^{15}N signatures do not reflect body condition in Arctic ground squirrels

M. Ben-David, C.J. McColl, R. Boonstra, and T.J. Karels

Abstract: Studies using stable-isotope analysis documented an enrichment in $\delta^{15}N$ values in nutritionally stressed animals. Investigators suggested that changes in $\delta^{15}N$ values measured in urine, hair, and blood may be a good indicator of lean-tissue losses. During our investigations into the effects of population density on body condition and reproduction of female Arctic ground squirrels (*Spermophilus parryii plesius*) near Kluane Lake, Yukon, Canada, we examined the relations between body condition and $\delta^{15}N$ values. Data obtained from 20 livetrapped female ground squirrels suggested that reproductive females from a population with moderate density and low food availability experienced a reduction in body condition, as indicated by mass loss and changes in blood urea nitrogen (BUN) and glucose concentrations. In contrast, those from a population that failed to reproduce successfully and had high density and low food availability experienced no nutritional stress. Similarly, those females from a high-density population with high food availability (i.e., supplemented food) that reproduced successfully suffered no noticeable nutritional stress. In contrast to our prediction, $\delta^{15}N$ values did not show a decline with increasing body mass, and animals in poor and excellent body condition had similar $\delta^{15}N$ values. In addition, female ground squirrels from the same group with access to similar types of food (natural or supplemented) and with similar body masses, BUN, and blood glucose concentrations showed a difference of up to 1.8‰ in $\delta^{15}N$ values. Thus, our results suggest that the ecological process (i.e., diet selection) may have obscured the physiological one (i.e., recycling of nitrogen). Therefore, we recommend that field ecologists studying animal diets using stable-isotope analysis use alternative techniques when attempting to evaluate the body condition of their subjects.

Résumé : Des études basées sur l'analyse des isotopes stables ont conclu à une augmentation des concentrations de $\delta^{15}N$ chez les animaux soumis à un stress alimentaire. Les chercheurs ont suggéré que les changements des concentrations de $\delta^{15}N$ mesurées dans l'urine, les poils et le sang pourraient être de bons indicateurs des pertes de tissu maigre. Au cours de nos recherches sur les effets de la densité des populations sur la condition physique et la reproduction chez les femelles du Spermophile arctique (*Spermophilus parryii plesius*) près de Kluane Lake, Yukon, Canada, nous avons examiné les relations entre la condition physique et les concentrations de $\delta^{15}N$. Les données obtenues sur 20 femelles capturées vivantes ont indiqué que les femelles reproductrices d'une population de densité moyenne exposées à des conditions de nourriture limitées avaient une condition physique moins bonne, tel qu'indiqué par la perte de masse et par les changements dans les concentrations d'azote de l'urée sanguine (BUN) et dans les concentrations de glucose sanguin. En revanche, les femelles d'une population de densité élevée exposée a une disponibilité de nourriture peu élevée et qui n'a pas réussi à se reproduire n'ont subi aucun stress alimentaire. De même, les femelles venant d'une population de densité élevée à disponibilité de nourriture élevée (suppléments alimentaires) et qui se sont reproduites avec succès n'ont pas subi de stress alimentaire appréciable. Contrairement à nos prédictions, les valeurs de $\delta^{15}N$ n'ont pas baissé en fonction d'une augmentation de masse et les animaux en mauvaise condition physique avaient les mêmes valeur de $\delta^{15}N$ que les animaux en bonne santé. En outre, les femelles d'un même groupe avec accessibilité aux mêmes types d'aliments (naturels ou en supplément), de même masse corporelle, à concentrations semblables de la BUN et de glucose sanguin, avaient des valeurs de $\delta^{15}N$ différentes et la différence pouvait atteindre 1,8‰. Nos résultats indiquent donc que le processus écologique (i.e., la sélection d'aliments) a pu masquer le processus physiologique (i.e., le recyclage de l'azote). Il est donc recommandé que les écologistes qui étudient l'alimentation des animaux en nature par analyse d'isotopes stables évaluent la condition physique de leurs sujets au moyen de plusieurs techniques.

[Traduit par la Rédaction]

Introduction

The reproductive success of many female mammals is related to body condition (Kennedy and Mitra 1963; Clutton-Brock et al. 1986; Caro 1994; Ruthven et al. 1994; Gerhart et al. 1997). These studies indicate that in many species the female must reach a critical body mass or accumulate a critical amount of fat in order to ovulate, implant, complete the

Received December 11, 1998. Accepted May 28, 1999.

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gestation period, and successfully lactate (Bronson 1989; Butler and Whelan 1994). Therefore, reproductive performance (i.e., fecundity, fertility, age at first reproduction, and survival of offspring) of females of many mammalian species is reduced as resources diminish (Berger 1986; Cheney et al. 1988; Clutton-Brock 1988; Ramsay and Stirling 1988; Sutherland 1996).

Determining body condition of free-ranging animals can be problematic because the most reliable method, direct measurement of body composition, is destructive. Therefore, the use of indirect measures of body condition, such as bioelectrical impedance analysis, dilution of deuterium-labeled water, as well as precise serum and hematological indicators have become widespread (Delgiudice et al. 1987, 1990; Messier 1987; Gustafson et al. 1998; Gau and Case 1999). These numerous indicators also include examination of hematocrit (Franzmann and LeResche 1978), blood urea nitrogen level (BUN; Kirkpatrick et al. 1975), blood glucose concentration, and serum cholesterol level (Coblentz 1975). BUN provides an index of dietary protein and of protein metabolism. Diminishing protein in the diet decreases BUN during early undernutrition (e.g., cottontail rabbit, Warren and Kirkpatrick 1978; wolf, Delgiudice et al. 1987). However, as nutritional stress increases, the animal must increasingly rely on catabolism of its own protein stores, thus increasing BUN (e.g., white-tailed deer, Delgiudice et al. 1990, 1994). Hematocrit, measured as the packed red blood cell volume (PCV), can be used as an index of body condition, in which higher values have been linked to better condition. Nonetheless, extreme hematocrit levels are suggestive of dehydration (Franzmann and LeResche 1978; Lochmiller et al. 1986; Hellgren et al. 1993; Boonstra et al. 1998). Blood glucose levels respond dramatically after prolonged stress (Boonstra et al. 1998), but the effects of nutritional stress on glucose levels are equivocal (e.g., Seal and Hoskinson 1978; Delguidice et al. 1987).

Recently, studies using stable-isotope analysis indicated an increase in $\delta^{15}N$ values in nutritionally stressed as well as water-stressed animals (Ambrose and DeNiro 1986, 1987; Hobson and Clark 1992; Hobson et al. 1993). Hobson et al. (1993) demonstrated enrichment of $\delta^{15}N$ values in nutritionally stressed captive juvenile Japanese quails (*Coturnix japonica*) compared with quails fed ad libitum. Similarly, enrichment of $\delta^{15}N$ values occurred in incubating adult Ross' geese (*Chen rossii*). Three models were suggested to account for the observed increase in $\delta^{15}N$ values. Ambrose and DeNiro (1987) noted an enrichment in $\delta^{15}N$ values in drought-tolerant herbivores compared with water-dependent species in East Africa and postulated that it was due to the high concentration of ^{15}N-depleted urea excreted by the drought-tolerant species. Hobson et al. (1993) suggested that under conditions of fasting and nutritional stress, a greater proportion of nitrogenous compounds are derived from catabolism. Because this source is already enriched in $\delta^{15}N$ values relative to diet, additional enrichment in the new tissues will occur. Also, $\delta^{15}N$ enrichment can potentially result from changes in the amino acid composition of tissues under nutritional stress. Because amino acids differ in their ^{15}N signatures, such changes in composition may lead to enrichment of the tissue (Hobson et al. 1993). Based on these observations, Gannes et al. (1997) suggested that changes in values of $\delta^{15}N$ measured in urine, hair, and blood could be a good indicator of lean-tissue losses and provide a measure of body condition in wild animals.

A 10-year study (1986–1996) of the ecosystem dynamics of the boreal forest near Kluane Lake, Yukon, Canada, involved in part an examination of the role of food and predation on population regulation of several small-mammal species. At the end of this study we were able to demonstrate major differences in densities of Arctic ground squirrels (*Spermophilus parryii plesius*) in groups exposed to different regimes of food supplementation and predation pressure (Karels 1996; Byrom 1997). In 1996, we stopped the food supplementation and observed the responses of the different groups as they declined to control density levels. This provided an ideal situation in which to assess the relations between body condition and $\delta^{15}N$ values in animals from a variety of population densities and nutrition situations. In this study, we examined the relations between parameters of nutritional stress such as BUN, glucose concentration, and $\delta^{15}N$ value in an attempt to validate the use of the $\delta^{15}N$ value as an indicator of body condition. We predicted that with increasing body condition (or, alternatively, decreasing nutritional stress), the $\delta^{15}N$ value would decrease concurrently with an increase in glucose concentrations and hematocrit. In contrast, a mutual decrease in both BUN and $\delta^{15}N$ value will occur initially, followed by an increase in BUN and a continued decrease in $\delta^{15}N$ value.

Methods

Study area

The study was carried out at Kluane Lake, Yukon Territory (60°57′N, 138°12′W), in a 350-km^2 portion of the Skakwak Trench, a broad glacial valley bounded by alpine area to the northwest and southwest. The valley bottom averages about 900 m above sea level and is mostly covered with white spruce (*Picea glauca*) forest (62%) with an understory of willow (*Salix* sp.) and birch (*Betula glandulosa*). Other vegetation types include aspen forest (*Populus tremuloides*; 3%), shrub thicket (26%), and meadows (9%). The climate is cold continental, with the growing season extending from mid-May through mid-August and snow cover present from October through early May. Precipitation is low, with most falling as snow. Snow depths average about 55 cm by late winter (Krebs et al. 1986).

Experimental animals

The animals came from areas that had been part of large-scale manipulations (1 km^2) in the Kluane Boreal Forest Ecosystem Project (for details of the experimental treatments see Krebs et al. 1995). The experiments lasted from 1986 to 1995 and treatments ceased in early 1996, prior to ground squirrel emergence in spring. We obtained data on animals trapped in spring (early May) and summer (end of June to end of July) in 1996, using the following 3 treatments: (1) Moderate density – low food: these females (n = 6) came from the former food 2 grid (the second replicate of the food-addition treatment; commercial rabbit chow had been distributed every 5–6 days). Food supplementation resulted in a density of 6.0 squirrels/ha by summer 1996, approximately double control densities (range 2.4–3.0 squirrels/ha; n = 4 control grids; T.J. Karels, unpublished data). Though they received no supplemental food in 1996, 79% (n = 34) of the females were lactating in spring, and 55.6% (n = 9) of females followed closely by radiotelemetry successfully weaned offspring (T.J. Karels, unpublished data). (2) High density – low food: these females (n = 7) came from the for-

mer predator exclosure + food grid (food was added as above and mammalian predators were excluded by a large, electrified fence). This treatment resulted in densities of 21.4 squirrels/ha by summer 1996, approximately 8 times control densities (T.J. Karels, unpublished data). This group received no supplemental food in 1996, consequently only 57% (n = 76) of all females were lactating in spring and none successfully weaned offspring (n = 12; T.J. Karels, unpublished data). (3) High density – high food: these females (n = 7) also came from the former predator exclosure + food grid. For this group, however, supplemental feeding was continued in 1996 in the same pattern as in 1986–1995, but on an area of only 2.5 ha nested within the larger treatment area. This treatment resulted in densities of 26.4 squirrels/ha by summer 1996. All females appeared to have bred. Forty out of 42 females (95%) were lactating in spring, and 88.9% (n = 9) successfully weaned offspring (T.J. Karels, unpublished data).

Blood samples

Blood samples were obtained from females livetrapped between 26 June and 26 July 1996, after successful females had reared and weaned their young. Animals were captured in Tomahawk live traps baited with peanut butter and set adjacent to burrows (for details of trapping methods and grid set-up see Hubbs and Boonstra 1997). Traps were set between 08:00 and 09:30 and were checked hourly. All animals were sexed, aged, and weighed immediately upon release from the trap into a mesh trapping bag. Animals were anesthetized using a bell-jar technique with the inhalant anesthetic Metofane (methoxyflurane) prior to the collection of a single 500-L blood sample from the suborbital sinus. Blood was collected in heparinized 75 microhematocrit tubes (Red-Tips, Fisher Scientific Co.) and allowed to flow into 0.5-mL Eppendorf tubes.

Analysis of blood parameters

Glucose concentration, hematocrit, and BUN were measured for each animal. Glucose concentration (mg/dL) was measured by glucose oxidase–peroxidase reaction using an Accu-chek III analyzer (Mannheim-Boehringer, Mannheim, Germany). The accuracy of the device was compared using standard control solutions and values were within 20% for the low control solution (51 mg/dL) and 10% for the high control solution (292 mg/dL); these are within the admissible range. Hematocrit was measured as PCV in 75-L heparinized microhematocrit tubes. Hematocrit samples were centrifuged in a IEC microhematocrit centrifuge for 5 min at 6000 rpm. The remaining blood was centrifuged at 8800 × g for 8 min in an Eppendorf microcentrifuge. The separated plasma was frozen and stored at –20°C until it was transported to Toronto, where it was stored at –70°C until analysis. BUN was measured commercially by VITA-TECH (Veterinary Laboratory Services, Toronto, Ont.) on an automated Hitachi 717 analyzer using reagent kits.

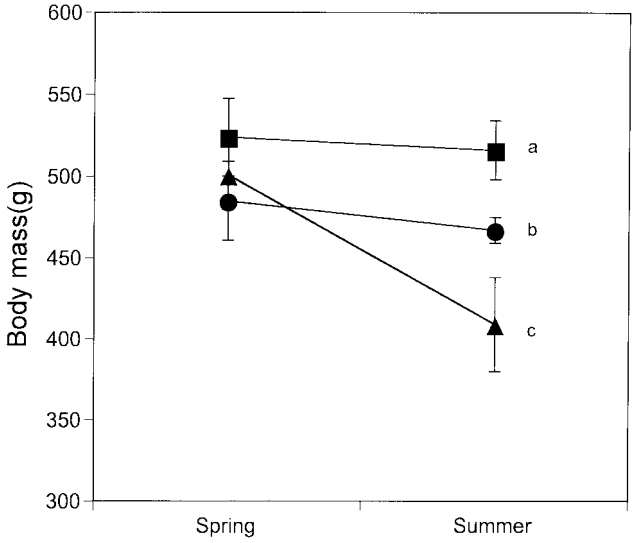
Analysis of stable-isotope ratios

Clotted blood cells were kept frozen until they were prepared for determination of stable isotope ratios. Samples were dried at 60–70°C for 48 h and then ground to a fine powder using a Wig-L-Bug grinder (Cresent Dental Co., Chicago, Ill.). Subsequently, a subsample (1–1.5 mg) was weighed into a miniature tin cup (4 × 6 mm) for combustion. We used a Europa C/N continuous-flow isotope-ratio mass spectrometer to obtain the stable-isotope ratios. Each sample was analyzed in duplicate and 3 peptone standards were analyzed with every 6 samples. Results were accepted only if the variance between the duplicates did not exceed that of the peptone standard ($\delta^{13}C_{std}$ = –15.8, $\delta^{15}N_{std}$ = 7.0; coefficient of variation = 0.1).

Statistical analysis

To determine the dynamics of body mass of females from the 3 treatments, we calculated percent mass change between spring and summer for each female. To test for differences in body mass between the 3 groups in spring (May) and summer (July), as well as the proportional change in mass, we used a Kruskal–Wallis test with multiple comparisons (Zar 1984; SPSS for Windows). We then employed the K nearest neighbor randomization test (Rosing et al. 1998) to investigate whether stable-isotope ratios for the 3 groups were significantly different from each other. In this test, the $\delta^{13}C$ and $\delta^{15}N$ values are considered simultaneously (Rosing et al. 1998). To determine if animals were nutritionally stressed, we plotted blood parameters against summer body mass and used nonlinear regression curve estimation to describe the relations between these variables. Similarly, we plotted $\delta^{15}N$ values against body mass and used nonlinear regression curve estimation to describe the relations between these variables (Zar 1984; SPSS for Windows). To test for a relation between $\delta^{15}N$ value and hematocrit and glucose concentration we used Spearman's rank-correlation analysis (Zar 1984; SPSS for Windows).

Fig. 1. Spring and summer body masses (g; mean ± SE) of female ground squirrels livetrapped in 3 experimental plots (high density – high food availability (■; n = 7); high density – low food availability (●; n = 7); moderate density – low food availability (▲; n = 6)) near Kluane Lake, Yukon, Canada, in 1996. Different letters represent significant differences among means at α = 0.05 (Kruskal–Wallis test with multiple comparisons).

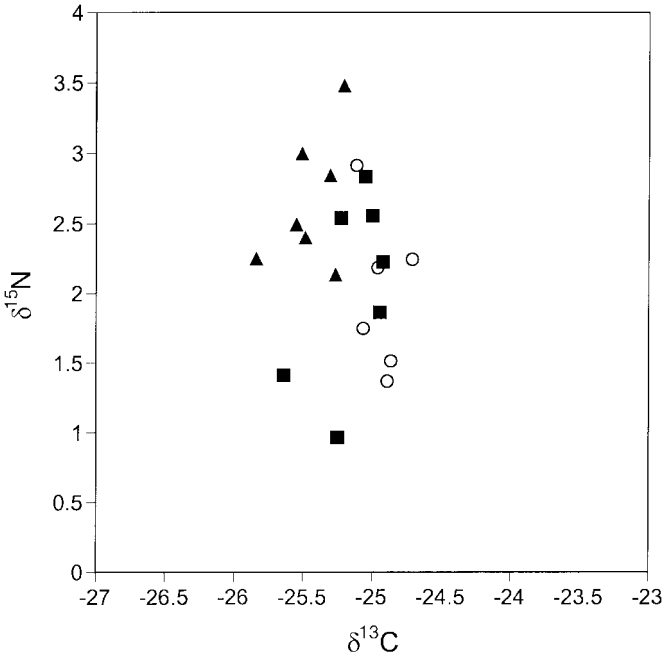


Results

Spring body masses of female ground squirrels from the 3 groups were similar (Fig. 1; Kruskal–Wallis test, P = 0.49). By summer, females from the moderate density – low food group (committed to reproduction and lactation) had lost 18.6 ± 3.6% (mean ± SE) of their spring body mass. Concurrently, females from the other two groups, which either deferred reproduction or reproduced under favorable conditions, lost 1.6 ± 2.2% of their spring mass (Fig. 1; Kruskal–Wallis test, P = 0.009). This resulted in a significantly lower body mass for females in the former group and a significantly higher body mass for females in the high density – high food group in summer (Fig. 1; Kruskal–Wallis test, P = 0.007). The stable-isotope ratios in summer did not differ significantly among the 3 groups (Fig. 2; K nearest neighbor randomization test, P = 0.96).

Summer hematocrit showed no relation to body mass, suggesting that there was no relation between body mass

Fig. 2. $\delta^{13}C$ and $\delta^{15}N$ levels (‰) for female ground squirrels livetrapped in 3 experimental plots (high density – high food availability (■); high density – low food availability (▲); moderate density – low food availability (○)) in Kluane National Park, Yukon, in July 1996.



(i.e., group association) and condition based on this parameter (Fig. 3*a*; $R^2 = -0.07$, $P = 0.98$). Similarly, hematocrit did not show extreme values (>50), suggesting that none of the experimental animals was dehydrated (Fig. 3*a*). Glucose concentration increased with body mass (Fig. 3*b*; exponential fit $R^2 = 0.49$, $P < 0.001$). The best fit for BUN was a second-degree polynomial curve (BUN = 95.9 – 0.36 × body mass + 0.0004 × (body mass)^2), similar to the expected curve for the different phases of nutritional stress (Fig. 3*c*; $R^2 = 0.70$, $P < 0.001$). No significant relation was found between $\delta^{15}N$ value and hematocrit (Spearman's rank correlation, $r = 0.24$, $P = 0.31$) or between $\delta^{15}N$ value and glucose concentration (Spearman's rank correlation, $r = -0.05$, $P = 0.85$). Although BUN followed the predicted pattern (initially decreasing with increasing body mass and later increasing), there was no significant relation between $\delta^{15}N$ value and body mass (Fig. 3*d*; $R^2 = 0.08$, $P = 0.24$).

Discussion

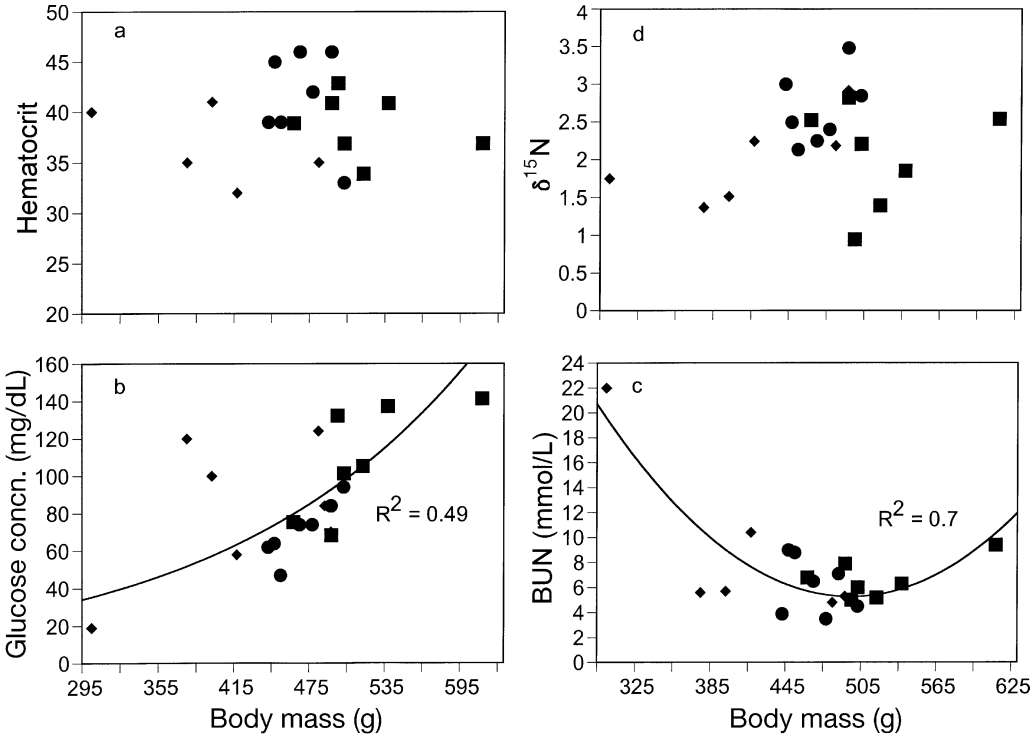
The commitment of a female to breed and the number of offspring she produces involve a trade-off between current and future reproductive effort in order to maximize overall reproductive fitness (Roff 1992; Stearns 1992). The effort needed to sustain a successful reproductive event may compromise future survival and fitness (e.g., Clutton-Brock et al. 1982). In our study, more females under the low food but moderate density conditions made a commitment to breed (79%) than those under the low food but high density conditions (56%). Although several factors may affect preweaning survival (e.g., infanticide, predation, unfavorable weather conditions), the ability of a female to successfully wean her young may largely depend on her body condition (Kennedy and Mitra 1963; Clutton-Brock et al. 1986; Caro 1994; Ruthven et al. 1994; Gerhart et al. 1997). By early summer, females from the moderate density – low food availability treatment lost 18.6% of their mass compared with a 1.6% loss, on average, by the other groups. We do not know how this breeding attempt affected subsequent survival or future reproduction of these females. Nor do we know the cues that governed the reproductive decisions of the females, though we suspect that less natural food was available in the high-density treatment than in the moderate-density one. Nonetheless, density alone may not have been the only variable, as those females in the high density – high food treatment bred (95%). Thus, the decision to breed and the ability to sustain a reproductive event must be related to food availability (see also Ricklefs 1990).

In combination, mass loss, summer body mass, glucose concentration, and BUN suggest that ground squirrels in the moderate density – low food treatment were experiencing a lower body condition than animals in the other two groups. Despite the good fit of the curve for BUN, it is clearly driven by the extreme points in the data set (Fig. 3*c*). Observing animals in the wild that are experiencing severe nutritional stress may be difficult, as the likelihood of survival for these animals is low. Therefore, our ability to detect actual nutritional stress in a single animal is not surprising. From our data, however, we can conclude that high population density, low food availability, and investment in lactation may leave animals in poor body condition.

That $\delta^{15}N$ values did not show a decline with increasing body mass, and animals that differed in body condition had similar isotope values, indicate that $\delta^{15}N$ value may not be a reliable indicator of body condition. In addition, female ground squirrels from the same group with access to similar types of food (natural or supplemented) and with similar body masses, BUN, and glucose concentrations showed a difference in $\delta^{15}N$ values of up to 1.8‰ (Figs. 2 and 3). Such a difference was considered a change in trophic position by several researches using stable-isotope analysis (DeNiro and Epstein 1981; Kline et al. 1993). Also, the fact that the $\delta^{15}N$ value for the animal which exhibited nutritional stress was not significantly higher than those for the other group members supports our conclusion. We suspect that the observed difference in $\delta^{15}N$ values between animals resulted from a difference in diet selection rather than from nutritional stress. Determining the diet of each ground squirrel from the stable-isotope ratios in our study, however, would have been impossible. The range of isotope values for the different plants and the overlap in signatures between species and plant parts in the region are too large for any of the existing isotope mixing models to be used (Ben-David et al. 1997*a*, 1997*b*; unpublished data).

The hematocrit for our study animals showed no relation to $\delta^{15}N$ values, suggesting that the large differences in isotope signatures within groups were not the result of water stress. Unfortunately, our data do not allow critical evaluation of the other two mechanisms proposed by Hobson et al. (1993) because we were unable to register an increase in $\delta^{15}N$ values. Our inability to detect such an increase may also be due to the choice of study animal. Hobson and Clark (1992), as well as Hobson et al. (1993), documented increase in $\delta^{15}N$ values with nutritional stress in several avian species in both field and laboratory studies. Mammals, espe-

Fig. 3. Hematocrit (% total volume) (*a*), glucose concentrations (*b*), BUN (*c*), and $\delta^{15}N$ values (‰) (*d*) plotted against body mass (g) for female ground squirrels livetrapped in 3 experimental plots (high density – high food availability (■); high density – low food availability (●); moderate density – low food availability (◆)) in Kluane National Park, Yukon, in July 1996. Nonlinear curve fitting was significant for glucose concentrations and BUN at $\alpha = 0.001$, but no significant relation was observed for hematocrit or $\delta^{15}N$ values ($P > 0.05$).



cially those species that go through natural cycles of starvation (i.e., marine or hibernating mammals), may possess biochemical pathways different from those of birds, in which no additional fractionation of nitrogen occurs. Several studies have demonstrated the ability of hibernating mammals, such as bears and ground squirrels, to recycle urea (Bintz and Torgerson 1981; Barboza et al. 1997). The connection between urea recycling and resulting $\delta^{15}N$ values merits further investigation under controlled conditions.

Gannes et al. (1997) recently emphasized that patterns of stable-isotope ratios are the result of an interaction between ecological, physiological, and biochemical processes, therefore a difference in ratios between two individuals does not necessarily indicate differences in diet, trophic level, or body condition. Our results support this conclusion and suggest that in this case the ecological process (i.e., diet selection) may have obscured the physiological one (i.e., recycling of endogenous nitrogen). In addition to joining Gannes et al. (1997) in their call for more laboratory experiments, we recommend that field ecologists studying animal diets using stable-isotope analysis use alternative techniques when attempting to evaluate the body condition of their subjects.

Acknowledgements

We thank H. Jewell and J. Hoyle for help in obtaining data in the field. N. Haubenstock and B. Barnette performed the stable isotope ratio analysis. We thank Dr. Alfred Choo of Vita-Tech for his help with the BUN analyses. We thank M.A. Castellini, W.J. King, D.M. Schell, and two anonymous reviewers for helpful comments on earlier versions of the manuscript. All procedures were critically reviewed and approved by an independent animal use and care committee at the University of Toronto in accordance with the "Guiding principles in the care and use of animals." Funding for the project was provided by Natural Sciences and Engineering Research Council of Canada as a Collaborative Special Project grant and by an individual operating grant to R.B. Additional funding was provided by the Water Research Center, University of Alaska Fairbanks. While preparing the manuscript, M.B.D. was supported by a grant from the USDA Forest Service, Pacific Northwest Research Station, Juneau, Alaska. This is Contribution No. 119 of the Kluane Boreal Forest Ecosystem Project.

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