty acids that are typically high in herring and were low in pollock decreased in the blubber of harp seals feeding on pollock, whereas fatty acids that are low in herring and high in pollock increased (Fig. 4a, 4b). These changes, reflecting dietary changes, occurred despite the fact that there was no net deposition of fat, and in fact, all animals lost fat.

If a seal is rapidly fattening on a high-fat diet, it is expected that a substantial amount of the fatty acids ingested above metabolic requirements will be deposited in blubber (see, e.g., Iverson et al. 1995). It is less clear to what extent deposition of dietary fatty acids would occur during periods of fat maintenance or loss. Presumably the animal would require all of the ingested fat for energy and metabolic requirements; however, it is not likely that simple. At each feeding, an animal consumes more than is required for immediate needs; hence, excess energy will be deposited in fat stores. Several hours later, in between meals, fat stores will be mobilized for metabolism. This process of deposition followed by mobilization will be

repeated at each subsequent feeding and nonfeeding interval. This pattern of catabolism may in part explain our findings that some turnover and net deposition of fatty acids take place regardless of whether the animal is fattening or not.

We used the initial blubber composition of harp seals to create a simple model of the changes in fatty acid composition expected on the new diet. As a first estimate of the proportion of dietary fatty acids deposited in blubber, our calculations of the maximum extent to which blubber could change were indeed very close to what was observed (Fig. 6). Our current model is limited in that it does not account for the physiological time scales of nutrient utilization discussed above. Nevertheless, the calculations provide a quantitative context in which to view fatty acid deposition or turnover. On average, seals started out with about 18 kg of body fat, mostly in the blubber layer. Seals fed below maintenance requirements hence catabolized dietary fat as well as body fat. In 30 d, seals consumed a total of only about 3 kg fat and lost about 6 kg body fat. Thus, blubber signatures could not have changed more substantially than what
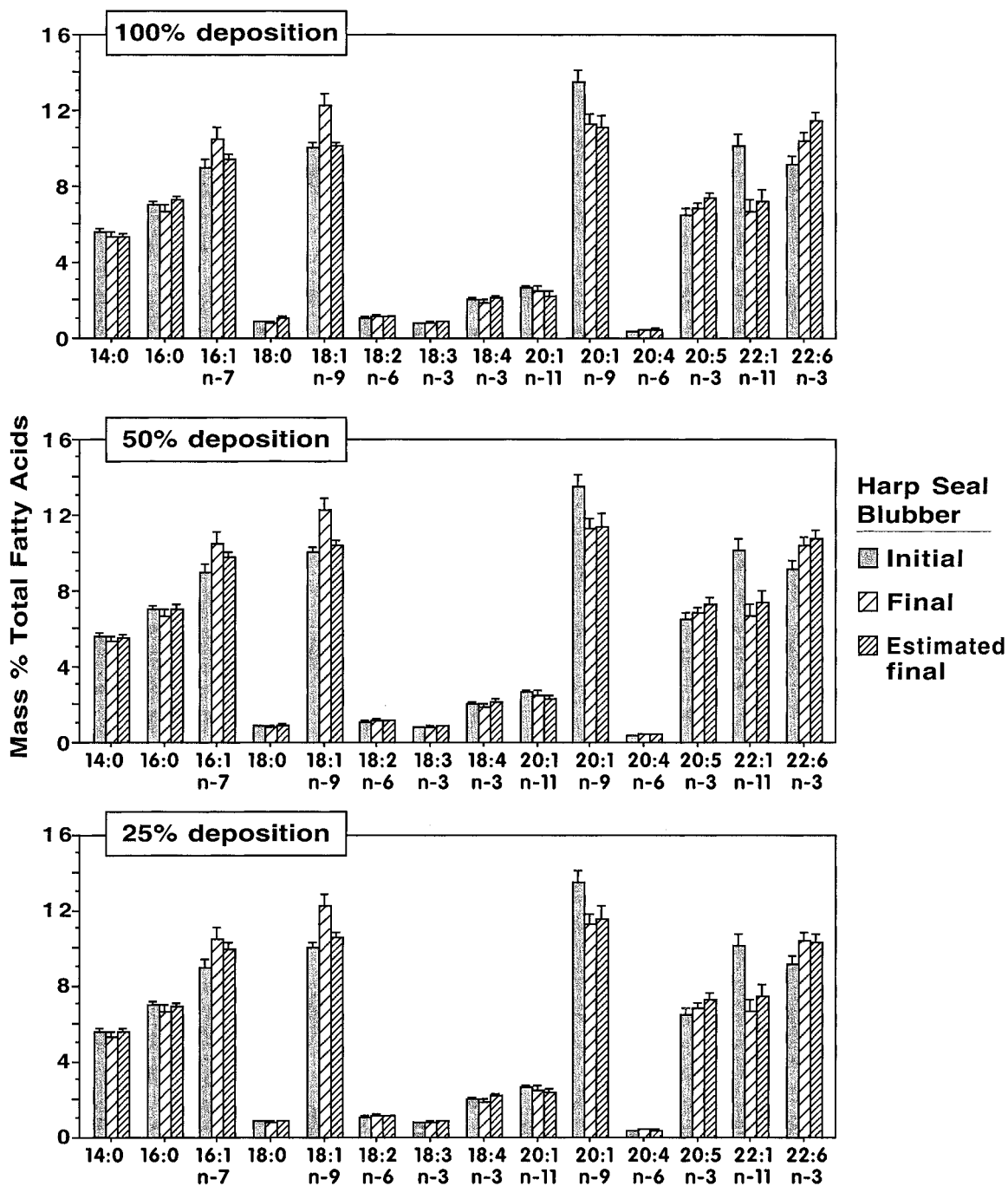
Figure 6. Initial (day 0) versus final (day 30) proportion of selected fatty acids in harp seal blubber in comparison to estimates of final proportions of these fatty acids deposited over the 30-d experiment. Estimated final amounts are based on scenarios of 100%, 50%, and 25% of available fatty acids (assuming 85% metabolizable energy) consumed by each seal being deposited in blubber. For these calculations we used total kilograms of body fat of each seal at day 0, fatty acid composition of that fat, total fat intake of each seal calculated from isotope dilution, fatty acid composition of pollock consumed, and the three rates of deposition. Based on forward Kullback-Liebler distance measures, the 50% or 25% scenarios corresponded most closely to the actual final composition (see text). Levels of individual fatty acids differed between observed final and estimated final for four components at 100% (18:0, 18:1n-9, 18:4n-3, and 22:6n-3), for three components at 50% (18:0, 18:1n-9, and 18:4n-3), and for one component at 25% (18:4n-3; $P < 0.05$, paired t-tests).

was observed. At various assumptions of 100%, 50%, and 25% of ingested fatty acids being available for deposition, in all cases, our estimated final blubber fatty acid signature was more similar to the observed final signature than to the initial signature, especially in the indicator fatty acids.

Although blubber fatty acids of harp seals changed to reflect the change in diet, they did not match that of diet. It is likely that blubber fatty acids would more strongly reflect dietary fatty acid signatures with a longer period on the diet or with higher daily intakes of fat. Nevertheless, it remains unlikely that blubber would ever exactly match diet signatures, especially on a low-fat, high-protein diet, because of some differential utilization of fatty acids or the synthesis of specific fatty acids. Although preformed dietary fatty acids are unlikely to enter typical lipid synthetic pathways (Nelson 1992), carbohydrates or amino acids consumed in excess of either metabolic or growth requirements are used to synthesize fatty acids in the liver, which are then deposited in blubber and other adipose tissue. The relative levels in blubber of these fatty acids, predominantly 16:0 and 18:0 (the products of mammalian fatty acid synthetase) and their unsaturates, 16:1n-7 and 18:1n-9, may consequently be largely independent of diet. Indeed, any 18:0 synthesized de novo is largely converted to 18:1n-9 (although this is not true for 18:0 consumed in the diet, Nelson 1992). This may also be the case for production of

16:1n-7 from biosynthesized 16:0 and the same common desaturase (the $\Delta 9$ desaturase). Given the large excess of protein consumed by harp seals in the present experiment, this would likely explain the consistently higher levels of 16:1n-7 and 18:1n-9 than would be predicted by dietary fatty acids alone (Fig. 6). In contrast, on high-fat diets, when adequate or excess energy is consumed, fatty acid synthesis is greatly reduced or absent, and dietary fatty acids tend to be stored directly in adipose tissue (Nelson 1992; Iverson et al. 1995). These types of differences in the metabolism of specific fatty acids can be taken into account when quantitatively modeling diets using blubber fatty acids (S. J. Iverson, C. Field, W. D. Bowen, and W. Blanchard, unpublished data). Additional studies are needed to better understand the turnover and deposition of fatty acids in the blubber of seals during periods both of maintenance and of rapid fattening. The latter case may be most pertinent to accurately understanding seal diets in the wild through fatty acid signatures, since seals typically go through annual periods of extensive depletion of fat stores during fasting, followed by intensive fattening in the few months before the next breeding season.

Acknowledgments

Literature Cited

Alaska Sea Grant College Program. 1993. Is it food? addressing marine mammal and seabird declines. Workshop summary. Alaska Sea Grant College Program, University of Alaska, Fairbanks. AK-SG-93-01, 59 pp.

Bigg M.A. and I.F. Fawcett. 1985. Two biases in diet determination of northern fur seals (*Callorhinus ursinus*). Pp. 284–291 in J.R. Beddington, R.J.H. Beverton, and D.M. Lavigne, eds. Marine Mammals and Fisheries. Allen & Unwin, London.

Bowen W.D. 1997. The role of marine mammals in aquatic ecosystems. Mar Ecol Prog Ser 158:267–274.

Bowen W.D. and G.D. Harrison. 1994. Offshore diet of grey seals *Halichoerus grypus* near Sable Island, Canada. Mar Ecol Prog Ser 112:1–11.

Bowen W.D. and S.J. Iverson. 1998. Estimation of total body water in pinnipeds using hydrogen-isotope dilution. Physiol Zool 71:329–332.

Condit R.S. and C.L. Ortiz. 1987. The physiological transition from fasting to feeding in weaned elephant seal pups. Mar Mamm Sci 3:207–219.

Cook H.W. 1985. Fatty acid desaturation and chain elongation in eucaryotes. Pp. 181–211 in D.E. Vance and J.E. Vance, eds. Biochemistry of Lipids and Membranes. Benjamin/Cummings, Menlo Park, Calif.

Dellinger T. and F. Trillmich. 1988. Estimating diet composition from scat analysis in otariid seals (*Otariidae*): is it reliable? Can J Zool 66:1865–1870.

Depocas F., J.S. Hart, and H.D. Fisher. 1971. Sea water drinking and water flux in starved and in fed harbour seals, *Phoca vitulina*. Can J Physiol Pharmacol 49:53–62.

Dos Santos J., I.C. Burkow, and M. Jobling. 1993. Patterns of growth and lipid deposition in cod (*Gadus morhua* L.) fed natural prey and fishbased feeds. Aquaculture 110:173–189.

Farley S.D. and C.T. Robbins. 1994. Development of two methods to estimate body composition in bears. Can J Zool 72:220–226.

Folch J., M. Lees, and G.H. Sloane-Stanly. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509.

Fraser A.J., J.R. Sargent, J.C. Gamble, and D.D. Seaton. 1989. Formation and transfer of fatty acids in an enclosed marine food chain comprising phytoplankton, zooplankton and herring (*Clupea harengus* L.) larvae. Mar Chem 27:1–18.

Gales R., D. Renouf, and E. Noseworthy. 1994. Body composition of harp seals. Can J Zool 72:545–551.

Graeve M., G. Kattner, and W. Hagen. 1994. Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: experimental evidence of trophic markers. J Exp Mar Biol Ecol 182:97–110.

Hilderbrand G.V., S.D. Farley, and C.T. Robbins. 1998. Predicting body condition of bears via two field methods. J Wildl Manag 62:406–409.

Hodder V.M., L.S. Parsons, G.H. Winters, and K. Spencer. 1973. Fat and water content of herring in Newfoundland and adjacent waters, 1966–1971. Fisheries Research Board of Canada Technical Report 365.

Iverson S.J. 1988. Composition, Intake and Gastric Digestion of Milk Lipids in Pinnipeds. PhD thesis. University of Maryland, College Park.

———. 1993. Milk secretion in marine mammals in relation to foraging: can milk fatty acids predict diet? Symp Zool Soc Lond 66:263–291.

Iverson S.J., J.P.Y. Arnould, and I.L. Boyd. 1997a. Milk fatty acid signatures indicate both major and minor shifts in the foraging ecology of lactating Antarctic fur seals. Can J Zool 75:188–197.

Iverson S.J., W.D. Bowen, D.J. Boness, and O.T. Oftedal. 1993. The effect of maternal size and milk energy output on pup growth in grey seals (Halichoerus grypus). Physiol Zool 66:61–88.

Iverson S.J., K.J. Frost, and L.L. Lowry. 1997b. Fatty acid signatures reveal fine structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska. Mar Ecol Prog Ser 151:255–271.

Iverson S.J. and O.T. Oftedal. 1992. Fatty acid composition of black bear (Ursus americanus) milk during and after the period of winter dormancy. Lipids 27:940–943.

Iverson S.J., O.T. Oftedal, W.D. Bowen, D.J. Boness, and J. Sampugna. 1995. Prenatal and postnatal transfer of fatty acids from mother to pup in the hooded seal (Cystophora cristata). J Comp Physiol B 165:1–12.

Iverson S.J., J. Sampugna, and O.T. Oftedal. 1992. Positional specificity of gastric hydrolysis of long-chain n-3 polyunsaturated fatty acids of seal milk triglycerides. Lipids 27:870–878.

Jobling M. 1987. Marine mammal faeces samples as indicators of prey importance—a source of error in bioenergetics studies. Sarsia 72:225–260.

Keiver K.M., K. Ronald, and W.H. Beamish. 1984. Metabolizable energy requirements for maintenance and faecal urinary losses of juvenile harp seals (Phoca groenlandica). Can J Zool 62:769–776.

Kirsch P.E. 1997. Dietary Effects on Fatty Acid Signatures in Marine Species: Five Captive Animal Studies. MSc thesis. Dalhousie University, Halifax, Nova Scotia.

Kirsch P.E., S.J. Iverson, W.D. Bowen, S.R. Kerr, and R.G. Ack-

man. 1998. Dietary effects on the fatty acid signature of whole Atlantic cod (Gadus morhua). Can J Fish Aquat Sci 55:1378–1386.

Klungsoyr J., S. Tilseth, S. Wilhelmsen, S. Falk-Petersen, and J.R. Sargent. 1989. Fatty acid composition as an indicator of food intake in cod larvae Gadus morhua from Lofoten, Northern Norway. Mar Biol 102:183–188.

Lee R.F., J.C. Nevenzel, and G.-A. Paffenhofer. 1971. Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. Mar Biol 9:99–108.

Nelson G.J. 1992. Dietary fatty acids and lipid metabolism. Pp. 437–471 in C.K. Chow, ed. Fatty Acids in Foods and Their Health Implications. Dekker, New York.

NRC (National Research Council). 1996. The Bering Sea Ecosystem. National Academy, Washington, D.C. 307 pp.

Oftedal O.T. and S.J. Iverson. 1987. Hydrogen isotope methodology for measurement of milk intake and energetics of growth in suckling seals. Pp. 67–96 in A.C. Huntley, D.P. Costa, G.A.J. Worthy, and M.A. Castellini, eds. Approaches to Marine Mammal Energetics. Allen, Lawrence, Kans.

Oftedal O.T., S.J. Iverson, and D.J. Boness. 1987. Milk and energy intakes of suckling California sea lion (Zalophus californianus) pups in relation to sex, growth, and predicted maintenance requirements. Physiol Zool 60:560–575.

Pierce G.J., P.R. Boyle, and J.S.W. Diack. 1991. Identification of fish otoliths and bones in faeces and digestive tracts of seals. J Zool (Lond) 224:320–328.

Pierce G.J., J.S.W. Diack, and P.R. Boyle. 1990. Application of serological methods to identification of fish prey in diets of seals and dolphins. J Exp Mar Biol Ecol 137:123–140.

Pond C.M., C.A. Mattacks, I. Gilmour, M.A. Johnston, C.T. Pillinger, and P. Prestrud. 1995. Chemical and carbon isotopic composition of fatty acids in adipose tissue as indicators of dietary history in wild arctic foxes (Alopex lagopus) on Svalbard. J Zool (Lond) 236:611–623.

Rau G.H., D.G. Ainley, J.L. Bengston, J.J. Torres, and T.L. Hopkins. 1992. $^{15}N/^{14}N$ and $^{13}C/^{12}C$ in Weddell sea birds, seals, and fish: implications for diet and trophic structure. Mar Ecol Prog Ser 84:1–8.

Reilly J.J. and M.A. Fedak. 1990. Measurement of living grey seals (Halichoerus grypus) by hydrogen isotope dilution. J Appl Physiol 69:885–891.

Ronald K., K.M. Keiver, F.W.H. Beamish, and R. Frank. 1984. Energy requirements for maintenance and faecal and urinary losses of the grey seal (Halichoerus grypus). Can J Zool 62:1101–1105.

Rouvinen K. 1992. Effects of fish fat feeding on body composition of foxes. Norw J Agric Sci Suppl 9:249–253.

Rouvinen K., M. Jaakko, T. Kiiskinen, and S. Nummela. 1992. Accumulation of dietary fish fatty acids in the body fat reserves of some carnivorous fur-bearing animals. Agric Sci Finl 1:483–489.

Rouvinen K. and T. Kiiskinen. 1989. Influence of dietary fat

source on the body fat composition of mink (*Mustela vison*) and blue fox (*Alopex lagopus*). Acta Agric Scand 39:279–288.

Sargent J.R. 1989. The lipids. Chap. 4 in J.E. Halver, ed. Fish Nutrition. Academic Press, Washington, D.C.

Sargent J.R., R.J. Parkes, I. Mueller-Harvey, R.J. Henderson. 1988. Lipid biomarkers in marine ecology. Pp. 119–138 in M.A. Sleigh, ed. Microbes in the Sea. Ellis Horwood, Chichester.

Schmidt-Nielson K. 1979. Animal Physiology: Adaptations and Environment. Cambridge University Press, Cambridge.

Sinclair E., T. Loughlin, and W. Pearcy. 1994. Prey selection by northern fur seals (*Callorhinus ursinus*) in the eastern Bering Sea. Fish Bull 92:144–156.

Smith R.J., K.A. Hobson, H.N. Koopman, and D.M. Lavigne. 1996. Distinguishing between populations of fresh- and salt-water harbour seals (*Phoca vitulina*) using stable-isotope ratios and fatty acid profiles. Can J Fish Aquat Sci 53:272–279.

Smith S.J., S.J. Iverson, and W.D. Bowen. 1997. Fatty acid signatures and classification trees: new tools for investigating the foraging ecology of seals. Can J Fish Aquat Sci 54: 1377–1386.

Speakman J.R. 1997. Doubly Labelled Water: Theory and Practice. Chapman & Hall, London.

St. John M.A. and T. Lund. 1996. Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea cod. Mar Ecol Prog Ser 131:75–85.

Worthy G.A.J. 1990. Nutritional energetics of marine mammals. Pp. 489–520 in L.A. Dierauf, ed. CRC Handbook of Marine Mammal Medicine: Health, Disease, and Rehabilitation. CRC, Boca Raton, Fla.

Yu T.C., R.O. Sinnhuber, and G.B. Putnam. 1977. Effect of dietary lipids on fatty acid composition of body lipid in rainbow trout (*Salmo gairdneri*). Lipids 12:495–499.