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Lipid and amino acid composition influence incorporation and discrimination of ^{13}C and ^{15}N in mink

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The incorporation of dietary macronutrients and associated isotopic signatures of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) into animal tissues is a result of the interaction between growth, nutritional status, and the composition of the diet. In mammalian carnivores incorporation is further complicated by lack of carbohydrates in the diet and allocation of large quantities of dietary macronutrients to fetuses and milk production. In this study, we explored the effects of diet composition, growth, pregnancy, and milk production on isotopic incorporation of ^{13}C and ^{15}N in captive mink (*Neovison vison*) fed 3 experimental diets (Beef, Fish, and a Mixture of the 2) that differed in lipid and amino acid composition. In nursing kits, growth was the main factor influencing isotopic incorporation rates into muscle. Similarly, in adults, changes in body mass influenced the dynamics of isotopic incorporation in red blood cells, although the rates differed for $\delta^{13}C$ and $\delta^{15}N$, as well as among the 3 experimental groups. Effects of allocation of dietary macronutrients to fetuses and milk did not differ from body mass changes, potentially because those macronutrients were assimilated in tissues other than blood cells. Although incorporation of $\delta^{13}C$ followed the expected exponential form, $\delta^{15}N$ incorporation lagged in the Beef and Mixture diet treatments, and both $\delta^{13}C$ and $\delta^{15}N$ incorporation rates differed substantially for the Fish-fed mink. These differences in isotopic incorporation can be attributed to the differential oxidation of dietary amino and fatty acids. Thus, we advocate the development of compound-specific isotopic analyses to estimate dietary contributions through the incorporation of essential dietary fatty and amino acids.

Key words: growth, kits, milk composition, *Neovision vison*, pregnancy, routing, tissue turnover rate

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Superficially, the incorporation of dietary macronutrients and associated isotopic signatures is simple. Proteins in animal tissues are most often derived from dietary amino acids (e.g., Jim et al. 2006), lipids are usually derived from dietary fatty acids (e.g., Howland et al. 2003), whereas most carbohydrates fuel respiration or when consumed in excess are converted to structural lipids (Kelly and Martínez del Rio 2010; Martínez del Rio and Wolf 2005). Therefore, isotopic ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in animal tissues should reflect the diet of individuals (Ben-David and Schell 2001; Caut et al. 2009; Felicetti et al. 2003; Hilderbrand et al. 1996). Nonetheless, the recent flurry of theoretical and experimental work has demonstrated that the incorporation of dietary macronutrients into animal tissues is a function of assimilation efficiencies of dietary macronutrients, rates of tissue catabolism and anabolism, and routing through various metabolic pathways (Ambrose and Norr 1993; Carleton and Martínez del Rio 2010; Fry and Arnold 1982; Gannes et al. 1997; Schwarcz 1991; Tieszen and Fagre 1993). Because growth and tissue catabolic turnover rate scale with body size (Hesslein et al. 1993), isotopic incorporation is highly dependent on this attribute (Martínez del Rio and Carleton 2012; Martínez del Rio et al. 2009).

Catabolic turnover rate (the replacement of material exported from tissues as a result of catabolism) in many cases scales allometrically. For example, the life span of red blood cells increases with mammalian body size (estimated at 45 days in mice, 60 in rats, 70 in rabbits, 82 in cats, 95 in dogs, 86 in pigs, 120 in humans, and 155 in horses—Carter et al. 1964). Thus, isotopic incorporation into blood cells will likely

be faster in smaller animals. Recently, Carleton and Martínez del Rio (2010) demonstrated that in growing tilapia (*Oreochromis niloticus*), the contribution of catabolism to incorporation rate of $\delta^{13}C$ was similar to that of growth (mass accretion rates) in muscle, but was 2.6 to 5 times higher in liver; with the latter exhibiting high tissue-specific catabolism (Sponheimer et al. 2006; Teiszen et al. 1983).

In younger indeterminate-growing ectotherms, growth can explain 30–100% of isotopic incorporation rates (Martínez del Rio et al. 2009; Reich et al. 2008 and references therein). For determinate-growing mammals (Sebens 1987), the effects of growth on isotopic incorporation is largely limited to the juvenile stage, a period initially characterized by suckling. In a semicontrolled study of several mammalian herbivores and carnivores, Jenkins et al. (2001) found no effect of age on incorporation of maternal milk $\delta^{13}C$ into neonate tissues, and a moderate effect on $\delta^{15}N$ in caribou (*Rangifer tarandus*) and brown bears (*Ursus arctos*), but not in moose (*Alces alces*). Nonetheless, in that study neonate sampling began 12–14 days after parturition, likely after the young had equilibrated on the lipid- and protein-rich milk diet (Oftedal 2004). Newsome et al. (2006) determined the age at weaning in northern fur seals (*Callorhinus ursinus*) and California sea lions (*Zalophus californianus*) from changes in values of $\delta^{15}N$ from sequential sampling of bone collagen and tooth dentin. In that study the effects of growth rate on isotopic incorporation, although postulated, could not be evaluated. Habran et al. (2010) demonstrated changes in $\delta^{13}C$ and $\delta^{15}N$ values in milk, blood cells, and serum of pup–mother pairs in northern elephant seals (*Mirounga angustirostris*) during lactation, but did not quantify the effects of pup growth. Similarly, Polischuk et al. (2001) described seasonal changes in both $\delta^{13}C$ and $\delta^{15}N$ of female polar bears (*Ursus maritimus*) and their cubs, but did not measure the effects of cub growth.

The effects of growth on isotopic incorporation of weaned juveniles were experimentally tested in only 3 studies. MacAvoy et al. (2005) found that growth accounted for only 10% of the incorporation rate of dietary $\delta^{13}C$ and $\delta^{15}N$ of mice (*Mus musculus*), probably because these animals were close to reaching adulthood. Caut et al. (2008) observed that in growing rats (*Rattus rattus*) variable isotopic incorporation rates largely reflected the difference between dietary $\delta^{13}C$ and $\delta^{15}N$ and the animals' initial signatures. Lecomte et al. (2011) detected differences in isotopic incorporation and discrimination between adult and yearling arctic foxes (*Vulpes lagopus*). In these studies, however, the effects of mass gain on the dynamics of isotopic incorporation were not assessed.

For adult mammals, tissue growth and associated changes in body mass largely result from fat deposition in adipose tissues and, to a certain extent, increases in muscle mass (Harris et al. 1986). This mass accumulation is reversible on a daily basis in bats and other small mammals (Cresswell 1998); on a seasonal basis as is common in temperate ungulates, hibernators, and marine mammals (Atkinson and Ramsay 1995); or during long-distance migration as in whales and some ungulates (Barboza and Parker 2008; Schell et al. 1988). The dynamics of isotopic incorporation in these animals will, therefore, depend on changes in mass. Unfortunately, most studies exploring the effects of mass changes in vertebrates use either indeterminate growers such as fish (Carleton and Martínez del Rio 2010; Gaye-Siessegger et al. 2004a, 2004b; Kelly and Martínez del Rio 2010) or design the experiment such that animals largely gain mass (Florin et al. 2011; Sick et al. 1997). Nonetheless, Hobson et al. (1993) found an enrichment in $\delta^{15}N$ values of nutritionally stressed, nest-tending, Ross's geese (*Chen rossii*), suggesting that mass loss affects isotopic incorporation and discrimination. In contrast, Ben-David et al. (1999) were unable to document a similar pattern in nutritionally stressed arctic ground squirrels (*Spermophilus parryii*). Thus, the effects of mass changes in adults on isotopic incorporation rates and discrimination remain unclear.

For female mammals, the effects of growth on isotopic incorporation may be further complicated by allocation of large quantities of dietary macronutrients to the nonreversible depositories of fetuses (once they develop past the resorption stage—Feldhamer et al. 2003) and milk (Oftedal 2004). Jenkins et al. (2001) observed changes in $\delta^{13}C$ and $\delta^{15}N$ values in maternal and offspring plasma of brown bears once mothers emerged from hibernation. Unfortunately, Jenkins et al. (2001) were unable to estimate isotopic incorporation rates because they sampled animals only once, 30 days after they began feeding. Polischuk et al. (2001) observed similar changes in maternal isotopic values during lactation and correlated $\delta^{15}N$ values with female body condition, but the dynamics of isotopic incorporation could not be determined because of the sampling schedule. In contrast, Habran et al. (2010) were unable to detect changes in $\delta^{13}C$ and $\delta^{15}N$ values in maternal tissue during lactation despite a significant change in the C:N ratios of the milk, representing increased nutrient allocation by the mother. Thus, the effects of pregnancy and lactation, unique cases of organismal growth, on isotopic incorporation and discrimination have not been fully assessed.

In addition to catabolic turnover rate and growth, numerous studies have shown the importance of diet composition to the dynamics of isotopic incorporation (Martínez del Rio and Carleton 2012). Kelly and Martínez del Rio (2010) demonstrated that incorporation of dietary $\delta^{13}C$ and $\delta^{15}N$ differed for diets containing 3.75–30% protein in growing tilapia, after accounting for the effects of differential growth among experimental groups. In that study, the difference in $\delta^{15}N$ of the fish relative to diet $\Delta^{15}N$ increased significantly with dietary protein content (Kelly and Martínez del Rio 2010). In contrast, Robbins et al. (2005), evaluating results of multiple studies, found no effect of protein intake on $\Delta^{15}N$ between animal tissues and diet. These authors demonstrated that discrimination between the animal $\delta^{15}N$ values and their diet decreased with an increase in biological value of the diet (the extent to which the essential amino acid composition of the absorbed proteins matches the animal's requirements). Indeed, Sick et al. (1997) and Florin et al. (2011) observed that $\delta^{15}N$ in rats varied with total protein intake as well as the biological value of the diet.

Table 1.—Proportion of protein and fat (of wet weight), percent carbon (%C) and nitrogen (%N), C to N ratio (C:N), and values of $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) for the preexperimental diet (Original), and 3 experimental diets (Beef, Fish, and Mixture) fed to captive mink. The isotopic values of Beef protein and Mixture protein were calculated using linear mixing models (Ben-David and Schell 2001).

Diet	Protein	Fat	%C	%N	C:N	$\delta^{13}C$	$\delta^{15}N$
Original	—	—	51.12	5.40	9.5	−23.3	8.8
Beef	0.12	0.12	43.20	4.22	10.2	−24.2	6.2
Mixture	0.16	0.08	49.30	6.18	8.0	−22.7	9.9
Fish	0.20	0.04	41.68	8.83	4.7	−20.5	11.7
Beef fat	—	—	—	—	—	−26.2	—
Beef protein						−22.2	
Mixture protein						−21.1	

Usually, controlled isotopic experiments of vertebrates use herbivores or omnivores as model systems (but see Lecomte et al. [2011], Roth and Hobson [2000], and Zhao et al. [2006]). We expect that these animals feeding on carbohydrate-rich diets would strictly route dietary protein to nonoxidative uses. Nonetheless, Kelly and Martínez del Rio (2010) and McCue et al. (2010) have shown that omnivores and herbivores feeding on carbohydrate-rich diets oxidize portions of the proteinaceous components (mainly glycine) of their food. Mammalian carnivores rarely consume large quantities of carbohydrates, thus we expect that these mammals primarily fuel respiration with a combination of lipids and proteins (Whiteman et al. 2012). The effects of oxidation of dietary protein and lipids on isotopic incorporation rates and discrimination in carnivores are unknown.

In this study we explored the effects of diet composition, pregnancy, and milk production on isotopic incorporation of dietary ^{13}C and ^{15}N in captive mink (*Neovison vison*). Through repeated sampling we described incorporation curves in blood cells while accounting for individual changes in body mass and determined discrimination factors at the end of the experiment for that tissue as well as liver, muscle, adipose fat, and bone collagen. Lastly, by mating half of the females in the experiment, we assessed the effects of pregnancy and milk production on maternal isotopic incorporation as well as the dynamics of isotopic incorporation in kits.

Materials and Methods

Animals and housing.—Thirty (6 males and 24 females) farmed mink (wild type) were purchased from the Oregon State University research center and housed at the animal quarters at the University of Alaska, Fairbanks, from February to May 1992. All animals were adults and ranged in age from 1 to 5 years. Mink were housed in individual wire cages (100 × 50 × 40 cm) in an environmentally controlled room at 17°C with artificial lighting. Day length was modified weekly to mimic conditions at the latitude of origin. Each cage was furnished with a straw-lined wooden sleeping box (20 × 15 × 15 cm). Individuals were allowed 3 weeks to acclimate to their new environment while feeding on the diet (a wet gruel, hereafter Original) they had received while at Oregon State University during the previous 4 months (Table 1). Food and water were provided daily ad libitum and cages were cleaned weekly.

Experimental design.—At the end of the 3-week acclimation period, all animals were sedated with a single intramuscular injection of ketamine hydrochloride (15 mg/kg body mass; Aveco, Fort Dodge, Iowa) and xylazine hydrochloride (3 mg/kg body weight; Aveco). Each individual was measured (to the nearest mm), weighed (to the nearest g), and marked subcutaneously with a passive integrated transponder microchip (Biosonics, Seattle, Washington). A blood sample of 2 ml was drawn from the jugular vein after which the animals were returned to their cages to recover from the anesthesia. During this procedure 4 older females (5 years old) died from seizures. Blood was spun at 3,000 rpm for 10 min within 2 h of collection, and serum was siphoned into a separate vial. Both serum and samples of clotted blood cells were frozen (−18°C) until analysis.

Following the initial handling (day 0), mink were randomly assigned to 3 groups such that each group consisted of 2 males and at least 6 females. Animals from each group were fed 1 of 3 experimental diets: marine-originated food (salmon [*Oncorhynchus*] hereafter Fish), terrestrial-originated food (Beef), and a mixture of the 2 (beef + salmon, hereafter Mixture) in equal proportions based on wet mass. Equal quantities of minerals and vitamins in powdered form and bone meal were added to all experimental diets; vitamin E was dissolved in oil. The Beef diet was composed of approximately 50% lipids, whereas the Fish diet was lean with less than 5% lipids yielding a disparate stoichiometry (C:N; Table 1). As previously, food and water were provided ad libitum.

Based on allometric relationships of longevity of red blood cells in mammals we estimated that all of these cells in the mink would be replaced in approximately 60–70 days (Carter et al. 1964). Therefore, we designed the experiment to last 77 days with weekly and bimonthly sampling sessions, except for the Beef group. For that group the experiment was terminated after 56 days because several individuals stopped eating. Members of this group were switched to the Mixture diet until the end of the experiment. At each sampling session animals were reweighed and blood samples were collected. During the course of the study, 1 female from the Mixture group and 1 male from the Beef group died (on days 22 and 31, respectively). At the end of 77 days, all remaining animals were euthanized with Halothane (Halocarbon, River Edge, New Jersey). Fat, muscle, liver, and bone samples were harvested from all 30 mink.

Mating and pregnancies.—On day 14 of the experiment (19 March), one-half of the females in each group were introduced to 1 of the males and allowed to mate for 2 h. Mating was repeated for 5 consecutive days or until the female copulated at least twice. We used serum samples collected throughout the experiment to determine the pregnancy status of all females. Progesterone assays were done using Diagnostic Products iodinated solid-phase RIA kits (R. A. Mead, University of Idaho, Moscow, Idaho). Intra- and intersample variation were 4.5 ± 1.8 ng/ml and 8.5 ± 11.1 ng/ml ($\bar{X}$ ± SD throughout; n = 38), respectively. Pregnancy status was determined based on progesterone levels reported in the literature (Allais and Martinet 1978), and ranged from 0.1–5.7 ng/ml in nonpregnant females ($\bar{X}$ = 1.7 ± 1.8 ng/ml) to 14.3–61.4 ng/ml in pregnant females ($\bar{X}$ = 33.8 ± 16.7 ng/ml). On days 59 and 60 of the experiment (1 and 2 May), 3 females, 1 from the Beef group and 2 from the Fish group, delivered 5 (total litter mass 26.12 g), 2 (11.60 g), and 7 (69.02 g) kits, respectively. Of these, 9 kits died within 2 days of parturition, 1 died at 7 days, 1 at 15 days, and 3 survived to the end of the experiment (21 days) and were euthanized with their mother, which belonged to the Fish dietary group. Muscle tissues were collected from all dead kits. None of the remaining mated females with progesterone levels > 14.3 ng/ml had palpable embryos, nor did they deliver young. These females were considered pseudopregnant. Milk was collected from 2 of the mothers and from 2 pseudopregnant females at the end of the experiment. All methods used in this research were approved by the Institutional Animal Care and Use Committees at the University of Alaska, Fairbanks, and all procedures adhered to guidelines for animal care and use adopted by the American Society of Mammalogists (Sikes et al. 2011).

Stable isotope analyses.—Samples of food (Original, Beef, Mixture, and Fish), blood clots, muscle, liver, and fat were dried at 60°C to 70°C for 48 h and then ground to fine powder using a Wig-L-Bug grinder (International Crystal Laboratories, Garfield, New Jersey). Subsequently, a subsample of 1–1.5 mg was weighed into a tin cup (4 × 6 mm) for combustion. To measure the isotopic ratios of lipids in the Beef diet, we extracted them using a standard Floch procedure (Floch et al. 1957) with chloroform–methanol solvent. The solvent was then rinsed with a 0.9% NaCl solution, and the remaining lipid phase was centrifuged and dried for 24 h before weighing. Bone collagen was extracted following protocols developed by Matheus (1997). In short, a segment of the femur was soaked in chloroform for two 8-h periods to remove lipids, air dried, and then soaked in weak hydrochloric acid (HCl) at low temperatures for 7 days (while refreshing the solution every 24 h) to remove bioapatites. The sample was then rinsed with dionized water to neutralize the acid. Subsequently, a small amount of HCl was added (until the pH reached between 3 and 4) and the sample was heated for 2–6 h. After dissolution, the supernatant (collagen) was centrifuged, separated, freeze-dried, and weighed.

We used a Europa C/N continuous-flow mass spectrometer (Shimadzu Europa, Berlin, Germany) to obtain the stable isotope ratios. Each sample was analyzed in duplicate and accepted only if isotopic variance between samples did not exceed that of a peptone standard ($\delta^{13}C_{std}$ = −15.8‰ and $\delta^{15}N_{std}$ = 7.0‰) and machine linearity did not deviate from 0.99.

Quantification of amino acids.—Amino acid concentrations of mink muscle, and Beef and Fish diets were determined using a reversed-phase high-performance liquid chromatography system with precolumn derivatization and α-amino adipic acid (Sigma; Sigma Aldrich, St. Louis, Missouri) as an internal standard (Zhao et al. 2006). Homogenized samples were hydrolyzed with 6 N HCl for 24 h at 110°C, dried under N_2 at 60°C, and filtered. We used a UVIS-200 UV detector (America Laboratory Trading, East Tyme, Connecticut), controlled by a Penelson NCI 900 network chromatography interface (Perkin-Elmer, Waltham, Massachusetts), to detect amino acid derivatives at 340 nm. Amino acid separation was achieved on an Allsphere ODS-II column (250 × 4.6 mm, 5-μm particle size; Alltech, Nicholasville, Kentucky) at a flow rate of 1.4 ml/min. Data were resolved with Turbochrom software (Perkin-Elmer). Results are reported as nM/100 μg dry matter.

Data analyses.—All analyses were conducted in SPSS for Windows version 15.0 (IBM, Armonk, New York). To explore the effects of age and sex we compared the isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of blood cells of all mink on day 0 of the experiment with a 2-way analysis of variance (ANOVA). We then evaluated the dynamics of isotopic incorporation for animals from the 3 experimental groups with repeated-measures nonlinear curve estimation procedures with body mass as an individual covariate. The inclusion of this variable was crucial because mass changes greatly varied among individuals (Fig. 1) and tissue growth is an important factor determining isotopic incorporation rates (Carleton and Martínez del Rio 2010). For each group and isotope, we constructed several competing models and evaluated fit based on R^2 values and parsimony (fewer terms in the equation). To determine whether an asymptote was reached and the number of elapsed days, we used a cutoff point when the change in value was less than machine error. To derive the most plausible tissue–diet discrimination factor for $\delta^{13}C$ and $\delta^{15}N$, we plotted the value derived from day 0 of the experiment and the values resulting from subtracting the blood cell value at each sampling point throughout the experiment.

To assess the effects of amino acid composition of the diet on isotopic incorporation, we compared concentrations of 16 amino acids in the diet to their concentration in mink muscle on the last day of the experiment for the Beef and Fish groups. Because we did not sample liver, muscle, and adipose fat on the same schedule as blood, we explored the relationships in $\delta^{13}C$ and $\delta^{15}N$ among these tissues and blood cells at the end of the experiment with a Pearson's correlation.

To evaluate the effect of fetuses' growth (i.e., nonreversible nutrient depository) on maternal isotopic incorporation, we calculated the mean $\delta^{13}C$ and $\delta^{15}N$ values for each diet group, excluding the mother, and then computed the difference between the mother value and the group mean (i.e.,

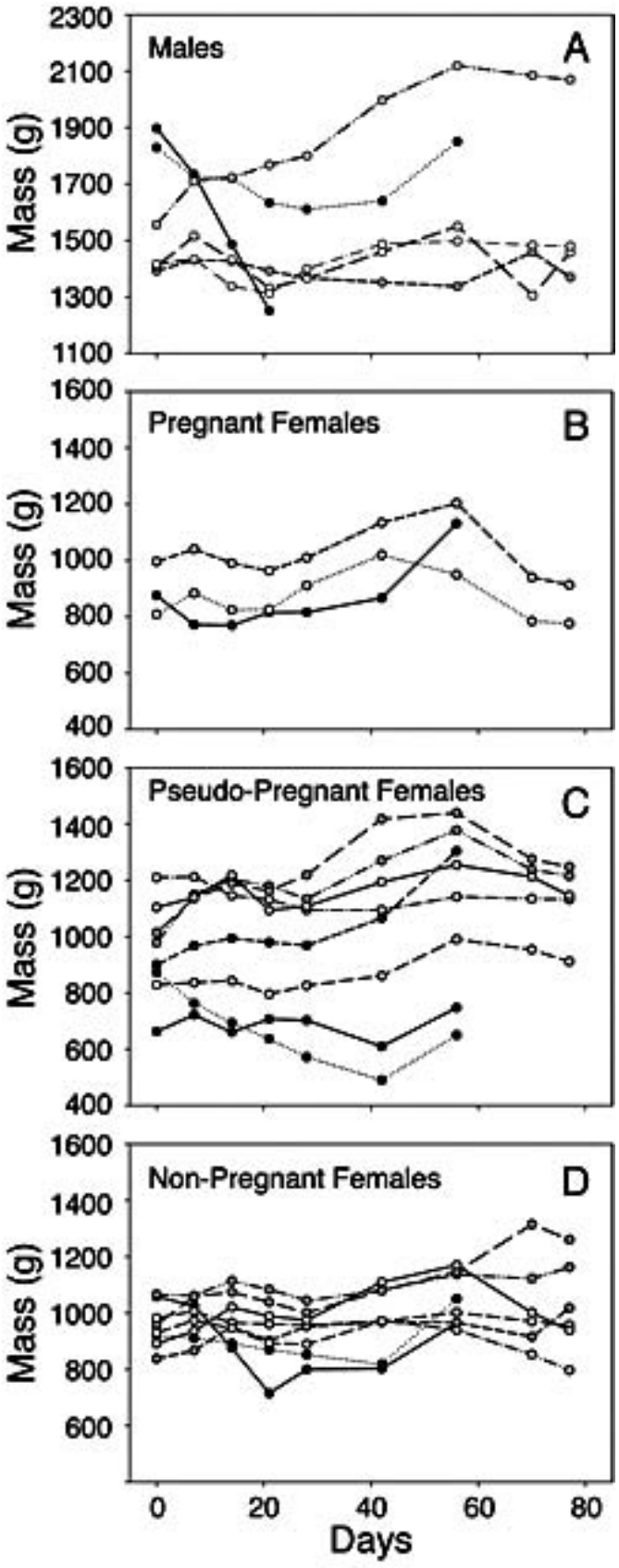

Fig. 1.—Body mass (g) of males, pregnant females, pseudopregnant females, and nonpregnant female mink during 77 days of feeding on experimental diets (Table 1). Black circles denote individuals fed on the Beef diet; gray circles represent the Mixture group; open circles indicate the Fish group. For the Beef group the experiment lasted only 56 days, after which they were switched to the Mixture diet. Mink were held in captivity at the Animal Quarters at the University of Alaska, Fairbanks, from February to May 1992.

$\Delta^{15}N_{mother-group\ mean}$). We also calculated the overall litter mass by multiplying the number by the mean mass of all kits that perished on days 1–2 after birth (9.8 g ± 0.2). We then used correlation to assess whether $\Delta^{15}N_{mother-group\ mean}$ was influenced by litter mass. Finally, we assessed isotopic incorporation rates of maternal milk into muscle tissue of kits in relation to kit growth with nonlinear curve estimation. Because we did not have repeated samples for the same individual we assumed that growth rate was similar for all kits. Similarly, we explored the relationship between isotopic incorporation and the relative change of mass of growing kits, or $\Delta w/w_t$, as suggested by Carleton and Martínez del Rio (2010). For initial mass we used the mean mass of their siblings that perished on days 1–2 after birth. We used linear models to describe these relationships.

Results

Stable carbon and nitrogen ratios in samples of red blood cells collected on day 0 of the experiment were not significantly influenced by sex (6 males, $\delta^{13}C = -19.2 \pm 0.06$ [‰; $\bar{X} \pm SD$] and $\delta^{15}N = 11.5 \pm 0.16$; 24 females, $\delta^{13}C = -19.2 \pm 0.20$ and $\delta^{15}N = 11.8 \pm 0.32$) or age (18, 1–2 years old, $\delta^{13}C = -19.1 \pm 0.18$ and $\delta^{15}N = 11.7 \pm 0.34$; 12, 3–5 years old, $\delta^{13}C = -19.2 \pm 0.17$ and $\delta^{15}N = 11.8 \pm 0.32$; 2-way ANOVA, $F_{2,27} = 0.83$, $P = 0.449$ for $\delta^{13}C$, and $F_{2,27} = 2.67$, $P = 0.069$ for $\delta^{15}N$). Individual changes in body mass were variable with the highest among-individuals dissimilarity observed in pseudopregnant females (Fig. 1). Despite the high lipid contents and C:N ratio of the Beef diet (Table 1), mass gains in mink fed on this diet were low, except for pregnant and pseudopregnant females (Fig. 1).

Exponential decay repeated-measures regression provided the best fit for $\delta^{13}C$ in the Beef group after accounting for individual differences in mass changes (Table 2; Fig. 2A). In that analysis the asymptote was reached by day 55. A similar equation provided best fit for ^{13}C incorporation into red blood cells for the Mixture group (Table 2; Fig. 2B). For that group, the asymptote was reached by day 54. In contrast, for the Fish group, isotopic incorporation showed a delay followed by a near-exponential increase with no indication of reaching an asymptote (Table 2; Fig. 2C).

The best repeated-measures regression fit for $\delta^{15}N$ in the Beef group, after accounting for individual differences in mass changes, was described by a 4-parameter Gompertz equation (Table 2; Fig. 2D), which captured the slight delay in isotopic incorporation of dietary ^{15}N. For that group, the asymptote was reached by 43 days. A similar equation provided the best fit for ^{15}N incorporation into red blood cells for the Mixture

Table 2.—Equations describing incorporation rates of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values in blood cells of captive mink fed Beef, Mixture, and Fish diets. The experiment lasted 77 days, except for the Beef group, for which the experiment ended after 56 days (see Fig. 2). Also provided are equations describing the incorporation of maternal milk isotopes into muscle of kits in relation to their growth rates and relative mass change ($\Delta w/w_t$; see Fig. 5).

Experimental diet	Equation	R^2	P
Beef	$\delta^{13}C = -20.23 + 1.21e^{(-0.08days)}$	0.96	<0.001
Mixture	$\delta^{13}C = -19.65 + 0.38e^{(-0.06days)}$	0.86	<0.001
Fish	$\delta^{13}C = -19.19 - 0.004(days) + 0.0001(days)^2$	0.97	<0.001
Beef	$\delta^{15}N = 10.41 + 1.35e^{\{-e[-(24.45 - days)/-10.39]\}}$	0.96	<0.001
Mixture	$\delta^{15}N = 11.87 + 0.97e^{\{-e[(54.5 - days)/28.08]\}}$	0.76	<0.001
Fish	$\delta^{15}N = 11.84 + 0.02(days) + 0.00005(days)^2$	0.99	<0.001
Kits	$\delta^{13}C = -1.995 + 3.008e^{(-0.043mass)}$	0.92	<0.001
	$\delta^{15}N = 2.64(1 - e^{(-0.54mass)})$	0.13	0.07
	$\delta^{13}C = -18.39 - 2.38\Delta w/w_t$	0.88	0.001
	$\delta^{15}N = 16.05 + 0.78\Delta w/w_t$	0.54	0.07

group, with the asymptote reached at 58 days (Table 2; Fig. 2B). In contrast, for the Fish group, isotopic incorporation showed near-linear increase but was best described by a polynomial equation (Table 2; Fig. 2C). An asymptote may have been reached at 77 days.

Values of $\delta^{15}N$ in all groups exhibited a clear trend of convergence on the 3‰ discrimination value recorded for the 4 individuals that perished on day 0 of the experiment, after feeding on the Original diet for more than 4 months (Fig. 3). The difference between blood and whole diet at the end of the experimental period ranged from 2.5‰ to 3.9‰ (Table 3). In contrast, the trend in convergence on the original 4‰ discrimination for $\delta^{13}C$ was slow and variable for the 3 groups (Fig. 3) and discrimination values ranged from 1.8‰ to 4.1‰ (Table 3). The discrimination value for $\delta^{13}C$ was significantly and positively correlated with the C:N ratio of the diet ($r = 0.99$, $P = 0.02$). No such relation existed for $\delta^{15}N$ ($r = 0.80$, $P = 0.41$), although the C:N ratio was highly correlated with time to full assimilation of dietary $\delta^{15}N$ ($r = 0.99$, $P = 0.03$). Discrimination values between blood cells and the proteinaceous component of the diet (or the computed, lipid-free $\delta^{13}C$ value; Table 1) was 2.1‰ for the Beef group and 1.5‰ for the Mixture group (Table 3).

Values of $\delta^{13}C$ and $\delta^{15}N$ for blood, muscle, and liver were highly correlated ($P < 0.01$), with $\delta^{13}C$ correlation coefficients of $r = 0.84$ for blood and liver, $r = 0.66$ for blood and muscle, and $r = 0.57$ for liver and muscle. For $\delta^{15}N$ blood and liver were correlated at $r = 0.91$, blood and muscle at $r = 0.91$, and liver and muscle at $r = 0.90$. These high correlations suggest that these tissues had incorporated the isotopic values of the diet at a similar rate to blood cells. Liver tissues were depleted in $\delta^{13}C$ relative to blood cells (-2.1 ± 0.76 [$\bar{X} \pm SD$]; $P < 0.01$; Table 4) and slightly enriched in $\delta^{15}N$ ($\bar{X} = 1.0 \pm 0.54$; $P < 0.01$; Table 4). Both muscle $\delta^{13}C$ and $\delta^{15}N$ were similar to blood cells (for $\delta^{13}C$, $\bar{X}\ \Delta = -0.3 \pm 0.4$; $P = 0.06$; for $\delta^{15}N$, $\bar{X}\ \Delta = 0.6 \pm 0.6$; $P = 0.21$; Table 4). Therefore, the isotopic signatures of muscle yielded similar discrimination values to blood cells, except for the Beef group, in which these values represent a 2nd dietary switch to the Mixture diet for the last 21 days of the experiment. Members of that group gained on average 131 g ($\pm$ 65 g) after the dietary switch.

Adipose fat $\delta^{13}C$ values were indistinguishable for the Beef and Mixture groups ($P = 0.07$; Table 4), but were significantly depleted relative to the Fish group ($P < 0.001$; Table 4). Values of $\delta^{13}C$ for adipose tissue of the Beef and Mixture groups were enriched relative to the lipid portion of the Beef diet by 2.2‰ (Table 3). Adipose $\delta^{15}N$ values also were indistinguishable for the Beef and Mixture groups ($P = 0.36$; Table 4), and were depleted relative to the Fish group ($P < 0.001$; Table 4). Bone collagen values of $\delta^{13}C$ and $\delta^{15}N$ were identical for all 3 experimental groups and those of the 4 individuals that perished on day 0 of the experiment (Table 4), reflecting the slow turnover rate of that tissue.

The amino acid composition of the Fish diet was more similar to that of mink muscle compared with the Beef diet (Fig. 4; Table 5). This was true for essential and nonessential amino acids, with glycine especially prevalent in the Fish diet and glutamic acid especially rare in the Beef diet (Fig. 4). This agrees with previous estimates of biological values of fish and domesticated animal meat (e.g., 92% and 78% similarity in concentrations of essential amino acids, respectively—Lewis and Morris 1983).

The isotopic values of red blood cells of pregnant and pseudopregnant females were indistinguishable; in addition, they did not significantly differ from those of males and nonpregnant females 16 days and 2 days prior to parturition (2-sample tests, $P > 0.08$). Nonetheless, we found a negative correlation between $\Delta^{15}N_{mother-group\ mean}$ and total litter mass ($r = -0.99$, $P = 0.03$), with the mother producing the largest litter (69.02 g) deviating least from her group mean (0.1‰) and the mother with the smallest litter (11.60 g) deviating the most (0.5‰).

The difference between isotopic values in kit muscle immediately after birth and maternal blood cells (i.e., in utero processes) suggested no discrimination in $\delta^{13}C$ but 2.5‰ for $\delta^{15}N$ (Table 6). This changed during lactation, when kits became depleted in $\delta^{13}C$ (Fig. 5A) and further enriched in $\delta^{15}N$ (Fig. 5B) relative to their mothers (Table 6). This change largely resulted from depletion in $\delta^{13}C$ and enrichment in $\delta^{15}N$ in milk (Table 6). The incorporation of milk $\delta^{13}C$ signatures into kit muscle in relation to kit growth was best described an exponential decay equation (Table 2; Fig. 5A). For $\delta^{15}N$ the nonlinear regression (Table 2; Fig. 5B) was not significant, likely because the difference between kit values as a result of in utero processes was only slightly lower than that resulting from discrimination from milk. Both curves describing the relation between $\Delta w/w_t$ and $\delta^{13}C$ and $\delta^{15}N$ were linear, suggesting that incorporation rates were largely a function of growth rather than catabolic turnover of their muscle (Figs. 5B and 5D).

Discussion

Growth was the main factor influencing isotopic incorporation in the muscles of nursing kits. Similarly, in adults, mass

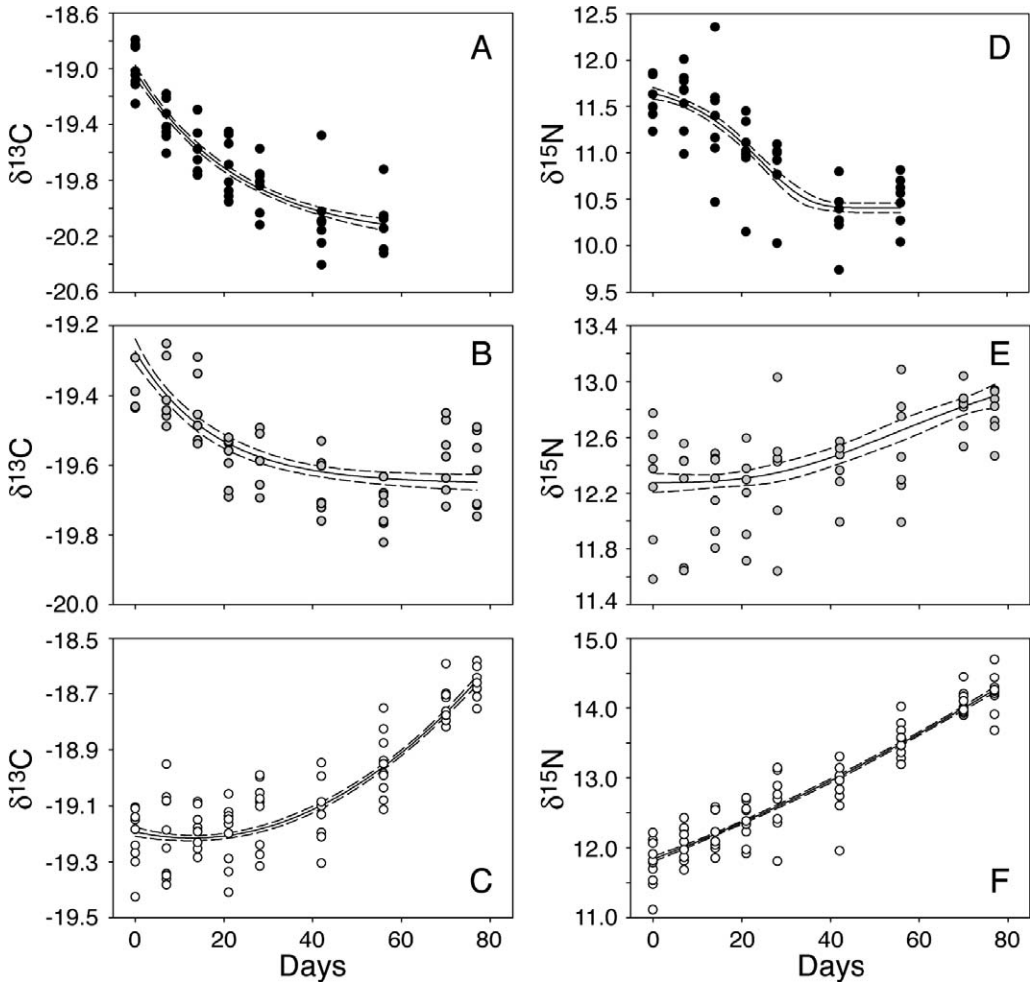

Fig. 2.—Values of A–C) $\delta^{13}C$ and D–F) $\delta^{15}N$ (‰) of clotted blood cells of captive mink during 77 days of feeding on experimental diets (Table 1). Black circles (A and D) denote values for individual animals in the Beef group, gray circles (B and E) represent the Mixture group, and open circles (C and F) indicate the Fish group. The regression lines and 95% confidence intervals were derived from repeated-measures curve estimation with body mass (Fig. 1) as an individual covariate. For the Beef treatment the asymptote was reached by day 55 for $\delta^{13}C$ and day 43 for $\delta^{15}N$. For the Mixture group $\delta^{13}C$ and $\delta^{15}N$ reached an asymptote on days 54 and 58, respectively. For the Fish group an asymptote was not reached for both $\delta^{13}C$ and $\delta^{15}N$.

changes influenced the dynamics of isotopic incorporation in red blood cells. The allocation of dietary macronutrients to fetuses and milk production (nonreversible depositories) resulted in similar isotopic incorporation dynamics to reversible mass changes, potentially because all occurred in tissues other than blood cells. Regardless, the most pronounced differences in isotopic incorporation and discrimination factors were related to the lipid and amino acid composition of the different diets. Although incorporation of $\delta^{13}C$ followed the expected exponential decay (or rise to a maximum), $\delta^{15}N$ incorporation lagged in the Beef and Mixture diet treatments. Further, $\delta^{13}C$ and $\delta^{15}N$ incorporation rates differed substantially for the Fish-fed mink despite the fact that all individuals were held under the same conditions, fed ad libitum, and theoretically the majority of their blood cells had been replaced over the course of the experiment. This disparity in isotopic incorporation can be attributed to the differential oxidation of amino and fatty acids in the diets.

Effects of mass change on incorporation rates and discrimination values.—Similar to previous studies (Habran et al. 2010; Jenkins et al. 2001; Polischuk et al. 2001; Stegall et al. 2008), we observed that kit muscles were enriched relative to milk in both $\delta^{13}C$ and $\delta^{15}N$, and that milk was depleted in $\delta^{13}C$ relative to maternal tissues (Table 5). Also, other studies (Fogel et al. 1989; Jenkins et al. 2001) found depleted milk $\delta^{15}N$ values relative to maternal tissues, and have attributed this to the presence of urea in milk, because urea is generally depleted in ^{15}N (Martínez del Rio et al. 2009; Sick et al. 1997). Dairy cows (*Bos primigenius*) and goats (*Capra hircus*) increase milk urea content with increasing dietary nitrogen (N) intake (Pfeffer et al. 2009). Because experimental diets in our study had high protein contents, we expected high urea excretion in milk and thus depleted $\delta^{15}N$.

Instead, similar to findings by Habran et al. (2010) in elephant seals, we observed an approximately 1.1‰ enrichment in milk $\delta^{15}N$ relative to maternal blood cells. We offer 3

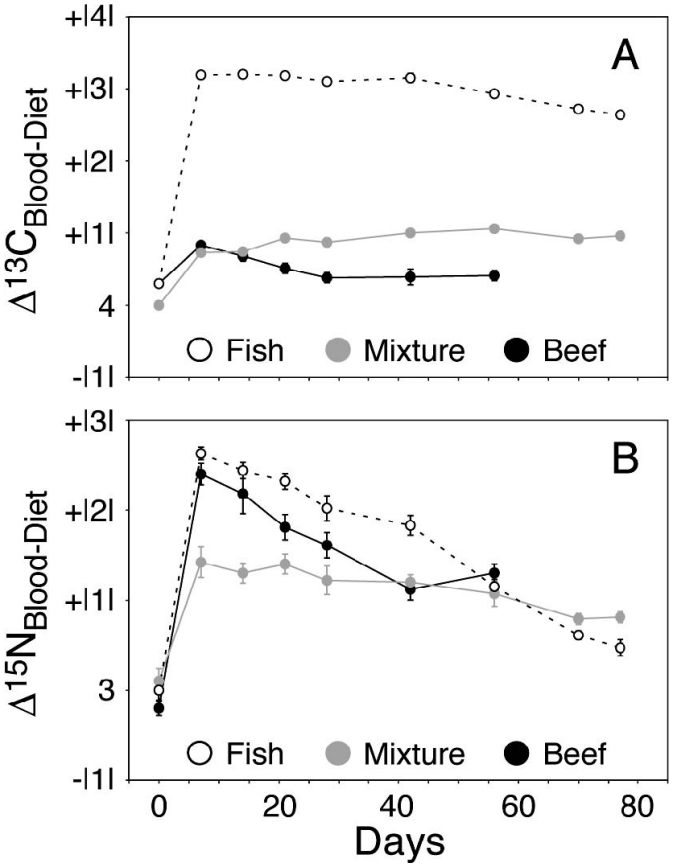

FIG. 3.—Discrimination (A, $\delta^{13}C$; B, $\delta^{15}N$; ‰) between the isotopic values ($\bar{X} \pm 95\%$ confidence intervals) of red blood cells of captive mink and the experimental diets they were fed during a 56-day trial (Beef—black circles) and 77-day trial (Mixture—gray circles; Fish—open circles). Discrimination values are presented as absolute values for comparison. Values of $\delta^{15}N$ in all groups exhibited a trend of convergence on the 3‰ discrimination value recorded for 4 individuals that perished on day 0 of the experiment after feeding on the Original food for more than 4 months. In contrast, a trend of convergence on the original 4‰ discrimination for $\delta^{13}C$ was slow and inconsistent among groups.

potential explanations: 1st, similar to the piscivorous elephant seals, 3 of the 4 females from which we collected milk consumed the Fish diet. This diet had a higher biological value, which is associated with decreased trophic enrichment of $\delta^{15}N$ (Florin et al. 2011; Robbins et al. 2005). Second, because the lactating mink were routing most amino acids to oxidation rather than biosynthesis, there was little preferential incorporation of ^{15}N into body tissues. Thus, it is possible that urea was not depleted in ^{15}N. Finally, Habran et al. (2010) reported that although milk was enriched in ^{15}N relative to blood cells, milk $\delta^{15}N$ values were similar (on day 5) or slightly depleted (on day 22) compared to serum. The vast difference in isotopic signatures of blood cells and serum in these wild-caught marine mammals could have resulted from a dietary shift or a difference in protein turnover rates between serum and red blood cells. In our captive mink, however, none of these processes could explain this pattern because the serum isotopic values, although not measured, must have equilibrated with the experimental diet prior to lactation. Therefore, there would be no opportunity for turnover rates to influence isotopic signatures. Whether routing of specific amino acids to milk production causes enrichment relative to blood cells is unknown. In addition, the identification of the biochemical pathways involved in urea synthesis that result in isotopic discrimination (Balter et al. 2006) merits further investigation.

The incorporation of milk macronutrients into muscles in kits followed the expected relation with growth (Carleton and Martínez del Rio 2010), especially for carbon. Although kits had attained less than 10% of their adult body mass at the end of the experiment, their isotope signatures had equilibrated with that of the milk. This rapid incorporation of milk isotopic values in kits may explain the constant difference between offspring, milk, and maternal serum through lactation reported by Jenkins et al. (2001). The effects of continued growth on isotopic incorporation and discrimination values in mammals, especially after weaning, should be the focus of future studies.

Although we found a strong correlation between total litter mass and isotopic incorporation rate for pregnant females, the effects of pregnancy were not substantially different from nonpregnancy mass gain. Similarly, we found no difference in isotopic incorporation patterns between lactating and non-lactating females. It is unlikely that N routing into muscle is similar to routing into developing fetuses. We found no difference in adult female $\delta^{15}N$ values between blood and muscle, yet observed a 2.5‰ enrichment for $\delta^{15}N$ in kit muscles shortly after birth, indicating some form of placental discrimination. To disentangle the effects of diet composition from tissue growth and nutrient routing, future studies should attempt to repeat our effort with a larger sample size. Nonetheless, we suggest that when sampling red blood cells, field researchers could account for the effects of pregnancy and lactation as they would for other changes in body mass.

Interpretation of the effects of mass changes on the dynamics of isotopic incorporation in adults requires further scrutiny. In adult mammals, blood cell volume and blood cell counts rarely vary among individuals of the same species under the same oxygen pressure (Crait et al. 2012; Storz et al. 2010). Thus, the observed changes in body mass of the experimental mink resulted from metabolism of fat and muscle (including the uterus and fetuses in pregnant females) rather than an increase in the number of red blood cells. Therefore, the incorporation of dietary isotopic signatures into blood cells is a function of the degree of nutrient routing to other tissues of the body. The negative correlation between $\Delta^{15}N_{\text{mothers–group means}}$ and litter mass supports the idea that growth or catabolic turnover in one tissue (or compartment) could influence the isotopic incorporation in another.

Recently, Cerling et al. (2007) and Carleton et al. (2008) discussed the merits of fitting 1-compartment or multi-compartment models to describe isotopic incorporation in different tissue types. Carleton et al. (2008) demonstrated that residence time slightly increased for liver, muscle, heart, and gizzard tissues in house sparrows (*Passer domesticus*) when isotopic incorporation into these tissues was modeled with 2-

Table 3.—Discrimination values (in ‰) between blood, liver, muscle, and adipose tissues and diet in captive mink that consumed the preexperimental diet (Original) and 3 experimental diets (Beef, Fish, and Mixture) that differed in C:N, isotope values (Table 1), and amino acid composition. The experiment lasted 77 days, except for the Beef group, for which the experiment ended after 56 days. For that group, liver, muscle, and adipose values represent a 2nd dietary switch to the Mixture diet for the last 21 days of the experiment. This is reflected by the high discrimination values for liver and muscle for the Beef group. For the Beef and Mixture dietary treatments discrimination values for $\delta^{13}C$ also were calculated in relation to the isotopic signatures of the proteinaceous component of the diet (see Table 1). For estimates of variance see Table 4.

Diet	*n*	Blood–diet	Blood–dietary protein	Liver–diet	Liver–dietary protein	Muscle–diet	Muscle–dietary protein	Adipose–dietary lipids
$\delta^{13}C$								
Original	4	4.1	—	2.3	—	4.0	—	—
Beef	7	4.1	2.1	1.8	−0.2	4.4	2.4	2.2
Mixture	7	3.1	1.5	0.8	−0.8	3.0	1.4	2.2
Fish	10	1.8	—	0.3	—	1.2	—	—
$\delta^{15}N$								
Original	4	2.9	—	3.7	—	3.8	—	—
Beef	7	3.9	—	6.9	—	6.2	—	—
Mixture	7	2.5	—	3.6	—	3.1	—	—
Fish	10	2.5	—	3.2	—	2.6	—	—

compartment models. Although the treatment of single tissues as multicompartment units may be superfluous, it is possible that assessment of isotopic incorporation into 1 tissue, such as blood cells, should be modeled while considering the entire body as a multicompartment unit. In such models, accounting for the effects of compartment-specific (e.g., muscle, liver, or adipose tissue) catabolic turnover rates and growth patterns will be needed. This will require measuring incorporation rates in all tissues simultaneously, a daunting task (but see Carleton et al. 2008) involving the sacrifice of multiple animals.

We did not expect changes in $\delta^{13}C$ and $\delta^{15}N$ for bone collagen over the short experimental period. Numerous studies have demonstrated that incorporation rate in bone collagen is slow even for small-bodied species (Carleton et al. 2008; Koch 2007; Tieszen and Fagre 1993). The similarity in bone collagen isotope values among young (1–2 years) and old (3–5 years) mink, however, suggested that they were fed the same diet for multiple years, although it may not have been the Original diet as indicated by the low $\Delta^{15}N_{bone–Original}$ (= 2.9‰) and high $\delta^{13}C_{bone–Original}$ (= 7.8‰). An older batch of food obtained from Oregon State University ($\delta^{13}C$ = −20.4‰; $\delta^{15}N$ = 6.5‰) yielded $\Delta^{15}N_{bone–old\ batch}$ = 5.2‰ and $\Delta^{13}C_{bone–old\ batch}$ = 4.9‰, which are similar to reported field values (Koch 2007; Murphy and Bowman 2006). Thus, the isotopic discrimination values we report here for bone collagen should be viewed with caution.

The correlations we observed in $\delta^{13}C$ and $\delta^{15}N$ between blood cells, muscle, and liver suggest that the latter 2 tissues were tracking the dynamics of isotopic incorporation of blood cells, especially that of N (more similar $\Delta^{15}N_{tissue–diet}$), and more so in the Fish diet (indistinguishable $\delta^{15}N$ values across tissues). In addition, if we assume that the liver and muscle $\delta^{15}N$ values of the 4 mink that died on day 0 of the experiment represent the initial values for all mink (given that all animals had the same values for red blood cells on that day) the change in $\delta^{15}N$ values after 77 days were similar among tissues in a given diet treatment ($\Delta^{15}N_{Original–Mixture}$ = 0.7 for blood, 1.0 for liver, and 0.4 for muscle, and $\Delta^{15}N_{Original–Fish}$ = 2.5 for blood, 2.4 for liver, and 1.7 for muscle). Although previous studies of determinate and indeterminate growers of various sizes have shown a slower turnover rate for muscle compared with blood cells (Carleton et al. 2008; Cerling et al. 2007), examination of our $\delta^{15}N$ data suggests that by the end of the experiment the isotopic signatures of the dietary macromolecules had been largely assimilated into muscle tissue. That no such assimilation was evident for $\delta^{13}C$ indicates that mink in the 3 diet treatments differentially oxidized lipids and amino acids.

Effects of dietary composition on incorporation rates and discrimination values.—Mink fed the Beef diet rapidly incorporated dietary N and reached a new isotopic equilibrium despite the lower biological value of that diet (Robbins et al. 2005). We suspect that oxidation of the energy-dense lipids (~50% of diet) allowed individuals to route most dietary amino acids to tissue synthesis, hence the rapid equilibration after approximately 43 days. In contrast, the biological value of the Fish diet was high, suggesting a rapid incorporation of dietary $\delta^{15}N$ and lower discrimination values (Florin et al. 2011; Kelly and Martínez del Rio 2010; Sick et al. 1997). Florin et al. (2011) observed that supplementation of diets containing more than 50% protein with carbohydrates and lipids, reduced the $\delta^{15}N$ between laboratory rats and their diet suggesting that most amino acids were routed to synthesis rather than oxidation. In our study, the low lipid content of the Fish diet likely forced the mink to oxidize some dietary amino acids (Sick et al. 1997). Because glycine dominated the proteinaceous component of the Fish diet (Fig. 4), its oxidation (McCue et al. 2010) instead of assimilation likely yielded the observed lag in $\delta^{15}N$ incorporation. In addition, the Fish-fed mink probably recycled endogenous fatty acids to metabolize new cell membranes (Yamashita et al. 1997), hence the similarly long delay in $\delta^{13}C$ incorporation in that group.

TABLE 4.—Mean stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values (‰ ± *SD*) for red blood cells, liver, muscle, adipose fat, and bone collagen in captive mink that consumed the preexperimental diet (Original) and 3 experimental diets (Beef, Fish, and Mixture) that differed in C:N, isotope values (Table 1), and amino acid composition. The experiment lasted 77 days, except for the Beef group, for which the experiment ended after 56 days. For that group, liver, muscle, and adipose fat values represent a 2nd dietary switch to the Mixture diet for the last 21 days of the experiment. Blood $\delta^{13}C$ for that group at 77 days was −19.9 (± 0.1) and $\delta^{15}N$ was 11.3 (± 0.2).

Diet	*n*	Blood	Liver	Muscle	Adipose fat	Bone collagen
$\delta^{13}C$						
Original	4	−19.2 (0.2)	−21.0 (0.5)	−19.3 (0.2)	−22.1 (0.1)	−15.5 (0.3)
Beef	7	−20.1 (0.1)	−22.4 (0.7)	−19.8 (0.3)	−24.0 (0.9)	−15.4 (0.2)
Mixture	7	−19.6 (0.1)	−21.9 (1.0)	−19.7 (0.2)	−24.0 (0.3)	−15.5 (0.2)
Fish	10	−18.7 (0.1)	−20.2 (0.4)	−19.3 (0.4)	−23.1 (0.7)	−15.3 (0.1)
$\delta^{15}N$						
Original	4	11.7 (0.3)	12.5 (0.2)	12.6 (0.1)	12.3 (0.8)	11.4 (0.8)
Beef	7	10.1 (0.1)	13.1 (0.5)	12.4 (0.2)	12.6 (0.6)	12.0 (0.3)
Mixture	7	12.4 (0.2)	13.5 (0.5)	13.0 (0.4)	12.9 (0.5)	11.7 (0.6)
Fish	10	14.2 (0.3)	14.9 (0.4)	14.3 (0.6)	14.0 (0.5)	12.1 (0.5)

Three concurrent lines of evidence support this conclusion. First, unlike previous studies (Podlesak and McWilliams 2007), we observed a 2‰ $\Delta^{13}C_{adipose–dietary\ lipids}$ in the Beef and Mixture groups. It is possible that some ^{13}C-depleted dietary fatty acids were routed to liver. The higher $\Delta^{13}C_{liver–dietary\ lipids}$ of ~4‰, however, is opposite of this expectation. Second, the correlation between dietary C:N and $\delta^{13}C$ between blood cells and diet suggests that when animals had high lipid availability, preferential oxidation of fatty acids resulted in high discrimination. Finally, the $\delta^{13}C$ between mink red blood cells and the proteinaceous component of the Beef diet was only 2.1‰, which is close to observed discrimination values for lipid-extracted tissues in other carnivores (Roth and Hobson 2000; Zhao et al. 2006). Despite the small difference in $\delta^{13}C$ and $\delta^{15}N$ between the Original and Mixture diet treatments, the time to full assimilation of dietary carbon isotopic signal was similar to that of Beef diet (55 and 54 days, respectively), suggesting that similar processes were occurring in these individuals.

The 2‰ enrichment in the adipose tissues of mink in the Beef and Mixture groups is opposite of the depletion commonly observed following the routing of dietary carbohydrates to lipid synthesis (DeNiro and Epstein 1977), or lack of change when endogenous fat is synthesized from dietary lipids (DeNiro and Epstein 1978; Podlesak and McWilliams 2007). This was unexpected, and could be explained by preferential oxidation of fatty acids. That is, if carnitine acyltransferase I (Kerner and Hoppel 2000) preferentially acts on ^{12}C atoms in the thioester bond of the acyl carrier molecule, similar to the preferential activity of pyruvate dehydrogenase on ^{12}C atoms in the carbonyl position (DeNiro and Epstein 1977), fatty acids bearing ^{13}C atoms would be more available for storage. Alternatively, differential oxidation and storage of

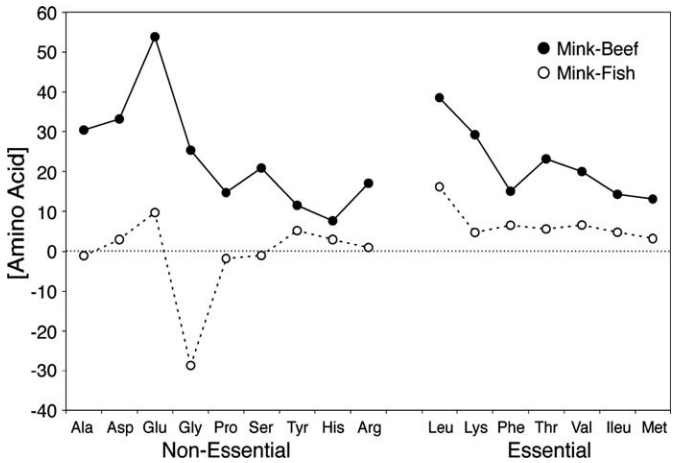

FIG. 4.—Difference in amino acid concentrations (nM/100 μg dry matter) between mink muscle and diet for Beef and Fish. Amino acid (AA) composition of Fish was closer to that of mink than Beef for both essential and nonessential amino acids. Glycine was particularly abundant in the Fish diet. Lines are drawn to highlight differences between the 2 treatments and are for illustration purposes only.

TABLE 5.—Amino acid concentrations (nM/100 μg dry matter) for mink muscle ($\bar{X}$ ± *SD*; $n = 4$), and Beef and Fish diets. The differences between mink muscle composition and amino acid concentration in the diets are illustrated in Fig. 4.

			Mink	
Amino acid	Beef	Fish	$\bar{X}$	*SD*
Alanine (ALA)	28.1	59.5	58.5	8.5
Arginine (ARG)	11.9	27.9	28.9	4.1
Aspartic acid (ASP)	21.0	51.2	54.1	8.8
Cysteine (CYS)	0.8	1.7	4.0	0.9
Glutamic acid (GLU)	37.3	81.5	91.2	18.8
Glycine (GLY)	44.2	98.2	69.5	11.8
Histidine (HIS)	4.7	9.4	12.3	7.4
Isoleucine (ILE)	7.5	17.1	21.8	3.4
Leucine (LEU)	18.6	41.1	57.2	4.4
Lysine (LYS)	18.0	42.6	47.2	11.8
Methionine (MET)	4.7	14.6	17.8	0.6
Phenylalanine (PHE)	7.2	15.7	22.2	2.0
Proline (PRO)	18.2	34.7	32.9	4.4
Serine (SER)	14.3	36.1	35.1	6.3
Threonine (THR)	12.3	29.9	35.4	4.3
Tyrosine (TYR)	5.1	11.4	16.5	1.6
Valine (VAL)	11.1	24.6	31.1	3.2

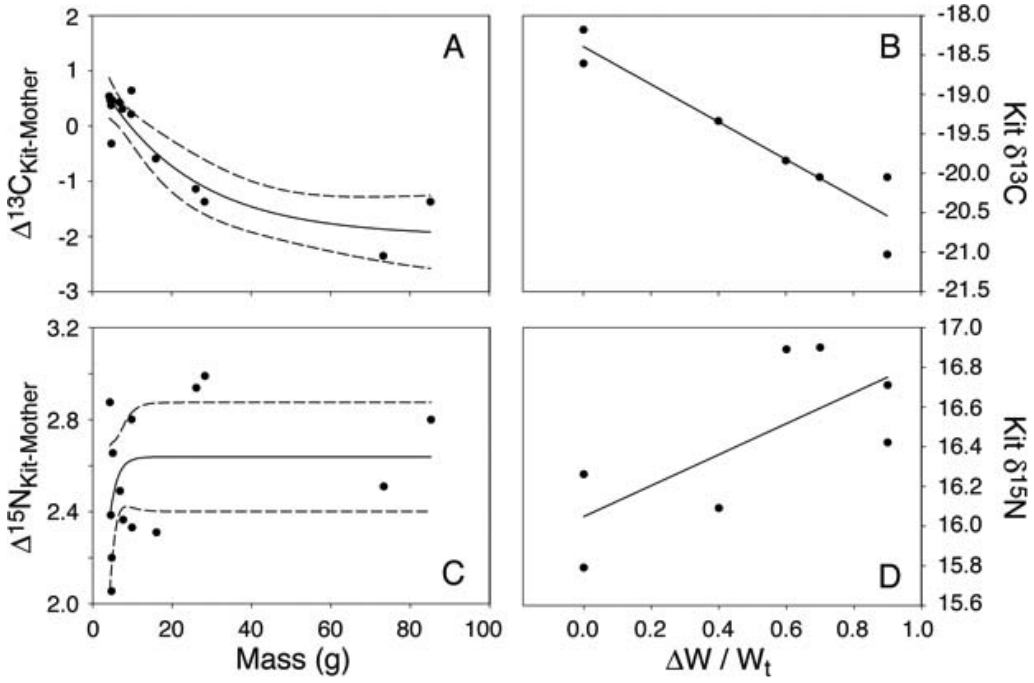

Fig. 5.—Difference between A) $\delta^{13}C$ and C) $\delta^{15}N$ (in ‰) of the kit muscle tissues and the mother red blood cells in relation to the kit body mass. The difference was calculated to account for changes in the mother isotopic blood values through time (over the 21 days since parturition) and the difference in isotopic values between experimental groups. Kit $\delta^{13}C$ and $\delta^{15}N$ values in the first 2 days after birth reflect in utero discrimination followed by incorporation of milk macronutrients (see equations in Table 2). Dashed lines represent 95% confidence intervals. Curves describing the relation between mass change ($\Delta w/w_t$) and B) $\delta^{13}C$ and D) $\delta^{15}N$ were linear (Table 2), suggesting that incorporation rates were largely a function of growth rather than catabolic turnover of muscle.

fatty acids could be related to carbon chain length and degree of saturation (Raclot and Groscolas 1994; Whiteman et al. 2012); if fatty acids differing in chemical identity also have distinct isotopic composition, discrimination could occur. Testing the effects of oxidation and enzymatic fractionation on $\Delta^{13}C_{adipose\text{–}dietary\ lipids}$ will likely require the application of compound-specific isotope analysis (Ben-David and Flaherty 2012; Evershed et al. 2007).

Conclusions.—Here we demonstrated that for carnivores, lipid content affects incorporation of carbon and nitrogen from dietary macronutrients even when the biological value of the food is high. Recently, Florin et al. (2011) demonstrated that fat contents of the diet may reduce the discrimination values of $\delta^{15}N$ in omnivores fed a high-quality protein diet, suggesting that lipid metabolism may be influential for isotopic incorporation in noncarnivores as well. Based on our results and those of Florin et al. (2011), we suspect, however, that for herbivores and in many cases for omnivores, lipid content of the diet may be less important than protein quality and quantity in determining incorporation rates and discrimination values (Robbins et al. 2010).

Similar to most recent studies (Florin et al. 2011; Kelly and Martínez del Rio 2010; Lecomte et al. 2011; Robbins et al. 2010), examination of our data suggests that because of oxidation of particular macronutrients, routing to different tissues (or compartments), and changes in body mass (or growth of nonsampled compartments), the reconstruction of diets with dual-isotope mixing models (Phillips 2012) may be problematic (e.g., Barnes et al. 2007; Kelly and Martínez del Rio 2010). To reconstruct diets from the isotopic signatures of bulk food and tissues it will be necessary to determine the macromolecular (i.e., lipid, carbohydrate, and protein) composition of the diet beyond C:N or percent protein, the growth status of the animal (if juvenile), or mass change (if an adult). Therefore, we join our colleagues in advocating the development of pertinent compound-specific isotopic analyses (Ben-David and Flaherty 2012; Evershed et al. 2007; Larsen et al. 2009; Newsome et al. 2011). We recommend conducting theory-based experiments that will provide knowledge on the

Table 6.—Mean (± *SD*) discrimination values (in ‰) between kit muscle and maternal blood cells and milk. Postpartum values were calculated for all kits that perished on days 1–2 after birth ($n = 9$) and represent in utero processes. Values at the end of the experiment (21 days after parturition) for kits were derived from incorporation equations listed in Table 2. Also reported are the differences in isotopic values of milk and blood cells for 2 of the mothers and 2 pseudopregnant females that produced milk.

Sampling	*n*	$\delta^{13}C$	$\delta^{15}N$
At birth			
Kit–maternal blood	9	0.3 (0.3)	2.5 (0.3)
After 21 days			
Kit–maternal blood	13	−1.5 (0.2)	2.8 (0.1)
Kit–maternal milk	13	1.5 (0.2)	1.6 (0.1)
Milk–maternal blood	4	−2.6 (0.4)	1.1 (0.2)

underlying dynamics of isotopic incorporation of essential fatty and amino acids that will be crucial for interpretation of these data. As a model for mammalian carnivores we recommend the mink, which can be easily maintained and bred in captivity.

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