Isotopic Incorporation and the Effects of Fasting and Dietary Lipid Content on Isotopic Discrimination in Large Carnivorous Mammals

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ABSTRACT

There has been considerable emphasis on understanding isotopic discrimination for diet estimation in omnivores. However, discrimination may differ for carnivores, particularly species that consume lipid-rich diets. Here, we examined the potential implications of several factors when using stable isotopes to estimate the diets of bears, which can consume lipid-rich diets and, alternatively, fast for weeks to months. We conducted feeding trials with captive brown bears (*Ursus arctos*) and polar bears (*Ursus maritimus*). As dietary lipid content increased to ~90%, we observed increasing differences between blood plasma and diets that had not been lipid extracted ($\Delta^{13}C_{tissue\text{-}bulk\ diet}$) and slightly decreasing differences between plasma $\delta^{13}C$ and lipid-extracted diet. Plasma $\Delta^{15}N_{tissue\text{-}bulk\ diet}$ increased with increasing protein content for the four polar bears in this study and data for other mammals from previous studies that were fed purely carnivorous diets. Four adult and four yearling brown bears that fasted 120 d had plasma $\delta^{15}N$ values that changed by $< \pm 2‰$. Fasting bears exhibited no trend in plasma $\delta^{13}C$. Isotopic incorporation in red blood cells and whole blood was ≥6 mo in subadult and adult bears, which is considerably longer than previously measured in younger and smaller black bears (*Ursus americanus*). Our results suggest that short-term fasting in carnivores has minimal effects on $\delta^{13}C$ and $\delta^{15}N$ discrimination between predators and their prey but that dietary lipid content is an important factor directly affecting $\delta^{13}C$ discrimination and indirectly affecting $\delta^{15}N$ discrimination via the inverse relationship with dietary protein content.

Keywords: bears, brown bears, carbon, nitrogen, polar bears.

Introduction

Natural variation in carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values among primary producers and trophic levels produces a natural marker for quantifying dietary contributions of plant versus animal or marine versus terrestrial food (Kelly 2000). However, a number of recent reviews have called for further experimentation to better understand the role that animal physiological state, dietary macronutrient composition, and species-specific physiological differences play in affecting isotopic incorporation (i.e., turnover rates) and trophic discrimination between consumer tissues and their diet. This information is required for accurate diet estimation (Bauchinger and McWilliams 2009; Martinez del Rio et al. 2009; Boecklen et al. 2011; Bowen and Iverson 2012). Furthermore, several studies have documented that dietary macronutrients, such as carbohydrates, proteins, and lipids, vary in their contribution to tissue synthesis and thereby affect isotopic incorporation and discrimination (Podlesak and McWilliams 2006; Federer et al. 2010; Budge et al. 2011; Cherry et al. 2011; Newsome et al. 2014). Better understanding of routing and incorporation of macronutrients into consumer tissues and their effects on isotopic discrimination is important for improving isotope-based dietary estimates.

Marine and terrestrial carnivores consume diets composed primarily of lipids and proteins, yet the majority of isotope-derived estimates of diet are typically based on nonlipid resources. Post et al. (2007) suggested that because lipids have lower $\delta^{13}C$ than proteins, samples should be lipid extracted when diets consist of >10% lipids. Following this guideline, potential prey sources

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are routinely lipid extracted before stable isotope analysis (e.g., Bentzen et al. 2007). In essence, removal of dietary lipids follows the assumption that consumers completely route dietary protein into structural proteinaceous tissues. While this assumption may be logical for those animals that feed primarily on carbohydrates and protein, inclusion of the ^{13}C-depleted dietary lipids into calculations of δ^{13}C trophic discrimination factors may increase the accuracy of diet estimation for carnivores that consume lipid-rich diets (Newsome et al. 2010, 2014; Ben-David et al. 2012; Parng et al. 2014; Wolf et al. 2015). Indeed, recent studies utilizing compound-specific isotope analysis indicate that the carbon skeletons of nonessential amino acids can be synthesized from both the protein and lipid portions of omnivore diets (Newsome et al. 2014). This finding is important because lipids typically have lower δ^{13}C values than associated proteins by as much as 6‰–8‰ (Cherry et al. 2011). Thus, excluding lipids may result in inaccurate tissue-diet isotopic discrimination factors, which could be a source of significant bias in diet estimation (Caut et al. 2009). However, discrimination factors that include the lipid fraction of the diet are currently available for a limited number of small-bodied carnivores (e.g., Ben-David et al. 2012; Stricker et al. 2015).

The accuracy of stable isotope–derived diet estimation for carnivores is also limited by our understanding of the role fasting may play in affecting tissue isotope values. Many large carnivores living in temperate to arctic environments often go days to weeks between meals (Smith et al. 2004; Sand et al. 2005; Cherry et al. 2009; Cavalcanti and Gese 2010). Indeed, several studies have documented changes in tissue isotope values and associated trophic discrimination during fasting (Hobson et al. 1993; Polischuk et al. 2001; Cherel et al. 2005; Fuller et al. 2005; Gaye-Siessegger et al. 2007). Ursids in particular are physiologically adapted to fast for long periods during which they metabolize lipid stores and minimize lean muscle loss by recycling urea nitrogen (Nelson 1987; Stenyinkel et al. 2013). This occurs throughout hibernation but also during periods of seasonal reductions in food availability (Polischuk et al. 2001).

When feeding selectively on marine mammals, particularly ringed (*Phoca hispida*) and bearded (*Erignathus barbatus*) seals that are accessed from sea ice (Thiemann et al. 2008), polar bears (*Ursus maritimus*) consume diets of up to 80% lipid (Best 1985; Cherry et al. 2011). Similarly, coastal brown bears (*Ursus arctos*) are largely carnivorous during certain periods of the year (Hilderbrand et al. 1999*b*) and often select for lipid-rich prey items (Gende et al. 2001, 2004). Both polar bears and brown bears also undergo seasonal fasts (Farley and Robbins 1995; Polischuk et al. 2001), although brown bears primarily fast during hibernation and consume plant-based diets when prey are not available (Hilderbrand et al. 1999*a*). Stable isotope studies of brown bears have focused more on estimating the contribution of meat versus plant or marine versus nonmarine food sources (Hilderbrand et al. 1999*a*; Ben-David et al. 2004; Edwards et al. 2011), while only a few studies have used δ^{13}C and δ^{15}N isotope values in polar bear blood and adipose tissue to estimate diets (Bentzen et al. 2007; Cherry et al. 2011). Many isotope-based dietary studies of ursids have used isotope discrimination factors from a study of yearling American black bears (*Ursus americanus*) fed omnivorous diets containing low lipid contents (Hilderbrand et al. 1996). These younger bears likely have shorter isotopic incorporation rates and lower isotopic discrimination factors (especially for δ^{15}N) because of their rapid growth and therefore greater retention of both carbon and nitrogen needed to synthesize new structural tissue in comparison to adult brown bears and polar bears (Lecomte et al. 2011). Further, trophic discrimination in bears fed omnivorous diets may differ greatly from those consuming a high-lipid and high-protein hypercarnivorous diet.

Therefore, we conducted studies with captive brown and polar bears to quantify (1) the effect of dietary lipid content on isotopic discrimination, (2) the effect of fasting on tissue isotope values, and (3) δ^{13}C and δ^{15}N discrimination and incorporation in a variety of tissues collected from bears fed high-lipid diets. We fed diets that varied in lipid content but mimicked the carnivorous marine-based diets consumed by wild polar bears and seasonally by brown bears. Effects of fasting were examined as captive brown bears entered and proceeded through hibernation.

Methods

Diets and Study Animals

Conducting feeding trials on large carnivores presented several challenges. Captive polar bears reside only in zoos, where the primary focus is exhibition and in which sedation for research-associated sample collection is rarely approved. We were able to collaborate with two zoos, one (Alaska Zoo) that agreed to sedate its polar bears ($n = 2$, 450-kg adult male and 300-kg female) at the beginning and end of feeding trials and the other (Oregon Zoo) that had trained polar bears ($n = 2$, 468-kg adult male and 210-kg adult female) for collection of blood and hair without sedation. We also conducted feeding trials with captive brown bears (four adult bears, four 1-yr-old bears, and four cubs-of-the-year) at Washington State University (WSU) Bear Research, Education, and Conservation Center, which similarly had limitations on the number of times bears could be sedated and the duration they could be fed experimental diets. Also, adult bears could not be kept in small concrete-floored pens for long periods of time per Institutional Animal Care and Use Committee (IACUC) approvals. Thus, younger bears were used in trials lasting more than ~40 d. Research was permitted under the Marine Mammal Protection Act and Endangered Species Act under US Fish and Wildlife Service permit MA95406A-0 and followed protocols approved by the IACUCs of the US Geological Survey's Alaska Science Center, the Alaska Zoo, the Oregon Zoo, and WSU.

A fixed diet ranging in dietary lipid content from 45% to 68% and composed of marine food sources was fed to two adult male and female polar bears at the Alaska and Oregon Zoos. The diet of polar bears at the Alaska Zoo consisted of pink salmon (*Oncorhynchus gorbuscha*), herring (*Clupea pallasii*), and herring oil. At the Oregon Zoo, polar bears were fed pink salmon, herring, capelin (*Mallotus villosus*), and salmon oil (table 1). Brown bears at WSU were fed diets composed of hatchery-produced Chinook salmon (*Oncorhynchus tshawytscha*) and salmon oil (Jedwards International bulk wild Alaskan salmon oil) or Hill's Science Diet Ad-

Table 1: Diets fed to captive brown and polar bears

Bears	*N*	Diet	% protein	% lipid	Trial length (d)	Bulk $\delta^{13}C$	Bulk $\delta^{15}N$	Lipid $\delta^{13}C$	Protein $\delta^{13}C$
Brown bear cubs-of-the-year	4	Dog chow	24.4	11	90	−17.6	3.7	…	−17.7
Brown bear cubs-of-the-year	4	Dog chow + salmon oil	14.9	39	89	−20.6	3.9	−24.0	−17.8
Brown bear yearlings	4	Dog chow + fish oil	16.4	43	79	−19.8	3.4	…	−17.4
Brown bears (age variable)	4	Salmon and salmon oil	43	49	15	−22.8	14.3	…	−19.4
Brown bears (age variable)	6	Salmon and salmon oil	38	59	15	−23.7	14.0	…	−19.4
Brown bears (age variable)	2	Salmon and salmon oil	28	68	15	−24.5	14.3	…	−19.4
Brown bears (age variable)	8	Salmon and salmon oil	62	25	15	−22.1	11.5	…	−19.6
Brown bears (age variable)	4	Salmon and salmon oil	7	92	10	−26.0	11.5	…	−19.6
Adult brown bears	4	Salmon and salmon oil	43	49	41	−23.0	13.5	…	−19.4
Adult polar bears (Alaska zoo)	2	Salmon, herring, and herring oil	27.3	68	100	−21.4	13.4	−24.9	−18.2
Adult polar bears (Oregon zoo)	2	Salmon, herring, capelin, and salmon oil	46.3	45	104	−21.0	13.2	−23.2	−18.8

Note. Captive brown bears included four adults, four 1-yr-olds, and four cubs-of-the-year, and polar bears included two adult males and two adult females. Protein and lipid composition is percent dry matter. Protein $\delta^{13}C$ is the lipid-extracted diet value. Bulk indicates that the sample was not lipid extracted.

vanced Fitness Original commercial dog chow (hereafter referred to as "chow") and salmon oil. These diets were chosen in an attempt to mirror the marine-based diet rich in unsaturated fat consumed by wild brown bears selectively eating salmon and wild polar bears consuming marine mammals, while simultaneously allowing for experimental manipulation of dietary protein-to-fat ratios (table 1). Chow was fed to brown bear cubs or yearlings only during long-duration (>70 d) trials to ensure that they received a nutritionally balanced diet. We strove to feed bears at or near weight maintenance. Experimental diets of seals or other marine mammals would have been ideal to duplicate the diet of wild polar bears; however, the US Marine Mammal Protection Act precludes the collection of seals and other marine mammals for the purpose of research on another species. Further, collection of dead, stranded marine mammals as a food source for polar bears could not be approved under IACUC due to the potential for disease transmission. Therefore, we used other marine-based food resources (e.g., marine fish and fish oils) to simulate the lipid-rich marine mammal diet of wild polar bears.

Isotopic Incorporation

"Isotopic incorporation" refers to the process by which isotopes get incorporated into tissues, which is often quantified with a half-life, defined as the median residence time (in days) of an element in a tissue (Martinez del Rio and Carleton 2012). We quantified isotopic incorporation for $\delta^{13}C$ and $\delta^{15}N$ in plasma, serum, red blood cells (RBC), whole blood, and hair for the two adult polar bears trained for voluntary blood sampling at the Oregon Zoo. These bears were fed a marine lipid-rich diet (table 1) for 104 d and sampled at days 0, 8, 14, 28, 41, 55, 83, and 104. Because hair is an isotopically inert tissue, incorporation represents the time required for the body pools used to synthesize new hair to equilibrate with the current diet after a dietary shift. We also estimated the half-life of plasma and serum for four adult brown bears (two females weighing 125 and 148 kg and two males weighing 292 and 300 kg) fed a 50 : 50 salmon : salmon oil diet for 41 d sampled weekly and of plasma and adipose tissue of four cub-of-the-year brown bears sampled monthly fed dog chow and salmon oil for 89 d (table 1). In all trials, a nonmarine pretrial diet consisting of vegetation, freshwater fish, and/or terrestrial meat was fed to all bears before the marine-based experimental diet to allow for a distinct isotopic shift in tissues. We fitted equilibration curves using equation (3) from Martinez del Rio and Carleton (2012): $\delta X(t) = \delta X_\infty - (\delta X_\infty - \delta X_0)e^{-\lambda t}$, where $\delta X(t)$ is the tissue isotopic ratio at time t, δX_∞ is the isotopic ratio at equilibration, δX_0 is the isotopic ratio at time 0, and λ is the isotopic incorporation rate. The half-life is then estimated as $\ln(2)/\lambda$.

Isotopic Discrimination

"Isotopic discrimination" refers to the difference in the isotopic composition between a tissue and the diet (Martinez del Rio et al. 2012). Using the data collected from the four adult brown bears fed experimental diets for 41 d, we estimated discrimination for $\delta^{13}C$ and $\delta^{15}N$. In addition, the following

year we conducted a set of four 15-d trials to estimate the effect of dietary lipids ranging from 25% to 68% on $\delta^{13}C$ and $\delta^{15}N$ discrimination in plasma using brown bears ranging in size from subadults to adult males (table 1; $n = 2–8$). A separate trial was conducted with four brown bears fed a diet with a lipid content of 92% for 10 d due to concerns about potential health effects of such a lipid-rich diet. Bears were sampled at the beginning and end of the trials. Similarly, we conducted trials with four first-year and second-year brown bear cubs on chow and salmon or fish oil diets lasting ≥79 d to estimate isotopic discrimination and half-lives. Tissues from these individuals were sampled monthly.

Brown bears were anesthetized with 2.5–3 mg/kg of tiletamine HCl and zolazepam HCl (Telazol, Pfizer Animal Health, New York) and 0.006–0.007 mg/kg of dexmedetomidine HCl (Dexdomitor, Pfizer Animal Health; Teisberg et al. 2014). Blood was collected from the jugular, cephalic, or femoral artery, and a fat biopsy was collected within 15 cm laterally from the base of the tail, where a fat deposit typically forms (Thiemann et al. 2008). For the trained polar bears housed at the Oregon Zoo, blood was collected from a vein in the arm. Hair was collected by shaving an area on the top of the forearm at the start of a trial and subsequently shaving the same location on each sampling date. The two Alaska Zoo polar bears were sedated and sampled at the beginning and end of the trials, which also allowed for collection of fat biopsies.

We report isotopic discrimination as $\Delta X = \delta X_{\text{tissue}} - \delta X_{\text{diet}}$, where X = either ^{15}N or ^{13}C, where tissue (blood, hair, adipose fat) and diet are derived from the specific feeding trials. In most cases, dietary items were analyzed separately for their isotopic composition because they could not be homogenized in the same proportion as fed. Composite diet isotope values were determined by summing the product of dry weight proportions of each item and corresponding isotope values. We calculated separate $\delta^{13}C$ values for the lipid and protein contributions by similarly summing the product of dry weight proportions of total dietary lipid or protein contributed by a dietary item and $\delta^{13}C$ of lipids or proteins in that dietary item. Mixed-effects linear models including individual bears as random effects were used to identify relationships between dietary lipid content and carbon discrimination values. To obtain a more comprehensive understanding of discrimination for $\delta^{15}N$, we also combined our data with those of several other studies (Hilderbrand et al. 1999*a*; Kurle 2002; Lecomte et al. 2011; G. Stenhouse, unpublished data). We used regression analyses to assess the relationship between percent dietary protein and $\delta^{15}N$ values in plasma.

Effects of Fasting

To determine the effect of fasting on $\delta^{13}C$ and $\delta^{15}N$ values in blood plasma, RBC, hair, and adipose tissue, four adult brown bears and four yearlings were sampled before and during hibernation. Hair and blood were collected from adults sampled at 0, 2, 4, and 16 wk. Food was removed on day 0. Hair, blood, and adipose tissue were collected from yearlings at 0, 4, 7, 12, and 20 wk. Bears hibernated either singly or in pairs in individual 3 × 3-m concrete dens and were given a bale of straw bedding. Bears were restricted to their dens and retained access to larger outside concrete runs. Studies during hibernation have demonstrated that activity levels can reach 2% of those during summer in December, January, and early February (Robbins et al. 2012). Beginning and ending $\delta^{13}C$ and $\delta^{15}N$ values for plasma and RBC were compared using paired *t*-tests across individual bears. A mixed-effects generalized linear model was used to identify trends in $\delta^{13}C$ and $\delta^{15}N$ across all samples collected during fasting to account for repeated measures of individual bears.

Tissue and Diet Stable Isotope Analyses

Blood was collected in purple-top ethylene-diamine-tetra-acetic acid whole-blood tubes and red-top no-additive tubes. After collection, blood was centrifuged, and plasma, serum, and RBC were separated. Samples of food items comprising experimental diets were collected at least once during each trial and multiple times throughout trials when the source or batch changed. Food and tissue samples were stored frozen and freeze-dried before laboratory processing. Hair samples were cleaned using a 2:1 chloroform:methanol solution and allowed to air dry. Dried blood samples were pulverized using a glass rod but not lipid extracted because we hypothesized that the high-lipid diets warranted including the lipid portion of samples in discrimination estimates. Adipose biopsies were transferred to glass vials and solvent extracted using 7 mL of a 2:1 chloroform:methanol solution. After 5 h, 5 mL of deionized water was added and vials were vortexed for 1 min. The samples were then centrifuged for 5 min, and the top layer was pipetted off and discarded. The remaining lipid residue was dried overnight in a vacuum oven set at ambient temperature to evaporate off the solvent. This residue was analyzed to determine adipose $\delta^{13}C$ values. With the exception of fish oils, food items were freeze-dried, ground in a cryogenic grinder, loaded into 10 × 50-mm cellulose thimbles, and lipid extracted by Soxhlet distillation for 5 h using a 2:1 chloroform:methanol solvent solution. The solvent and lipid residues were retained and dried in a vacuum oven maintained at ambient temperature. Isotopic discrimination and incorporation estimates were calculated using isotope data for bulk diets that were not lipid extracted, unless otherwise specified. Food samples were also analyzed for dry matter, gross energy density using bomb calorimetry, crude protein via a TruSpec carbon-nitrogen analyzer (Leco, St. Joseph, MO), and lipid content via ether extract at the WSU Wildlife Habitat and Nutrition Lab.

The $\delta^{13}C$ and $\delta^{15}N$ values were measured on tissue and diet samples by isotope ratio mass spectrometry. Approximately 1 mg of each sample was weighed into 4 mm × 6 mm tin capsules, crimp sealed, and combusted in an elemental analyzer (Carlo Erba NC2500) interfaced to a mass spectrometer (Micromass Optima) operated in continuous-flow mode (Fry et al. 1992). Isotopic data were normalized to internationally accepted scales using the primary standards USGS 40 (−26.24‰ and −4.52‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively) and USGS 41 (37.76‰ and 47.57‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively). Instrumental precision and sample reproducibility as assessed by replicate analysis were better than ±0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$.

Results

δ¹³C and δ¹⁵N Isotopic Incorporation

Although we attempted to feed bears at weight maintenance, bears gained on average 12.2 ± 17.4 kg on experimental diets. There was no relationship between weight change and dietary lipid content ($r = -0.016$, $n = 31$, $P = 0.93$).

Half-life estimates for ^{15}N were consistently longer than those for ^{13}C in the tissues we measured (i.e., serum, plasma, RBC, and whole blood; $n = 10$), including two adult polar bears, four adult

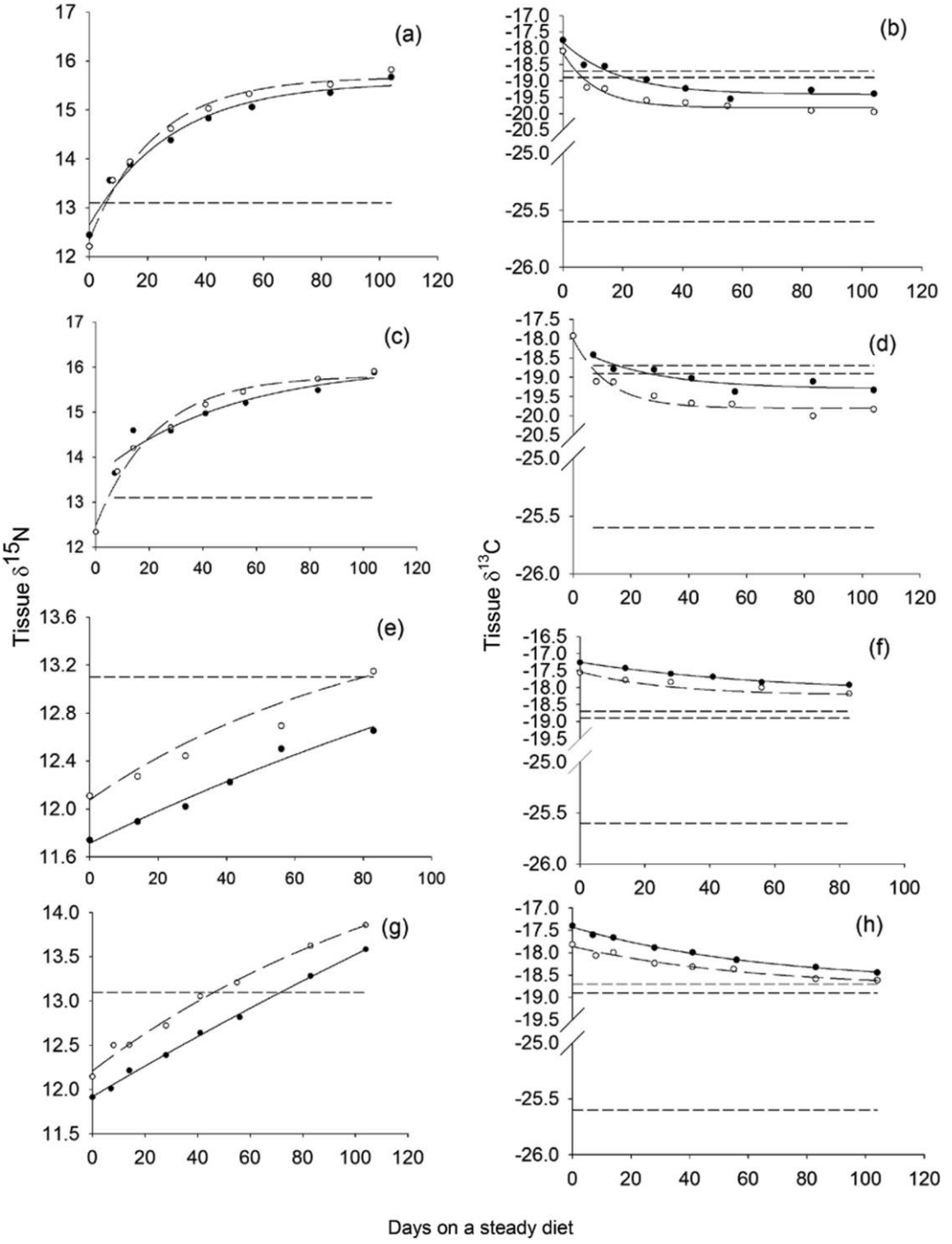

Figure 1. Change in plasma (*a*, *b*), serum (*c*, *d*), red blood cell (*e*, *f*), and whole blood (*g*, *h*) $\delta^{15}N$ (*left*) and $\delta^{13}C$ (*right*) for two adult polar bears fed lipid-rich (45%–68%) marine-based diet for 100–104 d. Data for the male are indicated by filled circles and solid line, data for the female by open circles and dashed line. Dashed lines in right panels represent $\delta^{13}C$ values of dietary protein (−18.7‰), bulk diet (−18.9‰), and dietary lipid (−25.6‰) sources. Dashed line in left panels represents the $\delta^{15}N$ of bulk diet (13.1‰).

Table 2: Instantaneous incorporation rates (λ; ^{15}N or ^{13}C per day) and half-lives (d) for ^{15}N and ^{13}C in bear tissues

	^{15}N λ		^{13}C λ			^{15}N half-life (d)		^{13}C half-life (d)		
Tissue	Adult polar bears	Adult brown bears	Adult polar bears	Brown bear cubs	Adult brown bears	Adult polar bears	Adult brown bears	Adult polar bears	Brown bear cubs	Adult brown bears
Plasma	.035/.044	.047 ± .025	.05/.09	.07 ± .02	…	19.6/15.7	14.7	7.7/12.8	9.9	…
Serum	.02/.04	.050 ± .029	.04/.08	…	…	34.7/17.3	13.8	8.4/18.0	…	…
Red blood cells	.004/.012	…	.016/.012	…	…	57.8/157.5	…	41.8/57.8	…	…
Whole blood	.0019/.0074	…	.016/.017	…	…	364.8/93.7	…	44.7/41.8	…	…
Hair	.068/.0068	…	.046/.011	…	.047[a]	NA	…	NA	…	NA
Adipose tissue	…	…	…	.029 ± .006	…	…	…	…	23.9	…

Note. Two adult polar bears (one male and one female) were maintained on a 48% lipid (on a dry matter basis) marine carnivorous diet for 104 d; λ and half-lives are provided for each individual polar bear. Four adult brown bears (two males and two females) were fed a salmon and salmon oil diet for 41 d (^{15}N in serum and plasma only), and four cub-of-the-year brown bears were fed chow and salmon oil for 89 d (adipose tissue and plasma only). Because brown bears were infrequently sampled, data were combined across individuals to estimate the values. In these cases λ is provided with the standard error. Half-life = ln(2)/λ (Martinez del Rio and Carleton 2012). Note that because hair is an inert tissue, isotopes are not turning over due to metabolic processes and half-lives are not reported. NA = not applicable.

[a]Equilibration curves converged for only two of the four adult brown bears with combined data.

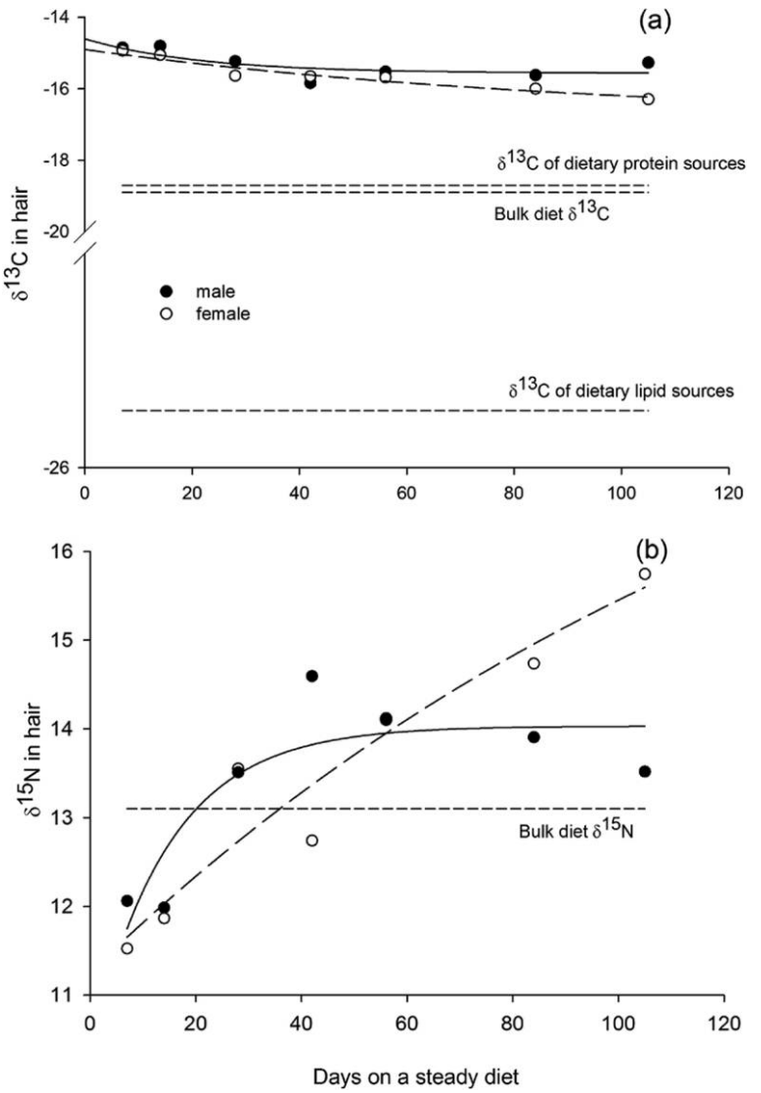

Figure 2. Change in hair $\delta^{13}C$ (*a*) and $\delta^{15}N$ (*b*) for two adult polar bears fed a lipid-rich (45%–68% of dry matter) marine-based carnivorous diet for 100–104 d. Equilibration curves exclude the initial hair sample taken at the beginning of the feeding trials. The $\delta^{13}C$ and $\delta^{15}N$ values of dietary components are provided as a dashed line.

brown bears, and four cub-of-the-year brown bears (fig. 1; table 2; apps. A, B). Plasma and serum had shorter half-lives (10–18 d; $n = 6$) for both isotopes than RBC, whole blood, and adipose tissue. Estimated ^{13}C half-lives for RBC and whole blood in the two polar bears were 50 and 43 d, respectively, but ^{15}N did not equilibrate in 104-d trials (fig. 1*e*, 1*g*).

Estimated half-lives of ^{15}N in RBC and whole blood were 108 and 229 d, respectively (table 2); however, these estimates should be applied with caution because these tissues did not completely equilibrate with experimental diets. The larger male polar bear had longer estimated half-lives of ^{15}N in both RBC and whole blood in comparison to the adult female. The ^{15}N and ^{13}C pools used to synthesize new hair took at least a month to equilibrate fully with the current diet for four adult brown and two adult polar bears (fig. 2). The half-life of ^{13}C in adipose tissue of four brown bear cubs was 24 d, which is likely an underestimate for adult bears (table 2; fig. 3; app. A).

Isotopic Discrimination

The $\delta^{13}C$ trophic discrimination (i.e., $\Delta^{13}C_{\text{tissue-bulk diet}}$) increased in plasma with increasing dietary fat content for brown bears and polar bears (fig. 4). The slope of this relationship was similar when including all trials ranging from 10 to 100 d in length (fig. 4*a*) and when including only those lasting >40 d (fig. 4*b*), where equilibration was more likely to have occurred. There was a moderate decrease in the difference between plasma $\delta^{13}C$ and that of lipid-extracted diets for bears that consumed purely carnivorous diets (fig. 5). The $\delta^{15}N$ trophic discrimination ($\Delta^{15}N_{\text{tissue-bulk diet}}$) for plasma was positively related to dietary pro-

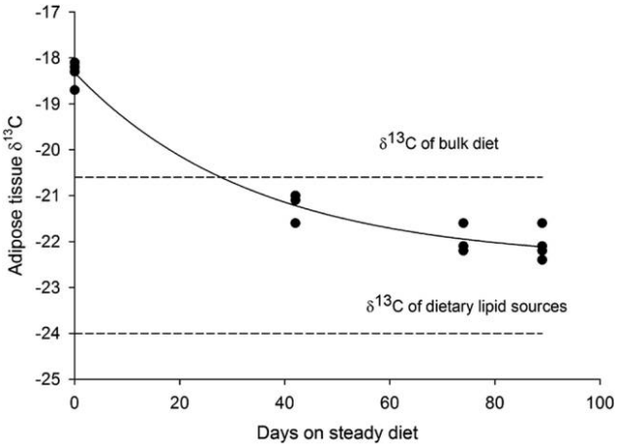

Figure 3. Change in adipose tissue $\delta^{13}C$ for four cub-of-the-year brown bears fed dog chow and salmon oil for 89 d. Bulk diet and dietary lipid $\delta^{13}C$ values are provided as a dashed line.

tein content for animals that consumed purely carnivorous diets, including polar bears in this study, brown bears (fed deer [*Odocoileus virginianus*], reindeer [*Rangifer tarandus*], or salmon [*Oncorhynchus* spp.]; G. Stenhouse, unpublished data), Arctic fox (*Vulpes lagopus*; Lecomte et al. 2011), black bears (Hilderbrand et al. 1996), and northern fur seals (*Callorhinus ursinus*; Kurle 2002; fig. 6). We included data only from trials where equilibration was likely reached.

Four brown bears fed salmon and salmon oil for ~40 d may not have reached equilibration for $\delta^{15}N$ (app. B) in plasma and serum, which could explain lower estimates of $\Delta^{15}N_{\text{tissue-bulk diet}}$ of $-0.3‰ \pm 0.8‰$ and $0.0‰ \pm 0.9‰$, respectively, compared to four polar bears fed for ~100 d (table 3; plasma: $F_{1,6} = 46.1$, $P = 0.001$; serum: $F_{1,6} = 38.0$, $P = 0.001$). After a ~100-d trial, $\Delta^{15}N_{\text{tissue-bulk diet}}$ in RBC and whole blood of bears consuming a salmon and salmon oil diet was $0.3‰ \pm 0.7‰$ and $0.7‰ \pm 0.4‰$, respectively, while estimates of $\Delta^{13}C_{\text{tissue-bulk diet}}$ were $3.1‰ \pm 0.2‰$ and $2.6‰ \pm 0.3‰$, respectively. Given our results for ^{13}C incorporation (see above), we are confident that the $\delta^{13}C$ values of RBC and whole blood had equilibrated with those of the diet after ~100 d; however, half-life estimates for ^{15}N suggest that nitrogen in these tissues had not equilibrated with diet by the end of these trials.

Equilibration times in hair were highly variable, particularly for $\delta^{15}N$ (table 2), making it similarly unclear whether pools incorporated into hair had equilibrated with the current diet. However, $\delta^{13}C$ in hair of the two polar bears appeared to reach equilibration with the experimental diet after 100 d (fig. 2). For the four polar bears fed the marine fish and fish oil diet, $\Delta^{13}C$ was $5.3‰ \pm 0.5‰$ and $\Delta^{15}N$ was $1.5‰ \pm 1.1‰$ after 100 d for bulk diets that were not lipid extracted.

The $\delta^{13}C$ of adipose tissue of four cubs of the year fed dog chow ($1.5‰ \pm 0.4‰$) or dog chow and salmon oil ($1.9‰ \pm 0.4‰$) and two polar bears fed marine fish and fish oil (0.9‰) was enriched relative to dietary lipid $\delta^{13}C$ sources. For the two polar bears, lipid content of dietary items had $\delta^{13}C$ values ranging from $-23.3‰$ to $-29.1‰$, resulting in $\delta^{13}C$ of dietary lipid sources estimated as $-24.9‰$ and of dietary protein sources as $-18.2‰$. The mean $\delta^{13}C$ for the two polar bears was $-23.0‰$, in between the $\delta^{13}C$ of dietary lipid and protein, suggesting contribution from both dietary lipid and protein. This resulted in an offset of $+0.9‰$ relative to dietary lipid and $-1.6‰$ relative to bulk diet.

Effects of Fasting on Tissue $\delta^{13}C$ and $\delta^{15}N$

The $\delta^{15}N$ increased by $1.5‰ \pm 0.4‰$ in RBC of four adult grizzlies after fasting 120 d (mixed-effects linear model: $\beta = 0.013 \pm 0.001$, $F_{1,11} = 118.5$, $P < 0.0001$; paired t-test between beginning and ending values: $t = -8.5$, $P = 0.003$) and by $1.2‰ \pm 0.2‰$ for four yearlings after fasting 138 d ($\beta = 0.007 \pm 0.001$, $F_{1,15} =$

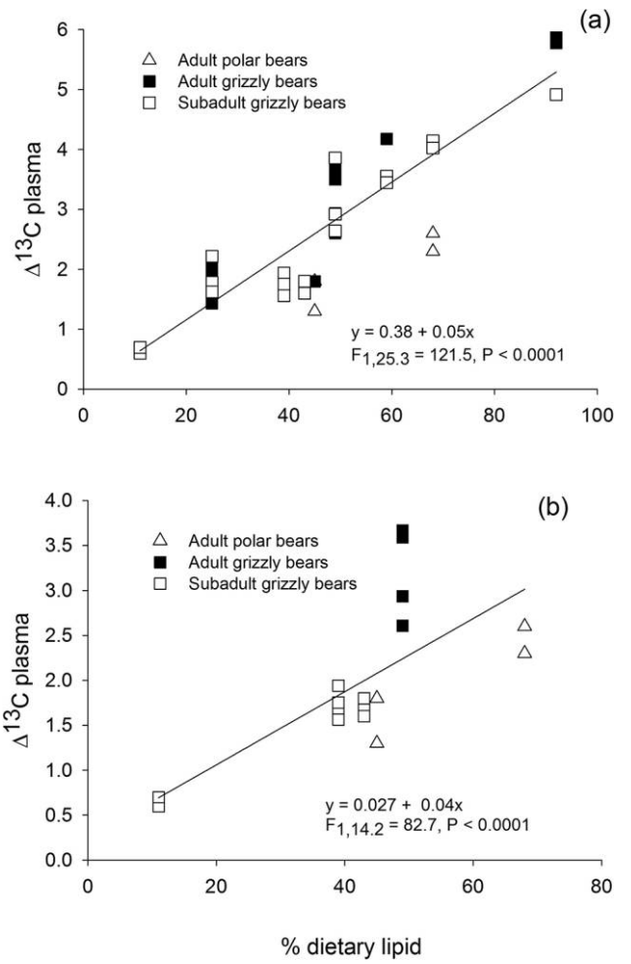

Figure 4. Relationship between percent dietary lipid (% dry matter) and carbon isotope discrimination between plasma and bulk diets that were not lipid-extracted ($\Delta^{13}C_{\text{tissue-bulk diet}}$) for brown bears and polar bears fed for 10–100 d (*a*) and for >40 d (*b*). Each data point represents an individual bear on an individual diet, with some bears repeated. Statistical results are from a mixed-effects general linear model including bear as a random effect. Random effects accounted for <1% of the variance in both relationships. Excluding random effects, the R^2 values were 0.79 and 0.58 for *a* and *b*, respectively.

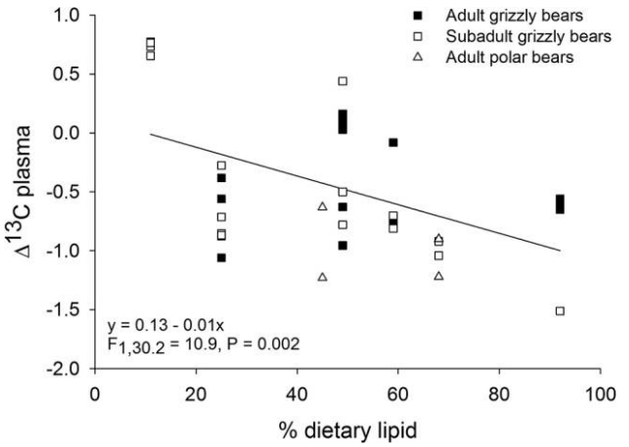

Figure 5. Relationship between percent dietary lipid (% dry matter) and carbon isotope discrimination between plasma and lipid-extracted diets for brown bears and polar bears fed for 10–100 d. Each data point represents an individual bear on an individual diet, with some bears repeated. Statistical results are from a mixed-effects general linear model including bear as a random effect. Random effects accounted for <1% of the variance in both relationships. Excluding random effects, the R^2 value was 0.23.

191.6, $P < 0.0001$; $t = 14.9$, $P = 0.001$; fig. 7*b*). Alternatively, δ^{15}N in plasma decreased 1.6‰ ± 0.6‰ after a 120-d fast for four adults (mixed-effects linear model: $\beta = -0.012 \pm 0.002$, $F_{1,11} = 64.1$, $P < 0.0001$; paired *t*-test: $t = 5.3$, $P = 0.013$) but increased in δ^{15}N of 1.8‰ ± 0.3‰ in the plasma of four yearlings after a 138-d fast ($\beta = 0.002 \pm 0.001$, $F_{1,15} = 4.6$, $P = 0.048$; $t = -5.5$, $P = 0.012$; fig. 7*a*). There was an increase in δ^{13}C of 0.3‰ ± 0.2‰ in RBC for four adult brown bears that fasted 120 d ($\beta = 0.002 \pm 0.001$, $F_{1,11} = 11.3$, $P = 0.006$; paired *t*-test: $t = -3.3$, $P = 0.05$) but no change in tissue δ^{13}C values for four yearlings ($\beta = -0.001 \pm 0.000$, $F_{1,15} = 15.0$, $P = 0.002$; $t = 0.29$; $P = 0.79$; fig. 8*b*). There was no change in plasma δ^{13}C values during fasting for four adult brown bears ($F_{1,11} = 0.12$, $P = 0.74$; paired *t*-test between start and ending values: $t = 0.60$, $P = 0.59$) or four yearlings ($F_{1,15} = 3.5$, $P = 0.08$; $t = -0.07$; $P = 0.95$; fig. 8*a*).

Discussion

δ^{13}C Discrimination

The $\Delta^{13}C_{\text{plasma-bulk diet}}$ increased by ~5‰ with increasing dietary lipid content from ~10% to ~90% (fig. 4). This relationship appeared to be largely driven by the predictable decrease in bulk diet δ^{13}C values as dietary lipids increased. We caution that in feeding trials lasting <40 d, the isotopic composition of bear plasma did not completely equilibrate with diet; however, the patterns described below warrant some discussion because they highlight physiological processes that contribute to isotopic variation and discrimination. The offset between bear plasma δ^{13}C values and those of lipid-extracted diet decreased slightly with dietary lipid content (fig. 5); however, plasma of brown and polar bears consuming purely carnivorous diets had consistently lower δ^{13}C values than the protein portion of the diet regardless of trial duration. For example, in the feeding trials on brown and polar bears lasting >80 d, in which we are confident blood plasma did equilibrate with diet, the difference between plasma and lipid-extracted diet was consistently negative (−0.6‰ ± 0.4‰). The negative δ^{13}C discrimination between plasma and lipid-extracted diet contrasts with other controlled feeding experiments on carnivores, which typically report positive δ^{13}C discrimination between plasma and dietary protein (Lesage et al. 2002; Lecomte et al. 2011). The relationship we observed in δ^{13}C discrimination between plasma and lipid-extracted diet could be the result of two processes. First, we did not lipid extract bear plasma before stable isotope analysis. Mean weight percent carbon-to-nitrogen (C:N) ratios of bear plasma were 4.4 ± 0.3, which is higher than the theoretical C:N ratio for pure proteins (~3.5; Ambrose and Norr 1992). Thus, the negative discrimination could be driven by the presence of lipids in bear plasma. A second explanation is that some ^{13}C-depleted dietary lipid carbon is being used to synthesize the proteins found in bear blood plasma. This explanation is supported by previous controlled feeding experiments on birds (Podlesak and McWilliams 2006) and mice (Newsome et al. 2014; Wolf et al. 2015) that show lipid-derived carbon is used by animals to synthesize nonessential amino acids that form the majority of proteinaceous tissues.

For feeding trials on polar bears lasting ~100 d in which hair likely equilibrated with diet (see below), mean (±SD) $\Delta^{13}C_{\text{hair-bulk diet}}$ was high (5.3‰ ± 0.5‰) but similar to δ^{13}C discrimination between keratinaceous vibrissae and bulk diet in Steller sea lions (*Eumetopias jubatus*; Stricker et al. 2015). In contrast, the mean (±SD) discrimination between polar bear hair δ^{13}C and lipid-extracted diets was lower (2.4‰ ± 0.8‰), which is consistent with δ^{13}C differences between keratins and lipid-extracted diets of other mammals (Hobson et al. 1996; Lesage et al. 2002; Caut et al. 2009; Lecomte et al. 2011; Tyrrell et al.

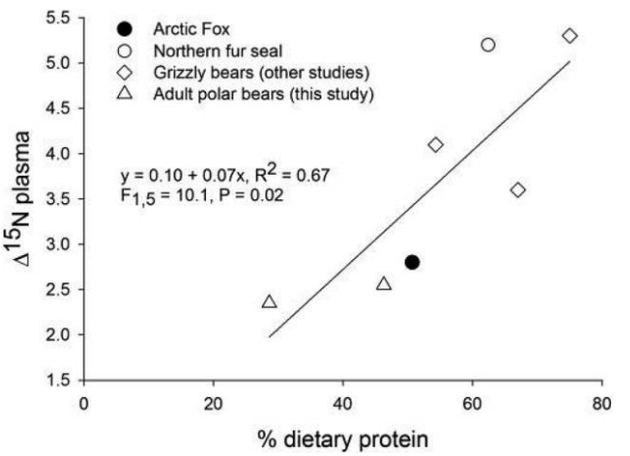

Figure 6. Relationship between percent dietary protein (% dry matter) and nitrogen isotope discrimination (Δ^{15}N) in plasma for Arctic fox (Lecomte et al. 2011), brown bears (this study with means and standard deviations; G. Stenhouse, unpublished data), black bears (Hilderbrand et al. 1996), polar bears (this study; means only because $n = 2$), and northern fur seals (Kurle 2002) fed purely carnivorous diets. Only trials that were long enough to reach equilibration are included.

Table 3: Isotope discrimination values (‰) from trials with four brown bear cubs on chow or chow with oil, four yearling brown bears fed dog chow and a mixed marine fish oil, adult brown bears consuming salmon and salmon oil, and polar bears consuming marine fish and fish oil (salmon or herring)

	Brown bear cubs on dog chow (90 d; $n = 4$)		Brown bear cubs on dog chow + salmon oil (89 d; $n = 4$)		Brown bear yearlings on dog chow + fish oil (79 d; $n = 4$)		Adult brown bears on salmon and salmon oil (41 d; $n = 4$)		Polar bears (100–104 d; $n = 4$)	
Fat content	11%		39%		43%		50%		45%–68%	
Isotope	$\Delta^{13}C$	$\Delta^{15}N$	$\Delta^{13}C$	$\Delta^{15}N$	$\Delta^{13}C$	$\Delta^{15}N$	$\Delta^{13}C$	$\Delta^{15}N$	$\Delta^{13}C$	$\Delta^{15}N$
Plasma	.6 ± .1	3.4 ± .1	1.7 ± .2	3.4 ± .2	1.8 ± .1	4.2 ± .2	3.2 ± .5	…	2.0 ± .6	2.5 ± .2
Serum	…	…	…	…	2.1 ± .1	4.5 ± .2	3.5 ± .5	…	2.1 ± .8	2.7 ± .1

Note. Fat content is percent dry matter. All discrimination values are for nonlipid extracted diets and tissues. Values are means ± standard deviation.

2013). This pattern suggests that dietary proteins are the primary source of carbon in the hair of polar bears fed lipid-rich diets.

Bear adipose tissue had lower $\delta^{13}C$ values than that of bulk diet by on average 1.5‰ at high lipid contents (30%–68%). Adipose tissue $\delta^{13}C$ (−23.0‰) was higher than that of dietary lipid sources (mean: −24.9‰, range −23.3‰ to −29.1‰). Ben-David et al. (2012) also reported higher $\delta^{13}C$ values of adipose tissue relative to dietary lipids for mink fed lipid-rich diets. Assuming there is little to no isotopic discrimination between dietary fats and adipose tissues, this pattern suggests that adipose tissue reflects some mixture of lipid- and protein-derived carbon and is consistent with results for yearling black bears (Hilderbrand et al. 1996). The potential for dietary protein carbon to contribute to fatty acid synthesis is supported by other studies on captive omnivorous mice fed lipid-rich diets (Newsome et al. 2014; Wolf et al. 2015).

$\delta^{15}N$ Discrimination

The observed increase in $\Delta^{15}N$ in plasma with increasing dietary protein content across five species consuming purely carnivorous diets (fig. 6) suggests that protein quantity, rather than protein quality (i.e., the complementation of amino acids consumed relative to an animal's metabolism; Robbins et al. 2010), may be the primary factor affecting $\Delta^{15}N$ in carnivores. Florin et al. (2010) documented that protein quality and quantity described most of the variation in $\Delta^{15}N$ for omnivores, but in carnivores, quality may have little or no effect on $\Delta^{15}N$ because this factor does not substantially vary across diets. Increased $\Delta^{15}N$ with increased dietary protein content has similarly been observed in the muscle of omnivorous fish (Kelly and Martinez del Rio 2010).

Results for feeding trials on four polar bears lasting ~100 d showed substantial variation in $\Delta^{15}N_{\text{hair-bulk diet}}$, ranging from 0.3‰ to 2.5‰. The highest discrimination estimate in this range was still lower than that for Arctic fox (3.3‰ ± 0.7‰) and brown bears (range: 3.2‰–5.0‰) but within the range reported for seals (~2‰; Hobson et al. 1996; Felicetti et al. 2003; Caut et al. 2009; Lecomte et al. 2011). Generally, lower $\Delta^{15}N$ discrimination may be a consequence of the lower protein contents in some of our feeding trials (fig. 6) or a result of a lack of equilibration (fig. 2). We contend that the diets used in our feeding trials are more similar to those consumed by wild polar bears; thus, lower $\Delta^{15}N$ values may be applicable to isotope-based dietary reconstructions for this species in the wild.

Effects of Fasting on Isotopic Discrimination

We found that plasma $\delta^{15}N$ values increased by 1.8‰ for four yearling brown bears after a 138-d fast but decreased by 1.6‰ in four adult female brown bears that fasted during hibernation for 120 d (fig. 7*a*). Plasma $\delta^{13}C$ values showed no trend during fasting for either age group (fig. 8*a*). The difference in the trend of $\delta^{15}N$ during fasting for the yearlings and adults may relate to the use of different endogenous energy reserves (Polischuk et al. 2001) by each age group or could result from yearlings and adults having consumed isotopically different diets when the mobilized tissue was formed. The rate of protein to lipid catabolism increases as nutritional stress increases during fasting, which likely impacts the concentration and isotopic composition of body nitrogen pools if nitrogen is being lost via urination or defecation. Animals that catabolize appreciable amounts of lean body mass during hibernation have shown increases in plasma $\delta^{15}N$ (Lee et al. 2012). Because brown bears urinate and defecate minimally if at all during hibernation, there is no mechanism for increasing/decreasing the concentration of ^{15}N, only modifying its distribution among endogenous nitrogen pools through urea recycling or other mechanisms (Lohuis et al. 2005). At present, we have no explanation for the different trends in plasma $\delta^{15}N$ values between yearling and adult brown bears.

Isotopic Incorporation

Isotopic incorporation rates inferred from $\delta^{13}C$ were longer for RBC (half-life = 50 d) and whole blood (half-life = 43 d) than serum (half-life = 13 d) and plasma (half-life: 10 d), which were much longer for the two adult polar bears in our study than previously reported for respective blood components of yearling black bears (Hilderbrand et al. 1996) or small carnivores (*Neovison vison*) that consumed lipid- and protein-rich diets (Ben-David et al. 2012). The $\delta^{15}N$ values in RBC and whole blood did not equilibrate with diet for the four adult polar bears in our feeding trials that lasted ~100 d, nor did they equilibrate in shorter ~40-d trials with four adult brown bears. Similarly, RBC of mink fed fish diets did not equilibrate during trials

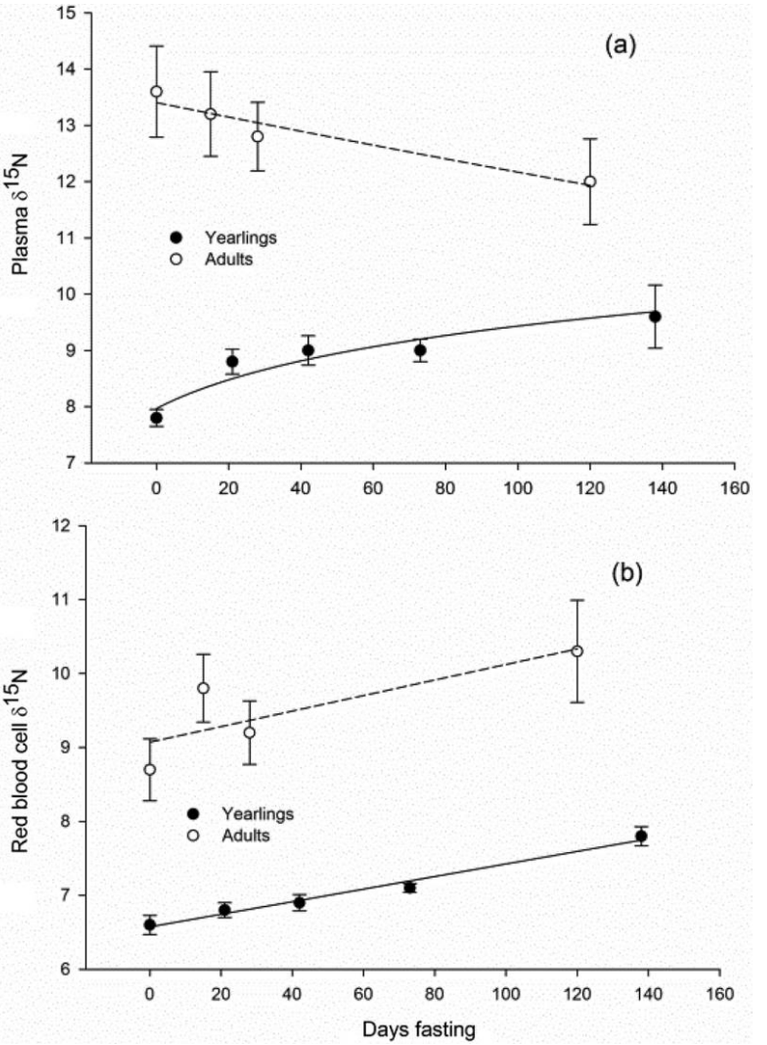

Figure 7. Mean and standard deviation in δ^{15}N in plasma (*a*) and red blood cells (*b*) of four adult and four yearling brown bears in hibernation fasting for 16 wk.

lasting 77 d, but one of the likely explanations offered by Ben-David et al. (2012) for longer equilibration times in RBC was the low lipid content of the diets, which is not a plausible explanation for the patterns found here. The modeled mean half-life (108 d) for RBC in the two polar bears was considerably longer than the 28-d half-life estimated for RBC of yearling black bears (Hilderbrand et al. 1996), which has been used in other studies as an estimate of isotopic incorporation in other ursids, including polar bears (Bentzen et al. 2007; Cherry et al. 2011) and brown bears (Ben-David et al. 2004). Longer isotopic incorporation rates observed in our study could be a result of larger body size of polar and brown bears relative to yearling black bears (Carter et al. 1964; Bauchinger and McWilliams 2009; Martinez del Rio and Carleton 2012), reduced protein turnover resulting from lipid-rich diets, or differences in the protein quality of diets used in different feeding experiments (Ben-David et al. 2012).

Isotopic incorporation for nitrogen was consistently longer in whole blood, RBC, plasma, and serum compared to carbon for four adult polar bears, a pattern that has been observed in other studies (Lecomte et al. 2011; Ben-David et al. 2012). Because of this elemental disparity in incorporation rates of tissues, two compartment models (Cerling et al. 2007) may better estimate rate processes for the tissues of bears and other species (Ayliffe et al. 2004; Ben David et al. 2012; Martinez del Rio and Carleton 2012). However, this approach requires simultaneous estimation of turnover in a variety of tissues, some of which may be inherently difficult to sample from live animals (e.g., liver).

Hair is a biologically inert tissue once synthesized, and isotopic composition should be reflective of diet during growth (Hobson 1999) if an animal is at isotopic equilibrium with its diet. We found that the carbon and nitrogen used to synthesize bear hair may take 50 d or more to reequilibrate after a diet shift occurs based on ~100-d feeding trials with two adult polar

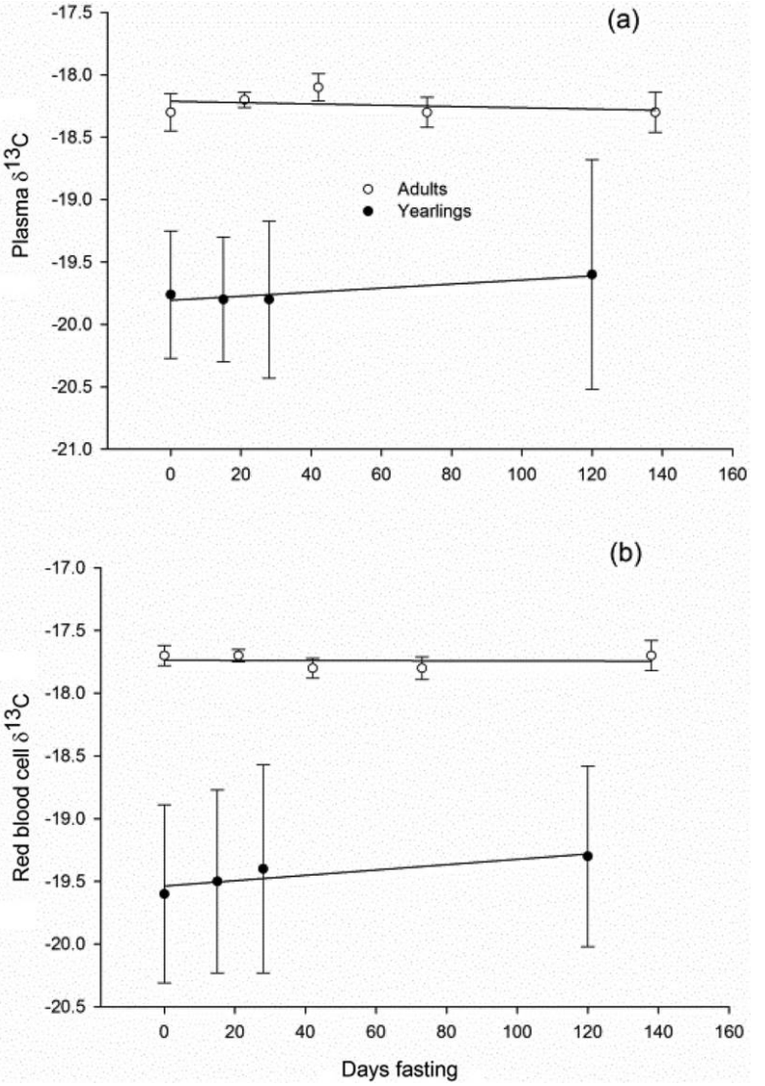

Figure 8. Mean and standard deviation in $\delta^{13}C$ in plasma (*a*) and red blood cells (*b*) of four adult and four yearling brown bears in hibernation fasting for 16 wk.

bears and ~40-d trials with four adult brown bears. This result is consistent with several other studies that demonstrate relatively long time periods for equilibration of carbon and nitrogen pools used to synthesize hair: 47.5 d for gerbils (Tieszen et al. 1983) and 21 wk for horses (Ayliffe et al. 2004).

In combination with other studies of terrestrial and marine carnivores, our results suggest that dietary lipid content is an important factor affecting isotopic discrimination (Budge et al. 2011; Cherry et al. 2011; Ben-David et al. 2012; Newsome et al. 2014; Wolf et al. 2015). It is also becoming clear that some animals may flexibly route macronutrients to fuel catabolism, build lean body mass, or store energy when excess fatty acids and nonessential amino acids are available. This has potential implications on conventional diet estimates using stable isotopes, particularly in the case of purely carnivorous predators. In future controlled feeding experiments, we advocate continuation and expansion of the topics presented here to provide further insight into isotopic routing and incorporation, including the implementation of experimental designs that emphasize a range of lipid and protein dietary contents, analysis of both bulk and lipid-free diets, and exploration of allometric relationships that influence isotopic incorporation in large carnivores.

Acknowledgments

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Geological Survey Fort Collins Science Center. We thank Cayce Gulbransen (USGS) for conducting the stable isotope analyses and Merav Ben-David, Todd Atwood, and several anonymous reviewers for their helpful comments on manuscript drafts. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US government. This article was reviewed and approved by USGS under its Fundamental Science Practices policy (http://www.usgs.gov/fsp).

APPENDIX A

Table A1: Equilibration curves

Bear	Tissue	Isotope	R^2	F	P	Equation
Male polar bear	Plasma	^{13}C	.95	48.5	.0005	$-19.4 - (-19.4 + 17.8)\exp^{-.054x}$
Female polar bear	Plasma	^{13}C	.96	61.7	.0003	$-19.8 - (19.8 + 18.1)\exp^{-.09x}$
Male polar bear	Plasma	^{15}N	.98	103.3	<.0001	$-15.6 - (-15.6 + 12.6)\exp^{-.035x}$
Female polar bear	Plasma	^{15}N	.99	191.3	<.0001	$-15.7 - (-15.7 + 12.3)\exp^{-.044x}$
Male polar bear	Serum	^{13}C	.85	11.7	.02	$-19.3 - (-19.3 - 18.2)\exp^{-.0386x}$
Female polar bear	Serum	^{13}C	.95	48.2	.0005	$-19.8 - (-19.8 + 18.0)\exp^{-.0822x}$
Male polar bear	Serum	^{15}N	.92	21.9	.007	$16.1 - (16.1 - 13.6)\exp^{-.0195x}$
Female polar bear	Serum	^{15}N	.98	153.7	<.0001	$15.8 - (15.8 - 12.5)\exp^{-.044x}$
Male polar bear	RBC	^{13}C	.99	197.2	.0007	$-18.1 - (-18.1 + 17.2)\exp^{-.0166x}$
Female polar bear[a]	RBC	^{13}C	.98	46.0	.02	$-18.5 - (-18.5 + 17.6)\exp^{-.012x}$
Male polar bear	RBC	^{15}N	.98	62.6	.003	$14.9 - (14.9 - 11.7)\exp^{-.0044x}$
Female polar bear	RBC	^{15}N	.83	8.0	.068	$13.7 - (13.7 - 12.1)\exp^{-.0117x}$
Male polar bear	Whole blood	^{13}C	.99	574.9	<.0001	$-18.7 - (-18.8 + 17.4)\exp^{-.0155x}$
Female polar bear	Whole blood	^{13}C	.97	77.4	.0002	$-18.8 - (-18.8 - 17.9)\exp^{-.0166x}$
Male polar bear	Whole blood	^{15}N	.99	1,020.3	<.0001	$21.2 - (21.2 - 11.9)\exp^{-.0019x}$
Female polar bear	Whole blood	^{15}N	.99	258.1	<.0001	$15.3 - (15.3 - 12.2)\exp^{-.0074x}$
Male polar bear	Hair	^{13}C	.71	6.2	.04	$-15.6 - (-15.6 + 14.6)\exp^{-.046x}$
Female polar bear	Hair	^{13}C	.93	38.5	.0009	$-16.9 - (-16.9 + 14.9)\exp^{-.011x}$
Male polar bear	Hair	^{15}N	.77	6.6	.05	$14.0 - (14.0 - 10.4)\exp^{-.068x}$
Female polar bear	Hair	^{15}N	.91	21.3	.007	$19.7 - (19.7 - 11.3)\exp^{-.007x}$
Adult brown bears	Plasma	^{15}N	.95	90.8	<.0001	$14.4 - (14.4 - 8.75)\exp^{-.0465x}$
Adult brown bears	Serum	^{15}N	.94	76.6	<.0001	$14.6 - (14.6 + 9.1)\exp^{-.0501x}$
Adult brown bears	Hair	^{13}C	.82	6.9	.076	$-17.8 - (-17.8 + 18.7)\exp^{.047x}$
Brown bear cubs	Adipose tissue	^{13}C	.97	237.0	<.0001	$-22.5 - (-22.5 + 18.3)\exp^{-.0287x}$
Brown bear cubs	Plasma	^{13}C	.98	418.4	<.0001	$-18.9 - (-18.9 + 17.0)\exp^{.0737x}$

Note. Equilibration curves are shown for two adult polar bears at the Oregon Zoo (one male, one female) fed a marine carnivorous diet for 104 d, four adult brown bears (two males, two females) fed salmon and salmon oil for 41 d, and four cub-of-the-year brown bears fed dog chow and salmon oil for 89 d. Diet information provided in table 1.

[a] A single outlier was removed.

APPENDIX B

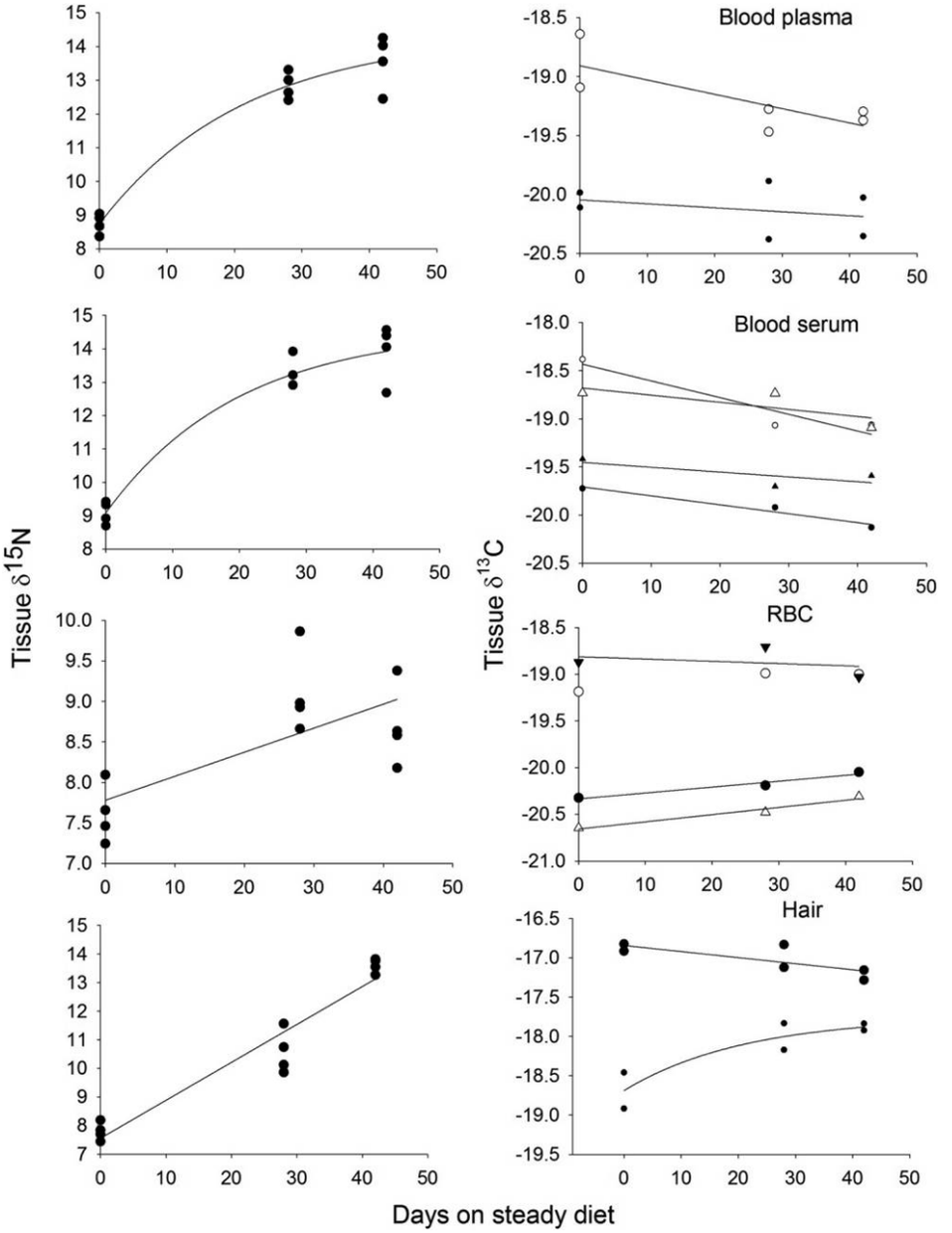

Figure B1. Tissue $\delta^{15}N$ and $\delta^{13}C$ values for four adult brown bears fed salmon and salmon oil (diet information provided in table 1) for 41 d.

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