The effect of metabolic rate on stable carbon and nitrogen isotope compositions in deer mice, *Peromyscus maniculatus*

J.J. Smith, J.S. Millar, F.J. Longstaffe, and R. Boonstra

Abstract: The stable isotopic compositions of nitrogen and carbon in animal tissues reflect diet. However, factors other than diet can also affect these stable isotopic ratios, leading to misinterpretations of diet composition. To test the hypothesis that variation in metabolic rate alters the isotopic compositions of tissues, deer mice (*Peromyscus maniculatus* (Wagner, 1845)) were kept at three temperatures (thermoneutral (23 °C), cool (5 °C), and cold (–10 °C)) and fed ad libitum. The changes in carbon and nitrogen isotope compositions of liver associated with the thermoneutral versus cool and cold conditions were very small in comparison with those arising from differences in diet. We conclude that temperature-induced variations in metabolic rate are insufficient to produce differences in the stable carbon or nitrogen isotope compositions that could be mistaken for changes in diet.

Résumé : Les compositions d’isotopes stables d’azote et de carbone dans les tissus animaux reflètent le régime alimentaire. Cependant, des facteurs autres que le régime peuvent affecter ces rapports d’isotopes stables, ce qui entraîne des interprétations erronées de la composition du régime alimentaire. Afin de tester l’hypothèse qui veut que la variation du taux métabolique affecte la composition isotopique des tissus, nous avons gardé des souris du crépuscule (*Peromyscus maniculatus* (Wagner, 1845)) à trois températures différentes, soit des régimes thermiques neutre (23 °C), frais (5 °C) et froid (–10 °C), et les avons nourries à volonté. Les modifications dans les compositions d’isotopes de carbone et d’azote dans le foie associées au régime thermique neutre par rapport à ceux des régimes frais et froids sont très petites par comparaison à celles qui proviennent de différences de régime alimentaire. Nous en concluons que les variations du taux métabolique causées par la température ne sont pas assez importantes pour produire des différences de composition des isotopes stables de carbone et d’azote qui pourraient être interprétées par erreur comme des changements de régime alimentaire.

[Traduit par la Rédaction]

Introduction

Measurement of stable carbon (13C/12C) and nitrogen (15N/14N) isotope ratios is commonly undertaken in ecological studies because the compositions of tissues reflect the composition of the diet (Kelly 2000). However, factors other than diet, such as nutritional stress (Hobson et al. 1993; Kelly 2000; but see Ben-David et al. 1999; Kempster et al. 2007), water stress (Ambrose and DeNiro 1986; Cormie and Schwarcz 1996; Schwarcz et al. 1999; Kelly 2000), and differential assimilation by tissues (Caut et al. 2008) may modify the tissue isotopic composition, potentially leading to misinterpretations about diet composition.

The effect of metabolic rate on stable isotope compositions is also unclear. MacAvoy et al. (2006) showed tissue isotopic turnover rates to be correlated with metabolic rate, and Yi and Yang (2006) suggested that the observed increase in δ15N values of plateau pika (*Ochotona curzoniae* (Hodgson, 1858)) muscle with increased altitude resulted from differences in metabolic rate. In contrast, Carleton and Martínez del Río (2005) found metabolic rate to have negligible effects on isotopic fractionation between diet and tissue.

We tested the hypothesis that higher metabolic rates caused by living at lower ambient temperatures alter the stable isotope compositions of nitrogen and carbon of liver tissue in deer mice (*Peromyscus maniculatus* (Wagner, 1845)). Liver tissue was sampled because it turns over more quickly than blood, muscle, or hair in deer mice (Miller et al. 2008). If these isotopic compositions are affected by an increase in metabolic rate at low ambient temperatures, we predicted that liver tissue should be enriched in 15N as a result of an increase in the preferential excretion of 14N. Because the demand for nitrogen increases with an increase in metabolic rate, the rate of protein turnover (MacAvoy et al. 2006) and the preferential excretion of 14N in urine may also increase.


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Furthermore, changes in carbon isotope composition could arise from variation in lipid content (Sotiropoulos et al. 2004), but deer mice typically do not exhibit seasonal fluctuations in lipid content (Millar and Schieck 1986). Thus, we predicted that there should be no difference in δ13C values of the liver tissue with increased metabolic rate.

We also compared the stable isotope results to measures of physiological condition (hematocrit — an index of condition; blood glucose concentration — an index of energy mobilization; concentration of plasma corticosterone — a measure of nutritional status and stress). Many processes are affected by physiological stress, including growth, reproduction, digestion, and immunity (Sapolsky et al. 2000), and these factors may also influence metabolic rate and the stable isotope compositions of tissues. Physiological stress including short-term exposure to cold is known to increase corticosteroid and glucose levels (e.g., Moshkin et al. 2002; Wingfield and Hunt 2002), and reductions in hematocrit are an indication of poor condition (e.g., Helgren et al. 1993; Boonstra et al. 1998). However, no difference was expected in the physiological stress response arising from increased metabolism caused by decreased temperature. This response is known to be desensitized in rodents after repeated exposure to certain stressors, including cold temperatures (Boonstra 2004).

Materials and methods

Study area and animals

Livetrapping was conducted in the Kananaskis Valley (51°2’N, 115°3’W) in Alberta, Canada, from May to June 2007. We captured 32 adult male deer mice using Longworth traps insulated with cotton nesting material and baits with sunflower seeds and oats. Only males were collected to avoid sex differences in the physiological stress response. Traps were set at 1800 and checked the following morning at 0630.

Animals were housed in 29 cm × 18.5 cm × 13 cm plastic cages with aspen woodchips and a plastic tube for shelter at the Biogeoscience Institute of the University of Calgary at Kananaskis, Alberta. We did not provide bedding during treatments to prevent the mice from making insulated nests. The daily photoperiod was set at 16 h light : 8 h dark. Animals were acclimated to laboratory conditions at 23 °C for at least 2 weeks. A transition diet of sunflower seeds and oats was provided for the first 2 days, along with standard rodent chow (LabDiet® 5001 Rodent Diet). Only the latter was provided throughout the remainder of the experiment. Animal care and treatment protocols were approved by the University Council on Animal Care (The University of Western Ontario, London, Ontario).

Temperature treatments

After acclimation, we randomly assigned animals to one of three temperature treatment groups to test the effect of metabolic rate on tissue isotopic compositions. Ten animals were housed at 23 °C, 11 at 5 °C, and 11 at –10 °C; these temperatures are all within maximum sustainable metabolism (Koteja 1996). The latter group was acclimated from 5 °C by reducing the temperature by 3 °C daily until the desired temperature of –10 °C was reached. Two individuals in the –10 °C treatment died during the treatment period; one died on day 1 and one died on day 4.

We provided commercial rodent chow to all animals ad libitum, and food consumption was monitored every 3 days. Mean daily food intake was calculated as a proportion of body mass (mean daily mass-specific food intake) for each animal at the end of the study. Daily mass-specific food intake was used as a surrogate measure of metabolic rate, assuming that diet digestibility was not affected by temperature treatment. All animals were provided with a constant supply of tap water. For animals held at subzero temperatures, we provided water in bowls twice daily, before and after the dark period. Animals in the other treatments were also disturbed at these times. All mice were held at the treatment temperatures for 28 days, which allowed sufficient time for liver tissue to turn over completely (Miller et al. 2008).

Collection of blood samples

After the treatment period, we obtained blood samples to test for glucose concentrations, hematocrit (packed red cell volume), and corticosterone concentrations. All blood samples were obtained between 1100 and 1400 to avoid time-of-day effects. Because corticosterone can increase within 3–5 min of handling (Brown and Martin 1974), animals were euthanized by carbon dioxide asphyxiation within 3 min of handling, and blood samples were obtained immediately by cardiac puncture using a heparinized syringe. We measured blood glucose using a glucose dehydrogenase reaction (Therasense Freestyle glucose monitor; Abbott Laboratories, Abbott Park, Illinois, USA). Blood samples were centrifuged in two 75 μL microhematocrit tubes at 8000 rev/min (5900g) for 5 min (Readacrit Centrifuge; Clay Adams, Division of Becton Dickinson, Parsippany, New Jersey, USA), and hematocrit was measured as a mean of the two readings. The separated plasma was stored at –80 °C and transported on ice to the University of Toronto for corticosterone radioimmunoassay.

Corticosterone radioimmunoassay

The amount of unbound (free) corticosterone was measured at the Centre for the Neurobiology of Stress at the University of Toronto at Scarborough. Critical to understanding the potential impact of changing corticosterone levels is knowing how much is actually free and not bound to its main carrier protein, corticosteroid-binding globulin (CBG), as it is primarily the free corticosterone which appears to be biologically active (Rosner 1990). Thus, it is critical to determine how much plasma corticosterone is free as opposed to the total present (R. Boonstra, unpublished data). However, some evidence suggests that CBG does not simply appear to act as a buffering mechanism for corticosterone in plasma but also acts as a carrier protein for corticosterone, interacts with cell surface receptors, and may be internalized in the cell (Rosner 1990).

To calculate the concentrations of free corticosterone, we used the calculation procedures outlined in Tait and Burstein (1964), and for these calculations, we need to know three values: the albumin concentration in plasma (albumin also binds corticosterone and has high capacity but low affinity), the ratio of albumin-bound to free corticosterone, and the af-
finity constant of CBG for corticosterone. Pure albumin was obtained through the trichloroacetic acid method described in Michael (1962). This albumin was then used as a standard to calculate the concentration of albumin in plasma by the chromographic method of Debroy et al. (1957). We calculated that deer mice have 5.11 g albumin per 100 mL plasma. The ratio of albumin-bound to free corticosterone in a 1% solution was 0.54. The CBG affinity constant for deer mice was measured in a microdialysis system (Englund et al. 1969) modified to 12 chambers (A.J. Bradley, personal communication) using 60 μL samples of plasma diluted 1:5 with a phosphosaline buffer (0.05 mol/L, pH 7.4). In this system, equilibrium was established in 12 h at 37 °C following which the specific activity of dialysate and sac contents was measured in a scintillation counter. The concentration of CBG bound and unbound corticosterone was calculated by the method described in Paterson and Hills (1967), and the CBG affinity constant calculated by the Scatchard analysis (Scatchard 1949). We calculated the CBG-binding constant of the deer mice to be 8.9 × 10^7 L/mol.

### Stable isotope analyses

Livers were removed from each animal upon euthanization and stored at −20 °C. All samples were transported on ice to the Laboratory for Stable Isotope Science at The University of Western Ontario for stable isotope analysis. Liver samples were lyophilized for 24 h and ground to a fine powder using a mortar and pestle. We extracted the lipids from half of each sample using three sequential soakings in a 2:1 chloroform to methanol solution (Hobson et al. 2002). Subsamples were air-dried and weighed (0.50 mg) into tin capsules (C = 0.50 mg; N = 0.50 mg), and also analyzed for nitrogen and carbon isotope ratios following combustion using a Costech elemental analyzer coupled to a Thermo Finnigan DeltaPlus XL stable isotope mass spectrometer in continuous flow mode. Given that the differences in stable carbon and nitrogen isotope compositions between liver samples with and without lipids extracted were similar among treatments (Table 1), only results for nonlipid extracted samples are used in the subsequent descriptions. Samples of the standard rodent diet were oven-dried, ground to a fine powder using a mortar and pestle, weighed into tin capsules (C = 0.50 mg; N = 1.50 mg), and also analyzed for stable carbon and nitrogen isotope compositions.

The δ15N values were calibrated to atmospheric nitrogen using IAEA-N1 (accepted value = 0.4‰) and IAEA-N2 (accepted value = 20.3‰). A δ15N value of 6.3‰ ± 0.1‰ (mean ± SD) was obtained for 37 measurements of an internal keratin standard (accepted value = 6.4‰). The mean reproducibility of duplicate nitrogen isotope analyses of samples was 0.02% ± 0.02% (n = 8). The δ13C values were calibrated to VPDB using NBS-22 (accepted value = −30.0‰) and ANU-Sucrose (accepted value = −10.5‰), which yields the accepted value of 1.9 ‰ for NBS-19 (Coplen 1994). A δ13C value of −24.0‰ ± 0.2‰ was obtained for 27 measurements of the internal keratin standard (accepted value = −24.0‰). The mean reproducibility of duplicate carbon isotope analyses of samples was 0.03‰ ± 0.03‰ (n = 8).

### Statistical analyses

We conducted all statistical analyses using SPSS version 16.0 for Microsoft Windows (SPSS Inc., Chicago, Illinois, USA). The data were checked for normality of residuals and homogeneity of variances and log-transformed when necessary. All values are reported as mean ± SD. Significance was evaluated using α = 0.05. The effects of location of capture and (or) temperature treatment on mean daily mass-specific food intake, measures of body condition (i.e., blood glucose concentration, hematocrit, and free corticosterone concentration), and stable carbon and nitrogen isotope compositions were assessed using analyses of variance (ANOVA). When ANOVA indicated significant variation (p < 0.05), Tukey’s honestly significant difference (HSD) test was used. Outliers were not removed from the analysis. Pearson product-moment correlation was used to determine if there were any significant effects arising from the number of days in the laboratory, time of day of blood sampling, and (or) mean daily mass-specific food intake on measures of body condition and stable carbon and nitrogen isotope compositions.

### Results

#### Background

Animals were captured at six different field sites within the Kananaskis Valley, but location of capture had no significant effect on mean daily mass-specific food intake (F[5,24] = 0.58, p = 0.56), blood glucose concentration (F[5,24] = 0.55, p = 0.74), hematocrit (F[5,24] = 1.50, p = 0.24), free corticosterone levels (F[5,24] = 1.23, p = 0.33), or stable carbon (F[5,24] = 0.41, p = 0.84) and nitrogen (F[5,24] = 0.59, p = 0.71) isotope compositions.

Total amount of time held in the laboratory ranged from 44 to 82 days. Number of days in captivity did not affect daily mass-specific food intake (r < 0.01, n = 30, p = 1.00), blood glucose concentration (r = 0.30, n = 30, p = 0.11), free corticosterone (r = 0.14, n = 26, p = 0.48), or stable carbon (r = −0.05, n = 30, p = 0.81) and nitrogen (r = 0.06, n =

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**Table 1.** Mean stable carbon and nitrogen isotope compositions of liver tissue in deer mice (*Peromyscus maniculatus*) held in one of three temperature treatments for 28 days.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Mean δ15Nair (%)</th>
<th>Mean δ13CVPDB (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without lipids</td>
<td>With lipids</td>
</tr>
<tr>
<td>23 (n = 10)</td>
<td>7.2</td>
<td>7.3</td>
</tr>
<tr>
<td>5 (n = 11)</td>
<td>7.0</td>
<td>7.2</td>
</tr>
<tr>
<td>−10 (n = 9)</td>
<td>7.1</td>
<td>7.3</td>
</tr>
<tr>
<td>Mean</td>
<td>7.1</td>
<td>7.3</td>
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30, \( p = 0.77 \) isotope compositions of liver. Hematocrit was positively related to number of days held in the laboratory \((r = 0.44, n = 25, p = 0.03)\). Blood glucose \((r = -0.04, n = 30, p = 0.83)\), hematocrit \((r = 0.12, n = 25, p = 0.57)\), and free corticosterone \((r = -0.07, n = 26, p = 0.72)\) were not affected by time of day of blood sampling.

Mean daily mass-specific food intake (log-transformed), which was used as a measure of metabolic rate, was significantly different among temperature treatments \((F_{[2,27]} = 7.05, p = 0.003)\) and was lower at 23°C than either 5°C (Tukey’s HSD: \( p < 0.001 \)) or –10°C (Tukey’s HSD: \( p < 0.001 \); Fig. 1); both the latter were similar.

Blood analyses

The concentration of glucose in the blood (log-transformed) differed among temperature treatments \((F_{[2,27]} = 7.05, p = 0.003)\) and was lower at –10°C than at 5°C (Tukey’s HSD: \( p = 0.006 \)) or at 23°C (Tukey’s HSD: \( p = 0.011 \); Fig. 2). However, glucose concentration was not related to mean daily mass-specific food intake \((r = -0.22, n = 30, p = 0.24)\). Hematocrit did not differ among treatments \((-10^{\circ}C: 0.54 \pm 0.02; 5^{\circ}C: 0.57 \pm 0.08; 23^{\circ}C: 0.55 \pm 0.05; F_{[2,22]} = 0.75, p = 0.81)\), but was positively related to mean daily mass-specific food intake \((r = 0.44, n = 25, p = 0.026; F_{[3,26]} = 3.97, p = 0.031)\).

Stable isotope analyses

The standard rodent chow had a \(\delta^{15}N\) value of 3.4% ± 0.4% and a \(\delta^{13}C\) value of –18.5% ± 0.7% \(n = 3\). The tissue to diet discrimination factor, which is the difference in isotopic composition between an animal and its diet, had a range of 2.9% to 4.2% for nitrogen \((\Delta^{15}N)\) and –2.8% to –0.9% for carbon \((\Delta^{13}C)\). The stable carbon and nitrogen isotope compositions of individual animals in each temperature treatment are listed in the Appendix Table A1.

Nitrogen isotope compositions differed slightly among temperature treatments \((F_{[2,27]} = 3.97, p = 0.031)\). The \(\delta^{15}N\) values of liver tissue for the 5°C treatment were slightly but significantly lower than for the 23°C treatment (Tukey’s HSD: \( p = 0.030 \)), but neither was different from the \(\delta^{15}N\) values at –10°C (Fig. 4). However, the \(\delta^{15}N\) values of liver tissue were not related to mean daily mass-specific food intake \((r = -0.25, n = 30, p = 0.19)\). The \(\delta^{13}C\) values of liver tissue...
tissue also differed among treatments ($F_{2,27} = 4.44, p = 0.022$), with $\delta^{13}C$ values for the $-10\, ^\circ C$ treatment being significantly lower than for the $23\, ^\circ C$ treatment (Tukey’s HSD: $p < 0.05$), but neither was different from the $\delta^{13}C$ values at $5\, ^\circ C$ (Fig. 5). Stable carbon isotope compositions of liver tissue were related but not significantly to mean daily mass-specific food intake ($r = -0.36, n = 30, p = 0.052$).

**Discussion**

The increase in metabolic rate produced by a decrease in temperature from warm ($23\, ^\circ C$) to cool ($5\, ^\circ C$) or cold ($-10\, ^\circ C$) resulted in almost a twofold increase in daily food intake; however, there was no difference in food intake between the $-10$ and $5\, ^\circ C$ treatments. Torpor was not observed in any of the treatments, but animals at the coldest temperature appeared to move about less than in other treatments. Blood analyses suggested that physiological stress did not confound the effect of metabolic rate, as temperature treatments did not significantly affect hematocrit or free corticosterone levels even though animals with a higher food intake had increased levels of free corticosterone. Furthermore, blood glucose concentration was lower, rather than higher as one would expect with increased levels of physiological stress, in animals in the $-10\, ^\circ C$ treatment relative to those in the other treatments. This outcome is most likely a result of increased costs in energy at this low temperature (Guezennec et al. 1988). Collectively, these results suggest that although stressed individuals were eating more, temperature treatment had no discernable impact on the physiological stress response. Therefore, the observed variations in isotopic composition were not likely a result of differences in physiological stress.

We predicted that the $\delta^{15}N$ values of tissues would increase with increasing metabolic rate as a result of an increase in the preferential excretion of $^{14}N$ in urine. Instead, a small but significant decrease in the $\delta^{15}N$ value of liver tissue from $23$ to $5\, ^\circ C$ was observed, which may be attributed to the increase in food intake. Gaye-Siessegger et al. (2003, 2004) have also reported a decrease in $\delta^{15}N$ values with increasing feeding rate in Nile tilapia ($Oreochromis niloticus$ (L., 1758)) and common carp ($Cyprinus carpio$ L., 1758). It is difficult to compare groups of animals with differences in nitrogen metabolism directly. That said, it remains a reasonable assumption that as feeding rate increases, the $\delta^{15}N$ value of tissues more closely resembles that of the diet because the portion of the diet being acquired through catabolism may decrease as food intake increases. Similarly, a recent model developed by Balter et al. (2006) examined transamination and ammonia detoxification during digestion and excretion and predicted that $\delta^{15}N$ values should decrease with increasing nitrogen intake. This outcome may arise from a decrease in urea recycling associated with increased nitrogen intake, which would produce greater $^{15}N$ depletion of tissues in those animals.

Despite these predictions, no overall relationship between mean daily mass-specific food intake and $\delta^{15}N$ values of liver tissue was found. Although animals experiencing a higher metabolic rate increased food consumption, they did not necessarily incorporate different amounts of $^{15}N$ in their tissues. Robbins et al. (2005) suggested that nitrogen isotope tissue–diet discrimination is related more to protein quality than to protein quantity. This is also consistent with the observations of Carleton and Martínez del Rio (2005), who did not find a difference in the tissue–diet nitrogen isotope discrimination factor in house sparrows ($Passer domesticus$ (L., 1758)) subjected to a cold-induced increase in metabolic
rate. Additionally, although an increase in food intake can result in a decrease in δ15N values, the small difference observed among temperature treatments in this experiment indicated that even a twofold difference in daily food intake in deer mice is not sufficient to affect interpretations of trophic level based on δ15N values of liver tissue.

While food intake was similar between the −10 and 5 °C treatments, no significant differences in δ13C values were observed between the coldest temperature treatment and the other treatments. It is possible that differences in water availability between the two colder temperature treatments may have led to a slight enrichment in 13N at −10 °C relative to that at 5 °C, which was counterbalanced by a depletion of 15N resulting from the increase in food intake relative to that at 23 °C. The animals held in the subzero treatment were provided with water in bowls twice daily in amounts that were more than enough to provide for their daily hydration requirements. However, access to water probably decreased when it froze. Although animals could chew ice for water, this effort may have been enough to decrease its availability. Water availability is known to affect δ13N values; Cormie and Schwarz (1996) and Schwarz et al. (1999) demonstrated that the tissues of animals subjected to water stress are more enriched in 15N and attributed this effect to the production of more concentrated urine.

Although it was predicted that δ13C values would not be affected by differences in metabolic rate, we found that the δ13C values of liver in deer mice decreased with decreasing temperature and increasing food intake. This outcome is consistent with the decrease in δ13C values of lipid-free matter with increasing feeding rate in Nile tilapia (Gaye-Siessegger et al. 2003), where similar to the decrease in 15N resulting from the increase in food intake relative to that at 23 °C. The animals held in the subzero treatment were provided with water in bowls twice daily in amounts that were more than enough to provide for their daily hydration requirements. However, access to water probably decreased when it froze. Although animals could chew ice for water, this effort may have been enough to decrease its availability. Water availability is known to affect δ13N values; Cormie and Schwarz (1996) and Schwarz et al. (1999) demonstrated that the tissues of animals subjected to water stress are more enriched in 15N and attributed this effect to the production of more concentrated urine.

In summary, we conclude that variations in metabolic rate, by themselves, are insufficient to produce differences in the stable carbon or nitrogen isotope compositions that could be mistaken for changes in diet.

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