Body Protein Stores and Isotopic Indicators of N Balance in Female Reindeer (*Rangifer tarandus*) during Winter

Perry S. Barboza[1,*]
Katherine L. Parker[2]
[1]Institute of Arctic Biology, Department of Biology and Wildlife, P.O. Box 757000, University of Alaska, Fairbanks, Alaska 99775-7000; [2]Natural Resources and Environmental Studies, University of Northern British Columbia, Prince George, British Columbia V2N 4Z9, Canada

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ABSTRACT

We studied bred and unbred female reindeer (*Rangifer tarandus tarandus*) during 12 wk of winter when ambient temperatures were low and nitrogen (N) demand for fetal growth is highest in pregnant females. Animals were fed a complete pelleted diet ad lib. that contained 2.54% N in dry matter that was 80% ± 2% ($\overline{X}$ ± SD) digestible. Female reindeer lost 64% ± 14% of body fat but gained 34% ± 11% of lean mass from 10 wk prepartum to parturition. These changes were equivalent to average balances of −14.14 ± 2.35 MJ d^{-1} and 10 ± 3 g N d^{-1}. Blood cells, serum, and urine declined in $^{15}N/^{14}N$ in late winter as body protein was gained from the diet. Blood cells of newborn calves were more enriched in ^{15}N and ^{13}C than that of their mothers, indicating the deposition of fetal protein from maternal stores. To quantify pathways of N flow in reindeer, N balance was measured by confining animals to cages for 10 d at 4 wk from parturition. N balance was inversely related to $^{15}N/^{14}N$ in urea-N but not related to $^{15}N/^{14}N$ of blood cells, creatinine, and feces. The proportion of urea-N derived from body protein increased above 0.46 as N balance fell below −200 mg N $kg^{-0.75}$ d^{-1}. Proportions of urea-N from body protein were −0.01 ± 0.21 in pregnant females before and after caging and were consistent with average body protein gain in winter. Storage of protein allows reindeer and caribou to tolerate diets that are low in N without impairing fetal development.

* Corresponding author; e-mail: ffpsb@uaf.edu.

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Introduction

Arctic ruminants such as reindeer and caribou (*Rangifer tarandus sp.*) consume winter forages that are typically low in N (less than 1% of dry food), limited in abundance, and often inaccessible under snow (Parker et al. 2005). Reindeer and caribou must maintain their body proteins to sustain normal functions such as thermoregulation and locomotion in winter. In addition, females may also support the growth of a fetus and the production of milk before plant growth resumes in summer. Although the importance of body fat as a store of energy for winter survival and reproduction is well established for arctic ruminants (White and Luick 1984; Tyler 1987; Adamczewski et al. 1993, 1995; Pond et al. 1993), the dynamics of body protein are not as well known. Seasonal changes in lean mass of adult reindeer and caribou suggest that protein in a variety of tissues from muscle to viscera may be used as stores for reproduction. Captive male reindeer gain body protein through summer but lose 25% of body protein during mating in September (Barboza et al. 2004). Wild female caribou apparently lose body protein at the end of winter because protein in visceral organs is lowest in May, when lactating females have 29% less body protein than nonlactating females (Gerhart et al. 1996). Consumption of low-N forages such as lichen can increase mass lost in winter (Bjarghov et al. 1976; Rominger et al. 1996) and reduce milk production and growth of calves (Jacobsen et al. 1981). Conversely, caribou fed high-N diets in early winter tolerate very low N intakes in late gestation and still deliver viable calves (Parker et al. 2005).

Caribou and reindeer can apparently conserve body protein in winter when intakes of N are low (Parker et al. 2005). The dynamics of body protein stores can be studied with a general model of the stages of N balance (Fig. 1). The model is derived from patterns of protein and energy deficit in mammals and birds (Young et al. 1981; MacRae and Lobley 1986; Barboza and Hume 2006). Dietary intakes of N that exceed urinary and fecal losses allow net retention of N in the body (positive N balance). Inadequate dietary N depletes body protein at rates that vary with the difference between intake and excretion. An intake of N below the endogenous rates of N loss in urine and feces requires the use of stored body N to maintain turnover of protein in critical tissues. Animals may sustain a stable negative N balance if there is a store of body protein and a supply of energy and other nutrients from the diet or from body stores such as fat (e.g., dormant bears; Barboza et al. 1997). The use of body protein as a source of energy is small when body stores

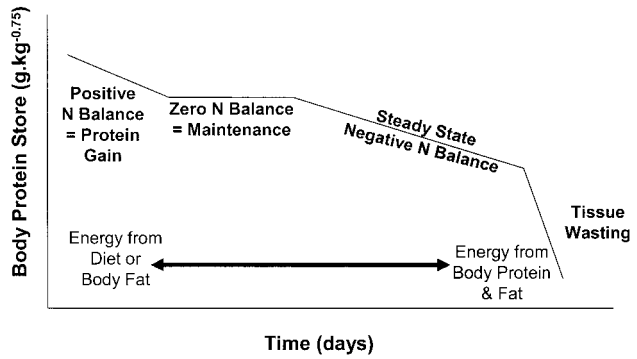

Figure 1. Conceptual model of the relationship between body protein stores (g $kg^{-0.75}$) and time (d) at different stages of daily N balance (g $kg^{-0.75}$ d^{-1}) in animals during winter. Duration of each stage and the slope of the relationship vary with N balance, the size of fat and protein stores, and the use of body protein for energy.

are lost at steady state. Duration of steady state N depletion therefore depends on the size of the body protein store and on the use of body protein as an energy substrate. Depletion of body fat when dietary energy intakes are low may result in tissue wasting when lean mass is oxidized to fuel energy expenditure (e.g., fasting geese; Le Maho et al. 1981). The transition from a steady state of negative N balance to an unsteady state of tissue wasting is therefore dependent on both energy expenditure and the composition of the body (Dulloo and Jacquet 1999). Pregnant female caribou and reindeer may be most vulnerable to N deficits in late winter because stores of body protein may have been progressively depleted through early winter and because reproductive demands for both fetal growth and lactation increase toward spring.

Rapid depletion of body protein during tissue wasting results in rapid mass loss, poor body condition, and mortality, but small changes in N balance are difficult to discern in captive and wild animals. The concentration of N metabolites such as urea and creatinine can provide an index of N catabolism that has been used to evaluate winter condition of several ruminants based on collection of urine in snow (DelGiudice et al. 1989, 2001; Larter and Nagy 2001). This index assumes that with no change in the N content of winter forage, increases in the concentration of urinary urea-N relative to creatinine are due to increased oxidation of body protein. The method is vulnerable to changes in creatinine concentration that are probably related to changes in renal function of caribou (Parker et al. 2005) and reindeer (Sakkinen et al. 2001; Barboza et al. 2004). Furthermore, the method assumes that dietary N is always limiting, but it cannot discriminate between N from the diet and N from body protein. Stable isotopes in urea and other pools of nitrogenous metabolites may better indicate the relationship between dietary N supply and body protein stores (Parker 2003; Parker et al. 2005).

We studied bred and unbred female reindeer during 12 wk of winter when ambient temperatures were low and N demand for fetal growth is highest in pregnant females. Animals were fed a complete diet that was high in N to describe seasonal changes in body mass, body composition, and isotope ratios of blood, urine, and feces when food quality and abundance were not limited. We also measured mass balance of caged animals in late March to relate N balance to N excretion under stable conditions of intake. We used $^{15}N/^{14}N$ ratios of diet, blood cells, and urea to calculate the proportion of urea-N derived from body protein. Patterns of $^{15}N/^{14}N$ in urea-N were compared with N balance in cages and with the average N balance indicated by the net change in body protein over winter.

Material and Methods

Animals and Experimental Design

We used reindeer from a herd maintained at the Institute of Arctic Biology for more than 20 yr. This herd is derived from Siberian stocks of semidomesticated reindeer (*Rangifer tarandus tarandus*) that were introduced to western Alaska during the early 20th century. Experiments and handling procedures were approved by the Institutional Animal Care and Use Committee, University of Alaska, Fairbanks, under protocol 02–04.

We studied three unbred and seven pregnant female reindeer (5.4 ± 1.9 yr old) between December 2002 and April 2003. The unbred group included two animals that were surgically prepared with ruminal cannulas more than 3 yr before this experiment. Measurements were made for approximately 12 wk before the average date of parturition on April 15, 2003. This study therefore included the final 40% of an average pregnancy lasting 30 wk. Reindeer were previously trained to halters that allowed them to be led by handlers each week and to be tied safely in handling stalls or cages. Chemical immobilants were not required for any measurements of these trained animals.

Reindeer were held together in outdoor pens of 0.5–1.0 ha. Ambient air temperatures ranged from 4°C to −39°C with a monthly average temperature of −15° ± 3°C during the study (University of Alaska, Fairbanks, Geophysical Institute). Animals were provided with ad lib. water as snow and with a complete pelleted diet. We used a complete formulation (D-Ration, Alaska Pet and Garden, Anchorage, AK) that was based on cereal grains (corn, barley) and roughage (alfalfa meal, beet pulp), with protein and sugar concentrates (soybean meal, molasses) and premixes of minerals and vitamins. This diet has been fed to reindeer and caribou from weaning through growth, breeding, pregnancy, and lactation for 6 yr without apparent ill effects.

Samples of spontaneously voided urine and feces were collected into clean plastic cups from animals tied at halter. Blood was withdrawn from the jugular vein with 18-gauge needles into evacuated tubes without additive (serum) or with Na-Heparin (plasma; Vacutainer Systems, Becton Dickinson, Franklin Lakes, NJ). Whole blood (10 mL) was centrifuged at 1,000 *g* for 10 min to separate plasma and serum. Clotted blood cells, serum, plasma, feces, and urine were stored at −20°C for analysis.

Body mass was recorded to the nearest 0.5 kg on an electronic scale in a handling chute (model 703 scale, Tru-Test, San Antonio, TX). The handling chute was also used for ultrasonic measures of subcutaneous fat at the rump. We shaved 8 × 3 cm with surgical clippers (#40 blade) to prepare a measurement site at 35% of the distance from the ilium to ischial tuberosity (Rombach et al. 2002). Subcutaneous fat was measured to the nearest 1 mm with a 7-cm probe (UST-S813-5, SSD10DX monitor, Aloka, Wallingford, CT) by using corn oil as a contact medium on the skin. Ultrasound measures were validated against direct measures of fat depth by dissection of three nonreproductive females in June 2004. We measured depths at four sites along the standard transect (medio-distal plane) and at another four sites that bisected the standard transect in the dorsoventral plane. Fat depths were more variable in the dorsoventral plane (1.55 ± 0.72 cm) than in the medio-distal plane (1.74 ± 0.20 cm). Because changes in the stance of the live animal can shift the position of the shaved ultrasound site in the dorsoventral plane, we repeated ultrasonic measures until animals maintained a standard posture in the handling chute. Ultrasonic measures were linearly related to direct measures with calipers:

$$\text{ultrasound measure} = 0.640 \times \text{direct measure} \pm 0.49 \text{ cm}$$

($R^2 = 0.946$, $P < 0.001$). Subcutaneous fat depths are reported as ultrasonic measures without correction for this relationship.

Subcutaneous fat was determined in nine of 10 animals because one animal would not remain still in the chute for more than the time required to measure body mass. We used the same nine animals for measures of body composition and for the experiment on N balance (see below). Body composition was determined by dilution with tritiated water at 10 wk (February) before and within 1 wk after the average date of parturition (April 15). Each animal received a single intrajugular dose of 1.5 mL sterile saline (0.9 g NaCl L^{-1}). Doses contained 109 μCi (152 GBq) ^3H_2O in February and 9 μCi (13 GBq) ^3H_2O in April (Sigma, Milwaukee, WI). Blood was sampled before dosing and at 3 and 24 h after the dose. February doses were also followed by blood samples at 6, 48, and 120 h to evaluate equilibration of the dose within 3 h. Access to food and water or snow was removed in the first 3 h after dosing to minimize changes in water flux during equilibration of the dose.

Calves were able to follow their mothers ($n = 7$) and to suckle before the first handling within 24 h of birth. Calves were weighed on a spring scale (±0.1 kg). Blood cells and serum were separated from 5 mL of whole blood sampled from the jugular vein at the first handling. Postpartum handling of mothers was minimized to reduce the risk of abandonment of calves. Suckling was disrupted for only 3 h to minimize errors in equilibration of tritiated water in the mothers.

Nine of the 10 reindeer were held indoors in cages (180 cm long × 60 cm wide) for 10 d to measure total food intake and total excretion of feces and urine in a N balance experiment. Animals were acclimated to cages for 6 d before measuring balance for four consecutive days at 4 wk from the average date of parturition. Ventilation with 100% external air flow kept indoor air temperatures between −3.3° and 5.9°C. We used incandescent lights and natural incident light from windows to mimic the photoperiod outdoors (sunrise at 8:00 a.m. to sunset at 8:00 p.m.). Closed-circuit cameras were used to monitor the behavior of animals in cages and to ensure that they were calm and safe. Feces were collected onto metal screens beneath each cage. Urine passed through screens onto metal chutes that drained into open plastic trays. Feces and urine were removed and weighed each morning before providing fresh food and water ad lib. Preservatives were not added to urine collectors because we determined that urea and ammonia were not lost from saline solutions or fresh urine held in the same trays under the same conditions. Feces and urine were stored at −20°C for analysis. Blood was sampled from all nine animals at the start and at the end of the period in cages.

Urea kinetics was measured in two unbred and two pregnant females for 24 h at the end of the balance experiment (3.8 wk from parturition). Each animal received a single intrajugular dose of 4 mL sterile saline (0.9 g NaCl L^{-1}) containing 82 μCi (183 GBq) ^{14}C-urea at 58 mCi $mmol^{-1}$ urea (Sigma, Milwaukee, WI). Blood was sampled before dosing and at 2, 4, 5, 6, and 23 h after the dose. Urine was removed from collection trays before dosing and at 2, 4, 6, 7, 9, 11, 13, 15, 18, and 22 h after the dose.

Chemical Analyses

Food and feces were dried at 55°C to constant mass for measures of dry matter (DM) content and in preparation for subsequent analyses. Ash was determined by combustion in a muffle furnace at 500°C. Dried samples were ground through a #20 (1.25 mm) screen in a Wiley mill before analysis. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin were extracted sequentially (Van Soest et al. 1991): NDF was extracted with Na_2SO_3 and amylase; lignin was extracted in H_2SO_4 without permanganate. Total C, N, and sulfur (S) were determined with an elemental analyzer (CNS2000, LECO, St. Joseph, MI). Gross energy content was determined by bomb calorimetry (Parr Instruments, Boleen, IL).

Blood samples from animals in the N balance experiment were analyzed with a chemanalyzer at Fairbanks Memorial Hospital (VITROS 950, Ortho Clinical Diagnostics, Raritan, NJ) that was certified for clinical diagnostics. This system delivered samples of plasma and serum onto glass slides for colorimetric reactions with reagents that were embedded in semipermeable membranes. The standard diagnostic panel included the following assays: glucose by glucose oxidase linked to colorimetric reaction with hydrogen peroxidase and dye; creatinine by creatine production with creatinine amidohydrolase; blood urea-N (BUN) by production of ammonia complex with dye in the presence of urease; lactate dehydrogenase (LDH) by disappearance of NADH in the presence of pyruvate as a substrate; alanine amino transferase (ALT) by pyruvate production from alanine as a substrate; aspartate amino transferase (AST) by oxaloacetate production from aspartate as a substrate; and creatine kinase by creatine production from creatine-phosphate. Plasma sampled from animals throughout the study was assayed for total cortisol by solid-phase radioimmunoassay with iodinated controls and rabbit antisera (Diagnostic Products, Los Angeles; Barboza et al. 2004).

Urea in plasma and urine was measured by the diacetylmonoxime method (procedure 535; Sigma, St. Louis, MO; Marsh et al. 1965). Urinary protein was assayed with Bradford reagent against bovine serum albumin (Sigma, Milwaukee, WI). Urinary ammonia was volatilized by steam distillation, collected into boric acid (4% w/v), and quantified by titration against HCl. Urinary urea-N was collected after ammonia was removed. Ammonia was released from urea by treatment with urease (Type IX, from jack bean, U-4002, Sigma, Milwaukee, WI) in 0.2 M phosphate buffer at pH 7 and collected by distillation into boric acid (Parker et al. 2005). Titrated solutions of ammonium borate from urea-N were overacidified with HCl and dried at 65°C for mass spectrometry.

Urinary creatinine was assayed by high-performance liquid chromatography (Xue et al. 1988) that was verified with the colorimetric alkaline picrate reaction (Heinegard and Tiderstrom 1973). Creatinine was separated by cation exchange at 25°C on a silica bonded-phase column (Spherisorb S10 SCX, Waters, Milford, MA) in an isocratic gradient of acetonitrile (15% v/v) and 0.2 M phosphate buffer at pH 2.5 (85% v/v). Eluted creatinine was detected by absorbance at 242 nm, identified by retention time of standards, and quantified by integration (Empower Software, Waters, Milford, MA). Eluted fractions containing creatinine were dried at 65°C for mass spectrometry.

Radioisotopes (^{3}H and ^{14}C) in plasma and urine were assayed by liquid scintillation and corrected for quench and background (LS 6000, Beckman Coulter, Fullerton, CA). Serum, blood cells, and urine were sampled into tin cups and dried at 65°C for mass spectrometry. Isotope ratios ($^{15}N/^{14}N$ and $^{13}C/^{12}C$) were assayed in dry food, blood, urine, feces, ammonium borate, and creatinine with the Europa Scientific 20-20 Continuous Flow IRMS (Europa Scientific, Chestershire). Accuracy of standard assays for peptone from meat (P7750, Sigma, Milwaukee, WI) were within 0.40‰ $\delta^{15}N$ (reference value 7.00‰) and 0.15‰ $\delta^{13}C$ (reference value −15.80‰) in this system.

Calculations and Statistics

Specific activity (SA) of labeled water ($^{3}H_2O$) was expressed as dpm g^{-1} to calculate maternal body composition (Barboza et al. 2004). Water space was calculated as the total dose (dpm) divided by either the SA at 3 h from dosing or the zero-time intercept of the regression of ln (SA) against time from dose. Log-linear, least-squares regression of SA against time from dose was significant in all animals ($n = 9$, $P < 0.05$, $R^2 > 0.90$) and indicated equilibration of the dose in a single pool within 3 h. Estimates of water space derived from plasma at 3 h were similar to those derived from the zero-time intercept of the regression. Because the average difference between these estimates of water space in February was only 0.14% ± 2.2%, we used the water space based on equilibration at 3 h from dose for subsequent calculations of body composition.

Water space was discounted by 10% to correct for overestimation of the tissue water pool and for digesta water as described by Barboza et al. (2004). We used data from dissections of 16 adult caribou and two reindeer fed pelleted diets in June 2002 and June 2004 to estimate the contents of the digestive tract. Digesta mass (kg) was calculated from body mass (kg) as

$$\ln(\text{digesta mass}) = 0.6437 \times \ln(\text{body mass}) - 0.7277$$

($n = 18$, $R^2 = 0.34$, $P = 0.011$). Digesta water was subsequently calculated with the average water content of digesta in the whole tract (86.29%). Tissue water was the discounted water space minus digesta water. Lean mass was tissue water divided by the moisture content of lipid-free tissue (0.6862 g g^{-1}; Gerhart et al. 1996). Body fat was the difference between ingesta free-mass (body mass − digesta mass) and lean mass. We used equations for juvenile caribou and reindeer from Gerhart et al. (1996) to calculate the ingesta-free mass (kg) and the mass of lean and fat tissues in calves. Body protein (kg) of adults and calves was derived from the equation for lean mass,

$$\text{body protein} = (0.246 \times \text{lean mass}) - 0.345$$

(Gerhart et al. 1996), and converted to N at 0.16 g N g^{-1} protein (Robbins 1993). The equivalent energy in protein and fat was estimated from the caloric values for depot fat (39.3 kJ g^{-1}) and muscle protein (23.7 kJ g^{-1}) in ruminants (Blaxter 1989). Net loss of N in the balance experiment was converted to daily changes in protein (g d^{-1}). Energy from protein loss was ex-

pressed as a percentage of the average change in body energy over winter (MJ d^{-1}).

Calculations of urea kinetics were based on declines in SA (dpm $mmol^{-1}$ urea) and the total dose of labeled urea (dpm) in plasma (Nolan and Leng 1972; Barboza et al. 1997). Linear regressions of ln(SA) against time from dose were used to calculate the size (dose/intercept = g urea-N) and turnover time ($slope^{-1}$; h) of the urea pool. The rate of urea entry was the pool size divided by the turnover time (g N h^{-1}). Urea excretion was the rate of appearance of urea in urine collected for 24 h after the dose. Urea degradation was the difference between the rates of entry and excretion of urea.

Urinary excretion of N and C was divided among ammonia, urea, and creatinine by calculating the daily excretion in total urine outflow. Whole-body balances of N and C compounds assumed that differences between dietary intake and fecal excretion were apparently digested. Digestible intakes were expressed as a proportion of total intake to express digestibility. Urinary losses were deducted from digestible intakes to derive metabolizable intakes or apparent balances. Fecal losses of N in NDF were assumed to be undigested dietary N with the remainder composed of metabolic fecal N that included both unresorbed secretions and microbial N. Truly digestible intake of N was the sum of digestible N intake and metabolic fecal N. Minimal excretion of N was estimated from the intercept of the regression of urinary N loss on truly digestible N intake (Barboza et al. 1993).

Concentrations of stable isotopes were expressed in relation to references ($R_{standard}$) that were 0.0036765 $^{15}N/^{14}N$ (air) and 0.0112372 $^{13}C/^{12}C$ (PDB limestone; Ehleringer and Rundel 1988). Isotope ratios of samples (R_{sample}) were expressed as

$$\delta(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right)1{,}000$$

(Wolfe 1992). These expressions were rearranged to derive SA (^{15}Ng g^{-1} total N or ^{12}C g g^{-1} total C) as follows:

$$SA = \frac{(\delta/1{,}000 + 1)}{[1/R_{standard} + (\delta/1{,}000 + 1)]}.$$

Mass balance for stable isotopes was determined as the product of SA and the corresponding flux of total N or C in food, feces, and urine. The sum of isotopic excretions in urine and feces was used to calculate the average isotope ratio of N in excreta. Calculations of the source of urea-N were based on an algebraic model with two pools of contrasting $\delta^{15}N$, that is, body protein and diet (sensu Wilson et al. 1988; Tayasu et al. 1994; O'Brien et al. 2000). We assumed that blood cells represent body protein stores because $\delta^{15}N$ of muscle and clotted blood are similar in caribou and reindeer as well as other species (Hobson et al. 1993).

We also assumed that degraded and recycled urea-N were similar to dietary N and therefore did not constitute an isotopically distinct pool. Urea-N was modeled as

$$\delta^{15}\text{N-urea} = p(\delta^{15}\text{N-blood cell} + \Delta) + (1 - p)(\delta^{15}\text{N-diet} + \Delta),$$

where p was the proportion of urea-N from body protein ($p$urea-N) and Δ was the discrimination or overall fractionation of N in urea. Discrimination of N during synthesis of urea (Δurea-N) was estimated from the difference between $\delta^{15}N$-urea and $\delta^{15}N$-diet for animals in positive N balance:

$$\Delta\text{urea-N} = \delta^{15}\text{N-urea} - \delta^{15}\text{N-diet}.$$

We used programs in SYSTAT 11.0 (SYSTAT, Richmond, CA) to calculate and analyze statistics. Repeated measures of body mass, body composition, and $\delta^{15}N$ and $\delta^{13}C$ in blood, metabolites, feces, or urine were compared by ANOVA with time (weeks from parturition) as a factor within animals and reproductive state (unbred or pregnant) as a factor between animals. Balance measures such as food intake were tested for the effect of reproductive state with body mass (kg) as a covariate. Mass-specific rates were subsequently calculated with metabolic body mass ($kg^{0.75}$). Pairwise contrasts were performed with Bonferroni's adjustments for multiple comparisons. Data expressed as proportions or percentages (e.g., urinary N components) were transformed to the arcsine of the square root to meet assumptions of normality for ANOVA and paired t-tests (Zar 1999). Means are reported ± 1 SD. Coefficient of variation (CV) was the SD divided by the mean. Statistical significance was determined as <5% probability of Type I error ($P < 0.05$).

Results

Body Mass and Composition

Female reindeer lost body mass and subcutaneous fat over winter from 10 to 2 wk before the average date of parturition (Fig. 2). Unbred females began to gain body mass, while pregnant females lost 6.1% ± 2.4% of body mass when calves were born at 6.6 ± 0.7 kg or 6.2% ± 0.9% of maternal mass (108.1 ± 12.7 kg). All pregnant females delivered healthy calves. Neonatal calves were estimated to contain only 0.14 ± 0.2 kg fat with 6.13 ± 0.58 kg lean tissue that included 1.16 ± 0.13 kg protein or 186 ± 20 g N. Young reindeer represented an investment of 7.3% ± 0.7% of lean mass and 0.9% ± 0.7% of fat mass in the summed tissues of the mother and calf (Fig. 3). Lactating and unbred reindeer contained similar amounts of lean mass (78.7 ± 10.5 kg) and fat (17.3 ± 8.4 kg). Female reindeer lost 64% ± 14% (29.1 ± 3.9 kg) from total body fat (46.4 ± 6.7 kg) but gained 34% ± 11% (19.4 ± 6.0 kg) from total lean mass

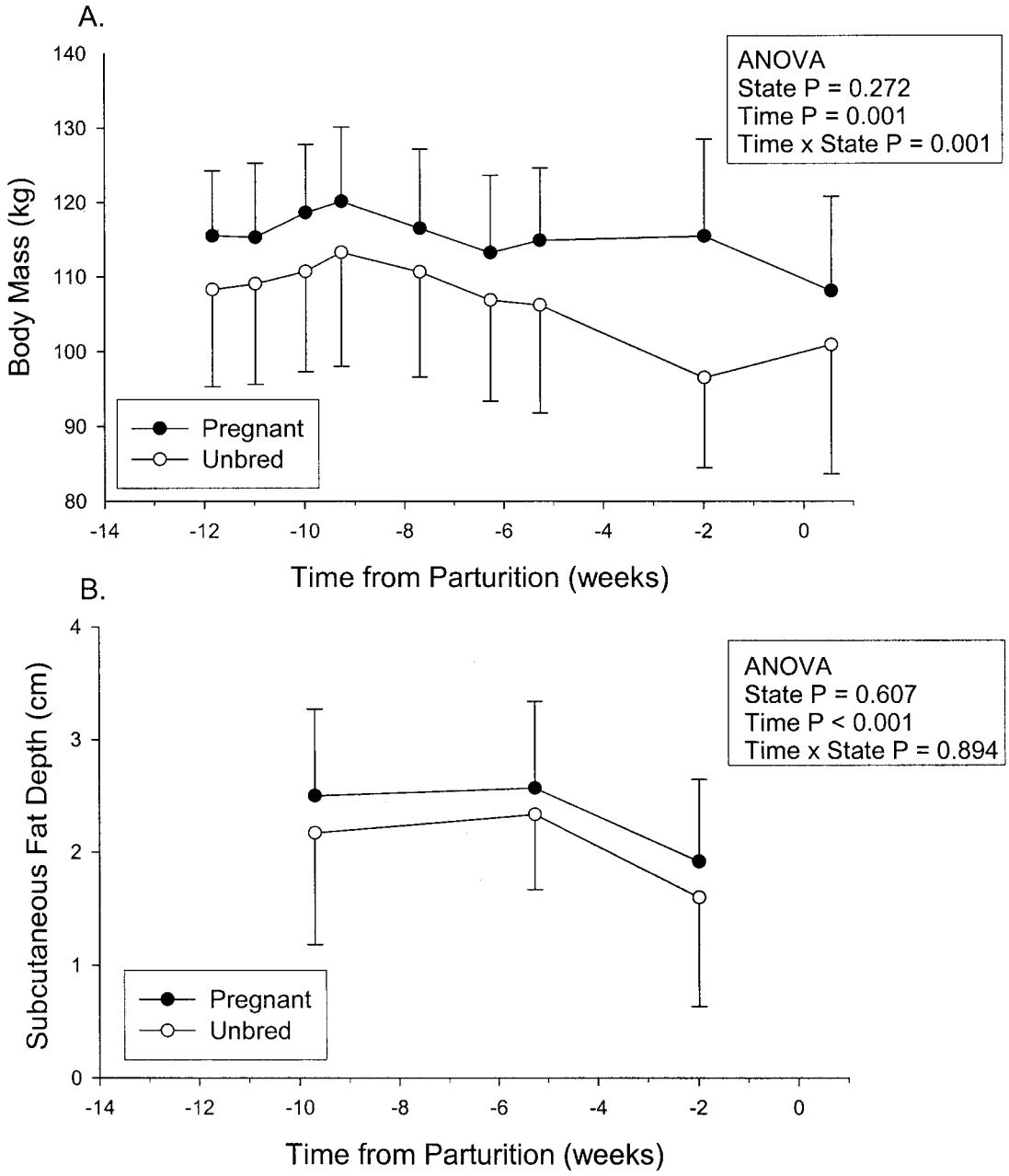

Figure 2. Mean (±SD) body mass (*A*) of pregnant (*filled symbols*; $n = 7$) and unbred (*open symbols*; $n = 3$) female reindeer during winter (weeks from the average date of parturition on April 15, 2003). *B*, Rump fat depth in six pregnant and three unbred females.

(59.3 ± 7.5 kg) over 74 ± 7 d between February and April. These changes were equivalent to an average loss of 14.14 ± 2.35 MJ d^{-1} with a gain of 10 ± 3 g N d^{-1}.

Blood $\delta^{15}N$ was higher than diet (1.24‰ ± 0.35‰) for both cells (6.54‰ ± 0.41‰) and sera (6.79‰ ± 0.55‰) at all times (Fig. 4). The isotopic difference between blood and dietary N decreased as $\delta^{15}N$ declined in blood cells of pregnant and unbred reindeer by 0.59‰ ± 0.26‰ over winter. Declines of $\delta^{15}N$ in sera were most pronounced in pregnant females during the last 4 wk of gestation (Fig. 4*B*). Isotope ratios of urinary N also declined through winter albeit at much lower $\delta^{15}N$ than for serum (2.06‰ ± 1.82‰ vs. 6.79‰ ± 0.55‰; Fig. 4*C*). Similarly, $\delta^{13}C$ in urine (−21.33‰ ± 0.91‰) and sera (−20.79 ± 0.20‰) also exceeded the diet (−23.19‰ ± 0.87‰; $P < 0.05$). Feces was higher in $\delta^{15}N$ (4.92‰ ± 2.21‰) than urine or diet. Blood cells in the newborn calf were enriched

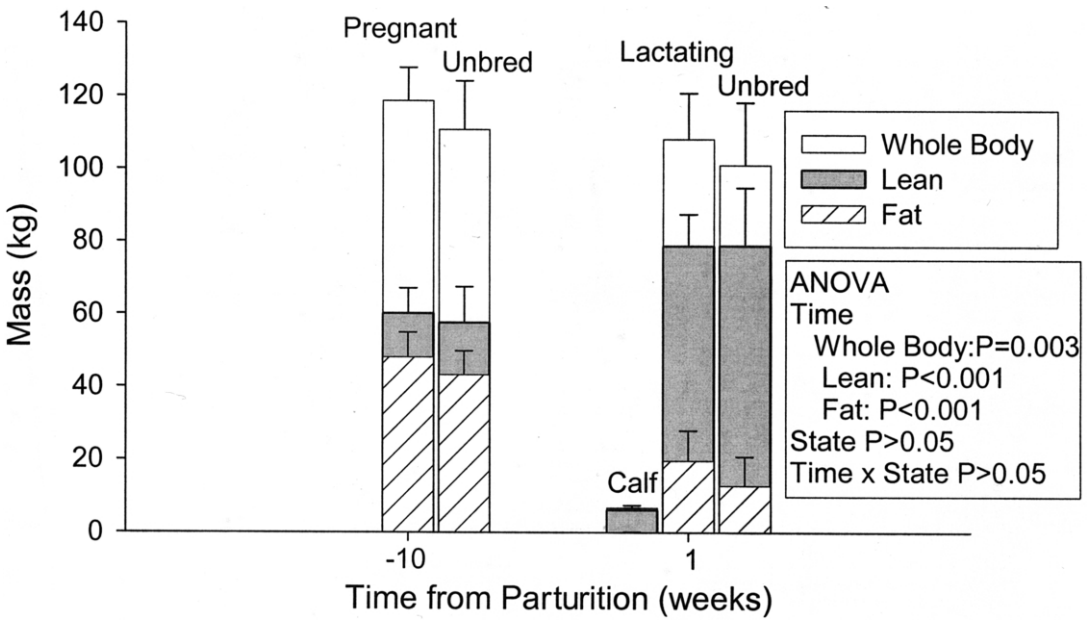

Figure 3. Mean (±SD) body composition of unbred females ($n = 3$) and reproductive females and their calves ($n = 6$; mean ± SD). Pregnant females were studied 10 wk from the average date of parturition (April 15) and within 1 wk (3.3 ± 2.6 d) after parturition. Composition was measured by dilution with tritiated water in adults and estimated from body mass of calves. Masses (kg) of lean (*shaded bars*) and fat tissue (*crosshatched bars*) are superimposed on whole body mass (*open bars*).

in both ^{15}N and ^{13}C when compared with blood from their mother (Fig. 5; $P < 0.05$). Serum values from calves were, however, isotopically similar to the serum from their mothers ($P > 0.05$).

Balance Measures

All animals remained calm and rested normally after 6 d of acclimation to cages. Although food intake varied widely between animals (15–1,551 g DM d^{-1}), each animal maintained a stable daily intake (7% ± 7% CV) over 4 d during the balance experiment. Reindeer lost 0.8 ± 0.4 kg d^{-1} from a median mass of 106 ± 12 kg during the balance experiment. Six animals were in negative N balance whereas three remained in positive N balance in cages. Net losses of N in six animals were equivalent to 593–2,634 kJ d^{-1} as protein, which was 12% ± 7% of the average daily loss of body energy between February and April.

During the experiment, the balances for energy and N were consistent with a steady state of depletion in body reserves (Fig. 1). Animals in cages did not show responses to stress or trauma. Plasma cortisol remained relatively constant through winter (1.67 ± 1.37 μg dL^{-1}), including the period from the start (1.93 ± 1.57 μg dL^{-1}) to the end (1.30 ± 1.17 μg dL^{-1}) of the balance experiment. Cortisol was not related to body mass loss or food intake in cages for either pregnant or unbred females ($P > 0.05$). Similarly, diagnostic parameters of blood sampled at the end of the period in cages were not significantly related to food intake ($P > 0.05$) for BUN (range: 13–27 mg dL^{-1}), creatinine (range: 1.3–2.4 mg dL^{-1}), serum protein (range: 7.6–8.6 g dL^{-1}), creatine kinase (range: 38–205 IU L^{-1}), glucose (range: 47–116 mg dL^{-1}), or ALT (range: 38–72 IU L^{-1}). Two serum enzymes, AST (range: 43–171 IU L^{-1}; $P = 0.014$) and LDH (range: 616–2,068 IU L^{-1}; $P = 0.016$), however, were inversely related to food intake in the cages. Nitrogen balance was directly related to food intake in cages ($P = 0.001$) and inversely related to serum activities of AST ($P = 0.014$) and CPK ($P = 0.015$): AST was 50 ± 10 versus 116 ± 48 IU L^{-1}, whereas CPK was 41 ± 5 versus 129 ± 51 IU L^{-1} for positive versus negative N balances, respectively.

Four animals (two pregnant and two unbred; 112 ± 17 kg) in the balance experiment consumed food (1,218 ± 446 g DM d^{-1}) at similar rates to reindeer fed pelleted diets outdoors during March 2004 (1,550 ± 361 g DM d^{-1}; 21 animals in two pens for three consecutive weeks; P. S. Barboza and K. L. Parker, unpublished data). We therefore report data for those four animals as the best representation of digestive responses, urea kinetics, and N flows in animals feeding outdoors (Fig. 6). Dry matter (DM) of the diet was composed of 7.67% ash, 43.59% C, 0.29% S, 34.40% NDF, 17.25% ADF, and 2.73% lignin. Digestibilities for four animals in cages were 80% ± 2% of DM, 43% ± 6% of ash, 81% ± 2% of C, 72% ± 3% of S, and 63% ± 6% of ADF. Gross energy content of the diet was 18.36 kJ g^{-1} DM, which was 81% ± 2% digestible. Total dietary N

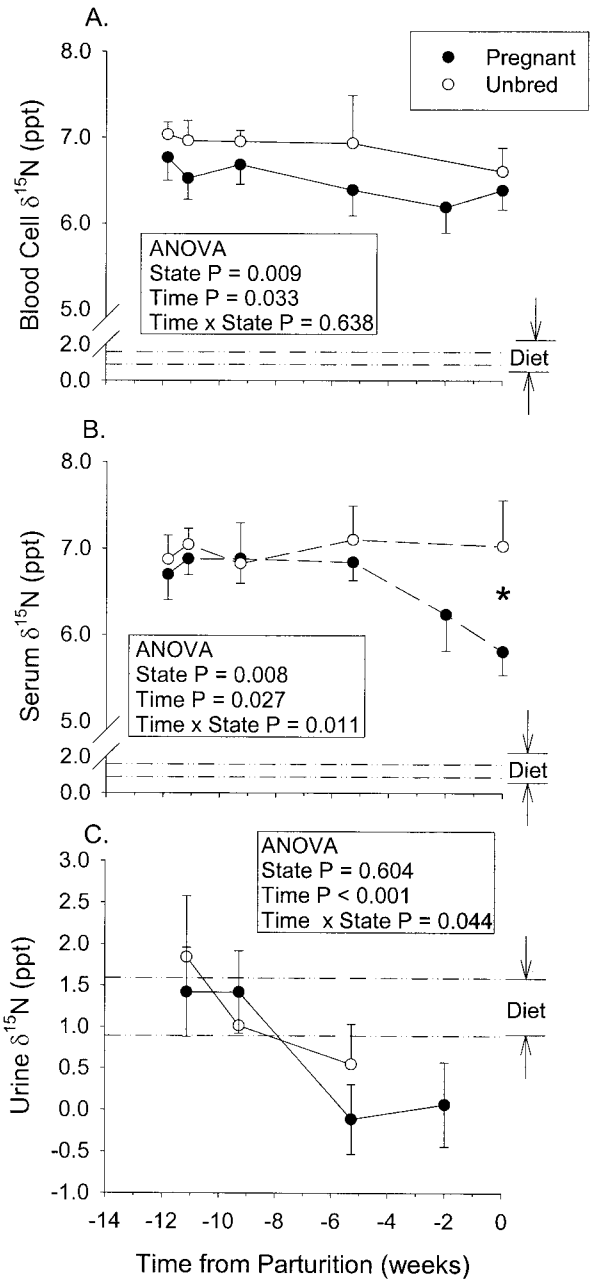

Figure 4. Isotope ratio of N (δ^{15}N ‰; mean ± SD) in blood cells (*A*), blood serum (*B*), and urine (*C*) of pregnant (*filled symbols*; $n = 7$) and unbred (*open symbols*; $n = 3$) female reindeer against time from the average date of parturition (April 15). Dashed lines indicate the range of dietary enrichment (1.24‰ ± 0.35‰). Asterisk indicates a significant difference between pregnant and unbred females at the average date of parturition.

was 2.54% of DM, with 1.25% DM in the NDF fraction (NDF-N). Fiber-bound N was more digestible (NDF-N, 72% ± 4%) than NDF (69% ± 4%; $P = 0.022$) but less digestible than total N in the diet (80% ± 2%; $P = 0.016$). True digestibility of N was 95% ± 1% after correcting for metabolic fecal N, which was 75% ± 4% of the fecal N loss.

Concentrations of urea-N in plasma (5% ± 3% CV) and urine (14% ± 4% CV) were stable over the 24-h urea kinetics trial and accompanied by linear declines in ln (SA) of labeled urea (all $R^2 > 0.995$, $n = 4$). The urea-N pool turned over every 11.28 ± 1.72 h, while excretion of 7.32 ± 3.24 g urea-N d^{-1} indicated that 70% ± 14% of the urea entering the pool was degraded during isotope dilution (Fig. 6). Synthesis of urea-N was equal to 88% ± 27% of the truly digestible N intake (Fig. 6). Urinary excretion of urea accounted for 32% ± 12% of the truly digested N with only 4.7% ± 1.4% in ammonia and 2.9% ± 1.4% in creatinine.

All data from the caged animals ($n = 9$) were used to test relationships with N balance. Intakes of DM, N, and energy were similar ($P > 0.05$) between unbred and pregnant females after accounting for body mass ($P < 0.05$). The minimal loss of N was 14.0 ± 1.3 g d^{-1} based on the following relationship:

$$\text{urinary N (g d}^{-1}) = 0.247(\text{truly digestible N intake}) + 14.0 \pm 1.2$$

($R^2 = 0.71$; $P = 0.001$). Corresponding minimal urinary loss of ^{15}N was 52 ± 5 mg ^{15}N d^{-1} based on a similar regression with truly digestible N intake ($P < 0.001$ for intercepts and slopes). Isotope ratios of urine increased as urinary losses of C and N declined with N balance for both δ^{15}N (range: −1.89 to 3.78; $P = 0.001$) and δ^{13}C (range: −22.3 to −20.0; $P = 0.003$). Isotope ratios in feces were not affected by N balance

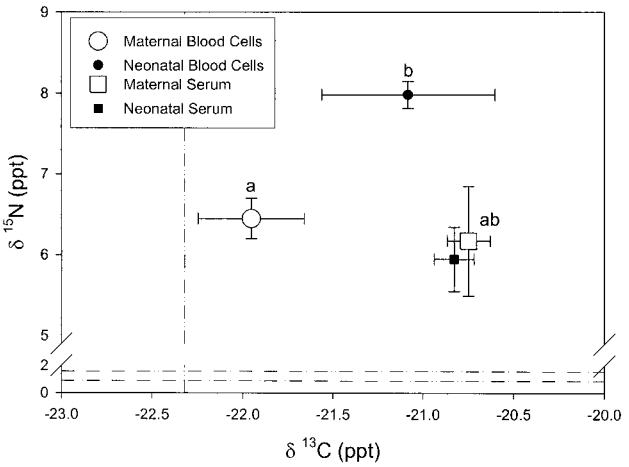

Figure 5. Postpartum isotope ratios of blood cells (*circles*) and sera (*squares*) from mothers ($n = 7$; *open symbols*) and their calves (*filled symbols*). Blood was sampled from calves within 24 h of birth, whereas maternal samples were drawn 3.3 ± 2.6 d from parturition. Dashed lines indicate the range of dietary isotope ratios for C (*vertical*; −23.19 ± 0.87) and for N (*horizontal*; 1.24‰ ± 0.35‰).

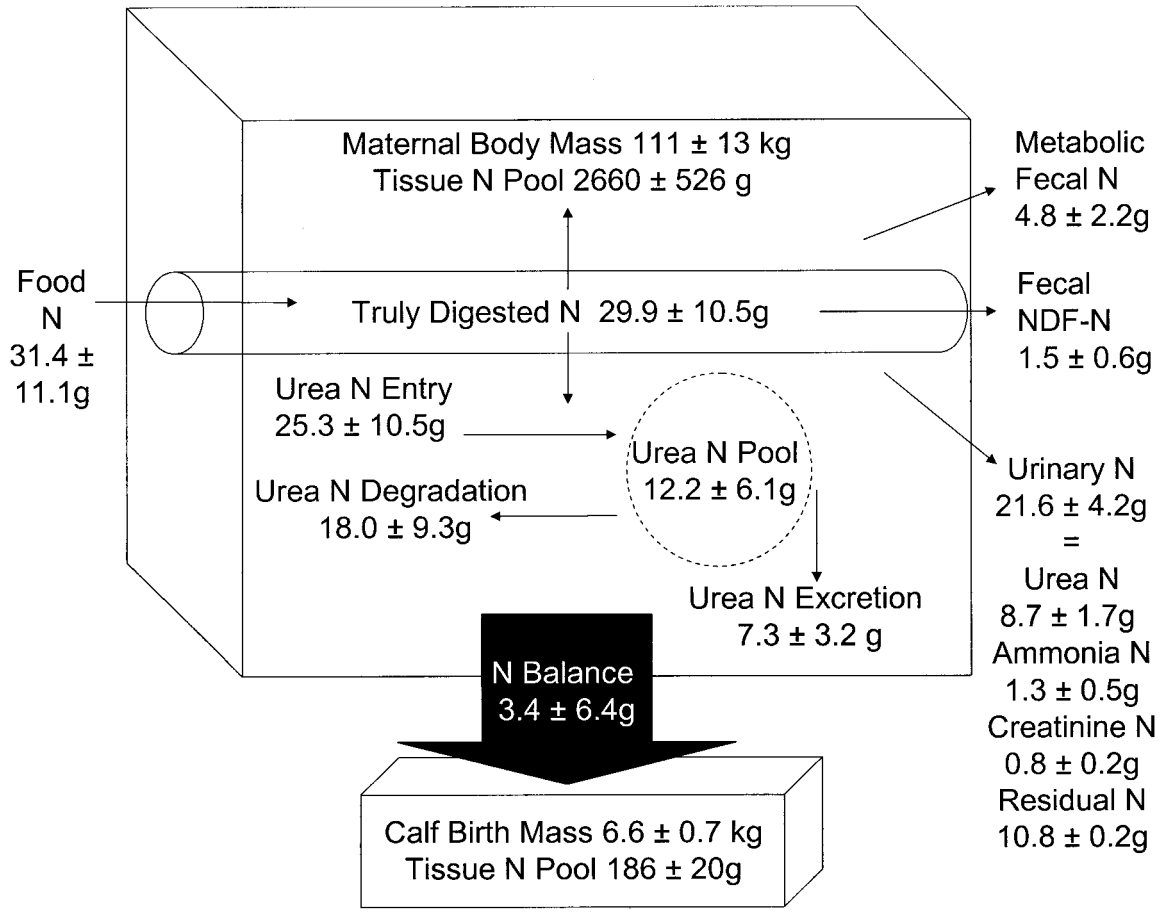

Figure 6. Flow of total N (g) through reindeer in late winter at 4 wk from the average date of parturition (April 15). Tissue N is the mean (±SD) body composition of reproductive and unbred adult females ($n = 9$; for February and for April) and calves ($n = 7$). Food intake, fecal, and urinary losses are from the balance experiment (average of 4 d) with two unbred and two pregnant females in March. Parameters for urea-N kinetics are means from the same four animals for 24 h after dosing with ^{14}C-urea.

for δ^{15}N (6.12 ± 2.09; $P = 0.294$) or δ^{13}C (−25.16 ± 0.41; $P = 0.696$). Average δ^{15}N of total excreta (range: 2.61 to 5.39) was higher than the diet but lower than blood cells at both positive and negative N balance. Consequently, ^{15}N/^{14}N retained in the body would have declined as dietary N was incorporated during positive N balance. Conversely, excretion of N that was depleted in comparison with blood cells would increase ^{15}N/^{14}N that remained in the body during negative N balance.

Declines in N balance were also related to decreasing proportions of urinary N in urea (range: 42%–32%; $P = 0.039$) and ammonia (range: 7.0%–1.5%; $P = 0.045$). Urinary pH (range: 8.7–5.9) decreased with N balance ($P = 0.008$) in direct relation to the decreasing proportion of urinary N in ammonia ($P = 0.016$). Creatinine-N excretion (0.76 ± 0.22 g d^{-1}) was not related to either N balance or urinary N loss at 3.9% ± 1.0% of urinary N. Urinary protein was only 12 ± 12 μg N dL^{-1} and just 0.12% ± 0.16% of urinary N. The proportion of unaccounted or residual urinary N increased as N balance declined (range: 47%–62%; $P = 0.024$). Urea and creatinine accounted for 16% ± 1% and 58% ± 5% of urinary C losses, respectively, across the range of N balance.

N Balance and Urea-N

Isotope ratios for ^{15}N/^{14}N in creatinine and blood cells were not affected by N balance ($n = 9$, $P < 0.05$; Fig. 7*A*). Carbon isotopes in blood cells (−21.9‰ ± 0.2‰) and creatinine (−22.6‰ ± 0.7‰) were similar and also unaffected by N balance ($P > 0.05$). Conversely, urea-N increased in δ^{15}N as N balance declined (Fig. 7*A*). Urea-N was depleted by 1.88‰ ± 1.01‰ relative to diet when N balance was positive in three females. Discrimination of N in urea synthesis was therefore estimated as −2.89‰ (Δurea-N; mean − SD) to calculate the

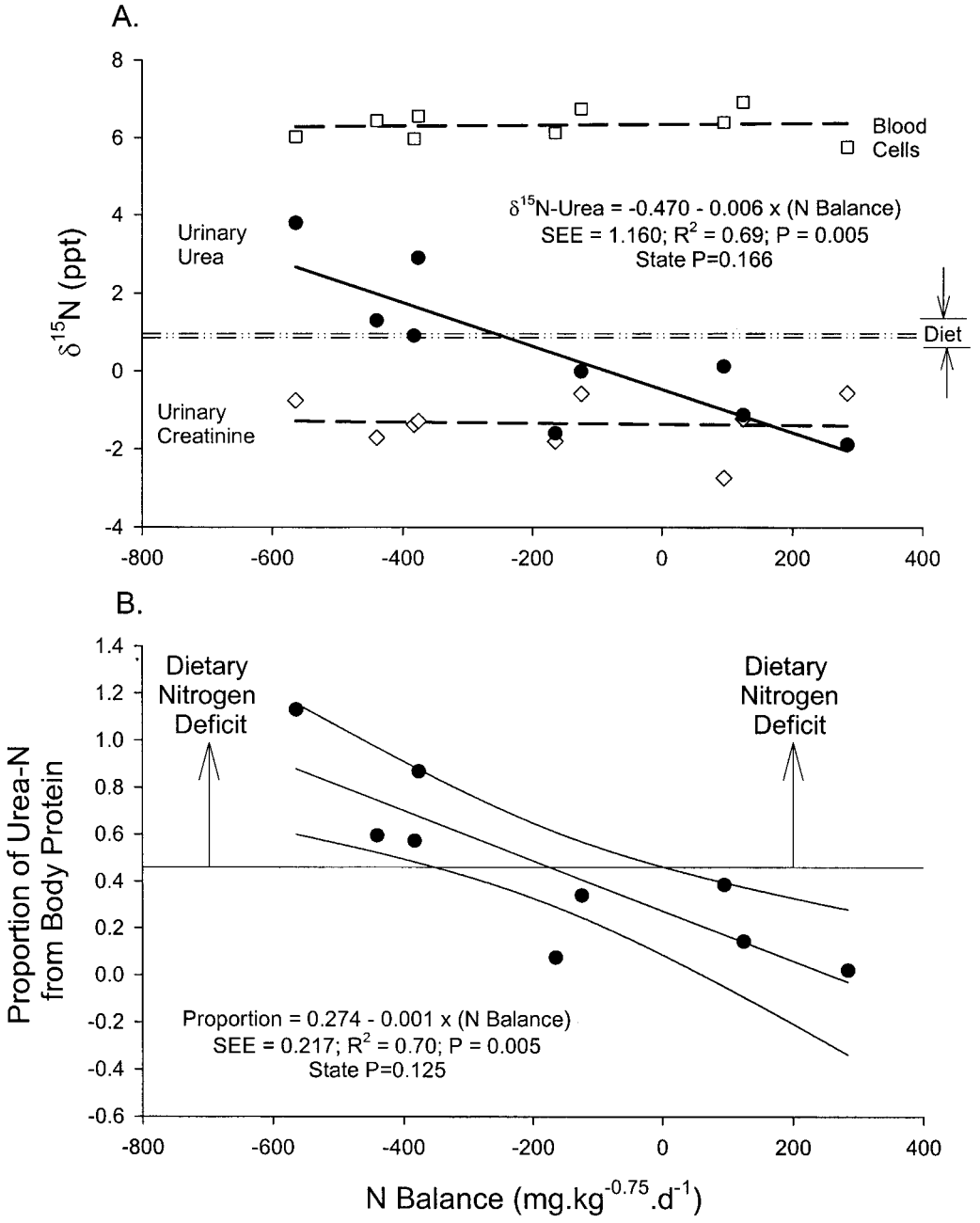

Figure 7. Comparisons of N balance (mg $kg^{-0.75}$ d^{-1}) with isotope ratios of body N pools (*A*) and the source of urea-N (*B*) in adult female reindeer. *A*, $\delta^{15}N$ (‰) in blood cells (*open squares*), urea (*filled circles*), and creatinine (*open diamonds*). Dashed horizontal lines indicate the range of dietary $\delta^{15}N$ in cages (0.91‰ ± 0.05‰). Lines through each series of points are least-squares regressions. *B*, Proportion of urea-N derived from body protein regressed against N balance (±95% confidence intervals). Proportions above 0.46 (*horizontal line*) indicate N balances below −200 mg $kg^{-0.75}$ d^{-1}.

proportion of urea-N from body protein (*p*urea-N). N balance was linearly related to *p*urea-N (Fig. 7*B*), which exceeded 0.46 when N balance was less than −200 mg N $kg^{-0.75}$ d^{-1} (Fig. 7*B*) and indicated that dietary N intakes were inadequate.

Plasma urea declined in uncaged animals held outdoors between 11 and 5 wk before average date of parturition (Fig. 8*A*). Isotope ratios of urea-N were variable in early winter but declined consistently through winter in pregnant females (n =

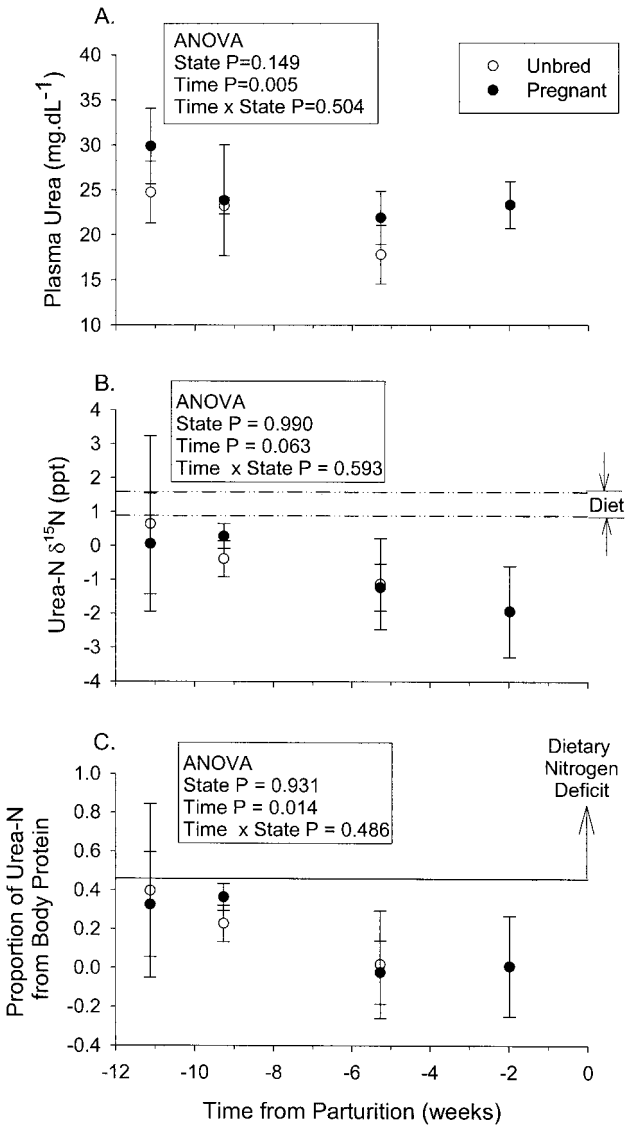

Figure 8. Urea dynamics over late winter in pregnant (*filled symbols*; $n = 7$) and unbred (*open symbols*; $n = 3$) female reindeer (mean ± SD) against time from the average date of parturition (April 15). *A*, Urea-N concentration (mg dL^{-1}) in blood plasma. *B*, Isotope ratio of urea-N ($\delta^{15}N$‰); dashed lines indicate the range of dietary $\delta^{15}N$ (1.24‰ ± 0.35‰). *C*, Proportion of urea-N derived from body protein; proportions above 0.46 (*horizontal line*) indicate N balances below −200 mg $kg^{-0.75}$ d^{-1}.

7, $P = 0.004$; Fig. 8*B*). Consequently, *p*urea-N was low for late gestation; that is, N balances were positive before and after animals were held in cages (Fig. 8*C*).

Discussion

Fat stores of ruminants are influenced by current energy demands (e.g., thermal, locomotor, productive) as well as endogenous regulation of appetite to replenish the body fat from dietary energy (Rhind et al. 2002). Fat stores were large in captive reindeer (40% ± 4% of body mass in February) and greater than those of adult female reindeer in the wild on Svalbard (33.6% body mass) at the start of winter (Reimers et al. 1982). Similarly, captive reindeer ended winter with more fat for spring (16% ± 7% of body mass in April) than adult females on Svalbard (9.86% body mass) in late winter (Reimers et al. 1982).

The loss of body fat in captive reindeer was probably due to low food intakes in both outdoor pens and indoor cages. Young reindeer held outdoors decrease food intake from January to March even when fed pelleted diets ad lib. (Ryg and Jacobsen 1982; Mesteig et al. 2000). Inappetence by some of the captive reindeer in cages was probably due to a decline in both locomotor and thermal demands (−15°C outdoors to 1°C indoors) as well as the availability of large stores of energy in fat. Abundant fat stores may allow female reindeer to minimize foraging when conditions are unfavorable. Food intake by individuals in cages was not related to plasma cortisol, a common indicator of stress in mammals (Reeder and Kramer 2005). The activity of AST and LDH in serum may be a correlate of energy deficits in reindeer. Our observation of negative correlations between food intake and these serum enzymes are supported by similar elevations of AST and LDH in pregnant and lactating females when feeding conditions are poor during late winter and spring (Nieminen 1980).

Protein deposition in female reindeer apparently follows the seasonal pattern of growth and metabolic rate in reindeer and caribou (Ryg and Jacobsen 1982; Fancy 1986; White et al. 1987; Mesteig et al. 2000). Most body protein was probably gained in the last 6 wk of gestation (Fig. 2), when $\delta^{15}N$ in blood cells, serum, and urine declined as N was incorporated from the diet (Fig. 3). Low food intakes can only support body protein gains when the dietary content of digestible N and energy are high. Net gains of N by females over winter (10 g d^{-1}) can be added to the rate of intake near N balance (31 g d^{-1}; Fig. 6) to estimate the average total dietary intake of 1,542 mg N $kg^{-0.75}$ d^{-1} by animals in outdoor pens. The corresponding average DM intake of 61 g $kg^{-0.75}$ d^{-1} for the high-N diet is similar to that of young reindeer fed pelleted foods outdoors in winter (70 g DM $kg^{-0.75}$ d^{-1}; Storeheier et al. 2003). This estimate of DM intake in female reindeer was equal to 855 kJ $kg^{-0.75}$ d^{-1} metabolizable energy, which exceeds total energy expended by free-living reindeer in winter (738 kJ $kg^{-0.75}$ d^{-1}; Gotaas et al. 2000) and was probably sufficient for body maintenance at the standard metabolic rate (SMR = 403 kJ $kg^{-0.75}$ d^{-1}; Fancy and White 1985). Diets with low digestible contents of N and energy may not support N gains in winter, even though reindeer are able to deposit protein. Food intakes required to gain the same amount of lean mass on lichens and senescent browse that contain only 0.4%–1.0% N (Parker et al. 2005) are predicted at 385 to 154 g DM $kg^{-0.75}$ d^{-1} if digestibilities are as high as the diets fed to

captive animals in this study. Those predictions exceed the upper limits of food intake in free-ranging animals (123–189 g $kg^{-0.75}$ d^{-1}; White et al. 1975; Holleman et al. 1979; Barboza and Bowyer 2001). Low dietary N may therefore prevent body N gains in wild reindeer and caribou at the end of winter. This suggestion is supported by reports of high calf production in herds from regions with mild winter weather and high plant production (Post and Klein 1999; Reimers 2005).

Dietary energy intakes below SMR may not support protein deposition and thus retention of N. Female reindeer in positive N balance in cages ($n = 3$; 168 ± 102 mg N $kg^{-0.75}$ d^{-1}) digested 586 ± 62 kJ $kg^{-0.75}$ d^{-1}, which was 145% of SMR, whereas animals in negative N balance ($n = 6$) digested less than 80% of the SMR. Energy intakes below maintenance increase N loss because protein synthesis declines and because amino acids from degraded body protein may be used for gluconeogenesis and energy (Young et al. 1981), even if body fat is the principal source of energy. Zero balance of N is therefore achieved at higher apparent intakes and losses of N when energy intakes are below maintenance. Consequently, the urinary loss of N predicted at zero N intake for captive reindeer in cages (422 ± 39 mg N $kg^{-0.75}$ d^{-1}) was greater than the endogenous urinary N loss for white-tailed deer (115–145 mg N $kg^{-0.75}$ d^{-1}; Robbins et al. 1974; Asleson et al. 1996) and other ruminants (Robbins 1993). Estimates of minimal urinary loss at submaintenance intakes of both energy and N are more representative of fasting and can be best used to predict the value of body stores. For example, if female reindeer lose N at the minimal excretion rate of 14 g N d^{-1}, they could fast for 54 d if the body store was equal to the net gain in body N between February and April (765 g N). The corresponding loss of energy from body fat at SMR would be 19 kg over 54 d, which was only 41% of total body fat in February. Protein storage with fat therefore allows reindeer and caribou to tolerate food shortages.

Protein storage in winter may be most important for reproduction in reindeer and caribou. Nonpregnant females can regain body mass and condition, while pregnant females acquire reserves for lactation when diets are adequate in late winter (Fig. 2). Pregnant females may require higher food intakes than unbred females to support protein storage and fetal growth. Increased N intakes are indicated by declines in serum $\delta^{15}N$ in pregnant females during the last 6 wk of gestation (Fig. 4*B*). The similarity in sera $\delta^{15}N$ between mothers and their calves is consistent with the transfer of milk to the calf because $\delta^{15}N$ of milk is directly related to maternal plasma in several species (Jenkins et al. 2001). The enrichment in both $\delta^{13}C$ and $\delta^{15}N$ of neonatal blood cells over that of their mother suggests that maternal protein, not dietary N, is the principal source of N for the fetus in reindeer. Maternal protein stores may be the most reliable source of fetal protein if N intakes are variable in caribou and reindeer during winter. Low stores of maternal protein have been proposed as a cause of embryonic mortality in caribou (Cameron et al. 1993; Russell et al. 1998). Although the investment of maternal N in the calf is small (Fig. 6), fetal development may be sensitive to small perturbations in the supply of amino acids. Maternal protein restriction during pregnancy can alter amino acid supply to the fetus (Wu et al. 1998), impair the growth of organs and muscle, and adversely affect postnatal growth and reproduction of the offspring (Sams et al. 1995; Robinson et al. 1999; Buttery et al. 2000; Langley-Evans 2000; Rees et al. 2000; Dwyer et al. 2003). The contribution of diet and maternal stores to N deposited in the calf during gestation and lactation awaits further study of reindeer and caribou.

Caribou and reindeer probably require as much N as other cervids in winter. The average dietary N requirement for adult male and female caribou (381 mg N $kg^{-0.75}$ d^{-1}; McEwan and Whitehead 1970) is similar to that of white-tailed deer (Asleson et al. 1996) and consistent with urinary losses of female caribou fed low-N diets in winter. Female caribou were probably in negative N balance at intakes of 315 ± 121 mg N $kg^{-0.75}$ d^{-1}, even though digestible energy intakes were 167% of SMR (676 ± 109 kJ $kg^{-0.75}$ d^{-1}; Parker et al. 2005). This suggestion is supported by the excretion of urea, ammonia, and residual N by caribou in similar proportions to reindeer in negative N balance. Furthermore, urinary urea-N was similar to or greater than diet $\delta^{15}N$ in caribou (Parker et al. 2005) and mostly derived from body protein (*p*urea-N = 1.05 ± 0.46 based on the same Δurea-N as reindeer).

N balance ultimately affects body $^{15}N/^{14}N$ ratios through the rate of elimination of N. We used mass balance to project the effect of positive N balance, negative N balance, and parturition on $\delta^{15}N$ of the body in reindeer by assuming that blood cells represented the average $\delta^{15}N$ of body tissues (Table 1). Projected loss of 16% body N would enrich body $\delta^{15}N$ by 0.19‰ in negative N balance, whereas a gain of only 7% body N predicted a decrease in body $\delta^{15}N$ of 0.96‰ (Table 1), even though total excreta was more enriched than diet. These changes in $\delta^{15}N$ of the body are consistent with the general pattern of "depletion in gain" and "enrichment in loss" observed and predicted in fed and food-restricted animals (Hobson et al. 1993; Ponsard and Averbuch 1999).

The rate at which body $^{15}N/^{14}N$ changes is dictated by the amount of N ingested and excreted and by the rate at which tissues are deposited or mobilized in relation to the size of the body pool (Ponsard and Averbuch 1999). Isotopic changes in the tissues of reindeer are small because the body N pool is large when compared with intake and excretion (Fig. 6). Furthermore, the rate of turnover of N in blood cells is much slower than that of the exchangeable pools of N in urea and other intermediary metabolites. Consequently, the decline in urinary $\delta^{15}N$ preceded those of blood cells and serum during winter (Fig. 4). Blood cells continued to decline in $\delta^{15}N$ from 6.58 ± 0.46 to 6.03 ± 0.35 ($P = 0.002$) during the 10 d of the balance experiment, even though N balance varied greatly dur-

Table 1: Projected effect of isotopic N balance on average δ^{15}N of female reindeer fed a high-N diet during winter

Condition	Total N (g)	^{15}N (g)	^{14}N (g)	Time (d)	Average δ^{15}N Body (‰)
Initial mass[a]	2,659	9.8	2,650	0	6.58
Final mass after:					
N loss[b]	2,235 (−14.134)	8.2 (−.052)	2,227 (−14.082)	30	6.77
N gain[c]	2,844 (+6.170)	10.5 (+.022)	2,834 (+6.148)	30	5.62
Parturition[d]	2,473 (−186)	9.2 (−.687)	2,464 (−185)	1	6.45

Note. Values in parentheses are rates (g d^{-1}).

[a] Initial mass was the mean body N of unbred and bred females between −10 and 1 wk of average parturition date. Initial ^{15}N and ^{14}N were calculated from the average δ^{15}N of blood cells at the start of the balance experiment (5.3 wk from parturition).

[b] N loss was calculated as ^{15}N and ^{14}N balance of four females in negative N balance (−441 ± 87 mg N kg$^{-0.75}$ d^{-1}).

[c] N gain was calculated as ^{15}N and ^{14}N balance of three females in positive N balance (168 ± 102 mg N kg$^{-0.75}$ d^{-1}).

[d] Parturition loss was calculated from average body N of calves and δ^{15}N of blood cells on day of birth.

ing this brief interval (Fig. 7). Differences between tissues in cell and protein turnover partition N exchanges within the body. Fetal N is apparently separate from the pools of N that involve much of the dietary and intermediary exchanges of N in the mother (Fig. 5). Maternal blood cells increased in δ^{15}N (6.18 ± 0.30 vs. 6.38 ± 0.23; $P = 0.004$), while serum was unchanged between 2 wk prepartum and the week of birth. Maternal blood did not therefore reflect the decrease in the average δ^{15}N of the body predicted by mass balance at parturition (Table 1).

Urea-N is sensitive to small changes in N flux in reindeer because the pool turns over twice each day near zero balance (Fig. 6). Urea production in the liver is closely related to dietary N intake in mammals (Waterlow 1999), but that dietary source is modified by fermentation in ruminants. Ruminal microbes produce ammonia, protein, and nucleic acids from dietary N as well as endogenous secretions (Morrison 2000). High δ^{15}N of feces compared with diet in reindeer was probably due to microbial contributions that accounted for 75% of fecal N. In positive N balance, urea is mainly formed from ammonia and amino acids absorbed from the diet (Lobley et al. 2000). Urea δ^{15}N in positive N balance is therefore an outcome of microbial N flow as well as hepatic ureagenesis, both of which may vary with energy and N intake (Nolan 1993). Isotopic variance associated with C and N supplies has been demonstrated in the growth of microbes (Macko et al. 1984) and is well demonstrated among invertebrates (Adams and Sterner 1984; Scrimgeour et al. 1995; Webb et al. 1998; Fantle et al. 1999). The residual fraction of urinary N in reindeer was probably also related to microbial flow because purine derivatives would account for the remaining 50% of N and 26% of C. Urinary excretion of purines is related to energy and N intake through the flow of microbial cells from the rumen in cervids and domestic ruminants (Garrott et al. 1996; Morrison and Mackie 1997). Consequently, the difference between urea-N and dietary δ^{15}N (Δurea-N) may vary with seasonal changes in intake of energy and N in reindeer and caribou.

Urea produced in negative N balance would be derived from free amino acids returning to the liver (Lobley et al. 2000). Concerted changes in both δ^{15}N and δ^{13}C of blood cells and sera (Figs. 4, 5) suggest that many of the changes in these lean tissues are due to exchanges of amino acids in reindeer. Ureagenesis from amino acids uses ammonia from deamination and aspartate from transamination (Jackson and Golden 1981). Transfer of amino groups by glutamate-oxaloacetate transaminase can discriminate against ^{15}N to produce depleted aspartate or glutamate (Macko et al. 1986) and subsequently depleted urea-N. The source of amino-N for urea synthesis may also change with time during negative N balance. Prolonged fasting in a steady state of negative N balance can alter plasma amino acid concentrations in mammals (Wright et al. 1999; Mustonen et al. 2004). Amino acids used for urea-N may vary in ^{15}N/^{14}N based on their essentiality or routes of oxidation (Gaebler et al. 1966), the turnover of proteins in the catabolized tissues and the complement of acids needed for continued synthesis of protein (Reeds et al. 1994). Although δ^{15}N of urea in female reindeer did not change with the short time in cages, the prolonged isotopic effects of negative N balance (Fig. 1) require further testing.

Reutilization of urea-N in amino acids may have only a small effect on tissue δ^{15}N because N is returned to a large exchanging pool of nonessential amino acids. The contribution of urea-N to amino acids is probably only 60% of the degradation rate because 40% of the degraded urea-N is resynthesized as urea across a wide range of food intakes in sheep (Sarraseca et al. 1998). Reutilization of urea-N, however, would contribute to tissue enrichment indirectly by sparing oxidation of amino acids and thus promoting amino acid reutilization from degraded protein. Without urea degradation, urinary N losses would more than double in female reindeer near N balance (Fig. 6).

Urea recycling may also reduce the cost of water and energy for renal excretion in reindeer when water is only available as snow (Hove and Jacobsen 1975). Costs of renal excretion and acid-base regulation probably increase with N intake in reindeer. Deamination of glutamine at the kidneys was the likely cause of increases in ammonia-N with N intake in reindeer (Munro 1969; Groff et al. 1995).

Ratios of $^{15}N/^{14}N$ in urea, blood cells, creatinine, and fecal N may be used to monitor N losses in relation to tissue and diet of wintering ruminants. The largest source of variation in $^{15}N/^{14}N$ is probably associated with changes in food intake and food selection that can change both nutrient and isotopic composition of the diet (Staaland et al. 1988; Barnett 1994; Jorgenson et al. 2002). Subsequent variance in the discrimination of N during ureagenesis (Δurea-N) affects the estimate of the proportion from body protein (*p*urea-N). We used a conservative estimate for Δurea-N (mean − SD) because *p*urea-N is increased by small values for the discrimination. Estimates of Δurea-N in this study, however, were similar to those of caribou fed high-N diets in early winter (−2.54 vs. −2.89; Parker et al. 2005).

Our isotopic approach to monitoring N balance can be used to test the functional limits of protein stores for reproduction in reindeer and caribou in both wild and captive populations. The importance of protein stores for reproduction in reindeer is consistent with high fecundity and abundance of insular populations when mild temperatures provide abundant food (Klein 1987; Swanson and Barker 1992) and with reproductive pauses in mainland populations exposed to variable conditions of food and weather (Cameron et al. 2002; Griffith et al. 2002).

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