GROWTH AND NUTRITIONAL DEVELOPMENT OF REINDEER (*RANGIFER TARANDUS*) AND MUSKOXEN (*OVIBOS MOSCHATUS*)

By

Katrina Kay Knott

RECOMMENDED:

Advisory Committee Chair

Chair, Department of Biology and Wildlife

APPROVED:

Dean, College of Science, Engineering and Mathematics

Dean of the Graduate School

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Date

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A

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Katrina Kay Knott, BA

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ABSTRACT

Young ruminants must grow and develop digestive function during brief summers in the Arctic. I examined growth and development of nutritional organs in reindeer and muskoxen as neonates (1 d), during transition from milk to forage (30-60 d) and at maturity. Reindeer and muskoxen gave birth to relatively smaller offspring than ruminants from more temperate regions. Costs of small birth mass are likely offset in neonates by an increase of thyroid hormones to enhance thermogenesis and hepatic reserves that provide additional nutrients during early development. Body mass gains during the neonatal period (1-30 d) were associated with well-developed abomasa that allow young to utilize milk immediately after birth. Transition to forage coincided with mass gains of the rumen, small intestine and colon. Digestive morphology also was modified to facilitate fermentation of plants and enhance digestion and absorption of nutrients by 60 days of age. Digestive anatomy of young reindeer and muskoxen also indicated that feeding strategies of adults may be determined from birth. Growth of reindeer and muskoxen, therefore, is dependent upon an endogenous sequence of nutritional development that allow young to take advantage of concentrated milk after birth and time fermentative function to plant emergence at high latitudes. These advances permit young to meet requirements of growth and establish reserves before winter.

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INTRODUCTION

Growth is determined by phylogenetic attributes of taxa that affect life-history components, and physical demands for development of digestive function (Dunham et al., 1989; Ricklefs, 1991; Ricklefs et al., 1998). Life history events such as growth, organ development and nutritional strategy are regulated by the endogenous (genetic) plan inherent of the species. If life events are "plastic", such that they can be modified by environmental effects, divergences of the endogenous plan could be evident at environmental extremes (West-Eberhard, 2003). The Arctic is a variable environment that has large annual fluctuations in photoperiod, temperature, and plant availability (Pielou, 1994). Arctic ruminants have shown a remarkable synchrony plant phenology in these environments by timing parturition to precede plant productivity for sufficient consumption of plants by both mother and offspring to enhance survival during austere winters (Cameron et al., 1993; Rachlow and Bowyer, 1994; Adamczewski et al., 1997; Bowyer et al., 1998).

Synchrony of birth with plant emergence is important because early plant phenologies hold the greatest nutrient contents and highest potential digestibility (Klein and Bay, 1990; Rachlow and Bowyer, 1994; Jorgenson et al. 2002; Johnstone et al., 2002). Although studies have discussed the importance of abundance and quality of plants for maternal investment to the fetus and in milk (Crete and Huot, 1993; Rachlow and Bowyer, 1994; White et al., 1997; Rachlow and Bowyer, 1998), few studies have discussed the relationships among diet, growth and development of offspring. Arctic ruminants are born in early spring and are quickly weaned (White and Luick, 1984;

Parker et al., 1990; Lavigueur and Barrette, 1992) to decrease lactational demands of the mother. Survival of young ruminants, therefore, likely depends on a rapid transition to high-quality plants to meet the requirements of growth and establish energy reserves before winter (Keech et al., 2000).

At birth, ruminants do not yet have digestive systems capable of fermentation of cellulytic plants (Church et al., 1961; Lyford and Huber, 1988); therefore, young arctic ruminants must also undergo a rapid transition in digestive function during their first summer. Digestive development of young ruminants includes an increase in the capacity and function of viscera to support increased somatic demands and also establish fermentative function (Batt 1980; Janssens and Ternouth 1987; Lyford 1988; Owens et al. 1993). Digestive function, however, must complement the composition of the diet to maximize utilization of nutrients for growth. Nutritional development, therefore, may be part of an endogenous program in ruminants to anticipate the changes from milk to forage consumption.

Differences in dietary consumption and digestive anatomy among ruminants have led to their classification as grazers that consume greater amounts of fibrous plants, browsers that select more digestible forages, and intermediate feeders that select a combination of forbs, woody browse, and grasses (Hofmann, 1988; Hofmann, 2000; Clauss et al., 2003). Differences in diet and morphology also may reflect greater body size of grazers that can further enhance the capacity to consume and degrade fibrous plants (Illius and Gordon, 1999; Barboza and Bowyer, 2000). Seasonal fluctuations and reproductive demands can further stimulate changes in digestive morphology in response

to nutritional value of the diet (Staaland et al., 1995; Lentle et al., 1996; Hofmann and Nygren, 1992; Barboza and Bowyer, 2001). Digestive plasticity, however, can only act as a secondary response to modify the ontogenetic program. Development of digestive function that allow young ruminants to utilize plants for nutrition, therefore, may also include specialized digestive structures and allow the greatest adaptation to their nutritional niche as adults.

My research investigated two subspecies of *Rangifer tarandus* (reindeer, *R. t. tarandus*; caribou, *R. t. grantii*) and *Ovibos moschatus* (muskoxen) that are endemic to the arctic and sub-arctic regions of Alaska to examine how environmental extremes may affect growth and nutritional development of their offspring. This study was conducted under captive conditions at the R. G. White Large Animal Research Station and the Biological Reserve maintained by the Institute of Arctic Biology, University of Alaska Fairbanks (65° N, 146° W). Captive conditions allowed for investigation of growth in young ruminants under similar environmental conditions and without nutritional constraints on growth. Young ruminants were also provided diets with similar composition to examine development of feeding strategy in each species. My research, therefore, attempted to limit the exogenous cues, such as diet, nutritional status, and climate, to investigate how the endogenous program of reindeer and muskoxen drives growth and determines the nutritional development under the maximum potential of each species.

In chapter 1, I studied growth of the body and nutritional organs in reindeer and muskoxen in three stages: the neonatal period (1 - 30 d), during the transition from milk

to forage (30 - 60 d), and at maturity (> 7 yrs). Lipid concentrations in the liver and muscle were also examined at each stage, as well as levels of thyroid hormones, to follow deposition of mass and determine signals for growth. I hypothesized that earlier reproductive age in reindeer and caribou in comparison to muskoxen would be associated with more rapid somatic and visceral growth. I also discuss the interplay of endogenous and exogenous cues that may act to regulate development. Growth and dietary transitions of young reindeer and muskoxen during brief summers also are compared with domestic and more temperate counterparts that may have less environmental constraints on growth.

In chapter 2, I continue my examination of growth in reindeer and muskoxen by focusing on nutritional development of feeding strategies. I measured the structural morphology and rates of cell division of the abomasum, rumen, small intestine and liver in young and adult animals to follow the digestive advances that occur during development *in utero* and during the transition from milk to forage. In addition, I measured the composition of the ingesta from the rumen and abomasum to determine if morphological changes were associated with differences in diet between ages and species. I hypothesized that the digestive morphology of young (< 2 mo) reindeer and muskoxen would anticipate dietary transitions and determine the adult feeding strategy of muskoxen as grazers and reindeer as intermediate feeders. I predicted that young muskoxen would exhibit digestive morphologies that would favor a better utilization of fibrous plants when compared with reindeer. Conversely, young reindeer were predicted to show specialized digestive morphology that would relate to their consumption of more digestible forages.

Literature cited

Adamczewski, J. Z., P. F. Flood, and A. Gunn.1997. Seasonal patterns in body composition and reproduction of female muskoxen (*Ovibos moschatus*). Journal of Zoology (London) 241: 245-269.

Barboza, P. S. And R. T. Bowyer. 2000. Sexual segregation in dimorphic deer: a new gastrocentric hypothesis. Journal of Mammalogy 81: 473-489.

Barboza, P. S. And R. T. Bowyer. 2001. Seasonality of sexual segregation in dimorphic deer: extending the gastrocentric model. Alces 37: 275-292.

Batt, R. A. L. 1980. Influences on animal growth and development. Studies in Biology no. 116. Camelot Press, London.

Bowyer, R. T. 1998. Timing and synchrony of parturition in Alaskan moose: long-term versus proximal effects of climate. Journal of Mammalogy 79: 1332-1344.

Cameron, R. D., W. T. Smith, S. G. Fancy, K. L. Gerhart, and R. G. White. 1993. Calving success of female caribou in relation to body weight. Canadian Journal of Zoology 71: 480-486.

Church, D. C., G. L. Jessup, and R. Bogart. 1961. Stomach development in the suckling lamb. American Journal of Veterinary Research 23: 220-225.

Clauss, M., M. Lechner-Doll and W. J. Streich. 2003. Ruminant diversification as an adaptation to the physiomechanical characteristics of forage. A reevaluation of an old

debate and a new hypothesis. Oikos 102: 253-262.

Crete, M. and J. Huot. 1993. Regulation of a large herd of migratory caribou: summer nutrition affects calf growth and body reserves of dams. Canadian Journal of Zoology 71: 2291-2296.

Dunham AE, BW Grant and KL Overall. 1989. Interfaces between biophysical and physiological ecology and the population ecology of terrestrial vertebrate ectotherms. Physiological Zoology 62: 335-355.

Hofmann, R. R. 1988. Morphophysiological evolutionary adaptations of the ruminant digestive system. In: Aspects of Digestive Physiology in Ruminants (A. Dobson and M. J. Dobson, eds.). Comstock Publishing Associates, Ithaca and London, pp. 1-20.

Hofmann, R. R. 2000. Functional and comparative digestive system anatomy of Arctic ungulates. Rangifer 20: 71-81.

Hofmann, R. R. and K. Nygren. 1992. Ruminal mucosa as indicator of nutritional status in wild and captive moose. Alces Supplement 1: 77-83.

Illius, A. W. and I. J. Gordon. 1999. The physiological ecology of mammalian herbivory. In: Nutritional Ecology of Herbivores. (H. J. G. Jung and G. C. Fahey, eds.). American Society of Animal Science, Savoy, Illinois, pp 71-96.

Janssens, P. A. and J. H. Ternouth. 1987. The transition from milk to forage diets. In: Nutrition of Herbivores. Academic Press, Australia, pp. 281-303.

Johnstone, J., D. E. Russell and B. Griffith. 2002. Variations in plant quality in the range of the Porcupine caribou herd. Rangifer 22: 83-91.

Jorgenson, J. C., M. S. Udevitz and N. A. Felix. 2002. Forage Quantity and Quality. In: Arctic Refuge Coastal Plain Terrestrial Wildlife Research Summaries (D. C. Douglas, P. E. Reynolds and E. B. Rhode, eds.). U.S. Geological Survey, Reston, Virginia, pp. 46-50.

Keech, M. A., R. T. Bowyer, J. M. Ver Hoef, R. D. Boertje, B. W. Dale, and T. R. Stephenson. 2000. Life-history consequences of maternal condition in Alaskan moose. Journal of Wildlife Management 64: 450-462.

Klein, D. R. and C. Bay. 1990. Foraging dynamics of muskoxen in Peary Land, northern Greenland. Holarctic Ecology 13: 269-280.

Lavigueur, L. and C. Barrette. 1992. Suckling, weaning, and growth in captive woodland caribou. Canadian Journal of Zoology 70: 1753-1766.

Lentle, R. G., I. M. Henderson and K. J. Stafford. 1996. A multivariate analysis of rumen papillary size in red deer (*Cervus elaphus*). Canadian Journal of Zoology 74: 2089-2094.

Owens, F. N., P. Dubeski, and C. F. Hanson. 1993. Factors that alter the growth and development of ruminants. Journal of Animal Science 71: 3138-3150.

Parker, K. L., R. G. White, M. P. Gillingham and D. F. Holleman. 1990. Comparison of

energy metabolism in relation to daily activity and milk consumption by caribou and muskox neonates. Canadian Journal of Zoology 68: 106-114.

Pielou, E. C. 1994. A Naturalist's Guide to the Arctic. The University of Chicago Press, Chicago, Illinois.

Rachlow, J. L. and R. T. Bowyer. 1994. Variability in maternal behavior by Dall's sheep: environmental tracking or adaptive strategy? Journal of Mammalogy 75: 328-337.

Rachlow, J. L. and R. T. Bowyer. 1998. Habitat selection by Dall's sheep (*Ovis dalli*): maternal trade-offs. Journal of Zoology (London) 245: 457-465.

Ricklefs RE, Stark JM and M Konarzewski. 1998. Internal constraints on growth in birds. In: Avian Growth and Development: Evolution within the Altricial-Precocial Spectrum. (JM Stark and RE Ricklefs, eds.). Oxford University Press, Inc., New York, pp. 266-287.

Ricklefs RE. 1991. Structures and transformation of life histories. Functional Ecology. 5: 174-183.

Staaland, H., J. Z. Adamczewski, and A. Gunn. 1995. A comparison of digestive tract morphology in muskoxen and caribou from Victoria Island, Northwest Territories, Canada. Rangifer 17: 17-19.

West-Eberhard, M. J. 2003. Developmental plasticity and evolution. Oxford University

Press, New York, New York.

White, R. G. And J. R. Luick. 1984. Plasticity and constraints in the lactational strategy of reindeer and caribou. Symposium of the Zoological Society of London 51: 215-232.

White, R. G., J. E. Rowell, and W. E. Hauer. 1997. The role of nutrition, body condition and lactation on calving success in muskoxen. Journal of Zoology (London) 243: 13-20.

CHAPTER 1. GROWTH IN ARCTIC UNGULATES: POSTNATAL DEVELOPMENT AND ORGAN MATURATION IN *RANGIFER TARANDUS* AND *OVIBOS MOSCHATUS*[1]

Abstract

Young arctic ungulates must grow quickly to use forages available during brief summers. We measured growth and organ maturation in *Rangifer tarandus* (reindeer, caribou) and *Ovibos moschatus* (muskoxen) at 3 stages: the neonatal period (1-30 d old); the transition from milk to plants (30-60 d old); and at maturity (7 yrs), to examine whether arctic ungulates exhibited attributes of their life history that were unique to high latitudes. Although *Rangifer* gave birth to proportionately larger young than muskoxen (6.9% vs. 4.8% maternal mass), both species grew at similar rates (0.002 vs. 0.005 d^{-1}). High thyroid secretion and large hepatic lipid reserves in neonates indicated high rates of energy metabolism for thermogenesis and use of nutrients sequestered *in utero*. Mass gains during the neonatal period were associated with large gastric abomasa that would allow young ungulates to digest milk soon after birth. Body growth continued during the transition to forage (60 days of age) and was accompanied by a disproportionate expansion of rumen, small intestine, and colon. Growth and organ maturation among ungulates, therefore, emphasizes maternal investment *in utero* and the duration of growth, with little variation in growth rate during both neonatal and transitional phases, regardless of environmental restrictions.

[1] Knott, K. K., P. S. Barboza, and R.T. Bowyer. In Review. Growth in arctic ungulates: postnatal development and organ maturation in *Rangifer tarandus* and *Ovibos moschatus*. Journal of Mammalogy.

Introduction

Plasticity of growth and digestive morphology could allow animals to respond to environmental fluctuations, which may ultimately modify the evolutionary direction of species by increasing the fitness of those individuals that can meet nutritional demands and successfully complete life history events despite those fluctuations (West-Eberhard 2003). The Arctic is a variable environment with large annual cycles of temperature, photoperiod and plant availability (Pielou 1994). Arctic ungulates, such as *Rangifer tarandus* (reindeer, *R. t. tarandus;* caribou, *R. t. grantii*) and *Ovibos moschatus* (muskoxen) time reproduction, birth, and growth to coincide with increased photoperiod and plant availability during brief summers (Suttie and Webster 1995). Although studies have investigated the importance of habitat and quality of plants used by females during fetal development and milk production (Crete and Huot 1993; Rachlow and Bowyer 1998; Rachlow and Bowyer 1994; White et al. 1997), few studies have examined how the quality of diet can affect growth and development in offspring. Limited quality and quantity of forage plants can constrain growth in young arctic ungulates (McEwan 1968; Olesen et al. 1991; Reimers et al. 1983). If growth is plastic among ruminants, arctic ungulates would be predicted to mature more rapidly than species from temperate regions where the season for plant growth is longer. Furthermore, species such as reindeer and caribou that mature earlier (18 months in captivity; see also Chan-McLeod et al. 1994) also would grow faster than muskoxen that reproduce at an older age in the same arctic climate (>2 years in captivity; White et al. 1998).

Timing and synchrony of parturition in ungulates are critical life-history characteristics that affect the survival of neonates (Bowyer 1991; Bowyer et al. 1998; Keech et al. 2000; Rachlow and Bowyer 1991). Reindeer and muskoxen are born in mid-April to early May (Adamczewski et al. 1997; White and Luick 1984) before the peak in plant growth, whereas caribou are born in early June near the time of plant emergence (Gerhart et al. 1996; Griffith et al. 2002). Young arctic ungulates use energy from milk and plants for growth during their first summer, but grow slowly or lose muscle and fat during their first winter (Adamczewski et al. 1987; Eastland et al. 1989; Peltier and Barboza 2003). Adequate nutrition during development, therefore, is essential to meet the high demands of growth during the first summer and establish reserves before winter.

Although neonatal ruminants are well developed somatically, young must expand the capacity and function of visceral systems and digestive tract to transition from milk to forage diets (Batt 1980; Janssens and Ternouth 1987; Lyford 1988; Owens et al. 1993). Young reindeer and muskoxen have similar intakes of highly concentrated milk after birth, but time-to-weaning may be greater in muskoxen (8 months) than reindeer (5 months; Parker et al. 1990). Transition from milk to plants increases the load of structural carbohydrates that require fermentative degradation in the rumen (Van Soest 1994). Maturation of nutritional organs, such as the digestive tract, liver, and kidneys, also includes enlargement of viscera to reach the functional morphology of adults and support of greater food intakes (Lyford 1988). Differences among species in the morphological development of nutritional organs may also relate to the differences in the characteristics of the diet (e.g., fiber, secondary compounds, nutritional content;

Hofmann 2000; Ihl and Klein 2001; Klein and Bay 1990; Staaland et al. 1995). Because early phenologies of plants hold the greatest nutritional value and are the most digestible (Johnstone et al. 2002; Jorgenson et al. 2002), young arctic ungulates must also coincide maturation of nutritional organs with growth of plants in the Arctic and precede senescence of those plants as forage quality declines into winter.

Arctic ungulates likely respond to environmental fluctuations through endogenous signals that integrate cues of photoperiod and temperature with systemic changes in growth and metabolism. Elevated concentration of thyroid hormones (T4 and T3) have been associated with increases in feed intake, body mass, antler development, and mating in ruminants at high latitudes (Loudon et al. 1989; Shi and Barrell 1992). Thyroid hormones may promote similar metabolic responses to the demands for rapid growth in the Arctic (Ryg and Jacobson 1982).

In this study, we compared growth of the somatic and visceral components of muskoxen with reindeer and caribou (hereafter referred collectively by their genus, *Rangifer*) in the neonatal period (1-30 days), during the transition to forage consumption (30-60 days), and at maturity. We measured the mass and composition of organs, as well as the concentrations of thyroid hormones, to assess functional changes in morphology and endocrine correlates of metabolism. Changes in viscera were compared with the ingested diet and with growth of the whole body to evaluate the interplay between exogenous dietary input and endogenous demands of somatic tissue in each species. We hypothesized that early sexual maturation in *Rangifer* is associated with more rapid

growth in comparison with muskoxen, and that postnatal growth is accompanied by the maturation of nutritional organs that support a transition to forage consumption.

Materials and methods

Animals and feeding practices. This study used only captive animals from the same location to minimize environmental differences between species and to remove any nutritional constraints on growth. All *Rangifer* and muskoxen were from captive herds at the R. G. White Large Animal Research Station and the Biological Reserve. Herds were maintained by the Institute of Arctic Biology, University of Alaska, Fairbanks (65° N, 146° W) for >15 years. Young animals remained with their mothers throughout the study, and were able to consume milk naturally. All animals had access to fresh grass (*Bromus* sp.) and browse (*Betula* sp., *Salix* sp.) in their enclosures. Adult muskoxen were given grass hay (*Bromus* sp.; Peltier et al. 2003) and a pelleted supplement of minerals (Muskox supplement; Rombach et al. 2002). Young muskoxen could access adult foods and a complete diet formulated from barley, corn, alfalfa, soy meal, and fish meal with minerals and vitamins (dry matter basis: lipid, 3.2%; neutral detergent fiber, 28%; lignin, 1%; carbon, 44.5%; nitrogen, 2.7%—Rombach et al. 2002). *Rangifer* were provided with a complete pelleted ration similar to that of young muskoxen. These feeds provided nutrient compositions similar to sedges, grass, and browse consumed by animals in the wild, and thus provided captive animals with stimuli similar to their wild counterparts for development and growth (Jorgenson et al. 2002; Ohlson and Staaland 2001). Animals were weighed (± 0.1 kg) on electric scales at regular intervals to monitor health and growth.

Total body growth. We used repeated measures of body mass from birth to maturity to assess overall growth in each *Rangifer* subspecies (reindeer and caribou) and muskoxen. Data on the body mass of caribou (n = 3) and reindeer (n = 3) were available at 90 day intervals from birth (caribou, 18 points, castrated < 5 months; reindeer, 30 points, castrated at 1 year). Weights from muskoxen (n = 5) were collected weekly from birth (1464 points, castrated at 1 year). Body mass included the mass of antlers in *Rangifer* and horns in muskoxen.

Growth of body mass was described by fitting mass to age (days) with the Gompertz growth equation (Zullinger et al. 1984) through a 3 parameter nonlinear model: $M(t) = A * e^{-e^{-K(t - I)}}$, where t = age (days), M(t) = mass (g), A = asymptotic mass, K = growth constant (day^{-1}), and I = inflection point. Age at inflection was the point at which mass gains begins to slow, and therefore provides an index of the duration of maximal postnatal growth. The Gompertz equation was chosen because it provides less biased estimates of growth in mammals when compared with von Bertalanffry and logistic equations (Zullinger et al. 1984). Data were fit using the nonlinear model function in Systat 10.2 (SPSS Inc., Chicago, Illinois). Iteration starting points were set to 0 for estimation of parameters A, K and I. Calculation of the growth equations were performed through least-squares estimation by the Guass-Newton method.

Growth of body components. Growth of body components and hormones in young muskoxen were compared with young reindeer only, because both species were born during the same month and therefore experienced the same window of growth until the first winter. We also compared reindeer with caribou at the same age (30 days) to

assess any differences between the subspecies. Animals were selected for necropsy after birth to assess postnatal development during the neonatal period (day 1 - 30), when milk was the primary food, and in the subsequent transition to forage (day 30 - 60). Males and females were included in each group of young animals. Because digestive morphology of adults could be altered by reproductive status in these species (Barboza and Bowyer 2000; 2001), castrated caribou and muskoxen, 7 ± 2.5 yrs (mean age ± SD) were used to represent the intermediate mature state between intact males and females. One-day-old muskoxen (n = 7) were from a cohort born April–June 1999 (Rombach et al. 2002), whereas other young muskoxen were born April 2001 (30 days, n = 4; 60 days, n = 4). Young reindeer (1 day, n = 4; 30 days, n = 4; 60 days, n = 3) were born April 2002, and caribou (30 days, n = 4) were born June 2002. Adult castrates were taken for necropsy at similar times to young animals to minimize effects of season (April 2002 for muskoxen, n = 5; June 2002 for caribou, n = 3).

All animals were immobilized with tiletamine hydrochloride and zolazepam (3 mg/kg; Telazol 100 mg/mL, Fort Dodge Animal Health, Fort Dodge, Iowa) for transport to the necropsy facility. Blood was collected from the jugular vein into sterile evacuated glass tubes (Vacutainer, Becton Dickinson, Rutherford, New Jersey). Plasma was separated by centrifugation at 1,000 g and stored at -20° C for subsequent hormone analyses. Thirty and 60 day-old-animals were maintained under anesthesia with isoflurane in oxygen for 2 hours prior to euthanasia. The anesthetic period was used to equilibrate a dose of labels for measures of cell division for another study. Animals were euthanized by overdose of barbiturates (Euthasol, Delmarva Laboratories, Midlothian,

Virginia) at the jugular vein. All procedures were approved for humane treatment of animals by the University of Alaska Fairbanks Institutional Animal Care and Use Committee under protocol #01-21.

Dissections of tissues commenced immediately after cardiac arrest and the loss of the corneal reflex. Digestive tract, liver, kidneys, and thyroids were excised and depleted of connective and adipose tissues before being weighed (± 0.01 g) on an electronic balance. Mass of paired organs (e.g., kidneys) was recorded as the sum of the pair. The digestive tract was ligated and measured as segments of the forestomach, small intestine and hindgut. The forestomach was further divided into the reticulo-rumen (rumen), omasum and abomasum. Samples were taken from the ingesta of the rumen and abomasum to describe the transition in dietary material (milk, forage or pelleted supplement). The proximal colon and cecum (cecum) was the region from the ileo-cecal junction to the spiral colon. Distal colon (colon) included the tissue from the spiral colon to the rectum. Segments of the digestive tract were weighed with and without digesta to estimate the mass of contents. Ingesta-free body mass was determined by subtracting the sum of digesta content from body mass.

Lipid content was measured in liver and skeletal muscle to evaluate lipid storage during maturation of tissues. Liver samples were collected from the caudate lobe in each animal. Samples of skeletal muscle were taken from the superficial gluteus of all animals except 1 day muskoxen, which was collected from the latisimus dorsi. Samples from both latisumus dorsi and superficial gluteus in adult muskoxen were used to assess differences in lipid content between muscle groups. All samples were stored at -20° C

prior to analysis. A portion of each tissue (1 g) was thawed, weighed, and dried to constant mass in a 55° C oven for estimation of dry matter. Remaining tissue (>20 g) was freeze-dried to constant mass and homogenized for analysis. Lipid content was determined by petroleum ether extraction (Soxtec No. 1043 Extractor, Tecator, Hoganas, Sweden).

Plasma was measured for total and free thyroxine (T4) and total and free tri-iodothyronine (T3) by solid-phase radioimmunoassay. Hormones bound to proteins in circulation, therefore, were the difference between total and free concentrations for T3 and T4. Concentrations of hormone were detected with rabbit anti-sera (Coat-A-Count®, Diagnostic Products Corporation, Los Angeles, California) with iodinated (^{125}I) tracers. Effects of immobilants on concentrations of thyroid hormones were likely negligible because plasma was sampled within 45 min of immobilization. Hormone assays were replicated to within a 10% CV for each blood sample. Each assay was validated for the recovery of iodinated hormone with plasma from each species containing hormone concentrations at the top and bottom of the calibration range. Recoveries of spiked assays were high and similar between species for total T4 (99%), total T3 (96%), free T4 (95%) and free T3 (91%). Consequently, we report hormone concentrations without adjustment for recovery in the assay.

Statistics. Statistical analyses were performed using Systat 10.2 (SPSS Inc., Chicago, Illinois) with $\alpha = 0.05$. Parameters of Gompertz growth curves were considered similar if 95% confidence intervals overlapped. Postnatal growth was linear ($R^2 > 0.92$, $P < 0.001$, $n = 26$) over the first 60 days; therefore, daily mass gain (g/day) of

components was estimated from neonatal (1-30 days) and transitional (30-60 days) periods. Daily mass gains of body and organs during each period were calculated by subtracting the mean mass from all animals within a species' age group by the mean mass of the preceding age group. Differences in daily mass gains between species and periods, therefore, were compared using chi-square statistics with Yates correction for 1 *d.f.* (Fowler et al. 2003) because variance between individual animals was unavailable. Maturation of the forestomach was determined through examination of the proportions of rumen, omasum, and abomasum within each age group. Proportions of forestomach segments were normalized with an arsine-square root transformation (Zar 1999). Only liver and kidney mass increased with body mass; therefore, we tested whether species and ages differed in the mass of liver and kidney while accounting for differences in body mass with analysis of covariance (ANCOVA; covariate = ingesta-free mass – organ; Christians 1999). We used analysis of variance (ANOVA) to test effects of species and age on the mass of gastrointestinal segments, hormone concentrations, and lipid in tissues. Post-hoc tests for species differences within an age group were performed using Bonferroni adjustments for multiple comparisons. Data are shown as mean ± SD.

Results

Both subspecies of *Rangifer* grew at similar rates (reindeer: K = 0.004 d^{-1}, *CI* = 0.001 - 0.005 d^{-1}; caribou: K = 0.003 d^{-1}, *CI* = 0.003 - 0.005 d^{-1}) with inflection of growth occurring during the first winter (reindeer; I = 172 days, *CI* = 79 - 264 days; caribou: I = 165 days, *CI* = 111 - 219 days; Fig. 1.1A). Although mature reindeer were marginally heavier than caribou, this difference was not significant (reindeer: A = 165 kg, *CI* = 125 -

205 kg; caribou: A = 132 kg, *CI* = 120 - 144 kg). Similarities between reindeer and caribou in body growth and development of organs at 30 days of age (all organs, $P > 0.05$), indicated that young grew consistently even though birth dates differed between subspecies. Growth rate in muskoxen was also within the 95% confidence interval of *Rangifer* during postnatal growth ($K = 0.002\ d^{-1}$, *CI* = 0.002 - 0.003 d^{-1}). Growth inflection, however, was later (I = 520 days, *CI* = 511 - 528 days), and asymptotic mass was greater (A = 299.3 kg, *CI* = 297 - 301 kg) than that for *Rangifer* (Fig. 1.1B).

Although total body and ingesta-free body mass of young and adult animals showed that muskoxen were larger than *Rangifer* overall ($F = 228.04$, *d.f.* = 3, 30, $P < 0.001$; Table 1.1), young muskoxen and *Rangifer* at each postnatal age group were similar in size (Bonferroni adjusted $P > 0.9$; Table 1.1). However, in proportion to maternal mass, *Rangifer* gave birth to larger young than did muskoxen (6.9 ± 1.0% vs. 4.8 ± 0.3%; $t = 61.8$, *d.f.* = 19, $P < 0.001$). Birth mass of *Rangifer* was also proportionately greater to muskoxen (5.4% vs. 3.5%) in relation to the mass of adult castrates. *Rangifer* continued to be proportionately larger than muskoxen (23 vs. 13% of adult castrate mass) at 60 days-of-age (Table 1.1).

Young animals gained ingesta-free mass and increased digesta content during the neonatal period from 1 to 30 days, and in the transitional period from 30 to 60 days (Fig. 1.2A). Daily gains of ingesta-free mass were similar between species during the neonatal period (mean = 348.1 g/day; $X^2 = 3.2$, *d.f.* = 1, $P > 0.05$). Muskoxen increased gains of ingesta-free mass during the transitional period, whereas reindeer continued to grow at the same rate as in the neonatal period (*Rangifer* = 353.8 g/day, muskoxen = 401.2 g/day;

$X^2 = 6.2$, *d.f.* = 1, $P < 0.05$). Muskoxen also gained more digesta mass than *Rangifer* during the transitional period (*Rangifer* = 35 g/day; muskoxen = 96 g/day; $X^2 = 6.2$, *d.f.* = 1, $P < 0.05$). Adult muskoxen also had greater digesta mass than adult *Rangifer*, resulting in an ingesta-free mass that was lower than total body mass by 17% and 7%, respectively (Table 1.1).

Gains of ingesta-free mass in young *Rangifer* and muskoxen were mainly associated with gains in lean rather than lipid mass. Newborn animals of both species had low concentrations of intramuscular lipid (1.0 g/100 g wet muscle), and continued to be lean to 30 days-of-age (0.7 g/100 g wet muscle; Fig. 1.3). Although superficial gluteus contained more lipid than the latisimus dorsi in adult muskoxen (5.8 vs. 3.5 g/100 g wet muscle; $F = 5.72$, *d.f.* = 1, 8, $P = 0.04$), lipid concentration of the gluteus in newborn muskoxen was similar to the low concentrations observed for the latisumus. Both species increased intramuscular lipid concentrations from 30 to 60 days during the transition to forage (1.7 g/100 g wet muscle; Bonferroni adjusted, $P < 0.006$; Fig. 1.3). Muscle lipid of 60 day old *Rangifer* and muskoxen was similar to adult *Rangifer* (Bonferroni adjusted $P > 0.9$); however, adult muskoxen had a greater intramuscular lipid content than all young animals (Bonferroni adjusted $P < 0.001$).

Although intramuscular lipid was low and similar between species in newborn animals, lipid concentration of the liver in newborn animals was higher than in muscle, and greater in muskoxen than *Rangifer* (8.7 vs. 5.1 g/100 g wet liver; Bonferroni adjusted $P < 0.008$; Fig. 1.3). Lipid content of the liver declined by more than one-half in each species by 30 days (muskoxen = 3.9, *Rangifer* = 2.6 g/100 g wet liver), and was similar to

adult levels (Bonferroni adjusted $P > 0.9$; Fig. 1.3). Absolute lipid content of the liver, however, increased with age and was greater in muskoxen than *Rangifer* (1 d to adult: 16.8 to 52.9 versus 8.9 to 43.6 g/whole wet liver).

High lipid concentration of the liver in newborn animals corresponded to elevated levels of hormones in both species. Concentrations of T4 and free T3 were greater in newborn *Rangifer* than in muskoxen ($P < 0.001$; Fig. 1.4A,B,D), even though thyroid mass in young animals did not differ between species (Bonferroni adjusted $P > 0.9$; Table 1.1). Although thyroidal mass was greater in adult muskoxen than in adult *Rangifer* (Bonferroni adjusted $P < 0.05$; Table 1.1), total concentrations of T4 were still higher in mature *Rangifer* than in muskoxen ($P = 0.015$; Fig. 1.4A,B). Thus, plasma hormone levels were greater in *Rangifer* than in muskoxen overall (species effect: $F > 6.2$, $d.f. = 1, 32$, $P < 0.02$) and declined with age in both species (age effect: $F > 9.9$, $d.f. = 3, 30$, $P < 0.001$; Fig. 1.4). Most circulating T4 and T3 were bound to carrier proteins (Total – Free) with <1% in the free form for both species at all ages. Concentrations of free T3, which would be most active at target tissues, were greater in newborn *Rangifer* than muskoxen (1.7 ± 0.10 vs. 0.06 ± 0.02 nmol/L; Bonferroni adjusted $P < 0.05$), but declined to similar levels during the transitional period (0.06 nmol/L) and into adulthood (0.02 nmol/L; $P < 0.001$; Fig. 1.4D).

Young *Rangifer* and muskoxen showed a similar transition from milk to forage diets during the postnatal period. One day old animals had empty rumens and abomasa that were filled with curdled milk. Ruminal ingesta at 30 days consisted of soil and hair with remnants of milk, whereas the ingesta from the abomasum contained milk curds.

Ingesta of the rumen and abomasum at 60 days appeared similar to adult ingesta, which contained pelleted ration and pasture plants. The appearance of adult food items at 60 days coincided with gains of digesta mass during this transitional period (Fig. 1.2A).

The transition in diet also was also reflected in the maturation of the forestomach, which was similar between species. The rumen and abomasum in 1-day-old animals were similar in mass (Table 1.1) and contributed equal proportions to the forestomach (Fig. 1.5). Mass of the omasum was absolutely (species effect: $F = 335.2$, *d.f.* = 1, 32, $P < 0.001$; Table 1.1) and proportionately (species effect: $F = 37.8$, *d.f.* = 1, 32, $P < 0.001$; Fig. 1.5) larger in muskoxen than in *Rangifer* at birth and all subsequent ages. Mass gains of the omasum and abomasum were similar between species and periods ($X^2 < 0.2$, *d.f.* = 1, $P > 0.05$; Fig. 1.2B). Although gains of ruminal mass (5 g/day) were also similar between species, ruminal mass exceeded rates of omasal (< 0.9 g/day) and abomasal (< 2.4 g/day) growth during the neonatal period. Ruminal growth was further enhanced as digesta mass increased during the transitional period (> 15 g/day; $X^2 > 15$, *d.f.* = 1, $P < 0.01$; Fig. 1.2B). Thus, the abomasal proportion of the forestomach declined from 1 day to 60 days (50 to 10%; $F = 233.7$, *d.f.* = 3, 30, $P < 0.001$) while the ruminal proportion increased through the transitional period (50 to 70%; $F = 228.2$, *d.f.* = 3, 30, $P < 0.001$; Fig. 1.5).

Growth of intestinal mass also was high and associated with the transition to forage in both species. Although muskoxen had greater small intestinal mass than *Rangifer* (species effect: $F = 128.1$, *d.f.* = 1, 32, $P < 0.001$; Table 1.1), daily gains of the small intestine were similar between species during the neonatal period (12 g/day; $X^2 =$

0.8, *d.f.* = 1, $P > 0.05$; Fig. 1.2B). Muskoxen increased mass of the small intestine during the transitional period (23.4 g/day; $X^2 = 6.2$, *d.f.* = 1, $P < 0.05$) as digesta mass increased, while *Rangifer* intestine continued to grow at a rate similar to the neonatal period (3.9 g/day; $X^2 = 3.4$, *d.f.* = 1, $P > 0.05$; Fig. 1.2B). Cecal and colonic masses also were greater in muskoxen than *Rangifer* during neonatal and transitional periods (Bonferroni adjusted $P < 0.001$; Table 1.1). Daily mass gains of the cecum did not differ between periods or species (< 2 g/day; $X^2 < 0.4$, *d.f.* = 1, $P > 0.05$). Colon mass gain of muskoxen doubled during the transitional period (8.3 g/day) and was greater than colonic gains in *Rangifer* (4 g/day; $X^2 = 5.2$, *d.f.* = 1, $P < 0.05$), whereas *Rangifer* had similar mass gains of the colon between periods ($X^2 < 0.1$, d.f. = 1, $P > 0.05$).

Growth rate of liver and kidneys did not differ between species or between neonatal and transitional periods (Fig. 1.2C). Rather, mass of the liver and kidneys followed increases in body mass (net ingesta-free mass as covariate, $F > 14.0$, *d.f.* = 1, 32, $P < 0.001$), and age ($F > 8.9$, *d.f.* = 3, 30, $P < 0.001$; Table 1.1). *Rangifer* had larger kidneys relative to their body mass than muskoxen overall ($F = 8.0$, *d.f.* = 1, 32, $P < 0.009$), but liver mass was similar between species at all ages ($F = 2.4$, *d.f.* = 1, 32, $P = 0.131$; Table 1.1).

Discussion

Our hypothesis of more rapid somatic development in *Rangifer* than muskoxen was supported by the proportionately greater birth mass of *Rangifer* (6.9 vs. 4.8% maternal mass). This proportionately larger birth mass indicates that *Rangifer* invest relatively more energy and nutrients to the fetus during gestation. Size of *Rangifer*

neonates may ultimately reflect the minimal body size for thermoregulation and the greater activity levels in *Rangifer* than muskoxen shortly after birth (Klein 1991). High maternal investment of *Rangifer*, therefore, is consistent with the close relationship between maternal body mass and the likelihood of parturition, as well as perinatal survival of offspring (Cameron et al. 1993; Whitten et al. 1992). Lower maternal investment in muskoxen could be related to the low-quality diet of sedges consumed by muskoxen in the wild during early gestation, especially during winter when maternal demands for energy and nutrients greatly exceed food intake (White et al. 1989). Female muskoxen, therefore, may allocate less energy reserves toward gestation than *Rangifer*, even when not limited by nutrition, as an inherent physiological mechanism to conserve available energy and increase individual survival and future reproductive success. Low plant availability, then, could be detrimental to fetal growth and survival of offspring because gestational costs and gains of fetal mass in muskoxen are greatest in late winter (Rombach et al. 2003). The combined effects of austere winters and short summers likely constrain maternal investment in both arctic species because ruminants from more temperate regions give birth to proportionately larger offspring (% maternal mass: white-tailed deer, *Odoecoileus virginianus*: 11%; red deer, *Cervus elaphus*: 7.5%; African buffalo, *Syncerus caffer*, 7.5%; Robbins and Robbins 1979). Thus, selection for survival and future reproductive output by females may outweigh immediate investment in the fetus in arctic versus more temperate ruminants.

Low birth masses, coupled with low ambient temperatures of the arctic environment, likely increase the thermogenic demands of neonatal *Rangifer* and

muskoxen. High levels of thyroid hormones in both species at birth are consistent with elevated rates of energy metabolism that may help to meet this thermogenic demand. Elevated thyroid hormones of neonates have been linked with development and maintenance of endothermy in several vertebrates (Laburn et al. 2000; McNabb et al. 1998), and are likely associated with the wide distribution of brown adipose tissue in young arctic ungulates (Blix et al. 1984; Soppela et al. 1992). High proportions of protein-bound hormone in *Rangifer* and muskoxen provide a large reservoir for release of free thyroidal hormones that could continue to influence metabolism throughout summer to increase growth and food intake at all ages (Nilssen et al. 1994; Ryg and Jacobson 1982). Smaller absolute birth mass, however, may increase demands for thermogenesis in *Rangifer* above those for muskoxen. Levels of T4 at 30 and 60 days also indicate that metabolic rates in *Rangifer* may exceed those of muskoxen during growth. Those differences, however, may also reflect the kinetics of interconversion and excretion of thyroidal hormones, and await direct confirmation from studies of receptor kinetics and binding of labeled T3 and T4 in these ruminants.

The large decline in hepatic lipid was probably associated with a change in metabolic function within the first days after birth. *In utero* sequestration of nutrients such as copper (Rombach et al. 2003) are consistent with the role of the liver as a reservoir of nutrients to supplement early growth and development. Large deposits of hepatic lipid of neonatal ruminants have not been recorded in other species and therefore could implicate an adaptation of *Rangifer* and muskoxen to offset their relatively smaller mass at birth. The greater concentration of lipid in liver of muskoxen compared with

Rangifer supports that smaller proportionate birth mass could be related to greater requirements of hepatic reserves supplied *in utero*. Greater hepatic lipid in neonatal muskoxen also may indicate disparities between the *Rangifer* and muskoxen in intermediary metabolism and in the mobilization of lipids (Soppela and Nieminen 1998). Low maternal nutrition, therefore, would likely decrease hepatic reserves of neonates, reduce initial growth and development, and therefore impact survival of offspring.

Our hypothesis of more rapid growth in *Rangifer* than muskoxen was not supported by gains in body and organ mass because both species grew at similar rates. Similarities in growth rate to 30 days of age probably reflect consumption of similar amounts of energy and protein in milk (Parker et al. 1990). High mass-specific milk intakes by *Rangifer* in the first week of life (Parker et al. 1990) may reflect high metabolic rates associated with elevated thyroid hormones. Therefore, early growth of both *Rangifer* and muskoxen can be limited by the lactational output of their mothers, even though arctic ungulates produce concentrated milks that are high in lactose and lipid (Oftedal 1984; White and Luick 1984). These similarities are also probably associated with the consistent size of the abomasum at birth since that segment is a prerequisite for processing the first load of dietary protein and lipid (Thivend et al. 1984). Growth of the liver, as well as kidneys, however, reflect changes in metabolic demands to support greater body mass (Adamczewski et al. 1987; Gerhart et al. 1996), in addition to the genetically programmed size of the organ.

Growth of the reticulo-rumen and the intestines in both *Rangifer* and muskoxen may be influenced most by the fill and composition of digesta that change dramatically in

the transition from milk to a diet high in vegetation. Ingestion of hair and soil by both species at 30 days could be an inadvertent consequence of suckling behavior, but also may stimulate the differentiation of mucosa before ingestion of plant fiber. Gains in ruminal mass of both species coincide with the presence of forages in ruminal digesta and an apparently active fermentation system. Physical stimulus of ingesta combined with the chemical stimuli of fermentation products would promote further expansion of the rumen as young animals consume greater amounts of forage (Faurie and Perrin 1995; Lentle et al. 1996; Soveri and Neiminen 1995; White et al. 1984). Therefore, rapid growth rates of *Rangifer* and muskoxen after the transition to forage are likely dependent on exogenous factors of the diet that stimulate adequate digestive development, as well as the quality and abundance of forage plants that provide nutrition for further growth (Lindsay et al. 1993; McEwan 1968; Olesen et al. 1991; Reimers et al. 1983).

Maximal size and structure of the forestomach are also driven by endogenous factors that are species-specific (Langer 1988), and ultimately determine the capacity for ruminal fermentation as the animal grows (Clauss et al. 2003). The relative functions of the rumen, omasum, and abomasum are well established by 60 days old in both *Rangifer* and muskoxen because segmental proportions were similar to adults. The early establishment of foregut may be a common feature among ruminants because forestomach proportions of domestic ruminants are similarly advanced at this same age (Church et al. 1961; Lyford 1988). However, the absolute capacity of the rumen in relation to body size at 60 days of age is small (Barboza and Bowyer 2001; Barboza and Bowyer 2000). Body size and ruminal mass of *Rangifer* and muskoxen at 60 days old

indicate that these species may have similar abilities to use fiber at the end of the first summer. The greater selection of forbs by young muskoxen compared to adults reflects the use of more digestible species among the available plants (Cote et al. 1997; Oakes et al. 1991). Greater consumption of sedges and grass by muskoxen may only occur as young muskoxen continue to gain size and can increase fermentation of more fibrous forages. Large omasal mass in muskoxen at all ages also indicates a genetic attribute of the species (Langer 1988) that may, in part, determine their feeding strategy as grazers as adults.

Young arctic ungulates must transition to similar diets of growing plants well before those forages senesce, which would contribute to lean mass gains during summer. Small increments in muscle lipid during the first 60 days of life were probably associated with maturation of connective tissues and intracellular membranes (Dickerson and Widdowson 1960). Young arctic ungulates continue to remain lean throughout their first summer (< 5g lipid/100g muscle mass; Adamczewski et al. 1987; Adamczewski et al. 1995; Peltier and Barboza 2003). Additions of protein accrued through increased muscle mass during the first summer, therefore, must serve to support nutrient demands when food supply is limited in winter. A rapid transition from milk to forage consumption by 60 days of age would allow young *Rangifer* and muskoxen to consume plants at early phenological stages that contain the highest nitrogen content (Johnstone et al. 2002; Rachlow and Bowyer 1994) that could support these gains in lean mass. Early transition to forage would also enable young ungulates to consume forages during the entire growing season.

Birth date of *Rangifer* and muskoxen, therefore, must allow sufficient time for organ maturation to utilize emergent plants in arctic environments. Delays in parturition may shorten the window for the transition to forage and force young animals to switch from milk to senescing plants that are high in fiber and most difficult to digest (Bowyer 1991). Later births in caribou as compared to reindeer could suggest differences between the subspecies in maternal investment and the importance of calving grounds, or delays in plant emergence at high latitudes (Griffith et al. 2002; Loudon et al. 1989). Milk may also be a crucial supplement of energy during the transition to forage and in the first winter as young arctic ungulates may not digest winter forages as effectively as adults (Munn and Dawson 2003). The disparity between young and adult animals may however be smaller for more digestible foods such as lichen or browse that are favored by *Rangifer*. Longer lactation periods in muskoxen, therefore, may provide additional nutrition in a species that has a longer growth period and takes longer to reach the ruminal capacity for consumption of adult forages.

Growth rates of ruminants in the Arctic are similar to those of African species (e.g., wildebeest, *Connochaetes taurinus*: A = 220 kg, K = 0.002 d^{-1}; nyala, *Tragleaphus angasi*: A = 105 kg, K = 0.005 d^{-1}; Georgiadis 1985; see also Attwell 1982; Robbins and Robbins 1979). In fact, ruminant species are considered to have one of the most rapid growth rates among vertebrates (Case 1978). Differences between *Rangifer* and muskoxen in adult mass and age at sexual maturation, therefore, are a proximal outcome of the duration of growth and the number of growing seasons required to reach mature body mass (Adamczewski et al. 1997; Leader-Williams and Ricketts 1981). This

hypothesis is supported by longer growth curves in males compared with females in Arctic ruminants and in other sexually dimorphic species (Georgiadis 1985; Peltier and Barboza 2003; Spaeth et al 2001). Consequently, Zullinger et al. (1984) reported that female *Rangifer* and muskoxen had a similar growth constant (K; *Rangifer* = 0.005 d^{-1}, muskoxen = 0.004 d^{-1}), but earlier inflection points (*Rangifer* = 160 days, muskoxen = 360 days) than castrated males in this study. Later reproductive age in muskoxen may also be related to the more conservative strategy of this species by allocating more energy toward growth than to early reproduction, especially when forage is limited (White et al. 1989).

Arctic ungulates increase lifetime female fecundity by providing less maternal investment toward offspring than temperate species. Female *Rangifer* and muskoxen, however, provided large lipid reserves toward young *in utero* and produce concentrated milk to support initial growth. Neonates have high levels of thyroid hormones to increase thermogenesis, and well-developed abomasa to take advantage of milk immediately after birth. Rapid maturation of the rumen, small intestine and colon of young arctic ungulates, however, indicate an early dependence on forage for further growth. Growth of young *Rangifer* and muskoxen, therefore, is also dependent on birth date to allow sufficient development of nutritional organs that correspond to plant emergence. Muskoxen may be more limited in growth and development than *Rangifer* because of less maternal investment and longer time for expansion of specialized foreguts. Later age of first reproduction in muskoxen in comparison to *Rangifer,* therefore, may relate to the duration of the growth period to allocate greater energy toward growth than early

reproduction. Similar rates of growth and organ maturation among young ungulates, regardless of environment, likely reflect endogenous cues common to the taxa. Modification of the endogenous plan, however, can occur in fluctuating environments that alter maternal investment and duration of growth. Arctic ungulates likely capitalize on these modifications, which have allowed them to be successful in the Arctic.

Literature cited

Adamczewski, J. Z., P. F. Flood, and A. Gunn. 1997. Seasonal patterns in body composition and reproduction of female muskoxen (*Ovibos moschatus*). Journal of Zoology (London) 241:245-269.

Adamczewski, J. Z, P. F. Flood, and A. Gunn. 1995. Body composition of muskoxen (*Ovibos moschatus*) and its estimation from condition index and mass measurements. Canadian Journal of Zoology. 73:2021-2034.

Adamczewski, J. Z., C. C. Gates, R. J. Hudson, and M. A. Price. 1987. Seasonal changes in body composition of mature female caribou and calves (*Rangifer tarandus grownlandicus*) on an arctic island with limited winter resources. Canadian Journal of Zoology 65:1149-1157.

Attwell, C. A. M. 1982. Growth and condition of blue wildebeest in Zululand. South African Journal of Wildlife Research 12:63-70.

Barboza, P. S., and R. T. Bowyer. 2001. Seasonality of sexual segregation in dimorphic deer: extending the gastrocentric model. Alces 37:275-292.

Barboza, P. S., and R. T. Bowyer. 2000. Sexual segregation in dimorphic deer: a new gastrocentric hypothesis. Journal of Mammalogy 81:473-489.

Batt, R. A. L. 1980. Influences on animal growth and development. Studies in Biology no. 116. Camelot Press, London.

Blix, A. S., H. J. Grau, K. A. Markussen, and R. G. White. 1984. Modes of thermal protection in newborn muskoxen (*Ovibos moschatus*). Acta Physiologica Scandinavia

122:443-453.

Bowyer, R. T., V. Van Ballenberghe, and J. G. Kie. 1998. Timing and synchrony of parturition in Alaskan moose: long-term versus proximal effects of climate. Journal of Mammalogy 79:1332-1344.

Bowyer, R. T. 1991. Timing of parturition and lactation in Southern Mule Deer. Journal of Mammalogy 71:138-145.

Cameron, R. D., W. T. Smith, S. G. Fancy, K. L. Gerhart, and R. G. White. 1993. Calving success of female caribou in relation to body weight. Canadian Journal of Zoology 71:480-486.

Case, T. 1978. On the evolution and adaptive significance of postnatal growth rates in the terrestrial vertebrates. The Quarterly Review of Biology 53: 243-282.

Chan-McLeod, A. C. A., R. G. White, and D. F. Holleman. 1994. Effects of protein and energy intake, body condition, and season on nutrient partitioning and milk production in caribou and reindeer. Canadian Journal of Zoology 72:938-947.

Christians, J. K. 1999. Controlling for body mass effects: is part-whole correlation important? Physiological and Biochmical Zoology 72:250-253.

Church, D. C., G. L. Jessup, and R. Bogart. 1961. Stomach development in the suckling lamb. American Journal of Veterinary Research 23:220-225.

Clauss, M., M. Lechner-Doll, and W. J. Streich. 2003. Ruminant diversification as an adaptation to the physicomechanical characteristics of forage. A reeveluation of an old

debate and a new hypothesis. Oikos 102:253-262.

Cote, S. D., J. A. Schaefer, and F. Messier. 1997. Time budgets and synchrony of activities in muskoxen: the influence of sex, age, and season. Canadian Journal of Zoology 75:1628-1635.

Crete, M., and J. Huot. 1993. Regulation of a large herd of migratory caribou: summer nutrition affects calf growth and body reserves of dams. Canadian Journal of Zoology 71:2291-2296.

Dickerson, J. W. T., and E. M. Widdowson. 1960. Chemical changes in skeletal muscle during development. Biochemistry Journal 74:247-257.

Eastland, W. G., R. T. Bowyer, and S. G. Fancy. 1989. Effects of snow cover on selection of calving sites by caribou. Journal of Mammalogy 70:824-828

Faurie, A. S., and M. R. Perrin. 1995. Rumen morphology and volatile fatty acid production in the Blue duiker (*Cephalophus monticola*) and the Red duiker (*Cephalophus natalensis*). Zeitschrift Fur Saugetierkunde 60:73-84.

Fowler J., L. Cohen, and P. Jarvis. 2003. Practical Statistics for Field Biology. John Wiley and Sons Ltd., Chichester, England.

Georgiadis, N. 1985. Growth patterns, sexual dimorphism and reproduction in African ruminants. African Journal of Ecology 23:75-87.

Gerhart, K. L., R. G. White, R. D. Cameron, and D. E. Russell. 1996. Body composition and nutrient reserves of arctic caribou. Canadian Journal of Zoology 74:136-146.

Griffith, B. et al. 2002. The Porcupine Caribou Herd. Pp. 8-37 in Arctic Refuge Coastal Plain Terrestrial Wildlife Research Summaries. (D. E. Douglas, P. E. Reynolds, and E. B. Rhode, eds.). U. S. Geological Survey, Reston, Virginia.

Hofmann, R. R. 2000. Functional and comparative digestive system anatomy of Arctic ungulates. Rangifer 20:71-81.

Ihl, C., and D. R. Klein. 2001. Habitat and diet selection by muskoxen and reindeer in western Alaska. Journal of Wildlife Management 65:964-972.

Janssens, P. A., and J. H. Ternouth. 1987. The transition from milk to forage diets. Pp. 281-303 in Nutrition of Herbivores. Academic Press, Australia.

Johnstone, J., D. E. Russell, and B. Griffith. 2002. Variations in plant quality in the range of the Porcupine caribou herd. Rangifer 22:83-91.

Jorgenson, J. C., M. S. Udevitz, and N. A. Felix. 2002. Forage Quantity and Quality. Pp. 46-50 in Arctic Refuge Coastal Plain Terrestrial Wildlife Research Summaries (D. C. Douglas, P. E. Reynolds, and E. B. Rhode, eds.). U.S. Geological Survey, Reston, Virginia.

Keech, M. A., R. T. Bowyer, J. M. Ver Hoef, R. D. Boertje, B. W. Dale, and T. R. Stephenson. 2000. Life-history consequences of maternal condition in Alaskan moose. Journal of Wildlife Management 64:450-462.

Klein, D. R. 1991. Comparative ecological and behavioral adaptations *Ovibos moschatus* and *Rangifer tarandus*. Rangifer 12:47-55.

Klein, D. R., and C. Bay. 1990. Foraging dynamics of muskoxen in Peary Land, northern Greenland. Holarctic Ecology 13:269-280.

Laburn, H., A. Faurie, and D. Mitchell. 2000. The thermal physiology of the ruminant fetus. Pp. 295-310 in Ruminant Physiology. Digestion, Metabolism, Growth and Reproduction. (P. B. Cronje, ed.). CABI Publishing, New York, New York.

Langer, P. 1988. The Mammalian Herbivore Stomach: Comparative Anatomy, Function and Evolution. Gustav Fischer, New York, New York.

Leader-Williams, N., and C. Ricketts. 1981. Seasonal and sexual patterns of growth and condition of reindeer introduced into South Georgia. Oikos 38:27-39.

Lentle, R. G., I. M. Henderson, and K. J. Stafford. 1996. A multivariate analysis of rumen papillary size in red deer (*Cervus elaphus*). Canadian Journal of Zoology 74:2089-2094.

Lindsay, D. B., R. A. Hunter, C. Gazzola, W. G. Spiers, and M. N. Sillence. 1993. Energy and growth. Australian Journal of Agricultural Research 44:875-899.

Loudon, A. S. I., J. A. Milne, J. D. Curlewis, and A. S. McNeilly. 1989. A comparison of the seasonal hormone changes and patterns of growth, voluntary food intake and reproduction in juvenile and adult red deer (*Cervus elaphus)* and Pere David's deer (*Elaphurus davidianus*) hinds. Journal of Endocrinology 122:733-745.

Lyford, S. J. 1988. Growth and development of the ruminant digestive system. Pp. 44-63 in The Ruminant Animal: Digestive Physiology and Nutrition. (D. C. Church, ed.). Prentice Hall, Englewood Cliffs, New Jersey.

McEwan, E. H. 1968. Growth and development of the barren-ground caribou. II. Postnatal growth rates. Canadian Journal of Zoology 46:1023-1029.

McNabb, F. M. A., C. G. Scanes, and M. Zeman. 1998. Endocrine control of development. Pp. 174-202 in Avian Growth and Development: Evolution within the Altricial-Precocial Spectrum (J. M. Stark and R. E. Ricklefs, eds.). Oxford University Press, New York, New York.

Munn, A. J., and T. J. Dawson. 2003. How important is milk for near-weaned red kangaroos (*Macropus rufus*) fed different forages? Journal of Comparative Physiology B 173:141-148.

Nilssen, K. J., S. D. Mathiesen, and A. S. Blix. 1994. Metabolic rate and plasma T3 in ad lib. fed and starved muskoxen. Rangifer 14:79-81.

Oakes, E. J., R. Harmsen, and C. Eberl. 1991. Sex, age and seasonal differences in the diets and activity budgets of muskoxen (*Ovibos moschatus*). Canadian Journal of Zoology 70:605-616.

Oftedal, O. T. 1984. Milk composition, milk yield and energy output at peak lactation: a comparative review. Symposia Zoological Society, London 51:33-85.

Ohlson, M., and H. Staaland. 2001. Mineral diversity in wild plants: benefits and bane for moose. Oikos. 94:442-454.

Olesen, C. R., T. Henning, and P. Aastrup. 1991. Growth of wild muskoxen under two nutritional regimes in Greenland. Rangifer 14:3-10.

Owens, F. N., P. Dubeski, and C. F. Hanson. 1993. Factors that alter the growth and development of ruminants. Journal of Animal Science 71:3138-3150.

Parker, K. L., R. G. White, M. P. Gillingham, and D. F. Holleman. 1990. Comparison of energy metabolism in relation to daily activity and milk consumption by caribou and muskox neonates. Canadian Journal of Zoology 68:106-114.

Peltier, T. C., and P. S. Barboza. 2003. Growth in an Arctic grazer: effects of sex and dietary nitrogen on yearling muskoxen. Journal of Mammalogy 84:915-925.

Peltier, T. C., P. S. Barboza, and J. E. Blake. 2003. Seasonal hyperphagia does not reduce digestive efficiency in an Arctic grazer. Physiological and Biochemical Zoology 76: 471-483.

Pielou, E. C. 1994. A Naturalist's Guide to the Arctic. The University of Chicago Press, Chicago, Illinois.

Rachlow, J. L., and R. T. Bowyer. 1998. Habitat selection by Dall's sheep (*Ovis dalli*): maternal trade-offs. Journal of Zoology (London) 245:457-465.

Rachlow, J. L., and R. T. Bowyer. 1994. Variability in maternal behavior by Dall's sheep: environmental tracking or adaptive strategy? Journal of Mammalogy 75:328-337.

Rachlow, J. L., and R. T. Bowyer. 1991. Interannual variation in timing and synchrony of parturition in Dall's sheep. Journal of Mammalogy 72:487-492.

Reimers, E., D. R. Klein, and R. Sorumgard. 1983. Calving time, growth rate, and body

size of Norwegian reindeer on different ranges. Arctic and Alpine Research 15:107-118.

Robbins, C. T., and B. L. Robbins. 1979. Fetal and neonatal growth patterns and maternal reproductive effort in ungulates and subungulates. The American Naturalist 114:101-116.

Rombach, E. P., P. S. Barboza, and J. E. Blake. 2002. Utilization of copper during lactation and neonatal development in muskoxen. Canadian Journal of Zoology 80:1460-1469.

Rombach, E. P., P. S. Barboza, and J. E. Blake. 2003. Costs of gestation in an Arctic ruminant: copper reserves in muskoxen. Comparative Biochemistry and Physiology Part C 134:157-168.

Ryg, M., and E. Jacobson. 1982. Seasonal changes in growth rate, feed intake, growth hormone, and thyroid hormones in young male reindeer (*Rangifer tarandus tarandus*). Canadian Journal of Zoology 60:15-23.

Shi, A. D., and G. K. Barrell. 1992. Effects of thyroidectomy on seasonal patterns of live weight, testicular function, antler development and molting in red deer. Pp. 443-449 in The Biology of Deer (R. D. Brown, eds.). Springer-Verlag New York, Inc., New York, New York.

Soppela, P., and M. Nieminen. 1998. Polyunsaturated fatty acids in serum lipids of reindeer during the close postnatal period. Journal of Comparative Physiology B 168:581-590.

Soppela, P., R. Sormunen, S. Saarela, P. Huttunen, and M. Nieminen. 1992. Localization, cellular morphology and respiratory capacity of "brown" adipose tissue in newborn reindeer. Comparative Biochemistry and Physiology 101A:281-293.

Soveri, T., and M. Neiminen. 1995. Effects of winter on the papillar morphology of the rumen in reindeer calves. Canadian Journal of Zoology 73:228-233.

Spaeth, D. F., K. J. Hundertmark, R. T. Bowyer, P. S. Bowyer, T. R. Stephenson, and R. O. Peterson. 2001. Incisor arcades of Alaskan moose: is dimorphism related to sexual segregation? Alces 37:217-226.

Staaland, H., J. Z. Adamczewski, and A. Gunn. 1995. A comparison of digestive tract morphology in muskoxen and caribou from Victoria Island, Northwest Territories, Canada. Rangifer 17:17-19.

Suttie, J. M., and J. R. Webster. 1995. Are arctic ungulates physiologically unique? Rangifer 18:99-118.

Thivend, P., R. Toullec, and P. Guilloteau. 1984. Digestive adaptation in the preruminant. Pp. 561-585 in Digestive Physiology and Metabolism in Ruminants. AVI Publishing Company, Inc., Westport, Connecticut.

Van Soest, P. J. 1994. Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca, New York.

West-Eberhard, M. J. 2003. Developmental plasticity and evolution. Oxford University Press, New York, New York.

White, R. G., D. F. Holleman, C. C. Schwartz, W. L. Regelin, and A. W. Franzman. 1984. Control of rumen turnover in northern ruminants. Canadian Journal of Animal Science 64:349-350.

White, R. G., D. F. Holleman, and B. A. Tiplady. 1989. Seasonal body weight, body condition, and lactational trends in muskoxen. Canadian Journal of Zoology 67:1125-1133.

White, R. G., and J. R. Luick. 1984. Plasticity and constraints in the lactational strategy of reindeer and caribou. Symposiums from the Zoological Societies of London 51:215-232.

White, R. G., J. E. Rowell, and W. E. Hauer. 1997. The role of nutrition, body condition and lactation on calving success in muskoxen. Journal of Zoology (London) 243:13-20.

White, R. G., J. E. Rowell, J. E. Blake, and W. E. Hauer. 1998. Population structure and dynamics in captive muskoxen at the Large Animal Research Station, 1988-1994. Rangifer 18: 145-151.

Whitten, K. R., G. W. Gardner, F. J. Mauer, and R. B. Harris. 1992. Productivity and early calf survival in the Porcupine caribou herd. Journal of Wildlife Management 56:201-212.

Zar, J. H. 1999. Biostatistical Analysis. Prentice Hall, Upper Saddle River, New Jersey.

Zullinger, E. M., R. E. Ricklefs, K. H. Redford, and G. M. Mace. 1984. Fitting sigmoidal equations to mammalian growth curves. Journal of Mammalogy 65:607-636.

Table 1.1. Mean mass (SD) of total body (BM), ingesta-free mass (IFM), total digesta, and organs of young and adult *Rangifer tarandus* and *Ovibos moschatus*, Fairbanks, Alaska, 1999–2002. Different letters in each row indicate significant differences between species and among age groups (all ANOVA, but Liver, Kidney—ANCOVA; Bonferroni adjusted $p < 0.05$).

	Age group							
	1 day		30 days		60 days		Adult	
	Rangifer	Muskoxen	*Rangifer*	Muskoxen	*Rangifer*	Muskoxen	*Rangifer*	Muskoxen
BM (kg)	6.85a (0.9)	10.26a (0.3)	18.03a (1.2)	22.63b (1.9)	29.70b (5.9)	37.55b (2.8)	126.83c (16.6)	291.80d (13.6)
IFM (kg)	6.75a (0.9)	10.13a (0.9)	17.02ab (1.3)	21.44ab (2.0)	27.63ab (5.71)	33.47b (2.2)	115.52c (17.1)	253.70d (15.0)
Digesta (kg)	0.10a (0.1)	0.13a (0.1)	0.88a (0.3)	1.19a (0.1)	2.07ab (0.2)	4.08b (0.6)	11.31c (0.5)	41.77d (2.8)
Rumen (g)	32.45a (3.7)	59.07a (5.7)	189.50a (66.0)	214.75a (12.1)	668.00a (98.7)	652.25a (111.7)	3285.50b (933.8)	5640.00c (687.8)
Omasum (g)	6.15a (1.4)	11.36a (2.8)	17.15ab (2.7)	31.00ab (1.6)	37.77b (10.3)	56.33b (5.5)	146.83c (52.1)	799.60d (56.1)
Abomasum (g)	37.54a (1.4)	46.43a (11.3)	86.01a (14.3)	113.26a (12.0)	157.17b (33.3)	157.25b (5.2)	353.50c (43.8)	671.96d (104.0)
Small Intestine (g)	164.02ab (49.3)	206.41ab (58.4)	471.61bc (146.1)	616.20c (66.0)	587.37c (112.3)	1317.57d (15.9)	728.06c (213.8)	2056.45e (214.5)
Cecum (g)	13.06a (2.3)	30.93a (17.7)	62.00a (7.6)	53.25a (7.8)	83.90ab (12.8)	105.13ab (19.0)	270.83b (36.3)	584.50c (196.3)
Colon (g)	46.35a (12.2)	NA	136.62a (32.4)	219.52a (82.6)	243.23ab (21.6)	468.97ab (83.2)	1420.73b (456.6)	2044.17c (830.6)
Liver (g)	174.97a (34.1)	192.86ab (14.4)	416.25b (35.9)	483.48b (30.5)	681.33c (146.1)	707.13c (49.6)	1677.5abc (207.9)	2203.7abc (158.9)
Kidney (g)	41.32a (6.8)	41.64a (3.9)	87.86a (8.4)	116.31a (13.3)	118.57a (13.5)	143.73a (9.2)	361.75a (93.5)	410.28b (19.8)
Thyroid (g)	2.94a (0.5)	1.93a (0.4)	2.82a (0.3)	2.52a (0.3)	3.48a (0.6)	2.82a (0.3)	12.69b (3.5)	17.76c (1.4)

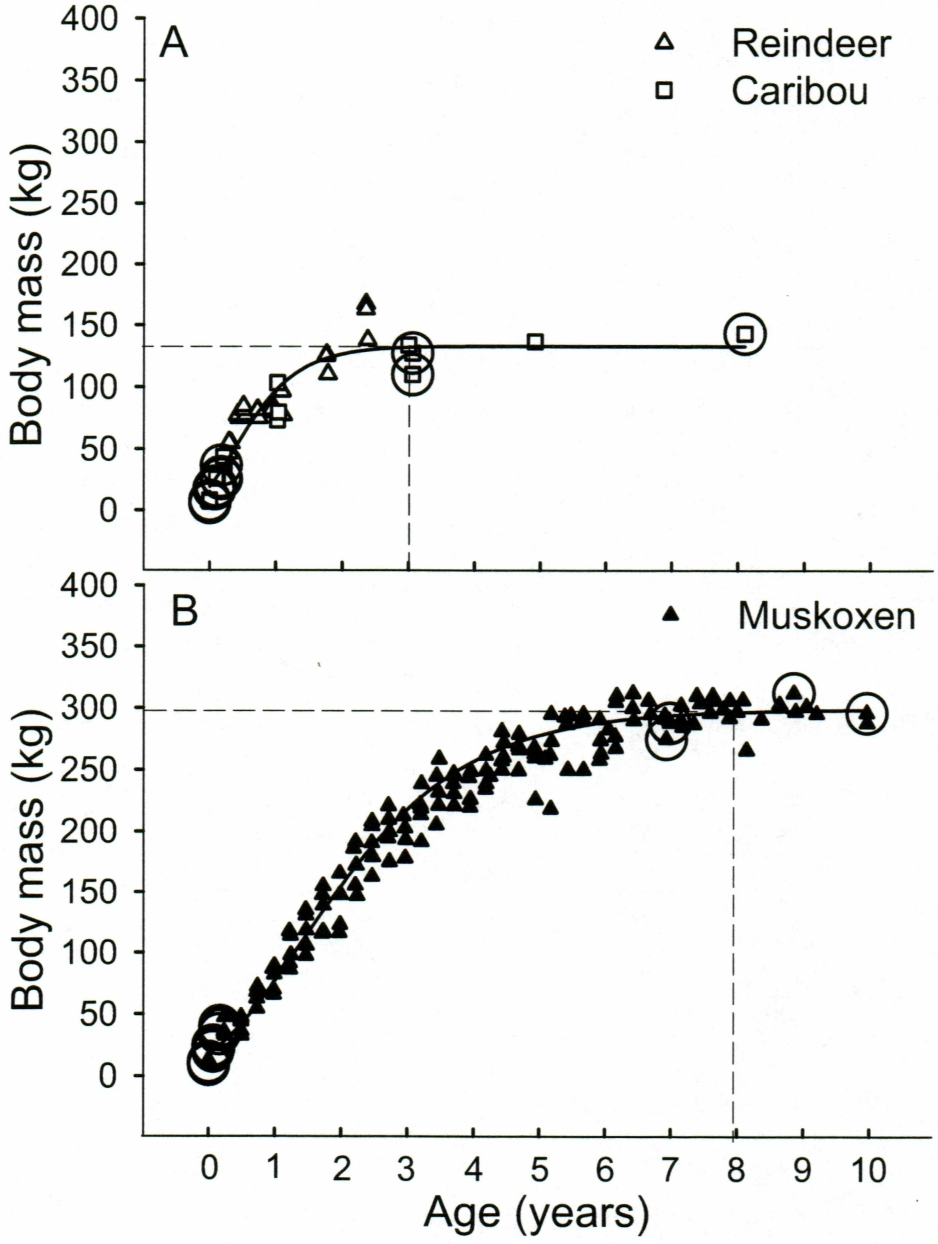

Figure 1.1. Growth of body mass (kg) of *Rangifer tarandus* (reindeer, caribou) and *Ovibos moschatus* (muskoxen) fitted to the Gompertz equation for age (years), Fairbanks, Alaska 1999-2002. Reindeer (n = 3) and caribou (n = 3); A = 133 kg, K = 0.005 day^{-1}, I = 0.4 years, R^2 = 0.958. Muskoxen (n = 5); A = 299 kg, K = 0.002 day^{-1}, I = 1.4 years, R^2 = 0.970. Body mass of muskoxen shown in 90 day intervals. Dashed line indicates time to asymptotic mass. Circles in each graph indicate animals used for measures of body components (*Rangifer* n = 14 and muskoxen n = 20).

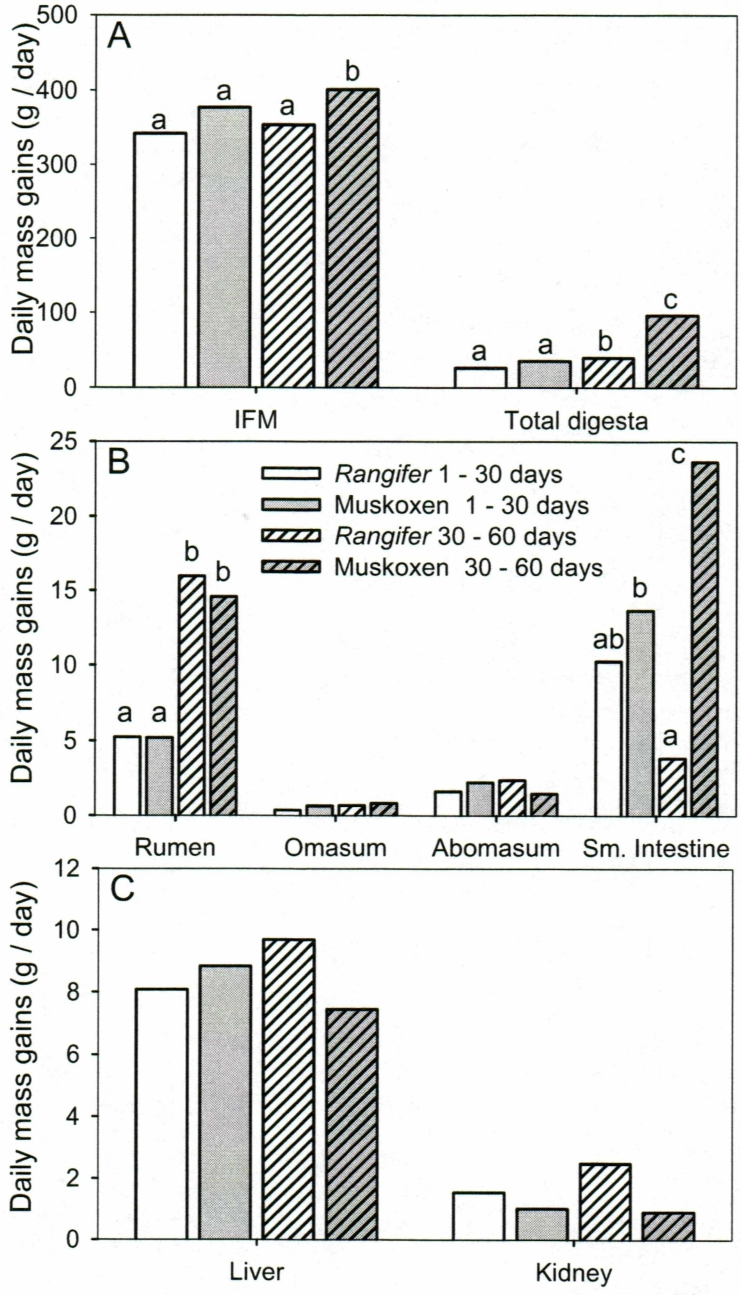

Figure 1.2. Daily mass gain (g/day) of body components of *Rangifer tarandus* (reindeer) and *Ovibos moschatus* (muskoxen) during milk consumption in the neonatal period (1 to 30 days) and while young transition to forage (30 to 60 days), Fairbanks, Alaska 1999-2002. Different letters within each group indicate differences in mass gained ($X^2_1 > 3.8$; $P = 0.05$).

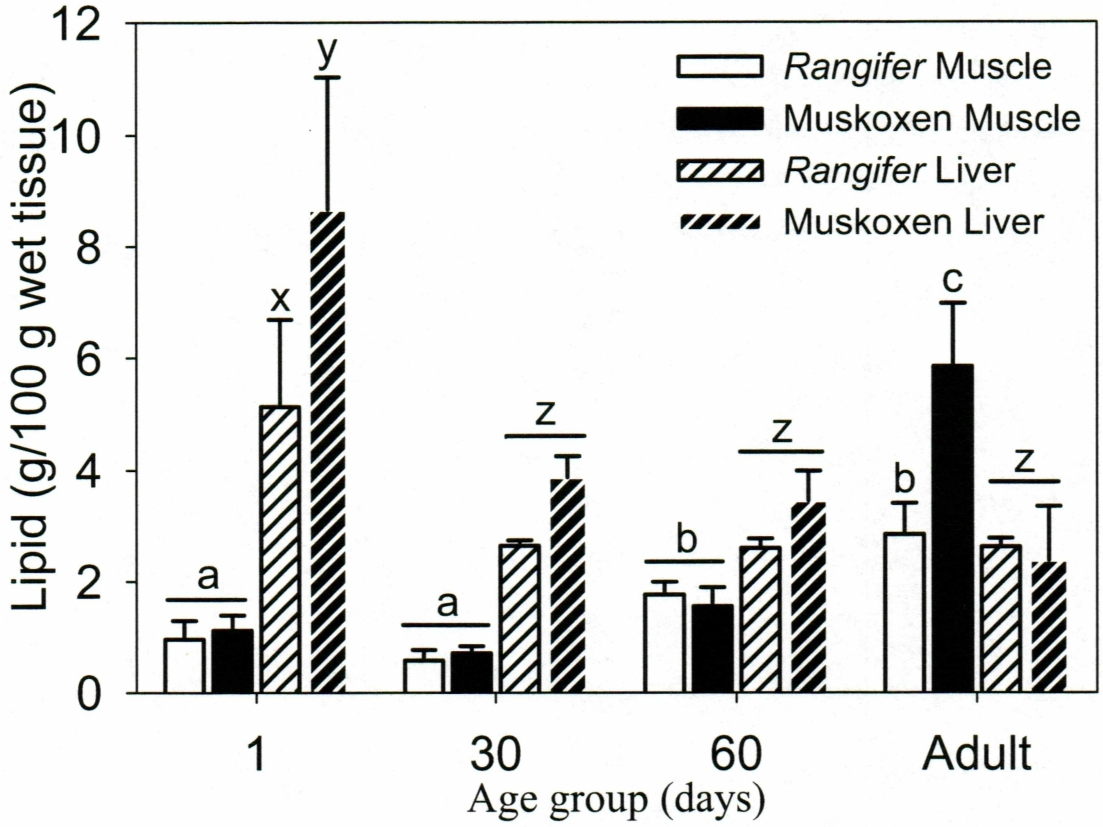

Figure 1.3. Lipid concentration (g lipid/100 g wet tissue, mean ± SD) of liver and skeletal muscle from young and adult *Rangifer tarandus* and *Ovibos moschatus,* Fairbanks, Alaska, 1999–2002. Different letters (a-c or x-z) indicate differences between species and age groups for each tissue (Bonferroni adjusted $P < 0.05$).

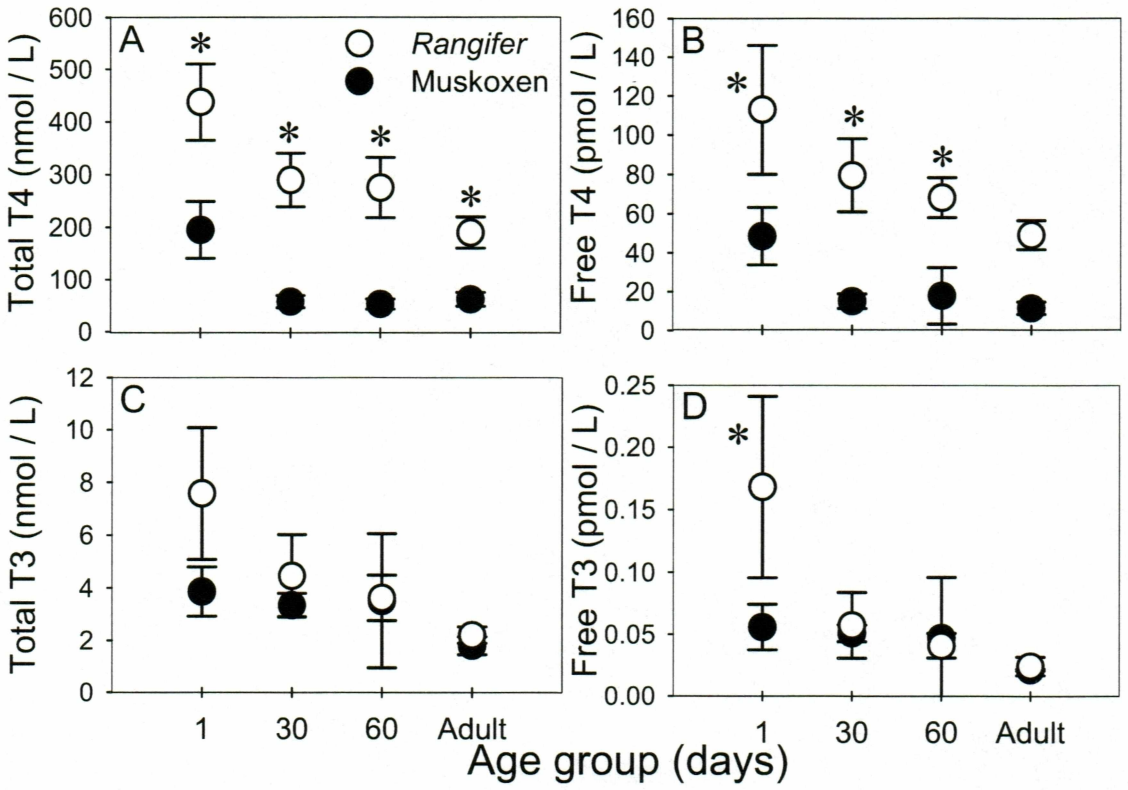

Figure 1.4. Thyroid hormone concentrations (mean ± SD) in plasma from young and adult *Rangifer tarandus* and *Ovibos moschatus*, Fairbanks, Alaska, 1999–2002. Asterisks indicate significant differences between species (Bonferroni adjusted $P < 0.05$) within age group.

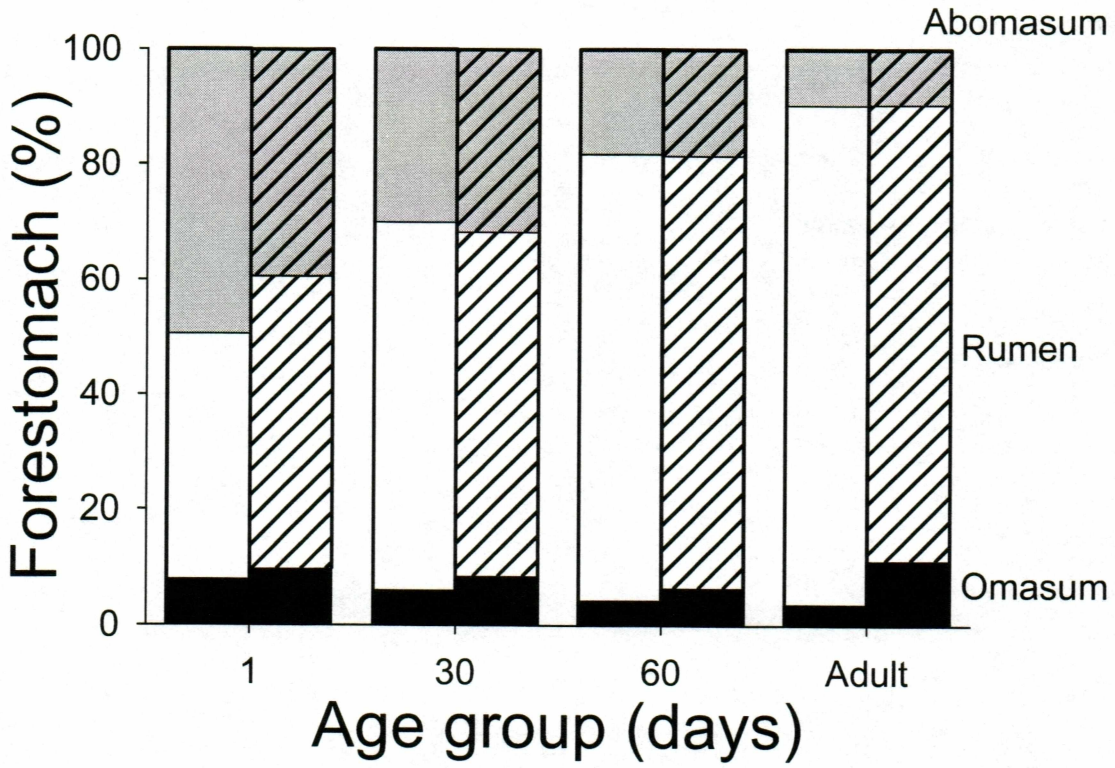

Figure 1.5. Proportional mass of the abomasum, rumen, and omasum (percent total forestomach mass) for young and adult *Rangifer tarandus* (solid bars) and *Ovibos moschatus* (hatched bars), Fairbanks, Alaska, 1999–2002.

CHAPTER 2. NUTRITIONAL DEVELOPMENT OF FEEDING STRATEGIES IN ARCTIC RUMINANTS: DIGESTIVE MORPHOMETRY OF REINDEER, *RANGIFER TARANDUS,* AND MUSKOXEN, *OVIBOS MOSCHATUS*[2]

Abstract

Consumption of more fibrous plants by muskoxen than by reindeer, and differences in digestive morphology, has led to classification of muskoxen as grazers and reindeer as intermediate feeders. We hypothesized that the digestive morphology of young (< 2 mo) reindeer and muskoxen anticipates transitions in diet and determines the feeding strategy of each species at adulthood. Development *in utero* provides the neonate with a functioning mucosa of the abomasum, and duodenal mucosa with high surface area for digestion and absorption of concentrated milks. Transition to forage is preceded by changes in ruminal papillae structure that increase surface area and likely contribute to active fermentation by 60 days of age. The abomasum also increases in acid-secreting parietal cells during the transition to forage, which may enhance digestion of plant and microbial proteins. Young muskoxen had thick cornified epithelia and muscle layers of the rumen that would provide ruminal mucosa greater protection from fibrous abrasion and enhance motility of bulky diets. Conversely, young reindeer had more complex papillary shapes in the rumen and more foliate villi in the duodenum, indicating a greater absorptive capacity of these structures in comparison with muskoxen. Endogenous patterns, therefore, play the primary role for digestive development of reindeer and muskoxen and determine nutritional strategy of adults.

[2] Knott, K. K., P. S. Barboza, R. T. Bowyer, and J. E. Blake. In Review. Nutritional development of feeding strategies in arctic ruminants: digestive morphometry of reindeer, *Rangifer tarandus,* and muskoxen, *Ovibos moschatus.* Zoology.

Introduction

Seasonal fluctuations in arctic environments favor ruminants that can respond quickly to short periods of plant production to meet the demands for reproduction and growth (Suttie and Webster, 1995; Adamczewski et al., 1997; Barboza and Bowyer, 2001). Reindeer (*Rangifer tarandus*) and muskoxen (*Ovibos moschatus*) are born in early spring (White and Luick, 1984; Adamczewski et al., 1997) shortly before the emergence of high-quality plants. Although reindeer give birth to proportionately larger young (relative to adult body mass) than muskoxen, growth rates during the first summer are similar between species as animals rapidly increase in lean mass (Knott et al., in press; see also Adamczewski et al., 1987; Adamczewski et al., 1995; Peltier and Barboza, 2003). Rapid growth rates are associated with consumption of concentrated milk, which reach peak intakes in both species within the first month of life (Parker et al., 1990). As milk quality declines, somatic growth and expansion of nutritional organs are associated with a transition to forages (Frisby et al., 1984; Oakes et al., 1991; Lavigueur and Barrette, 1992; Knott et al., in press). The sufficient consumption of forages during brief summers is an important component of the life history of Arctic and sub-Arctic ruminants to survive extreme winters (Cameron et al., 1993; Rachlow and Bowyer, 1994; Adamczewski et al., 1997; Bowyer et al., 1998). Forage plants in arctic environments contain the highest nitrogen content and greatest potential digestibility during July - August (Klein and Bay, 1990; Rachlow and Bowyer, 1994; Johnstone et al., 2002), which may present a critical opportunity for young arctic ruminants to meet the nutritional needs for growth as well as increase energy reserves before the onset of winter.

Digestive attributes of ruminants probably reflect their current diet as well as the functional anatomy of their species (Langer, 1988; Iason et al., 2000). Muskoxen are classified as grazers based on the gross anatomy of their digestive system (Staaland et al., 1995; Hofmann, 2000; Mathiesen et al., 2000) and on the inclusion of fibrous plants such as sedges and grasses in their diet throughout the year (Klein and Bay, 1990; Larter and Nagy, 1997). Although diets of reindeer can overlap with those of muskoxen, reindeer usually consume more digestible forages such as forbs, fungi, and lichens (Ihl and Klein, 2001), and they have been described as intermediate feeders (Hofmann, 2000). Although larger body size of ruminants can enhance the capacity to consume and degrade fibrous plants (Robbins et al., 1995; Illius and Gordon, 1999; Barboza and Bowyer, 2000; Perez-Barberia and Gordon, 2001), digestive morphology cannot be overlooked as an adaptation to feeding strategy. For example, Clauss et al. (2003) reported that thicker muscle layers of the rumen of grazers would allow for greater motility of larger forage particles and increase mixing of ruminal contents. If feeding strategy is related to morphology, then ruminants should be born, or quickly develop shortly after birth, specialized digestive structures that allow the greatest adaptation to their nutritional niche as adults. Consequently, development of digestive function may provide evidence of whether ontogeny determines the type of forage that ruminants consume as adults.

We compared morphological development of the nutritional organs of muskoxen with reindeer as neonates (1 d old), during the transition to forage consumption (30 - 60 d old), and at maturity (> 7 yrs). This study used only captive animals from the same location to minimize environmental differences between species and to remove any

nutritional constraints on growth. We measured tissue structural morphology and cell division rates to assess functional changes of rumen, abomasum, duodenum and liver, as well as composition of ingested milk and forage. Animals were provided similar diets to investigate the endogenous patterns that exist during nutritional development of reindeer and muskoxen that may determine the nutritional niche of adults. We predict that young reindeer and muskoxen would exhibit digestive morphology specific to their feeding strategy that precede the transitions to adult diet and proportional digestive capacity.

Materials and methods

Procedures for animal experimentation were approved by the University of Alaska Fairbanks Institutional Animal Care and Use Committee under protocol #01-21 and in keeping with the American Society of Mammalogists guidelines for research on mammals (ASM ACUC Guidelines). All animals were from captive herds at the R. G. White Large Animal Research Station and the Biological Reserve (65° N, 146° W). Herds were maintained with standardized husbandry procedures and feeding protocols developed by the Institute of Arctic Biology, University of Alaska Fairbanks over the past 15 yrs. Young animals remained with their mothers throughout the study, and were able to consume milk naturally. All animals had access to small amounts of fresh grass (*Bromus* sp.) and browse (*Betula* sp., *Salix* sp.) that were growing in their enclosures. Adult muskoxen were fed grass hay (*Bromus* sp.; Peltier et al., 2003) and a pelleted supplement of minerals (Muskox supplement; Rombach et al., 2002). Young muskoxen could access adult foods as well as a complete diet formulated from barley, corn, alfalfa, soy meal, and fish meal with minerals and vitamins (Rombach et al., 2002). Reindeer were provided with a complete pelleted ration similar to that of young muskoxen. These feeds provided nutrient compositions similar to sedges, grass, and browse consumed by animals in the wild, and thus

provided captive animals with stimuli similar to their wild counterparts for development and growth (Jorgenson et al. 2002; Ohlson and Staaland 2001). Animals were weighed (± 0.1 kg) on electric scales at regular intervals to monitor health and growth.

Animals were selected for necropsy at 1 day, 30 days and 60 days of age to assess nutritional development during the transition from milk to forage. Males and females were included in each group of young animals. Castrated caribou (127 ± 17 kg) and muskoxen (292 ± 14 kg) were used to represent mature adults, 7 ± 2.5 yrs (mean ± SD) in each species without confounding effects of reproduction on morphology. Caribou were used to represent adult morphology of *Rangifer tarandus* because growth patterns of whole-body and organs were similar between reindeer (*R. t. tarandus*) and caribou (*R. t. grantii*; Knott et al., in press). One-day-old muskoxen (n = 7, 10.3 ± 0.3 kg) were born April – June 1999 (Rombach et al., 2002), whereas other young muskoxen were born April 2001 (30 d of age, n = 4, 22.6 ± 1.9 kg; 60 d of age, n = 4, 37.6 ± 2.8 kg). Young reindeer (1 d of age, n = 4, 6.8 ± 0.9 kg; 30 d of age, n = 4, 18.0 ± 1.2 kg; 60 d of age, n = 3, 29.7 ± 5.9 kg) were born April 2002. Castrated adults were taken for necropsy at similar times to young animals to minimize effects of season (April 2002 for muskoxen, n = 5 and June 2002 for caribou, n = 3).

All animals were immobilized with tiletamine hydrochloride and zolazepam (3 mg/kg; Telazol 100 mg/mL, Fort Dodge Animal Health, Fort Dodge, Iowa) for transport to the necropsy facility. Thirty and 60 d old animals were intubated and maintained under gaseous anesthesia with halothane (3 %) in oxygen for 2 h prior to euthanasia. The anesthetic period in these animals was used for equilibration of a single intrajugular dose (30 d = 20 mL; 60 d = 40 mL) of bromodeoxyuridine (BrdU, 50mg/kg; Sigma-Aldrich, St. Louis, Missouri), a thymidine analogue that binds to DNA during the S phase of cell division. Animals were euthanized by overdose of

barbiturates (0.2 mL/kg body mass; Euthasol, Delmarva Laboratories, Midlothian, Virginia) at the jugular vein.

Dissections of tissues commenced immediately after cardiac arrest and the loss of the corneal reflex. Associated adipose and connective tissues were dissected from the digestive tract and organs before being weighed on an electronic balance (± 0.01 g or kg). Ingesta from rumen and abomasum were determined as the difference between intact and empty weights of each segment and stored at -20° C for analysis. Duodenum tissue was sampled from the first 12 cm of the small intestine within 5 min post mortem to limit autolysis. Remaining tissue samples were collected from each organ within 2 h post mortem. Tissues were sampled from the ventral mid-section of the rumen, abomasal fundus, and the caudate lobe of the liver. All tissues were placed immediately into zinc formalin for analysis of histomorphometry. A second tissue sample from 30 and 60 day-old animals also was fixed in methacarn (60% methanol, 30% chloroform, 10% glacial acetic acid, v/v) for immunohistochemistry (IHC).

Composition of ingesta. Samples of ruminal and abomasal ingesta were analyzed to assess amount and composition of dietary substrates for degradation in the segments of the forestomach. Ingesta were described qualitatively as milk, pelleted feed, or forage. Ingesta pH was measured with a standardized electrode (± 0.01 units) in young animals. Ingesta were dried at 55° C to constant mass for determination of dry matter (DM). Ruminal ingesta were ground in a Wiley mill (1.25 mm screen; Arthur Thompson Company, Philadelphia, Pennsylvania) and abomasal ingesta were processed in a Waring blender (Eberbach Corporation, Ann Arbor, Michigan) for homogenization of samples. Ash was determined by combusting 1 g of dried ingesta for 8 hours at 500° C in a muffle furnace (Barnstead International, Dubuque, Iowa). Organic matter was estimated as the

difference between DM and ash. Carbon and nitrogen content of ingesta was determined by an elemental analyzer (LECO; St. Joseph, Michigan). Neutral detergent fiber (NDF) of ingesta from 60 day-old and adult animals was estimated by extraction of plant cell walls with Na_2SO_3 (Van Soest et al., 1991).

Digestive morphometry. Tissues were fixed for 3 days, dehydrated, cleared, and infiltrated with paraffin following standard procedures. Tissues fixed in methacarn were prepared by the methods of McGinley et al. (2000). We sectioned tissues at 5-6 μm for analysis of histomorphometry and immunohistochemistry. Tubular organs (e.g., duodenum) were cut in cross section to discriminate between all layers of the alimentary canal.

Tissues fixed with zinc formalin were stained with hematoxylin and eosin (H&E) for histomorphometry and examined under light microscope. We captured digital images of each tissue with scale bars (μm) using Axiovision 3.0.6.1 software (ZeissVision GmbH, Germany). Morphological structures were quantified with Image-Pro Express v4.5 software (Media Cybernetics, Inc., Silver Spring, Maryland). We selected 3 - 5 fields per tissue using a random number assignment and a field finder (Lovin Microslide Field Finder, Gurley Precision Instruments, Troy, New York). Tissue morphology was based on structural criteria described by Ross et al. (1995) for mammalian gastrointestinal system and liver, and Langer (1988) for morphology of the ruminant forestomach. Surface enlargement was measured in ruminal and duodenal tissues by dividing the surface boundary by the basal boundary (Snipes, 1994). Appendix 1 provides a complete description of measurements of each tissue.

We also examined surface images of ruminal papillae with an ISI- 40 scanning electron microscope. Formalin–fixed samples of rumen (1 × 1 cm) from 30 and 60 day old animals were dehydrated to 100% ethanol and vacuum sublimated (Tousimis Samdri-790 Critical Point Dryer). Samples were mounted and sputter coated with gold palladium for 3 × 1 min intervals before viewing.

Immunohistochemistry. Dividing cells from 30 and 60 day-old animals were visualized by IHC on methacarn fixed tissue. Positive controls were included in each batch to monitor variation in staining. Tissues from reindeer and muskoxen within each age group were stained together to avoid artifacts in species comparisons through a method for BrdU detection modified from McGinley et al. (2000). Tissue sections were pretreated with 2 N HCl for 90 min to denature DNA before performing detection steps with the Microprobe Staining Station (Fisher Scientific, Pittsburgh, Pennsylvania). Slides were rinsed with 0.13M NaCl phosphate buffered saline solution (PBS; pH = 7.4) containing 10% Triton-X (Fisher Scientific, Pittsburgh, Pennsylvania) as a surfactant (5 mL/L PBS) between reaction steps. Endogenous peroxides were saturated with a peroxides block (0.03% hydrogen peroxide; DAKO Corporation, Carpenteria, California) to remove nonspecific staining with chromagen. Tissues were incubated for 30 min with mouse anti- BrdU (1:200; DAKO Corporation, Carpenteria, California), which binds to the in vivo labeled BrdU within the DNA in dividing cells and visualized with DAKO EnVision + System, Peroxidase, DAB-chromagen kit (anti-mouse; DAKO Corporation, Carpenteria, California). Tissues were rinsed in water and counterstained with

hematoxylin (liver) or H&E. After staining, slides were prepared and quantified following the same procedures for formalin fixed tissues.

Counts of dividing cells were performed after recoding sample labels to avoid observer bias of species and age. Rumen was examined for cell division for several regions (base, middle, tip, between) along the length of papillae. Cell counts were made on 3 – 5 randomly selected fields per tissue. Counts of BrdU positive cells were expressed as an average of the proportion of all cells in a region that included over 30 cells / field for duodenal crypts and over 100 cells / field in rumen, abomasum and liver. Appendix 2 provides a complete description of the method for quantification of BrdU positive cells and other prevalent features for each tissue.

Statistics. Statistical analyses were performed using Systat 10.2 (SPSS Inc., Chicago, Illinois) with $\alpha = 0.05$ to test effects of species and age by analysis of variance (ANOVA). Tissue measurements were averaged within each animal to avoid pseudoreplication. Proportional measures of cell division (percent BrdU positive cells) were normalized by an arcsine-square root transformation (Zar, 1999). Organ mass was included as a covariate (ANCOVA) in all analyses, but was only significant for the effect of abomasal mass on mucosal thickness. Post-hoc tests for species and age were performed using Bonferroni adjustments for multiple comparisons.

Results

Digestive morphology of neonates. The surface of ruminal mucosa in neonatal reindeer and muskoxen had short papillary nodes entering the lumen, thereby providing a low surface enlargement factor of only 2.6 (Fig. 2.1A and Fig. 2.4D). Papillae nodes,

however, were surrounded by "epithelial clear cells", which were more tightly packed together in reindeer than muskoxen. Epithelial clear cells had open cytoplasm that may have contained lipid or another material that was dissolved during processing of tissues (Fig. 2.1A). A single layer of large open nuclei surrounded the flanks of the papillary nodes, which would form the eventual stratified squamous epithelium. All neonatal reindeer and 1 neonatal muskoxen had papillary nodes that protruded above the clear cells of the rumen. The epithelial layer of papillary nodes was thin and similar between species (40 μm; Fig. 2.4C). Circular muscle and cornified layers of the rumen, however, were 2 fold thicker in neonatal muskoxen than in neonatal reindeer (Fig. 2.4A, B).

Ingested milk of neonatal reindeer and muskoxen bypassed the underdeveloped rumen (see *Ingesta substrate load*) and was digested in the abomasum. The abomasal mucosa was thin in both neonatal reindeer and muskoxen (367 μm; Fig. 2.5). The small number of acid secreting parietal cells in neonates (Fig. 2.2A) was reflected in the high pH of abomasal ingesta (pH 4; Fig. 2.5). Cells of the abomasal mucosa, therefore, were composed primarily of zymogenic chief cells and mucous surface cells that formed large gastric pits (Fig. 2.2A).

Duodenum of neonatal reindeer and muskoxen contained long (mean ± SD: 954 ± 295 μm) and narrow (mean ± SD: 115 ± 28 μm) villi (Fig. 2.3A). The duodenal surface enlargement factor was maximal in animals consuming milk diets ($P < 0.001$), and greater in muskoxen (16.8) than in reindeer at birth (8; $P < 0.05$; Fig. 2.6B). Duodenal crypts were short in neonatal animals (170 um) and occurred only in a single layer along the muscularis mucosa (Fig. 2.3A). The villar epithelium stained heavily with eosin in

neonatal muskoxen, but not in reindeer, suggesting a greater content of mucin in muskoxen. Although blood vessels were evident within the lamina propria of the villi of neonates of both species, lymphatic lacteals were only readily apparent in duodenum of reindeer.

Digestive development. Epithelial thickness ($P < 0.003$) and surface enlargement factor ($P < 0.001$) of ruminal mucosa increased similarly in reindeer and muskoxen as young animals transitioned to forage (age effect; Fig. 2.4C, D). Muskoxen, however, had thicker circular muscle ($P < 0.001$) and cornified ($P < 0.002$) layers than reindeer overall (species effect; Fig. 2.4A, B). Muscle layers of the rumen also increased as animals aged ($P < 0.001$; Fig. 2.4A), whereas the cornified layer remained at 10-13 μm in reindeer and 14-20 μm in muskoxen at all ages ($P = 0.21$; Fig. 2.4B). Width of ruminal papillae did not change after 30 days of age (334 μm, $P > 0.673$); therefore, increase of ruminal surface enlargement ($P < 0.001$; Fig. 2.4D) was likely because of the removal of the clear cells surrounding neonatal papillary nodes (Fig. 2.1A vs. 2.1B) and lengthening of papillae with age ($P < 0.001$). Consequently, cornified and epithelial layers surrounded the entire ruminal papillae shortly after birth. Thirty-day-old reindeer and muskoxen had uniformly spaced ruminal papillae that were conelike and circular at the base (Fig. 2.1B,C). Ruminal papillae became more spatulate at 60 days old in both species (Fig. 2.5C vs. 2.5D), further enhancing surface enlargement. BrdU positive cells were located at the base of papillary epithelium of all 30 and 60-day-old animals, and cell division rates did not differ between species, age or region of papillae (8% BrdU positive cells; $P > 0.55$; Fig. 2.7). Protozoa were associated with ruminal surfaces after 30 days of age in

reindeer and after 60 days of age in muskoxen, indicating the presence of a fermentation system. Ruminal pH in young animals (Fig. 2.1D) was within the range of ruminal pH noted in adults of these species (6.3-7.1; Barboza et al., unpublished data). Although papillae measurements were similar between species at each age group, ruminal papillae of reindeer were more complex and often bifurcated than the more uniform papillae in muskoxen. Adult muskoxen, however, showed a complete muscularis mucosa along the base of epithelial layers and within papillae structures, which was not present in adult deer.

The influx of forage in ruminal ingesta (see *Ingesta substrate load*) to the abomasum coincided with an increase in the thickness of the abomasal mucosa that followed abomasal mass in both species (age effect; ANCOVA; $P < 0.009$; Fig. 2.5). The abomasal mucosa thickness was also thicker in reindeer than muskoxen, independent of organ mass (species effect; ANCOVA; $P < 0.001$; Fig. 2.5). Thickening of the mucosa layer also corresponded with an increase in number of parietal cells in both species (Fig. 2.2B,C), although abomasal ingesta remained at pH 4 in all young animals (Fig. 2.5). Gastric glands increased in density after birth, as animals aged, but mucous surface cells and chief cells composed a smaller proportion of the mucosal depth (Fig. 2.2A vs. 2.2B). Mucous surface cells, however, contained mucinogen granules and appeared to be more columnar in 60-day-old animals than younger age groups. Adult reindeer had the thickest abomasal mucosa (1106 μm; age*species interaction; Bonferoni adjusted; $P = 0.005$; Fig. 2.5) because of an extension of the mucous surface cells above the parietal cells (Fig. 2.2C).

Abomasal mucosa cells differentiated as a band of BrdU positive cells, which migrated toward the lumen from 30 to 60 days old in both species (age effect; $P = 0.04$). Although the width of the band was similar between species and among age classes (175.5 um; age and species effects; $P > 0.56$), the band of BrdU positive cells was farther from the muscularis mucosa in reindeer than in muskoxen (species effect; $P = 0.001$). Total rates of cell division in the abomasal mucosa, however, did not differ between species or with age (3.4% BrdU positive cells; $P > 0.51$; Fig. 2.7).

The surface enlargement factor of the duodenum decreased similarly in reindeer and muskoxen (age effect; $P < 0.001$; Fig. 2.6B) as the quantity of milk declined, but villi increased in width (age effect; $P = 0.006$). Although reindeer had shorter duodenal mucosa than muskoxen (species effect; $P < 0.02$), the duodenal mucosa remained the same as animals aged (860 μm; $P = 0.16$). Circular muscle of the duodenum, however, was similar in young ruminants (150 μm), and increased after 60 days old in both species (age effect; Bonferoni adjusted; $P < 0.05$; Fig. 2.3C). Although lymphatic lacteals were present in reindeer from birth, muskoxen did not show evident lacteals within the duodenum until 60 days of age. Reindeer also had more foliate villi (Fig. 2.3B), often with flat tips at the luminal surface, than the more uniform villi of muskoxen. Villi from adult reindeer also had a greater number of goblet cells, more prominent lacteals, and less columnar crypts than muskoxen adults.

Duodenal crypt depth was greater in muskoxen than reindeer (species effect; $P < 0.001$), and increased with age in both species ($P < 0.001$; Fig. 2.6A). Rates of cell division within duodenal crypts of young reindeer and muskoxen were higher than cell

division rates of all other tissues (> 29 % BrdU positive cells; tissue effect; $P < 0.05$), but did not differ between species or age groups ($P > 0.82$; Fig. 2.7). BrdU positive cells were located within the whole expanse of circular crypts, but only in the bottom half (closer to muscularis mucosa) of the more tubular crypts occurring in 60 day-old reindeer and muskoxen. Similarly, species and ages did not differ in the level of BrdU positive cells within the lamina propria (10.5 cells / villi; $P > 0.06$) or in epithelial cells (1.4 cells / villi; $P > 0.20$) of duodenal villi.

Neonatal livers of reindeer and muskoxen contained the large vacuoles within the cytoplasm of hepatocytes that may have contained lipid or glycogen before tissue processing (Fig. 2.3C). The number of hepatocytes containing vacuoles declined as reindeer and muskoxen consumed greater amounts of forage. Although cell area was similar between species (204 μm^2), nuclear area of hepatocytes was larger in muskoxen (33 μm^2) than in reindeer (26 μm^2) at all ages (age effect; $P = 0.011$). BrdU positive cells were scattered throughout the parenchyma of the liver in both reindeer and muskoxen, but 30 day-old muskoxen had a greater rate of cell division in the liver than 30 day-old reindeer (2.2 vs. 0.8 %; species effect; $P = 0.039$). Species and age group, however, did not differ in the rates of cell division overall (1.2% BrdU positive cells; $P > 0.06$; Fig. 2.7). Liver development during the transition to forage also occurred through formation of more polygonal hepatocytes and an increase in the number of hepatic blood vessels in both species.

Ingesta substrate load. Mass of ruminal ingesta increased in both species as animals aged ($P < 0.001$), but muskoxen had greater ruminal ingesta than reindeer overall

($P < 0.001$; Table 2.1). The milk diet of neonatal and 30 day-old animals did not enter the rumen. The rumen, however, was filled with soil and hair at 30 days of age. Ingestion of soil and hair was associated with the highest concentrations of ruminal ash at 30 days-old in both species ($P < 0.04$; Table 2.1). Concentrations of ash decreased, while levels of carbon and organic matter increased, in ruminal ingesta during the transition to forage (plant and pelleted food) at 60 days of age. Reindeer increased ruminal mass from 4 % body mass at 60 days of age to 7 % body mass in adults (Table 2.1). Ruminal mass of muskoxen, however, was similar to adult reindeer by 60 days of age (6.9 % body mass) and was almost twice the capacity of adult reindeer as adults (13% body mass). Increases in ruminal ingesta mass corresponded to carbon concentration, which also increased from 60 days to adulthood. Ruminal ingesta at 60 days of age, however, was similar to adults in ash, organic matter, and DM ($P = 0.141$; Table 2.1). Although both species had similar fiber content within ruminal ingesta at 60 days of age (55 ± 5% DM), adult muskoxen continued to increase fiber content of the rumen (63.5 ± 1.5% DM), while adult reindeer sustained similar levels to 60 day old animals (49.6 ± 1.0% DM).

Abomasal ingesta of neonatal and 30 day-old reindeer and muskoxen contained curdled milk that was high in carbon, organic matter and nitrogen, but low in ash (Table 2.2). Although mass of abomasal ingesta did not increase during the transition from milk to forage diet (age effect; Bonferoni adjusted; $P > 0.05$), DM and nitrogen content decreased during this period (age effect; Bonferoni adjusted; $P < 0.001$; Table 2.2). Ash content of abomasal ingesta was higher in reindeer than muskoxen overall ($P = 0.03$), but did not increase with age ($P > 0.11$; Table 2.2).

Discussion

Our results indicate 3 overlapping endogenous patterns for nutritional development of reindeer and muskoxen that complement their requirements for growth and establish feeding strategies from birth. Digestive development *in utero* produces a precocial offspring that can maximize milk consumption, whereas postnatal development anticipates the transition forage. Anticipatory morphology, resulting from exogenous as well as endogenous stimuli, may permit young ruminants to time ruminal fermentation to the emergence of plants at Arctic latitudes. Differences in digestive anatomy between reindeer and muskoxen during development were also consistent with an endogenous pattern that could determine feeding strategies as intermediate feeders and grazers, respectively.

In utero digestive development. *In utero* development must result in offspring that can successfully utilize milk, especially the concentrated milk produced by arctic ruminants (>20% lipid; White and Luick, 1984; Oftedal, 1984; White et al., 1997). The abomasum of neonatal reindeer and muskoxen are large (50% of forestomach; Knott et al., in press) and include well-developed glandular mucosa (Fig. 2.2A). Low number of parietal cells and curdled milk at moderate pH (> 4) indicate that abomasal proteolysis involves chymosin, rather than pepsin typical of adults (Lyford and Huber, 1988). Low acid secretion is typical of young mammals and likely spares colostral immunoglobulins from proteolysis. Because the duodenal epithelium of ruminants is permeable to immunoglobulins for only 1-2 days after birth (Thivend et al., 1984; Lyford and Huber, 1988), large surface enlargement of duodenal mucosa, favors high rates of absorption of

immunoglobulins from the first ingestion of milk. Concentrations of lipid and lactose in milk also are highest at the start of lactation (White and Luick, 1984; White et al., 1987; Parker et al., 1990). Therefore, duodenal enlargement would contribute to the absorption of several products of digestion, including milk sugars (Scharrer et al., 1979).

Morphology of the liver of neonates (Fig. 2.3C) correlated with a high proportion of lipid in both reindeer and muskoxen (6.6g/100g wet tissue; Knott et al., in press). Liver reserves may be necessary to augment dietary supplies of lipid or glycogen when milk intakes are low during the first week of life (Parker et al., 1990). These hepatic reserves may also be the principal source of some nutrients that are low in milk such as copper (Rombach et al., 2002). Lipid reserves and consumption of colostrum are also associated with high thyroid secretions, which likely accommodate high thermogenic demands of small ruminants at birth (Robinson et al., 1999; Knott et al., in press). Consequently, development of the nutritional organs *in utero* allows reindeer and muskoxen to use concentrated milks immediately from birth and make use of reserves to support growth during the first 30 days of life.

Transition to forage. A rapid transition to forage is mutually beneficial to both mother and offspring because maternal demands for lactation are attenuated at weaning and both the young and the mother can use growing forages to support their own tissues (White and Luick, 1984; Lavigueur and Barrette, 1992). Although ruminal development is not used for consumption of milk, rumen mass is 50% of the forestomach tissue at birth (Knott et al., in press). Papillary nodes in the rumen, therefore, may be an anticipatory morphology to aid in the quick transition to forage. Ingestion of soil and hair at 30 days

of age may also be anticipatory to the consumption of forage as young animals seek new sources of food (Short, 1964; Lyford, 1988). Consumption of nonnutritive items and digestive aberrations have been reported in growing ruminants >49 days of age that were artificially held in the suckling stage (veal calves; Pearson et al., 1987; McFarlane et al., 1988) and were highest in those animals confined individually (Bokkers and Koene, 2001). It is unlikely that consumption of hair and soil by muskoxen and reindeer at 30 days was due to captive stress because animals were held in large enclosures and allowed interaction with mothers and other similar aged young of their species. Furthermore, ulcerations or other anomalies were not evident in the digestive tract at this age. Ingestion of soil and hair at 30 days, therefore, likely acts as a normal physical stimulus that is triggered by an endogenous cue to initiate development of the ruminal mucosa and provoke the infiltration of microbes.

Similar rates of ruminal cell division between 30 and 60 days of age, as well as increases in papillae formation and muscle layers, indicate high rates of tissue differentiation during the transition to forages that are consistent with high ruminal mass gains in young ruminants (reindeer and muskoxen, 15 g/d; Knott et al., in press; white-tail deer (*Odocoileus virginianus*), Short, 1964; domestic cattle and sheep, Lyford, 1988). Formation of ruminal papillae, however, may not be an increase in cell number, but a decrease in epithelial loss (Sakata, 1994), which may be associated with the formation of a resistant cornified layer in young animals (Fig. 2.1B and Fig. 2.4B). Increases in the surface area of the papillae were related with changes in both height and shape that would ultimately favor absorption of fermentation products, such as short-chain fatty acids (Van

Soest, 1982). The presence of protozoa and a stable pH near neutrality at 60 days of age also indicates active fermentation in both species. Subsequent mass gains of muskoxen and reindeer, therefore, likely result from a successful transition to forage, with only supplementary nutrients from milk, allowing young animals to consume plants at early phenological stages in July.

Post-ruminal changes during the transition to forage probably included an increase in abomasal acidity and enhanced absorptive efficiency in the small intestine. Increases in mucosal thickness of the abomasum were associated with a gain in the number of acid secreting parietal cells as well as surface cells containing mucinogen granules. Surface cells produce bicarbonate secretions, which can buffer the acidic conditions of the abomasal ingesta and prevent acid damage to gut mucosa (Ross et al., 1995). Increase in the acid secretion from abomasal mucosa allows for greater proteolysis of proteins from plants and from the ruminal influx of microbes (Karasov and Hume, 1997). Absorption of these nutrients in the small intestine was not increased through second order enlargement because surface area of villi decreased with age. This result could be expected since duodenal morphology of animals under sufficient nutrition also remained at a lowered surface enlargement compared to animals that were nutritionally limited (Konarzewski and Stark, 2000). The total absorptive surface of the small intestine, however, probably continued to increase with mass during transitional periods, providing additional absorption through elongation of the tract. Absorptive efficiency is likely sustained, as body mass gains were not altered by the transition to forages (>375 g/d; Knott et al., in press). Thin layers of duodenal muscularis in young

muskoxen and reindeer are consistent with high digestibility and low residual bulk of their diets that may be moved through the tract more easily than the fibrous materials consumed by adults. Organization of duodenal tissues probably contributed to absorptive efficiency through increases in crypt depth and subsequent high rates of cell division. These changes could be associated with differential expression of epithelial transporters that complements the changing substrates in the diet (Ferraris and Carey, 2000; Wood et al., 2000). High rates of cell division, however, may also demonstrate the normal range of cell division occurring in the small intestine, which consistently exhibits high turnover rates in comparison with other tissues (Ross et al., 1995; Stark, 1996). Post-ruminal advancements, therefore, would allow young reindeer and muskoxen to sufficiently digest and absorb nutrients from the fermentation of plants. The digestible energy and protein from forage, as well as microbial nutrients, would help to sustain continued growth and establish reserves before winter.

Ontogeny of Nutritional Niche. Digestive morphology of reindeer and muskoxen differed from birth, although young arctic ruminants consumed similar diets at each age group. Ruminal morphology of muskoxen exhibited an early development of thick muscle walls and cornified epithelium that is consistent with the feeding strategy of grazers (Clauss et al., 2003). Thick muscle walls would allow for greater mixing of bulky fibrous material (sensu, Hofmann). Furthermore, the rumen continued to increase in musculature as muskoxen aged, commensurate with fibrous fill, indicating motility even at the papillary level. A larger ruminal volume of muskoxen was evident from 60 days of age (Table 2.2), which would demonstrate a greater capacity to retain fibrous

ingesta (Demment and Van Soest, 1985; Barboza and Bowyer, 2000), even independent of body size. More cornified ruminal mucosa in muskoxen than reindeer also would protect the underlying absorptive epithelia from the abrasive effects of a more fibrous diet consumed by the grazer. Large omasa of muskoxen from birth (Knott et al., in press) also support a high capacity to filter the greater load of fluids and particulate matter from fibrous forages emerging from the rumen (Langer 1988). These attributes would support slower and more thorough fermentation of forages in muskoxen than in reindeer (Staaland and Thing, 1991; Klein, 1991; Adamczewski et al,. 1994). Thus, ontogenetic effects likely dictate the grazing morphology of muskoxen, especially by enhancing the time for microbial fermentation of large particles of fibrous forage through altering ruminal flow.

Young reindeer also had specialized digestive morphology indicating increased absorption rates that may be attributed to consumption of low-fiber diets by intermediate feeders. For example, ruminal papillae were bifurcated in young and adult reindeer, which would enhance absorption of short-chain fatty acids. Because enhanced surface area of ruminal papillae has been associated with high short-chain fatty acid production in reindeer (Soveri and Neiminen, 1995; Mathiesen et al., 2000), complex ruminal shapes at young and adult ages also may indicate an inherently greater rate of ruminal uptake in reindeer compared with muskoxen. Duodenal villi also were more complex in reindeer than in muskoxen, which infer greater post-ruminal absorption in the intermediate feeder. Furthermore, earlier development of vascular structures, such as lacteals, in reindeer than muskoxen is consistent with higher rates of absorption.

Differences in digestive morphology of the abomasum between reindeer and muskoxen also may reflect their feeding strategy. Although both species increased mucosal thickness and parietal cell number in the abomasum with age, abomasal mucosa thickened more quickly in reindeer than muskoxen, independent of abomasal mass. Early development of abomasal mucosa indicates an earlier differentiation of parietal cells to increase acidic secretion. The abomasal mucosa of reindeer compared with muskoxen highlights this earlier differentiation, because BrdU positive cells exhibited a further migration from the muscularis mucosa to the luminal surface. Adult morphologies continued this progression by creating a dense region of parietal cells below a large region of buffering surface cells, indicating that acid secretion of adult reindeer is likely high. Intermediate feeders such as reindeer and browsing ruminants are predicted to produce more acid (Hofmann, 1988), which would increase proteolytic capacity to meet the higher concentrations of protein typical of forbs and browse. Increased digestion and absorption of forages in reindeer support the greater passage rates and digestion of forages suggested for reindeer in comparison with muskoxen (White et al., 1987; Klein, 1991). Therefore, reindeer not only select highly digestible forage, but also likely digest and absorb nutrients more quickly than muskoxen.

The broad selection of forages of adult ruminants may further stimulate morphological changes of nutritional organs as seasonal and reproductive demands vary with nutritional value of the diet (Staaland et al., 1995; Lentle et al., 1996; Hofmann and Nygren, 1992; Barboza and Bowyer, 2000; Barboza and Bowyer, 2001). Digestive plasticity, however, can only act as a secondary response to modify the ontogenetic

program. Consequently, endogenous patterns established early in life play the primary role for digestive development of reindeer and muskoxen and determine nutritional strategy of adults.

Literature cited

ASM ACUC Guidelines. Guidelines for the capture, handling, and care of mammals as approved by The American Society of Mammalogists. Prepared by the Animal Care and Use Committee. http://www.mammalogy.org/pubsociety/index.html

Adamczewski, J. Z., C. C. Gates, R. J. Hudson, and M. A. Price. 1987. Seasonal changes in body composition of mature female caribou and calves (*Rangifer tarandus grownlandicus*) on an arctic island with limited winter resources. Can. J. Zool. 65: 1149-1157.

Adamczewski, J. Z., R. K. Chaplin, J. Schaefer and P. F. Flood. 1994. Intake, digestibility and passage rate of a supplemented hay diet in captive muskoxen. Rangifer 241: 245-269.

Adamczewski, J. Z, P. F. Flood, and A. Gunn. 1995. Body composition of muskoxen (*Ovibos moschatus*) and its estimation from condition index and mass measurements. Can. J. Zool. 73: 2021-2034.

Adamczewski, J. Z., P. F. Flood and A. Gunn. 1997. Seasonal patterns in body composition and reproduction of female muskoxen (*Ovibos moschatus*). J. Zool. (London) 241: 245-269.

Barboza, P. S. and R. T. Bowyer. 2000. Sexual segregation in dimorphic deer: a new gastrocentric hypothesis. J. Mamm. 81: 473-489.

Barboza, P. S. and R. T. Bowyer. 2001. Seasonality of sexual segregation in dimorphic

deer: extending the gastrocentric model. Alces 37: 275-292.

Bokkers, E. A. M. and P. Koene. 2001. Activity, oral behavior and slaughter data as welfare indicators in veal calves: a comparison of three housing systems. Appl. An. Beh. Sci. 75: 1-15.

Bowyer, R. T. 1998. Timing and synchrony of parturition in Alaskan moose: long-term versus proximal effects of climate. J. Mamm. 79: 1332-1344.

Cameron, R. D., W. T. Smith, S. G. Fancy, K. L. Gerhart and R. G. White. 1993. Calving success of female caribou in relation to body weight. Can. J. Zool. 71: 480-486.

Clauss, M., M. Lechner-Doll and W. J. Streich. 2003. Ruminant diversification as an adaptation to the physiomechanical characteristics of forage. A reevaluation of an old debate and a new hypothesis. Oikos 102: 253-262.

Demment, M. W., and P. J. Van Soest. 1985. A nutritional explanation for body-size patterns of ruminant and non-ruminant herbivores. Amer. Nat. 125: 641-672.

Ferraris, R. P. and H. V. Carey. 2000. Intestinal transport during fasting and malnutrition. An. Rev. Nutr. 20: 195-219.

Frisby, K., R. G. White and B. Sammons. 1984. Food conversion efficiency and growth rates of hand-reared muskox calves. Biol. Pap. Univ. Al. Sp. Rep. 4: 196-202.

Hofmann, R. R. 1988. Morphophysiological evolutionary adaptations of the ruminant digestive system. In: Aspects of Digestive Physiology in Ruminants (A. Dobson and

M. J. Dobson, eds.). Comstock Publishing Associates, Ithaca and London, pp. 1-20.

Hofmann, R. R. 2000. Functional and comparative digestive system anatomy of Arctic ungulates. Rangifer 20: 71-81.

Hofmann, R. R. and K. Nygren. 1992. Ruminal mucosa as indicator of nutritional status in wild and captive moose. Alces Supp. 1: 77-83.

Iason, G. R., D. A. Sim and I. J. Gordon. 2000. Do endogenous seasonal cycles of food intake influence foraging behavior and intake by grazing sheep? Funct. Ecol. 14: 614-622.

Ihl, C. and D. R. Klein. 2001. Habitat and diet selection by muskoxen and reindeer in western Alaska. J. Wild. Manag. 65: 964-972.

Illius, A. W. and I. J. Gordon. 1999. The physiological ecology of mammalian herbivory. In: Nutritional Ecology of Herbivores (H. J. G. Jung and G. C. Fahey, eds.). American Society of Animals Science, Savoy, Illinois, pp. 71-96.

Johnstone, J., D. E. Russell and B. Griffith. 2002. Variations in plant quality in the range of the Porcupine caribou herd. Rangifer 22: 83-91.

Jorgenson, J. C., M. S. Udevitz and N. A. Felix. 2002. Forage Quantity and Quality. In: Arctic Refuge Coastal Plain Terrestrial Wildlife Research Summaries (D. C. Douglas, P. E. Reynolds and E. B. Rhode, eds.). U.S. Geological Survey, Reston, Virginia, pp. 46-50.

Karasov, W. H. and I. D. Hume. 1997. Vertebrate gastrointestinal system. In: Handbook of Physiology. Section 13: Comparative Physiology (W. H. Dantzler, ed.). Oxford University Press, New York, New York, pp. 409-480.

Klein, D. R. 1991. Comparative ecological and behavioral adaptations *Ovibos moschatus* and *Rangifer tarandus*. Rangifer 12: 47-55.

Klein, D. R. and C. Bay. 1990. Foraging dynamics of muskoxen in Peary Land, northern Greenland. Hol. Ecol. 13: 269-280.

Knott, K. K., P. S. Barboza and R. T. Bowyer. In Press. Growth in arctic ungulates: postnatal development and organ maturation in Rangifer tarandus and Ovibos moschatus. J. Mamm.

Konarzewski, M. and J. M. Stark. 2000. Effects of food shortage and oversupply on energy utilization, histology, and function of the gut in nestling song thrushes (*Turdus philomelos*). Phys. Bio. Zool. 73: 416-427.

Langer P. 1988. The Mammalian Herbivore Stomach: Comparative Anatomy, Function and Evolution. Gustav Fischer, New York, New York.

Larter, N. C. and J. A. Nagy. 1997. Peary caribou, muskoxen and Banks island forage: Assessing seasonal diet similarities. Rangifer 17: 9-16.

Lavigueur, L. and C. Barrette. 1992. Suckling, weaning, and growth in captive woodland caribou. Can. J. Zool. 70: 1753-1766.

Lentle, R. G., I. M. Henderson and K. J. Stafford. 1996. A multivariate analysis of rumen papillary size in red deer (*Cervus elaphus*). Can. J. Zool. 74: 2089-2094.

Lyford, S. J. 1988. Growth and development of the ruminant digestive system. In: The Ruminant Animal: Digestive Physiology and Nutrition (D. C. Church, ed.). Prentice Hall, Englewood Cliffs, New Jersey, pp. 44-63.

Lyford, S. J. and J. T. Huber. 1988. Digestion, metabolism and nutrient needs in preruminants. In: The Ruminant Animal: Digestion, Physiology and Nutrition (D. C. Church, ed.). Prentice Hall, New Jersey, pp. 401-420.

Mathiesen, S. D., W. Sormo, O. E. Haga, H. J. Norberg, T. H. A. Utsi, N. J. C. Tyler. 2000. The oral anatomy of Arctic ruminants: Coping with seasonal changes. J. Zool. (Lond.) 251: 119-128.

Mathiesen, S. D., O. E. Haga, T. Kaino, and N. J. C. Tyler. 2000. Diet composition, rumen papillation and maintenance of carcass mass in female Norwegian reindeer (*Rangifer tarandus tarandus*) in winter. J. Zool. 251: 129-138.

McFarlane, J. M., G. L. Morris, S. E. Curtis, J. Simon and J. J. McGlone. 1988. Some indicators of welfare of crated veal calves on three dietary iron regimens. J. Anim. Sci. 66: 317-325.

McGinley, J. N., K. K. Knott and H. J. Thompson. 2000. Effect of fixation and epitope retrieval on BrdU indices in mammary carcinomas. J. Histo. Cyto. 48: 355-382.

Oakes, E. J., R. Harmsen, and C. Eberl. 1991. Sex, age and seasonal differences in the diets and activity budgets of muskoxen (*Ovibos moschatus*). Can. J. Zool. 70: 605-616.

Ohlson, M. and H. Staaland. 2001. Mineral diversity in wild plants: benefits and bane for moose. Oikos 94: 442-454.

Oftedal, O. T. 1984. Milk composition, milk yield and energy output at peak lactation: A comparative review. Symp. Zool. Soc. Lond. 51: 33-85.

Parker, K. L., R. G. White, M. P. Gillingham and D. F. Holleman. 1990. Comparison of energy metabolism in relation to daily activity and milk consumption by caribou and muskox neonates. Can. J. Zool. 68: 106-114.

Pearson, G. R., D. B. Welchman and M. Wells. 1987. Mucosal changes associated with abomasal ulceration in veal calves. Vet. Rec. 121: 557-559.

Peltier, T. C., and P. S. Barboza. 2003. Growth in an Arctic grazer: effects of sex and dietary nitrogen on yearling muskoxen. J. Mamm. 84: 915-925.

Peltier, T. C., P. S. Barboza, and J. E. Blake. 2003. Seasonal hyperphagia does not reduce digestive efficiency in an Arctic grazer. Phys. Bio. Zool. 76: 471-483.

Perez-Barberia, F. J. and I. J. Gordon. 2001. Phylogentic analysis of stomach adapations in digestive strategies in African ruminants. Oecologia 129: 498-508.

Rachlow, J. L. and R. T. Bowyer. 1994. Variability in maternal behavior by Dall's sheep:

environmental tracking or adaptive strategy? J. Mamm. 75: 328-337.

Robbins, C. T., D. E. Spalinger and W. van Hoven. 1995. Adaptation of ruminants to browse and grass diets: are anatomical-based browser-grazer interpetations valid? Oecologia 103: 208-213.

Robinson, J. J., K. D. Sinclair, R. D. Randel, and A. R. Sykes. 1999. Nutritional management of the female ruminant: mechanistic approaches and predictive models. In: Nutritional Ecology of Herbivores (H. J. G. Jung and G. C. Fahey, eds.). American Society of Animal Science, Savoy, Illinois, pp 550-604.

Rombach, E. P., P. S. Barboza, and J. E. Blake. 2002. Utilization of copper during lactation and neonatal development in muskoxen. Can. J. of Zool. 80: 1460-1469.

Ross, M. H., L. J. Romrell, and G. I. Kaye. 1995. Histology. A Text and Atlas. Williams and Wilkins, Baltimore, Maryland.

Sakata, T. 1994. Short-chain fatty acids as a physiological signal from gut microbes. In: The digestive system in mammals: food, form and function. (D. J. Chivers and P. Langer, eds.). Cambridge University Press, New York, New York, pp. 392-410.

Scharrer, E., H.-G. Liebich, W. Raab and N. Promberger. 1979. Influence of age and rumen development on intestinal absorption of galactose and glucose in lambs. A functional and morphological study. Zbl. Vet. Med. A 26: 95-105.

Short, H. 1964. Postnatal stomach development of white-tailed deer. J. Wild. Manag.

28: 445-458.

Snipes, R. L. 1994. Morphometric methods for determining surface enlargement at the microscopic level in the large intestine and their application. In: The Digestive System in Mammals: Food, Form and Function. (D. J. Chivers and P. Langer, eds.). Cambridge University Press, pp. 234-263.

Soveri, T. and M. Neiminen. 1995. Effects of winter on the papillar morphology of the rumen in reindeer calves. Can. J. Zool. 73: 228-233.

Staaland, H., J. Z. Adamczewski, and A. Gunn. 1995. A comparison of digestive tract morphology in muskoxen and caribou from Victoria Island, Northwest Territories, Canada. Rangifer 17: 17-19.

Staaland, H. and H. Thing. 1991. Distribution of nutrients and minerals in the alimentary tract of muskoxen, *Ovibos moschatus*. Bio. Phys. 98A: 543-549.

Stark, J. M. 1996. Phenotypic plasticity, cellular dynamics, and epithelial turnover of the intestine of Japanese quail (*Coturnix coturnix japonica*). J. Zool. (Lond.) 238: 53-79.

Suttie, J. M. and J. R. Webster. 1995. Are arctic ungulates physiologically unique? Rangifer 18: 99-118.

Thivend, P., R. Toullec, and P. Guilloteau. 1984. Digestive adaptation in the preruminant. In: Digestive Physiology and Metabolism in Ruminants (T. P. Ruckebusch, ed.). AVI Publishing Company, Inc., Westport, Connecticut, pp. 561-

585.

Van Soest, P. J. 1982. Nutritional ecology of the ruminant. Cornell University Press, Ithaca, New York.

Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

White, R. G., D. F. Holleman, M. E. Hubbert, and H. Staaland. 1987. Herbivores in cold climates. In: The Nutrition of Herbivores (J. B. Hacker and H. Ternouth, eds.). Academic Press, Australia, pp. 465-486.

White, R. G. and J. R. Luick. 1984. Plasticity and constraints in the lactational strategy of reindeer and caribou. Symp. Zool. Soc. Lond. 51: 215-232.

White, R. G., J. E. Rowell and W. E. Hauer. 1997. The role of nutrition, body condition and lactation on calving success in muskoxen. J. Zool. (Lond.) 243: 13-20.

Wood, I. S., R. R. Hofmann and S. P. Shirazi-Beechey. 2000. Expression of the Na+ / glucose co-transporter (SGLT1) in the intestine of domestic and wild ruminants. Europ. J. Phys. 441: 155-162.

Zar, J. H. 1999. Biostatistical Analysis. Prentice Hall, Upper Saddle River, New Jersey.

Table 2.1. Description of ruminal ingesta with the concentration of total mass, dry matter (DM), carbon, nitrogen and ash of young and adult reindeer (R) and muskoxen (M), mean (SD), Fairbanks, Alaska 1999-2002. Rumen of neonatal animals was empty. Different letters within rows indicate significant differences between age groups or between species ($P < 0.05$).

	Age						ANOVA	
	30 days		60 days		Adult			
	R	M	R	M	R	M	species	age
Description	soil, hair	soil, hair	forage	forage	forage	forage		
Wet ingesta, kg	0.63[a] (0.09)	0.44[a] (0.21)	1.26[a] (0.24)	2.61[a] (0.30)	9.25[b] (0.22)	38.93[c] (3.25)	< 0.001	< 0.001
DM, % wet	14.8 (1.2)	21.3 (10.9)	13.8 (3.2)	13.2 (0.7)	13.9 (5.4)	12.3 (1.0)	0.508	0.141
Carbon, % DM	33.9[a] (7.0)	27.3[a] (9.7)	42.4[b] (0.7)	41.0[b] (0.5)	43.2[c] (2.2)	44.8[c] (0.5)	0.336	< 0.001
Nitrogen, % DM	3.3 (0.7)	3.8 (2.2)	4.9 (0.2)	4.2 (0.1)	4.7 (0.3)	2.6 (0.2)	0.080	0.154
Ash, % DM	32.7[a] (16.8)	45.4[a] (20.8)	17.0[b] (2.1)	21.0[b] (1.2)	15.2[b] (6.2)	12.2[b] (0.9)	0.360	0.001

Table 2.2. Description of abomasal ingesta with the total mass, dry matter (DM), carbon, nitrogen, and ash in young and adult reindeer (R) and muskoxen (M), mean (SD), Fairbanks, Alaska 1999-2002. Different letters within rows indicate significant differences between ages or between species ($P < 0.05$). The amount of ingesta from neonatal muskoxen was insufficient for analysis.

	Age								ANOVA	
	1 day		30 day		60 day		Adult			
	R	M	R	M	R	M	R	M	species	age
Description	milk	milk, soil	milk, forage	milk	feed	feed, forage	feed, forage	feed, forage		
Wet ingesta, g	60.3[a] (56.6)	na	202.5[a] (32.7)	152.1[a] (75.5)	325.1[a] (125.4)	215.6[a] (80.0)	525.5[a] (45.4)	1036.4[b] (523.8)	0.500	< 0.001
DM, % wet	33.4[a] (10.6)	na	29.4[a] (6.2)	36.5[a] (8.9)	21.2[a,b] (5.7)	34.3[a] (7.7)	9.8[b] (2.8)	7.2[b] (4.3)	0.008	0.001
Carbon, % DM	57.8[a] (2.4)	na	54.8[a] (3.0)	58.5[a] (1.6)	51.3[a] (4.1)	49.4[b] (4.9)	40.9[c] (2.2)	43.0[b,c] (1.1)	0.338	< 0.001
Nitrogen, % DM	4.8[a] (1.1)	na	4.6[a] (0.4)	4.7[a] (0.4)	3.9[a] (0.3)	4.2[a] (0.6)	3.1[b] (0.4)	3.0[b] (0.3)	0.808	0.001
Ash, % DM	4.7 (0.0)	na	9.8 (3.4)	6.8 (1.4)	20.9 (19.6)	16.9 (8.4)	20.8 (2.7)	16.2 (4.1)	0.030	0.094

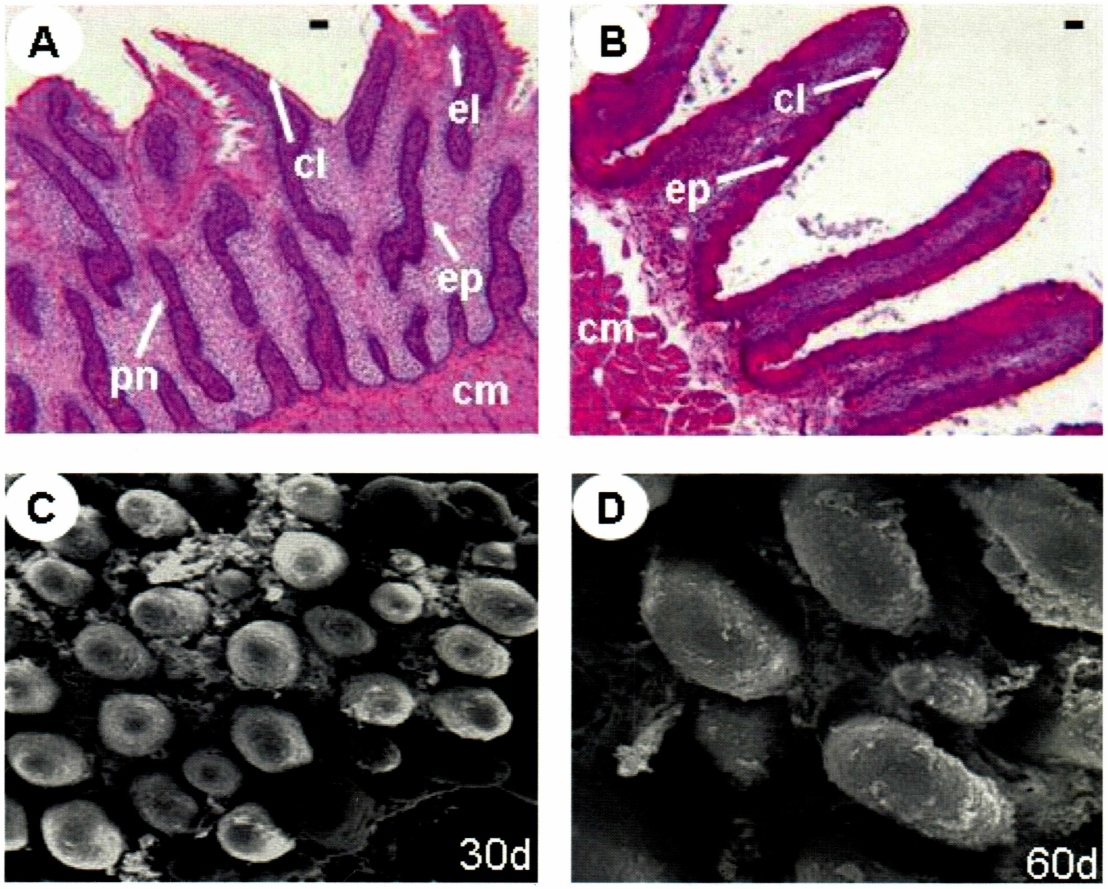

Figure 2.1. Light and scanning electron micrographs of the morphology of rumen in reindeer and muskoxen during nutritional development, Fairbanks, Alaska 1999 - 2002. **(A)** Rumen morphology of neonatal muskoxen showing papillary nodes surrounded by epithelial precursor cells. **(B)** Rumen of 30 day old reindeer showing formed papillae with an increase in cornified and epithelial layers. **(C,D)** Ruminal papillae in young muskoxen showing the transition from cylindrical to spatulate forms with increased surface area between 30 (SEM mag. = 20x) and 60 (SEM mag. = 60x) days. **Legend:** cl, cornified layer; ep, epithelial precursor cells; el, epithelial layer; pn, papillary node; cm, circular muscle. **Bars** = 100 μm.

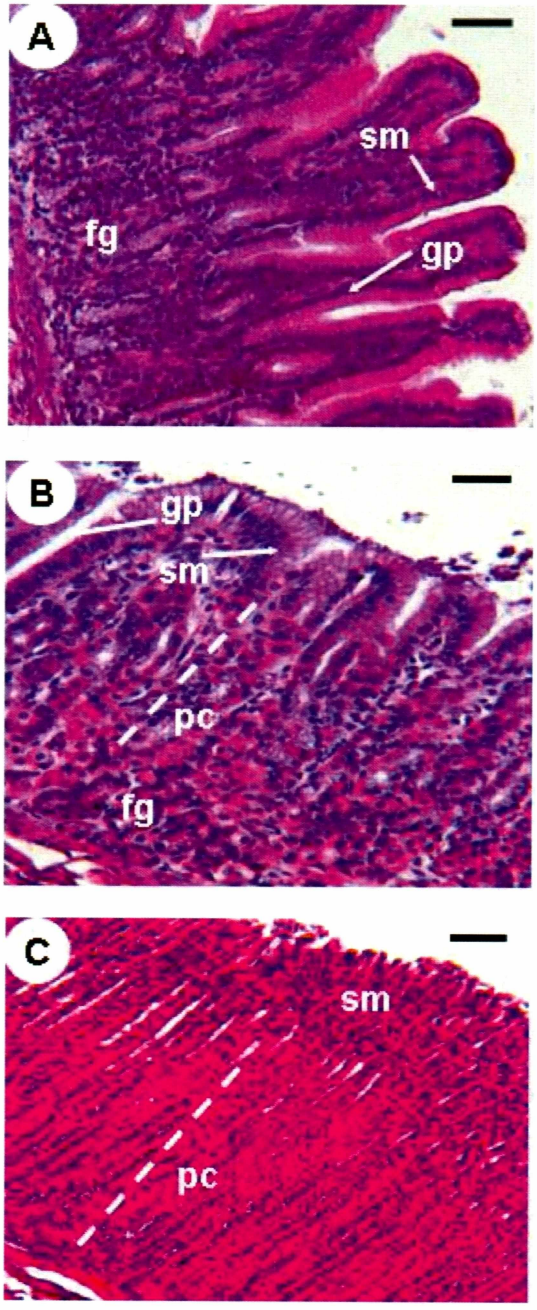

Figure 2.2. Light micrographs of the morphology of abomasum in reindeer and muskoxen during nutritional development, Fairbanks, Alaska 1999 - 2002. **(A)** Abomasum of neonatal muskoxen showing large gastric pits with fundic glands and few parietal cells. **(B)** Abomasum of 60 day old reindeer showing increased numbers of eosinophilic parietal cells. **(C)** Abomasum of adult reindeer showing the extension of the mucous surface cells and the tightly packed region of parietal cells. **Legend:** fg, fundic glands; sm, surface mucous cells; gp, gastric pits; pc, parietal cells. **Bars** = 50 μm in A, B; 100 μm in C.

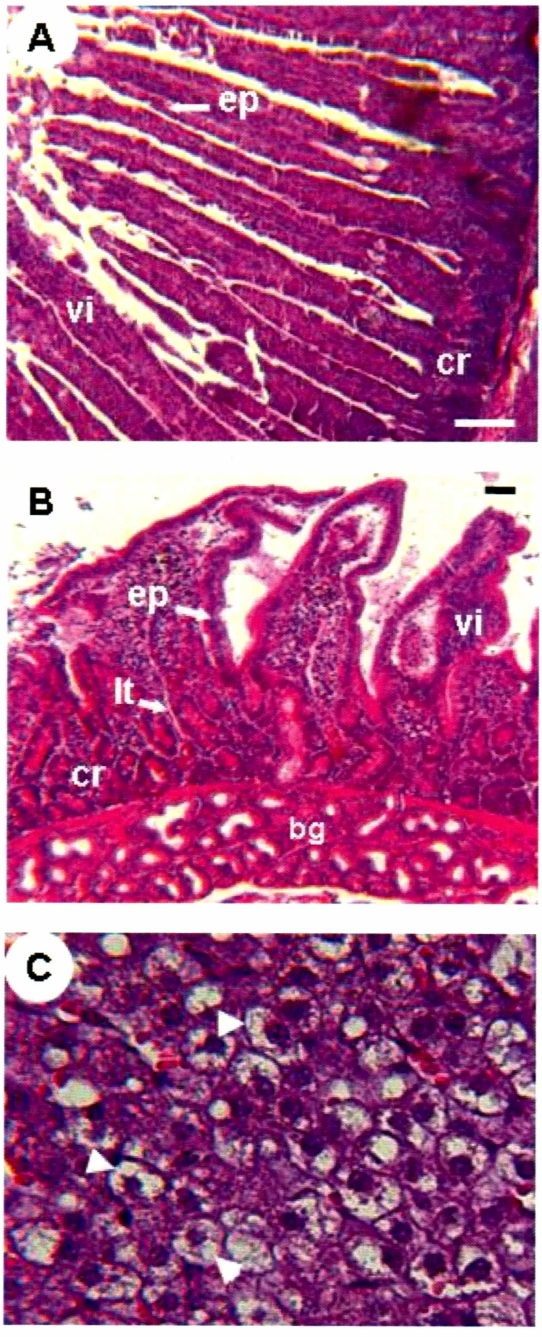

Figure 2.3. Light micrographs of the morphology of duodenum and liver in reindeer and muskoxen during nutritional development, Fairbanks, Alaska 1999 - 2002. **(A)** Duodenum of neonatal muskoxen showing large surface enlargement of the mucosa. **(B)** Large foliate villi of the duodenum of adult deer showing prominent lacteals. **(C)** Hepatocytes of neonatal muskoxen showing lipid droplets within the cytoplasm. **Legend:** vi, villi; cr, crypts; lt, lacteals; bg, Brunner's glands; arrow heads, lipid droplets. **Bar** = 100 μm in A, B; 20 μm in H.

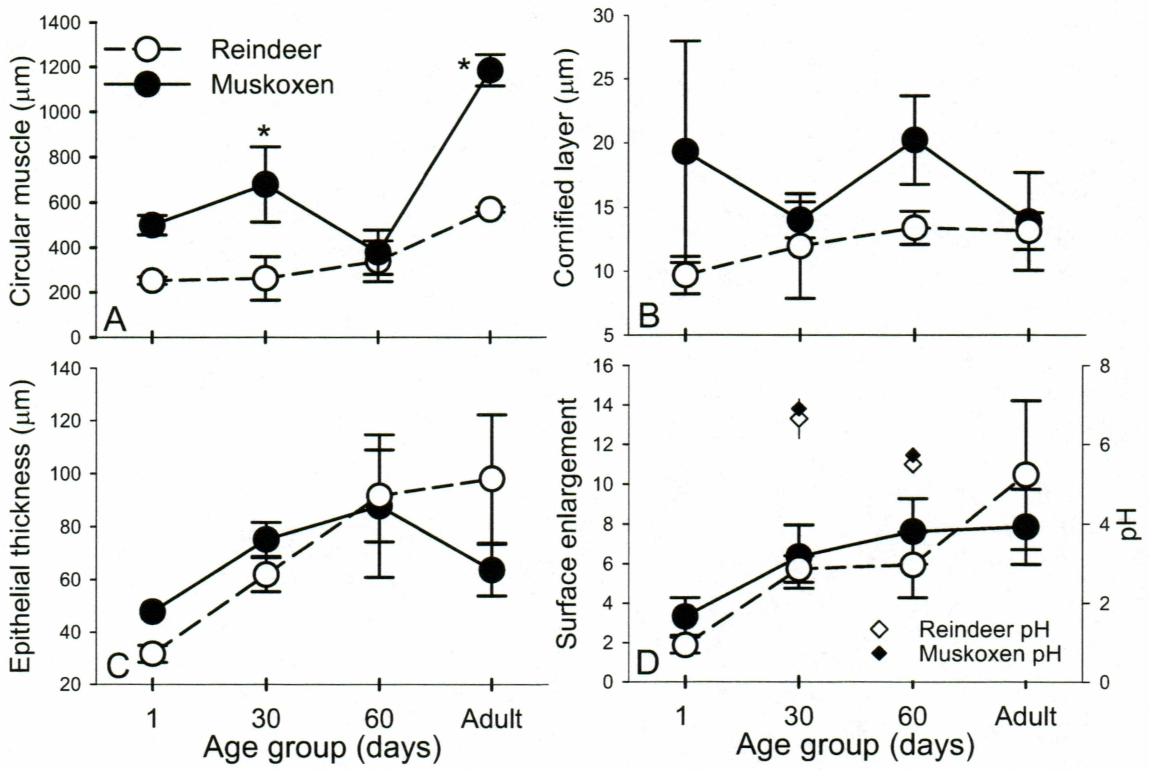

Figure 2.4. Measurements (mean µm ± SD) of ruminal morphology and pH in young and adult reindeer and muskoxen, Fairbanks, Alaska 1999 - 2002. **(A)** Circular muscle: *P* (species) < 0.001, *P* (age) < 0.001; **(B)** Cornified layer: *P* (species) = 0.002, *P* (age) = 0.205; **(C)** Epithelium: *P* (species) = 0.755, *P* (age) < 0.003; and **(D)** Surface enlargement: *P* (species) = 0.664, *P* (age) < 0.001 with pH. Asterisks indicate significant differences between species within age group, Bonferroni adjusted $P < 0.05$.

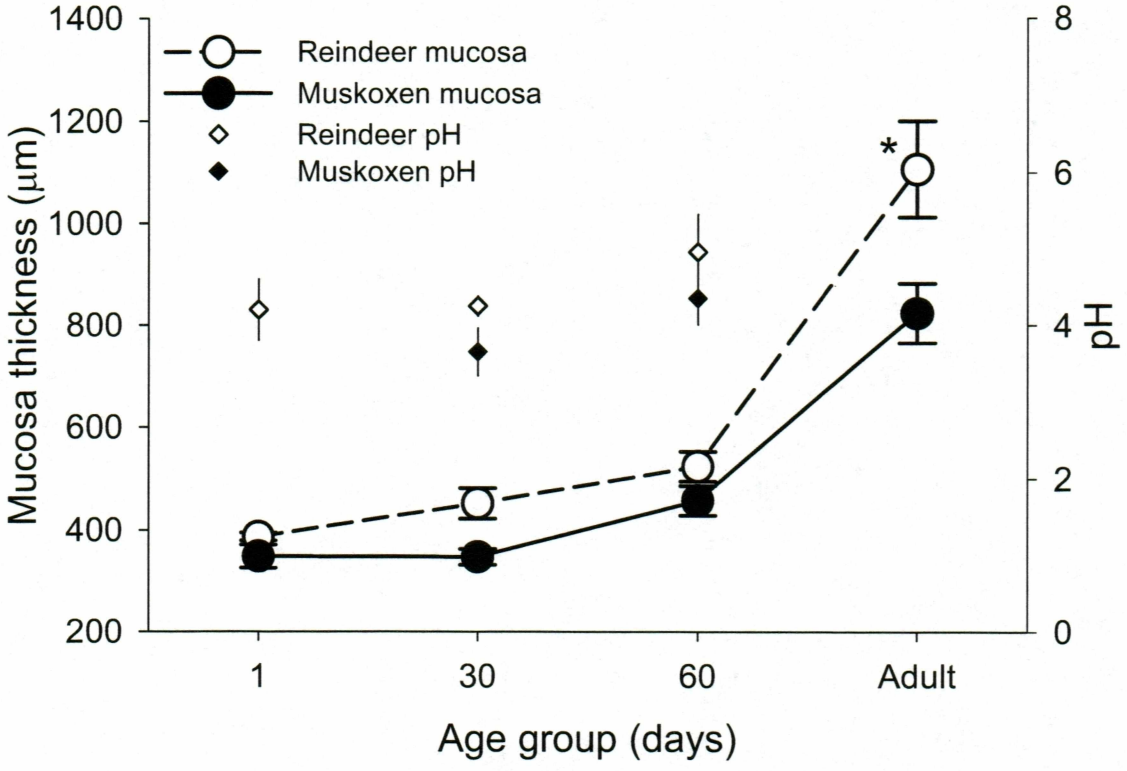

Figure 2.5. Mucosal thickness (mean µm ± SD) and ingesta pH of the abomasum in young and adult reindeer and muskoxen, Fairbanks, Alaska 1999 - 2002, P (species) < 0.001, P (age) = 0.550; covariate = abomasum mass, P = 0.009. Asterisk indicates significant differences between species within age group, Bonferroni adjusted $P < 0.05$.

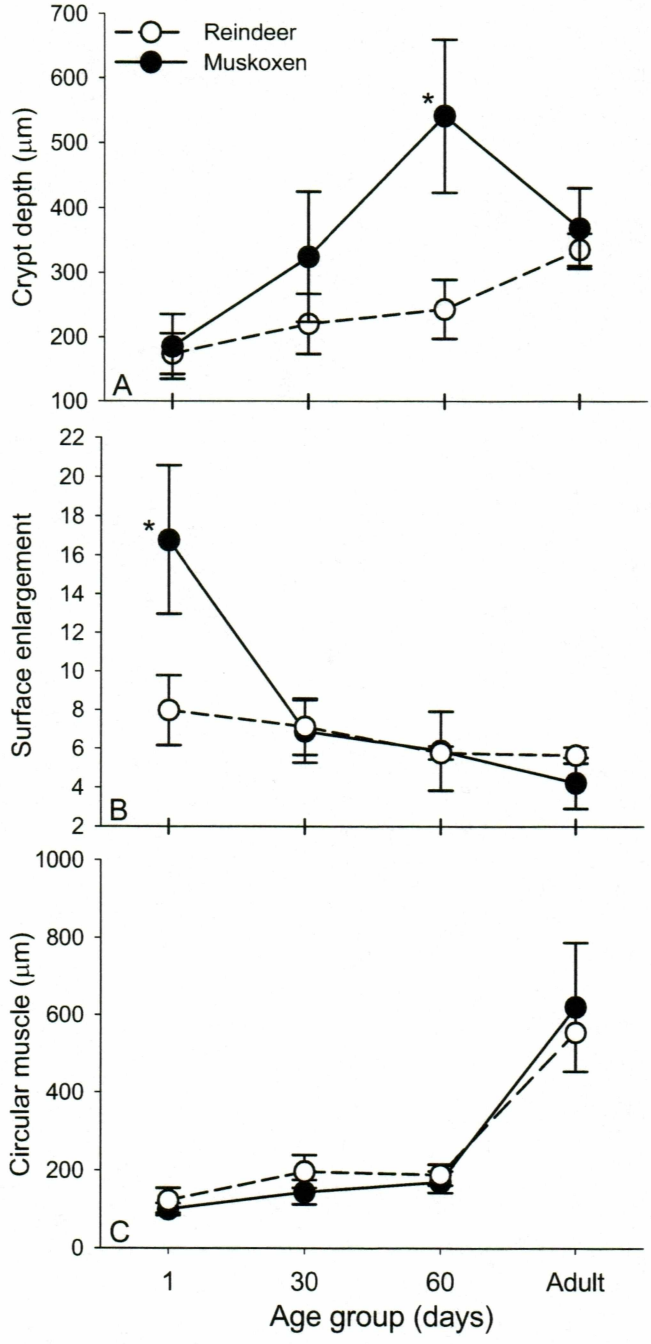

Figure 2.6. Measurements (mean µm ± SD) of duodenal morphology in young and adult reindeer and muskoxen, Fairbanks, Alaska 1999 - 2002. **(A)** Crypt depth: P (species) < 0.001, P (age) < 0.001; **(B)** Surface enlargement: P (species) = 0.014, P (age) < 0.001; and **(C)** Circular muscle: P (species) = 0.840, P (age) < 0.001. Asterisks indicate significant differences between species within age group, Bonferroni adjusted $P < 0.05$.

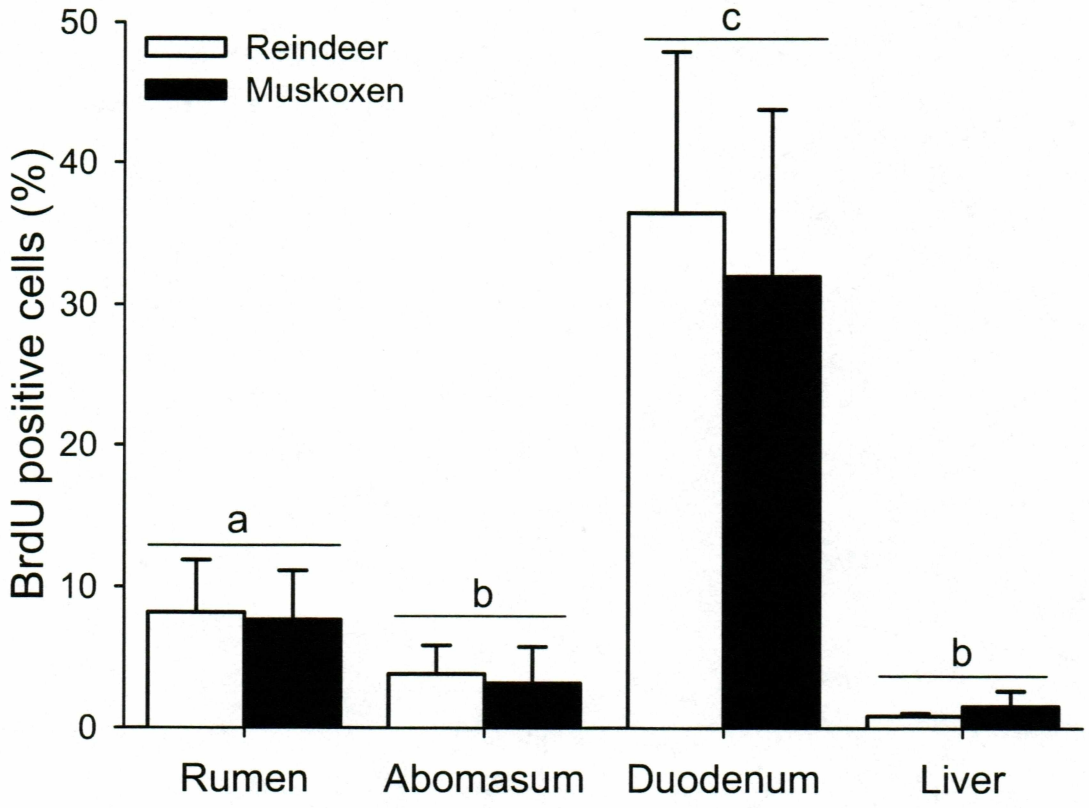

Figure 2.7. Rates of cell division (mean % BrdU positive cells) in rumen, abomasum, duodenum and liver of young reindeer and muskoxen (mean ± SD at 30 and 60 days of age), Fairbanks, Alaska 1999 - 2002. Different letters indicate significant differences between tissues (Bonferroni adjusted $P < 0.05$). Species and age groups were not significantly different ($P > 0.05$).

Appendix 2.1. Definitions of measurements made for morphometric analysis of tissue from reindeer and muskoxen. Measurements were made in microns (μm).

Abomasum

Five areas were randomly selected that contained a complete extension of the mucosa showing clear delineation of gastric pits.

mucosal thickness – distance of the gastric glands from the muscularis mucosa to the lumen surface

circular muscle thickness – distance across the inner (circular) layer of the muscularis externa

longitudinal muscle thickness -- distance across the outer (longitudinal) layer of the muscularis externa

Rumen

Three papillae per animal were selected based on full extension of the papillae height.

papillae height – distance from the base to the tip of papillae (determined as the extent of the mucosal layer within the rumen)

papillae width – distance across the papillae taken at the base of the papillae

stratified squamous epithelium (SSE) – width of the SSE taken at the base of the papillae from the submucosa within the papillae to the lumen surface not including the cornified layer

cornified layer – width of the keratin layer taken at the papillae base from the exterior of the SSE to the lumen surface

circular muscle thickness – distance across the inner (circular) layer of the muscularis externa

longitudinal muscle thickness -- distance across the outer (longitudinal) layer of the muscularis externa

papillae surface boundary – distance along the surface of 2 or more papillae that come into contact with the lumen

papillae basal boundary –distance along a reference line below the base of the papillae but above muscle bands, following contour of the cut section from the beginning to end of the measurement made for surface boundary

surface enlargement factor (SEF) -- surface boundary / basal boundary

Duodenum

Five fields were randomly chosen that included full extent of villi and inclusion of underlying layers.

mucosa height – distance of the length of villi from the muscularis mucosa to the lumen surface (includes crypts)

villi width – distance of the width of the villi taken above the crypt region

crypt height – distance of the crypt located at the base of the villi from the muscularis mucosa to the top of crypt region

gland height – distance across the width of Brunner's gland layer where present

circular muscle thickness – distance across the inner (circular) layer of the muscularis externa

longitudinal muscle thickness -- distance across the outer (longitudinal) layer of the muscularis externa

villi surface boundary – distance along the surface of 2 or more villi that comes into contact with the lumen (above the crypt layer)

villi basal boundary –distance along a reference line along the muscularis mucosa, following the contour of the cut section from the beginning to end of the measurement made for surface boundary

surface enlargement factor (SEF) -- surface boundary / basal boundary

Liver

5 fields were randomly selected per slide. Measurements were then performed on three random cells per field.

cell area – area of the whole cell circumscribed around the cytoplasm

nuclear area -- area of the nuclei

Appendix 2.2. Quantification of BrdU positive stained cells in tissue from 30 and 60 day old reindeer and muskoxen. Distance measurements were made as microns (μm).

Abomasum

Five fields were randomly selected that contained a complete extension of the mucosa showing clear delineation of gastric glands. Due to the large amount of cells within regions, the total number of cells in the region was not counted but percent BrdU was estimated as described below.

region area –area of the region measured for percent positive BrdU cells which included 2-3 gastric glands in each of the five areas measured per animal

cell area – area of 3 randomly selected cells within the mucosa in 3 random fields per animal

total cell count – region area / mean cell area, estimated total number of cells (positive and negative BrdU cells) within the region in each field in each animal

BrdU positive cell count – number of BrdU positive cells counted within the region

percent BrdU positive cells – BrdU positive cell count / total cell count * 100

BrdU distance – distance from the muscularis mucosa to the most external BrdU positive cell

BrdU band – distance of the width of the band of BrdU positive cells within the mucosa from the most external positive cell to the most luminal positive cell, identified as the zone of undifferentiated cells

Rumen

Three papillae were selected per animal based on full extension of papillae length. The papillae were divided into 4 regions (base, middle, tip and between papillae) to examine

differentiation of tissue between regions. A 10,000 μm^2 area spanning the width of the stratified squamous epithelium in each region was randomly selected using the grid function of Image-pro Express software.

BrdU positive cell count – number of BrdU positive cells counted within the region

BrdU negative cell count – number of BrdU negative cells counted within the region not including exterior epithelial cells that did not contain nuclei

Percent BrdU positive cells – BrdU positive / (BrdU positive + BrdU negative) * 100

Duodenum

Five fields were chosen randomly based on complete villus distinction and visibility of underlying layers. Two villi and 2 crypts per villi were examined for BrdU positive cells. Those crypts below each villi closest to the muscularis mucosa were chosen for evaluation. BrdU positive cell counts were performed on all features of duodenum tissue but percent BrdU positive cells were only calculated for crypts since this is the area that is considered to promote differentiation of the villi and thereby influence absorption.

Epithelium BrdU positive cell count – number of BrdU positive cells counted within the columnar epithelium surrounding the villi

Lamina propria BrdU positive cell count – number of BrdU positive cells counted within the lamina propria of the villi

Crypt BrdU positive cell count – number of BrdU positive cells counted within the crypt

Crypt BrdU negative cell count – number of BrdU negative cells counted within the crypt

Crypt percent BrdU positive cells – BrdU positive / (BrdU positive + BrdU negative) * 100

Liver

Five fields were selected randomly for each animal and pictures taken at 20x magnification, a 26,000 μm^2 area. Each field was counted for BrdU positive cell count. However, due to the low number of BrdU positive cells in each field (< 3%), data for percent positive BrdU is based on only the one field per animal with the highest count of BrdU positive cells.

BrdU positive cell count – number of BrdU positive cells counted within the field

BrdU negative cell count – number of BrdU negative cells counted within the field

Percent BrdU positive cells – BrdU positive / (BrdU positive + BrdU negative) in field with highest BrdU positive cell count per animal

CONCLUSION

Mass gains of reindeer and muskoxen indicate that maximal rates of growth are probably similar across environments for ruminants. Proportionate birth mass, however, can be variable since arctic reindeer and muskoxen have proportionately smaller young than more temperate counterparts. Reindeer give birth to proportionately larger young than muskoxen, which probably reflects the minimal body size for mobility and thermoregulation in an ungulate that is more active shortly after birth. Because reindeer and muskoxen grow at similar rates, muskoxen attain a greater body size by extending the period of growth. Muskoxen, therefore, reach reproductive maturity later than reindeer primarily due to the greater time it takes to reach their larger adult body mass.

Neonatal reindeer and muskoxen have digestive morphology that allow young ruminants to use concentrated milk supplied at birth and metabolic capacity to thermoregulate in cold and windy weather. Young ruminants gain little intramuscular fat for insulation and energy storage during the first 60 days of life. Lipid reserves established in the liver *in utero*, therefore, provide a large source of energy to aid in thermoregulation as well as provide additional nutrients that may be limiting in milk. Thermogenesis is probably initiated in young animals by high secretions of thyroid hormones that may also support growth and differentiation of tissues. Neonates are able to utilize their first ingestion of milk through well-developed abomasum and highly absorptive small intestine. These digestive organs maximize maternal transfer of nutrients and contribute to the rapid mass gains of young reindeer and muskoxen through the first 30 days of life.

Transition from milk to forage is anticipated in young reindeer and muskoxen by establishing fermentative structures that precede high ingestion of forages. Neonatal reindeer and muskoxen have proportionately large rumens that contain precursors to the production of ruminal papillae. As milk quality declines, young reindeer and muskoxen seek new sources of food that also may enhance ruminal development. By 30 days of age, the rumen of young reindeer and muskoxen exhibited full papillary structures although animals were still at peak consumption of milk. Post-ruminal advances during the transitional period included an increase in acid-secreting parietal cells in the abomasum. Absorption of nutrients is also enhanced by gains in digestive capacity and increases in surface area of mucosa during the transitional period. Further increases in tissue and body mass, therefore, correlate to the increased consumption of forage by 60 days of age. Rapid growth of young reindeer and muskoxen, therefore, is not only dependent upon nutritional input from the mother, but also on the adequate development of digestive function that must coincide with the abundance of high-quality plants.

Because postnatal development of digestive systems is rapid, reindeer and muskoxen also exhibit digestive structures specialized to their feeding strategy from birth. Grazing morphology of muskoxen was evident in the thick muscle and cornified layers of the rumen in both young and adult animals. Thick muscle walls would increase motility of fibrous forage, while cornified layers would protect the underlying absorptive epithelia from abrasion. Reindeer showed morphology that was adapted to more digestible forages, such as greater surface areas to increase absorption in the rumen and duodenum and earlier development of acidity in the abomasum that may help increase

proteolysis of plant browse. These data suggest that the feeding strategy of ruminants may not be simply a response to body size and plant availability, but determined at birth by their respective endogenous programs.

Maximal growth and nutritional development of young reindeer and muskoxen is likely determined by an endogenous program consistent among ruminants. Growth and nutritional development, however, can be hindered by the nutrition available to young ruminants to support this inherently rapid program. Young reindeer and muskoxen in the Arctic, therefore, are dependent upon the critical timing of birth and adequate nutrition from maternal and environmental sources. Sufficient nutrition would support a rapid transition to high-quality plants to meet the requirements of growth and establish energy reserves before winter.