Ecology, 86(1), 2005, pp. 125–139

PLANT SECONDARY METABOLITES COMPROMISE THE ENERGY BUDGETS OF SPECIALIST AND GENERALIST MAMMALIAN HERBIVORES

Jennifer S. Sorensen,[1] James D. McLister,[2] and M. Denise Dearing

Biology Department, University of Utah, Salt Lake City, Utah 84112 USA

Abstract. Ingestion of plant secondary metabolites (PSMs) presents a physiological and behavioral challenge for mammalian herbivores. Herbivores must not only detoxify PSMs, but they may also deal with energetic constraints such as reduced food intake, mass loss, increased excretion of energy, and increased metabolic demands. We hypothesized that the energetic consequences of consuming PSMs will significantly compromise apparent metabolizable energy intake (AMEI) and energy expenditure in mammalian herbivores. Furthermore, we hypothesized that foraging strategy would influence the degree to which plant consumption impacts energy budgets, such that dietary specialists would be less impacted than generalists when both are consuming the plant species preferred by the specialist. Hypotheses were tested by comparing AMEI and energy expended on basal metabolic rate (BMR) and locomotion in a juniper specialist (*Neotoma stephensi*) and generalist (*N. albigula*) woodrat fed control diet and diet containing juniper foliage (*Juniperus monosperma*). In general, the intake of PSMs in juniper increased the energy excreted in urine and feces in both specialist and generalist woodrats. Specialist woodrats minimized the costs associated with the intake of juniper by ingesting more juniper diet, thereby increasing energy intake, and reducing energy expended on BMR and locomotor activity. Generalist woodrats also decreased locomotor activity on a juniper diet but did not increase intake and maintained BMR. In turn, specialist woodrats had twice as much energy available for activities such as reproduction when consuming a juniper diet than generalists. These results suggest that the intake of PSMs impinges on AMEI and compromises energy expenditure, but that the impact of PSMs on energy budgets is relative to ecological experience with PSMs. Moreover, compensatory feeding, metabolic depression, and low activity may be strategies employed by specialist woodrats that can mitigate the cost of processing PSMs, but these strategies may be constrained in generalist woodrats.

Key words: AMEI; BMR; generalist; locomotion; mammalian herbivore; Neotoma albigula; Neotoma stephensi; *plant secondary metabolite; specialist.*

INTRODUCTION

Maintenance, growth, reproduction, and locomotion are considered the major activities influencing the energy budgets of mammals (Pianka 1981, McNab 2002). The ingestion of plant secondary metabolites (PSMs) may also present a significant energetic challenge to mammalian herbivores, yet has received little attention as a factor influencing energy budgets. PSMs can impact apparent metabolizable energy intake (AMEI), which is the energy consumed that is not excreted in the urine or feces. PSMs can impact AMEI by diluting food energy (Foley and McArthur 1994, Guglielmo and Karasov 1996) and decreasing the absorption of energy from food by inhibiting nutrient transport across cells (Robbins et al. 1991, Silverstein et al. 1996, Song et al. 2002). Absorbed PSMs can also reduce AMEI by increasing the excretion of endogenous energy. For example, one detoxification pathway, glucuronidation, results in the excretion of glucuronic acid, a derivative of endogenous glucose (Foley 1992, Guglielmo and Karasov 1996, Mangione et al. 2001). Energy availability may be further limited by increased metabolic expenditure associated with the process of detoxification and elimination of PSMs (Thomas et al. 1988, Iason and Murray 1996, Bozinovic and Novoa 1997). Thus, the ingestion of PSMs may significantly reduce available energy and influence the energy budgets of mammalian herbivores.

Animals can employ a variety of strategies to compensate for energy limitations caused by decreased AMEI or increased energy demands. Most animals respond to limited AMEI by increasing food intake (Hammond and Wunder 1991, Speakman and McQueenie 1996, Young Owl and Batzli 1998). However, herbivores may be unable to compensate for energy limitations caused by processing PSMs by increasing

Manuscript received 22 September 2003; revised 29 April 2004; accepted 12 May 2004; final version received 28 June 2004. Corresponding Editor: B. J. Danielson.

[1] Present address: Oregon State University–Cascades Campus, 234 Cascades Hall, 2600 NW College Way, Bend, OR 97701 USA. E-mail: jennifer.sorensen@oregonstate.edu

[2] Present address: Department of Biological Science, Indiana University, South Bend, Indiana 46634-7111 USA.

food intake due to concomitant increases in PSM loads that may exceed maximal tolerable levels (Stapley et al. 2000). Therefore, herbivores are expected to respond to energetic constraints imposed by PSMs by decreasing energy expenditure rather than by increasing food intake. Decreased expenditure may include a reduction in basal metabolic rate (Hill et al. 1985, Munch et al. 1993, Deerenberg et al. 1998), slowed growth rates (Derting 1989), delayed or reduced reproductive output (Wade and Schneider 1992), and/or down-regulated locomotor activity (Randolph et al. 1977, Handy et al. 1999). The high cost and flexibility of metabolism, growth, reproduction, and locomotion suggest that these activities may be influenced by the energy constraints associated with the intake of PSMs.

The degree of dietary specialization in herbivores may play an important role in the relative impact PSMs have on AMEI and energy expenditure. Specialist herbivores ingest higher quantities of their preferred plant than generalists (Atsatt and Ingram 1983, Lawler et al. 1998, Dearing et al. 2000, Marsh et al. 2003), which could result in greater intake of PSMs. Furthermore, specialist herbivores may employ mechanisms that minimize energy costs per unit PSM ingested and/or excrete PSMs more readily than generalists (Boyle et al. 1999, Sorensen and Dearing 2003, Sorensen et al. 2004). Specialist herbivores may also compensate for the elevated costs of PSM intake by expending less energy on BMR, locomotion, or other activities than generalists. Mammals consuming high levels of PSMs have lower than expected metabolic rates (McNab 1986) and several studies have demonstrated that metabolic rates of some specialists are lower than their generalist counterparts (Boyle and Dearing 2003, McLister et al. 2004). Researchers have also speculated that herbivores, and specialists in particular, have inherently low activity levels (McNab 2002). Although these studies have predicted that low BMR and activity levels are a response to the high costs of processing PSMs, this relationship has not been empirically investigated.

In this study, we examined the impact of a naturally consumed plant high in secondary metabolites, one-seeded juniper (*Juniperus monosperma*), on the energy budgets of a juniper ‘‘specialist’’ (*Neotoma stephensi*) and ‘‘generalist’’ (*N. albigula*) woodrat. *Neotoma stephensi* and *N. albigula* offer an exceptional opportunity to initially test how foraging strategy influences the energetic consequences of PSM intake, while minimizing confounding factors of phylogeny, size, and ecological experience with PSMs. These species are sympatric (Dial 1988), closely related (Edwards and Bradley 2002), and similar in body size. In addition, both species have ecological experience consuming *Juniperus monosperma*, but vary in degree of specialization on juniper. *Neotoma stephensi* feeds almost exclusively on juniper (80–95% juniper) across its range, whereas *N. albigula* consumes 17–33% juniper along with other plant species (Vaughan 1982, Dial 1988). Although it is has not been demonstrated in the laboratory that *N. stephensi* prefers juniper over other plant species, we designated juniper as the ‘‘preferred’’ plant species based on its preference under natural conditions. Based on foraging differences, *N. stephensi* will be referred to as a ‘‘specialist’’ and *N. albigula* will be referred to as a ‘‘generalist’’ for simplicity.

We carried out a series of experiments to examine the impact of juniper intake on energy budgets in specialist and generalist woodrats. Our experiments addressed the following questions: (1) Does the intake of juniper affect AMEI in woodrats? (2) Does the intake of juniper compromise energy expenditure in woodrats? (3) Do compromises in AMEI and energy expenditure associated with juniper intake differ between specialist and generalist woodrats?

METHODS

Woodrats and diet

Specialist (*N. stephensi*) and generalist (*N. albigula*) woodrats were trapped near Wupatki National Park, 45 km northeast of Flagstaff, Arizona, USA (35°30′ N, 111°27′ W) and transported to the University of Utah Animal Facility. Animals were housed individually in plastic cages (48 × 27 × 20 cm) with bedding and cotton batting and put on a 12:12 h light:dark cycle for at least 6 mo prior to experiments. Experiments took place at 28–29°C, which is within the thermoneutral zone of these woodrats (McLister et al. 2004). All animals were fed Harlan Teklad rabbit chow (formula 2031; Harlan Teklad, Madison, Wisconsin, USA) and water ad libitum prior to experimentation. Juniper foliage (*J. monosperma*) was collected randomly from eight to 10 trees at the trapping site and placed immediately on dry ice and stored at −20°C.

Fifteen specialist (seven male, eight female) and 13 generalist (six male, seven female) woodrats were given continuous access to a running wheel (Nalgene 640-0700; Mini Mitter Company, Bend, Oregon, USA) for at least 10 d prior to experimentation. One day prior to experimentation, woodrats were placed in ‘‘metabolic cages’’ that permitted separate collection of urine and feces and provided continuous access to wheels. Metabolic cages were standard shoebox cages fitted with a feeder (Nalgene 650-0104) and mesh stainless steel bottoms (Nalgene 676-2154) suspended over a stainless steel mesh platform and a Plexiglas ramp that allowed for fecal and urinary collections.

Woodrats were given a sequential series of control, acclimation, and juniper diets. Woodrats were maintained on the control diet for 11 d. The control diet was a formula designed to simulate the nutritional content of juniper, but without PSMs, following procedures in Dearing et al. (2000). All woodrats were then fed an acclimation diet containing a homogenous mix of 75% control and 25% ground juniper for 3 d, which allowed animals to become accustomed to a juniper diet. Immediately following the acclimation period, woodrats were fed a juniper diet containing 50% control and 50% juniper for 21 d. Although specialists readily consumed 50% juniper in their diet, this concentration was selected because it was the maximum concentration that generalists could tolerate without rapid and excessive loss of body mass ($<3\%$/d). We assumed that detoxification capacity of both species was fully induced by day 21 on a 50% juniper diet because both species consumed maximum quantities of juniper by day 13 and were able to maintain this intake for at least 5 d. For all juniper diet treatments, juniper foliage was crushed on dry ice to produce plant fragments that were <1.0 mm in size and added to the control diet. It was necessary to crush juniper to eliminate selective foraging. Juniper was kept on dry ice during diet preparation to minimize volatilization of terpenoids. Nutrient and chemical profiles of control and juniper diets were determined from a subsample of each diet treatment (see Appendix A).

All diets were prepared fresh daily in excess of intake requirements to maintain body mass. Diets were offered at approximately 16:00 each day for a 24-h period. Water was provided ad libitum throughout the experiment. Body mass was measured at the beginning of each diet treatment and every 5 d on the control diet and every 3 d on the juniper diet. Any animal losing more than 12% of body mass during the juniper diet treatment was removed from the experiment. Body mass measured on the last day of control and juniper diet treatments was used for analysis.

Energy budgets

Energy budgets were evaluated by comparing apparent metabolizable energy intake (AMEI) and energy expenditure between species of woodrats on control and juniper diets. AMEI was quantified as the energy ingested that was not excreted in feces or urine and was considered apparent rather than true metabolizable energy because there was no correction for endogenous energy lost in urine and feces. Energy expenditure was partitioned into two critical parameters: (1) basal metabolic rate (BMR) and (2) locomotion. We assumed that energy was not utilized for growth, reproduction, or thermoregulation, as all woodrats were nonreproducing adults within their thermoneutral zone. ''Surplus energy'' was estimated as the proportion of AMEI not expended on BMR and locomotion. Surplus energy was interpreted as the energy that could be used by woodrats for daily activities (i.e., remaining alert, grooming), reproduction, thermoregulation, specific dynamic action, and/or methane production. Furthermore, surplus energy not expended may be stored and represented by a gain in body mass.

Apparent metabolizable energy intake.—AMEI was measured in specialists and generalists on control and juniper diets. AMEI was averaged during 3-d experiments at the end of control and juniper diet treatments. Uneaten food and excreted feces and urine were collected daily on the last 3 d of each diet treatment. Fecal and urinary collections were combined separately and stored at $-20°C$. A subsample of each 3-d pooled sample was dried completely at 45°C to determine daily dry matter output of feces and urine. Energy content (kJ/g) of dry diet treatments, feces, and urine were measured with a microbomb calorimeter (Isoperibol Calorimeter 1261, Parr Instruments, Moline, Illinois, USA). Benzoic acid was used as the standard. Analyses were done in duplicate and repeated if values differed more than 5%. Digestible energy intake (DEI, kJ/d) was calculated as (energy intake) − (energy lost in feces) and AMEI (kJ/d) was calculated as (energy intake) − (energy lost in feces) − (energy lost in urine).

An important constituent of energy lost in the urine is glucuronic acid. Total glucuronic acid (mg/d) was measured in the urine of a subset of specialists ($n = 9$) and generalists ($n = 10$). Glucuronic acid was quantified with a colorimetric assay following techniques adapted from Blumenkrantz and Asboe-Hansen (1973). Glucuronic acid was used as the standard (Sigma G-5269; Sigma-Aldrich, St. Louis, Missouri, USA). Quantification of glucuronic acid was done in duplicate and repeated if values differed by more than 5%. The energetic cost of glucuronic acid excretion was determined using the energy content of 13.5 kJ/g for glucuronic acid (Guglielmo and Karasov 1996).

BMR.—Basal metabolic rates (BMR) were determined by measuring O_2 consumption of woodrats during the last day of control and juniper diet treatments. Woodrats were placed in open-circuit metabolic chambers at temperatures ~27–28°C. The BMR of all woodrats was collected between 09:00 and 14:00. Because woodrats are nocturnal and the majority of food consumption occurs at the beginning of the dark cycle (approximately between 18:00 and 22:00), the measurement period constituted the time when specific dynamic action is minimal and woodrats are in the resting phase of their activity cycle (J. S. Sorensen, *personal observation*).

Each animal was acclimated to the metabolic chamber for 30 min before commencing measurements of BMR. O_2 consumption was measured continuously for 30 min following the acclimation period. Measurements of BMR were taken following procedures in McLister et al. (2004). Body temperature was measured rectally following each measurement of BMR.

The energetic cost of BMR was estimated by converting measurements of O_2 consumption (mL O_2/min) to energy (kJ/d). Because the respiratory quotient (RQ, defined as the volume of expired CO_2 divided by the volume of O_2 consumed per unit time) of specialist and generalist woodrats ranges between 0.76 and 0.85 on diets containing various concentrations of juniper (M. D. Dearing, *unpublished data*), O_2 consumption was converted to energy expenditure as (liters of O_2 consumed/d) × (20.1 kJ/L oxygen) (Schmidt-Nielsen 1997). Available energy (kJ/d) for activities other than BMR was calculated as available energy = AMEI − BMR.

Locomotor activity.—Voluntary locomotor activity was measured as the average activity during the 3 d at the end of control and juniper diet treatments. Distance (km/d), time spent running (h/d), and maximum speed (km/h) were recorded daily using event recorders (TOMO Cat Eye model CC-ST200; Performance Bicycle, Chapel Hill, North Carolina, USA) attached to wheels.

The energetic cost of locomotion in woodrats was calculated using the equation for incremental cost of locomotion (ICL, kJ/km), where ICL = $10.679W^{0.70}$ (Garland 1983) and where W is body mass in kg. The daily cost of locomotion was then obtained by multiplying ICL by the daily distance run by each woodrat. Preliminary studies confirmed that woodrats conform to the costs of running used to generate the allometric equation for ICL (Taylor et al. 1970, Garland 1983).

''Surplus energy'' (kJ/d) that could be used for activities not accounted for in our study such as resting, grooming, immunocompetency, reproduction, or other non-locomotor activities was estimated by AMEI − BMR − (daily cost of locomotion).

Statistical analyses

All statistical analyses were performed using JMP software for Macintosh (SAS Institute 2003). In general, analyses were performed using repeated measures ANOVA or ANCOVA with species (specialist vs. generalist) as the between-subjects effect and diet treatment (control vs. juniper) as the within-subjects effect. Covariates for ANCOVAs are specified for each analysis. Insignificant interactions between the covariate and main effects were removed from final analyses. Planned comparisons were performed using least squares means contrasts whenever significant interactions were detected between species and diet treatment or when there was both a significant effect of species and diet treatment. Sample size in analyses may vary for several reasons. Only a subset of animals was used to analyze glucuronic acid excretion and body temperature. Two generalist woodrats were removed from the experiment due to a body mass decrease of >12% during the juniper diet treatment. All measures associated with urine excretion on the control diet (g/d, kJ/g, kJ/d, mg glucuronic acid/d, AMEI, available energy, and surplus energy) were removed for one specialist and one generalist. The specialist was removed because values for urinary mass, energy, and glucuronic acid were more than three standard deviations greater than the means for woodrats. The generalist was removed because this animal did not provide enough urine on control diet to run the analyses.

Variables of AMEI.—Body mass (g) was analyzed with a repeated-measures ANOVA. Energy intake (kJ/d) was analyzed with a repeated measures ANCOVA with body mass as the covariate. Energy intake was transformed (χ^2) to normalize data prior to analysis. The following variables were correlated with energy intake and were therefore analyzed by controlling for energy intake with a repeated measures ANCOVA: total fecal and urinary energy excretion (kJ/d), dry matter output of urine and feces (g/d), energy per gram dry mass of urine and feces (kJ/g), and glucuronic acid excreted in the urine (mg/d). Digested energy intake (kJ/d) and apparent metabolizable energy intake (kJ/d) were analyzed with separate repeated-measures ANOVAs.

BMR.—Whole-body resting metabolic rate (BMR, kJ/d) was analyzed with a repeated-measure ANCOVA with body mass as the covariate. BMR and body mass were log transformed prior to analysis. Because effects of diet treatment on BMR can be confounded by changes in body mass, a separate analysis was performed to isolate the effects of diet treatment on BMR. Independent regression analyses were performed within each species with change in BMR ([BMR on juniper diet] − [BMR on control diet]) as the dependent variable and change in body mass ([body mass on juniper diet] − [body mass on control diet]) as the independent variable. An intercept that differs from zero indicates that diet treatment has an effect on BMR that is independent of mass gain or loss. Body temperature (°C) and available energy (kJ/d) were analyzed with separate repeated-measures ANOVAs.

Locomotor activity.—Distance, time, maximum speed, and surplus energy were analyzed separately with repeated measures ANOVAs. Distance and time were log transformed to normalize data. Because we were interested in the effect of available energy on locomotor activity and because available energy differed between species, we also analyzed distance, time, and maximum speed with repeated-measures ANCOVAs with energy availability as the covariate.

RESULTS

Apparent metabolizable energy intake

The presence of juniper foliage in the diet affected body mass and energy intake differently in specialists and generalist woodrats (Table 1). Overall, there was no difference in body mass between species. However, specialists gained 6.2% body mass on a juniper diet compared to control ($P = 0.0001$), whereas generalists lost 9% body mass on a juniper diet compared to control ($P < 0.0001$) as indicated by a significant diet treatment effect and species-by-diet interaction. Body mass was positively correlated with energy intake. Energy intake did not differ between species when body mass was controlled, but diet treatment significantly increased energy intake in both specialists and generalists (specialist, $P < 0.0001$; generalist, $P = 0.007$). However, specialists increased energy intake to a great-

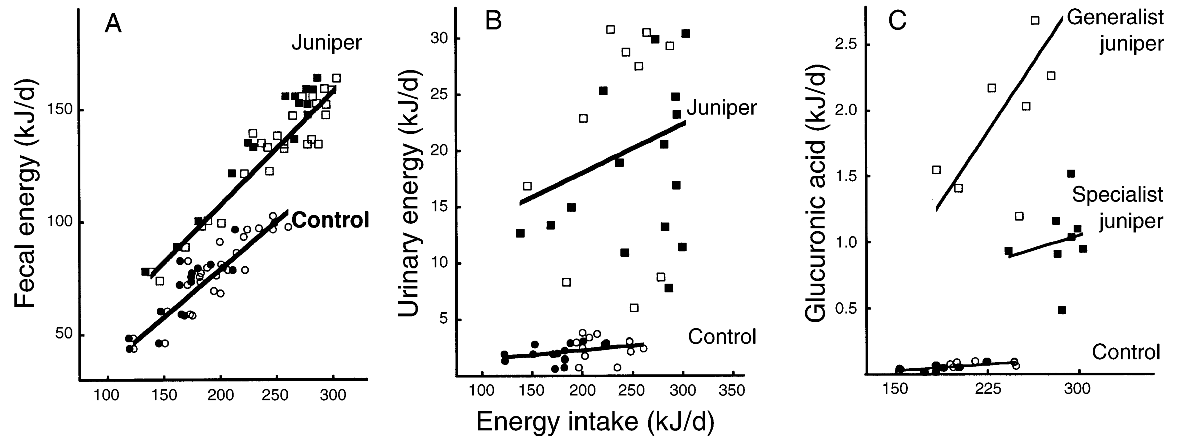

FIG. 1. The energy excreted in (A) feces, (B) urine, and (C) as glucuronic acid in the urine plotted against energy intake in woodrats on control and juniper diet treatments. Circles indicate control diet, and squares indicate juniper diet. Open symbols represent specialist woodrats, and filled symbols represent generalist woodrats. Because there was no significant species effect for fecal and urinary energy ANCOVAs, specialists and generalists were combined for each diet regression. For glucuronic acid ANCOVA, species did not differ on control diet and were combined, but species did differ on juniper diet and were separated. Linear regression results: (A) control diet, $y = -5.8 + 0.42x$, $P < 0.0001$, $R^2 = 0.82$; juniper diet, $y = 6.23 + 0.50x$, $P < 0.0001$, $R^2 = 0.91$; (B) control diet, $y = 0.61 + 0.008x$, $P = 0.11$, $R^2 = 0.1$; juniper diet, $y = 9.3 + 0.04x$, $P = 0.24$, $R^2 = 0.06$; and (C) control diet, $y = -5.07 + 0.05x$, $P = 0.005$, $R^2 = 0.45$; specialists on juniper diet, $y = 14.23 + 0.21x$, $P = 0.65$, $R^2 = 0.04$; and generalists on juniper diet, $y = -96.4 + 1.03x$, $P = 0.06$, $R^2 = 0.48$.

er extent than generalists as indicated by a significant species-by-diet interaction. Specialists and generalists consumed more energy on a juniper diet for a given body mass than on control diet as indicated by a significant diet by body mass interaction.

The intake of juniper foliage increased excretion of total fecal and urinary energy in specialists and generalists compared to control diet when energy intake was controlled (Table 1, Fig. 1). There was no difference between species in dry matter output of feces (g/d), energy content per gram feces (kJ/g), total fecal energy (kJ/d), dry matter output of urine (g/d), energy content per gram urine (kJ/g), or total urinary energy (kJ/d). Specialists and generalists excreted more fecal dry matter and had higher energy content per gram feces on juniper diet than control. Similarly, specialists and generalists excreted more urinary dry matter and had higher energy content per gram urine on juniper diet than control (Table 1). There were no interactions between species and diet treatment for any of the fecal or urinary parameters.

Specialists and generalists excreted more glucuronic acid per unit energy intake on juniper diet than control (Table 1). Moreover, glucuronic acid increased at a faster rate as energy intake increased on a juniper diet than control as indicated by a significant diet by energy intake interaction. Glucuronic acid excreted in the urine did not differ between species on control diet ($P = 0.99$), but generalist excreted nearly twice the glucuronic acid per unit energy intake as specialists on a juniper diet ($P < 0.0001$, Fig. 1).

Specialists and generalists digested and metabolized energy differently on control and juniper diets. There was no main effect of species or diet treatment on DEI (Table 1). However, specialists had a lower DEI on a control diet than on juniper ($P = 0.04$), whereas generalists had a higher DEI on control diet than on juniper ($P = 0.002$) as indicated by a significant species-by-diet interaction. Overall, AMEI did not differ between species. Although there was a significant diet treatment effect on AMEI, only the generalist changed AMEI. In specialists, AMEI was similar on control and juniper diets ($P = 0.23$), whereas generalists had a higher AMEI on control diet than on juniper ($P < 0.0001$, Fig. 2), as indicated by a significant species-by-diet interaction.

Energy expenditure

Basal metabolic rate.—BMR was positively correlated with body mass (Table 2). The intake of juniper affected BMR differently in specialists and generalists when controlling for body mass. Overall, specialists had a lower BMR than generalists when body mass was controlled. Although there was no main effect of diet treatment on BMR, specialists decreased energy expended on BMR on juniper diet compared to control ($P = 0.03$), whereas generalists expended similar amounts of energy on BMR on control and juniper diets ($P = 0.21$, Fig. 2) as indicated by a significant species-by-diet interaction. In addition, changes in BMR were positively correlated with changes in body mass ($F_{1,13} = 8.46$, $P = 0.01$, $R^2 = 0.39$) in specialists, but not in generalists ($F_{1,9} = 0.11$, $P = 0.75$, $R^2 = 0.01$). Therefore, the effects of juniper on BMR were confounded by mass gain in the specialists. However, correcting for the effects of mass gain still yields a significant

TABLE 1. (A) Means and (B) summary of ANOVAs and ANCOVAs for body mass, energy intake, parameters of fecal and urinary excretion, digestible energy intake (DEI), and apparent metabolizable energy intake (AMEI) in specialist and generalist woodrats on control and juniper diets.

A) Means

	Mean ± 1 SE			
	Specialist		Generalist	
Variable	Control diet	Juniper diet	Control diet	Juniper diet
Body mass (g)	179.6 ± 6.68	190.8 ± 7.14	195.2 ± 6.58	177.7 ± 5.38
Energy intake (kJ/d)	175.2 ± 7.61	253.8 ± 13.54	221.4 ± 6.51	236.4 ± 12.97
Fecal energy (kJ/d)	68.8 ± 3.96	136.0 ± 7.02	87.3 ± 3.26	123.2 ± 6.85
Fecal output (g/d)	3.9 ± 0.21	6.5 ± 0.34	5.0 ± 0.17	5.9 ± 0.33
Energy per gram feces (kJ/g)	17.5 ± 0.13	20.8 ± 0.12	17.5 ± 0.12	20.9 ± 0.06
Urinary energy (kJ/d)	2.0 ± 0.21	18.3 ± 1.84	2.5 ± 0.30	22.4 ± 3.21
Urinary output (g/d)	0.44 ± 0.03	0.94 ± 0.09	0.52 ± 0.06	1.19 ± 0.17
Energy per gram urine (kJ/g)	4.6 ± 0.37	19.3 ± 0.15	4.7 ± 0.24	18.8 ± 0.23
Glucuronic acid (kJ/d)†	0.05 ± 0.009	1.01 ± 0.10	0.07 ± 0.007	2.09 ± 0.18
DEI (kJ/d)	106.3 ± 4.63	117.8 ± 6.88	134.1 ± 4.08	113.2 ± 7.34
AMEI (kJ/d)	105.5 ± 4.73	99.5 ± 6.66	132.1 ± 4.44	90.8 ± 7.34

B) Summary of ANOVAs and ANCOVAs

	ANOVA/ANCOVA		
Source of variation	*F*	df	*P*
Body mass			
Species	0.0005	1, 26	0.98
Diet	4.87	1, 24	0.04
Species × diet	66.48	1, 24	<0.0001
Energy intake			
Species	1.50	1, 27	0.23
Diet	73.49	1, 21	<0.0001
Species × diet	10.04	1, 21	0.005
Covariate (body mass)	9.24	1, 21	0.006
Covariate (diet × body mass)	10.78	1, 23	0.004
Fecal energy			
Species	3.91	1, 27	0.06
Diet	142.8	1, 22	<0.0001
Species × diet	0.01	1, 22	0.92
Covariate (energy intake)	316.1	1, 22	0.001
Fecal output			
Species	1.71	1, 27	0.20
Diet	26.79	1, 22	<0.0001
Species × diet	0.83	1, 22	0.37
Covariate (energy intake)	279.2	1, 22	0.0001
Energy per gram feces			
Species	0.37	1, 27	0.55
Diet	1330.3	1, 22	<0.0001
Species × diet	1.64	1, 22	0.21
Covariate (energy intake)	0.06	1, 22	0.81
Urinary energy			
Species	0.86	1, 26	0.36
Diet	69.25	1, 21	<0.0001
Species × diet	3.16	1, 21	0.09
Covariate (energy intake)	3.46	1, 21	0.08
Urinary output			
Species	1.56	1, 26	0.22
Diet	24.49	1, 21	<0.0001
Species × diet	2.99	1, 21	0.10
Covariate (energy intake)	2.69	1, 21	0.12
Energy per gram urine			
Species	0.82	1, 26	0.37
Diet	2176.4	1, 21	<0.0001
Species × diet	0.18	1, 21	0.68
Covariate (energy intake)	3.32	1, 21	0.08

TABLE 1. Continued.

Source of variation	ANOVA/ANCOVA F	df	P
Glucuronic acid			
Species	28.17	1, 17	<0.0001
Diet	34.02	1, 9	0.0002
Species × diet	29.25	1, 9	0.0003
Covariate (energy intake)	5.99	1, 9	0.04
Covariate (diet × energy intake)	5.18	1, 9	0.05
DEI			
Species	2.43	1, 27	0.13
Diet	1.58	1, 23	0.22
Species × diet	16.81	1, 23	0.0004
AMEI			
Species	1.30	1, 27	0.26
Diet	34.88	1, 23	<0.0001
Species × diet	18.23	1, 23	0.0003

Notes: "Species" is the between-subjects effect, and "diet" is the within-subjects effect. Digestible energy intake (DEI) = (energy intake) − (fecal energy). Apparent metabolizable energy intake (AMEI) = (energy intake) − (fecal energy) − (urinary energy).

† Glucuronic acid is a component of urinary energy that directly links a detoxification pathway, glucuronidation, to excreted energy.

decrease in BMR for the specialist. Specialists decreased BMR when there was no change in body mass as indicated by a significant intercept term ($y = -9.06 + 0.56x$, $P = 0.005$), whereas generalists did not change BMR when there was no change in body mass ($y = -1.31 + 0.18x$, $P = 0.92$).

Differences in BMR between specialists and generalists are reflected in differences in body temperature (T_b) between species (Table 2). Overall, specialists had a lower T_b than generalists on both control and juniper diet. There was no diet treatment effect ($F_{1,14} = 0.25$, $P = 0.62$) or species-by-diet interaction for T_b.

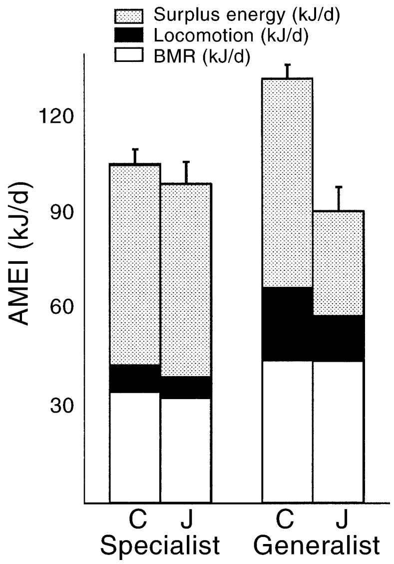

FIG. 2. Apparent metabolizable energy intake (AMEI) in specialist and generalist woodrats on control (C) and juniper (J) diet treatments. AMEI is partitioned into the energy expended on basal metabolic rate (BMR), locomotion, and surplus energy (AMEI − BMR − locomotion) available for activities such as reproduction, thermoregulation, and immunocompetency. Error bars represent +1 SE for AMEI.

Overall, available energy (i.e., AMEI − [energy cost of BMR]) did not differ between specialists and generalists (Table 2). Although there was a significant effect of diet treatment on available energy, only generalists changed available energy on juniper diet compared to control. Specialists maintained constant levels of available energy on control and juniper diets ($P = 0.89$), whereas generalists had twice as much available energy on control diet than juniper ($P < 0.0001$) as indicated by a significant species-by-diet interaction.

Locomotor activity.—The intake of juniper significantly reduced locomotor activity in specialist and generalist woodrats. Generalists ran over twice as far (km/d) and long (h/d) as specialists on control and juniper diets (Table 2). Woodrats reduced distance run and time spent running by 25–33% on juniper diets compared to control (Fig. 3). Woodrats expended 23–37% less energy on locomotion (kJ/d) on a juniper diet than control (Fig. 2). Maximum speed did not differ between specialists and generalists on control or juniper diets and was not affected by diet treatment. There was no significant species-by-diet interaction for distance, time, or maximum speed.

Available energy was positively related to distance and time, but was only marginally related to maximum speed (Table 2). Controlling for energy availability did not change species effects on locomotor activity, as specialists still ran less than generalists. However, controlling for available energy removed the effects of diet treatment on distance (Fig. 3) and time spent running.

TABLE 2. (A) Means and (B) summary of ANOVAs and ANCOVAs for basal metabolic rate (BMR), body temperature (T_b), available energy, locomotor activity, and surplus energy for specialist and generalist woodrats on control and juniper diets.

A) Means

	Mean ± 1 SE			
	Specialist		Generalist	
Variable	Control diet	Juniper diet	Control diet	Juniper diet
BMR (mL O_2/min)	1.19 ± 0.07	1.13 ± 0.09	1.53 ± 0.10	1.52 ± 0.13
T_b (°C)	38.9 ± 0.10	38.9 ± 0.09	39.5 ± 0.20	39.6 ± 0.13
Available energy (kJ/d)	71.3 ± 4.43	66.8 ± 5.08	85.5 ± 5.22	46.8 ± 7.29
Distance (km/d)	2.7 ± 0.56	2.0 ± 0.45	6.7 ± 1.16	4.5 ± 0.77
Time (h/d)	0.8 ± 0.18	0.6 ± 0.14	1.8 ± 0.29	1.2 ± 0.18
Maximum Speed (kJ/d)	8.7 ± 0.43	8.9 ± 0.42	9.6 ± 0.43	0.8 ± 0.31
Surplus energy (kJ/d)	62.1 ± 4.01	60.2 ± 4.20	64.5 ± 4.10	32.5 ± 5.66

B) Summary of ANOVAs and ANCOVAs

	ANOVA/ANCOVA		
Source of variation	*F*	df	*P*
BMR			
Species	14.78	1, 27	0.0007
Diet	0.14	1, 22	0.71
Species × diet	5.90	1, 22	0.02
Covariate (body mass)	30.15	1, 22	0.0001
T_b			
Species	11.69	1, 18	0.003
Diet	0.67	1, 12	0.43
Species × diet	1.20	1, 12	0.30
Covariate (body mass)	2.45	1, 12	0.14
Covariate (body mass × diet)	5.06	1, 12	0.04
Available energy			
Species	0.23	1, 26	0.63
Diet	26.66	1, 22	<0.0001
Species × diet	16.35	1, 22	<0.0005
Distance			
ANOVA			
Species	9.51	1, 27	0.005
Diet	7.88	1, 23	0.01
Species × diet	0.03	1, 23	0.87
ANCOVA			
Species	13.25	1, 26	0.001
Diet	0.04	1, 21	0.85
Species × diet	7.24	1, 21	0.01
Covariate (available energy)	18.80	1, 21	0.0003
Time			
ANOVA			
Species	8.12	1, 27	0.0008
Diet	10.70	1, 23	0.003
Species × diet	0.18	1, 23	0.68
ANCOVA			
Species	10.78	1, 26	0.003
Diet	0.03	1, 21	0.88
Species × diet	5.63	1, 21	0.03
Covariate (available energy)	17.45	1, 21	0.0004
Maximum speed			
ANOVA			
Species	0.59	1, 26	0.45
Diet	0.83	1, 23	0.37
Species × diet	1.69	1, 23	0.21

TABLE 2. Continued.

	ANOVA/ANCOVA		
Source of variation	*F*	df	*P*
ANCOVA			
Species	0.58	1, 26	0.45
Diet	0.05	1, 21	0.83
Species × diet	0.07	1, 21	0.79
Covariate (available energy)	2.78	1, 21	0.11
Surplus energy			
Species	6.41	1, 26	0.02
Diet	17.79	1, 22	0.0004
Species × diet	13.62	1, 22	0.001

Notes: ''Species'' is the between-subjects effect, and ''diet'' is the within subjects effect. Available energy = AMEI − (energy expended on BMR). Surplus energy = AMEI − (energy expended on BMR) − (energy expended on locomotion).

However, there was a significant species-by-diet interaction for both distance and time when available energy was controlled. Specialists decreased distance ($P = 0.04$) and time run ($P = 0.03$) per unit available energy on juniper diets compared to control, whereas generalists did not change distance ($P = 0.17$) or time spent running ($P = 0.30$). There was no effect of species, diet, or species-by-diet interaction on maximum speed when energy availability was controlled.

Juniper intake affected surplus energy differently in specialists and generalists. Overall, surplus energy was significantly affected by species and diet treatment (Table 2). However, surplus energy was not affected by diet treatment in specialists ($P = 0.87$), whereas generalists had half as much surplus energy on a juniper diet than control ($P = 0.0001$), as indicated by a significant species-by-diet interaction. Specialists had over twice as much surplus energy on a juniper diet than did generalists (Fig. 2).

DISCUSSION

We hypothesized that the energetic costs associated with consuming juniper foliage rich in secondary metabolites would play a central role in defining the energy budgets of herbivorous woodrats. Furthermore, we hypothesized that the energy budget of a juniper specialist would be less impacted by juniper intake than that of a generalist. We found that the energy budgets of both specialist and generalist woodrats were negatively impacted by juniper intake primarily through increased energy excreted in the urine and feces. Energy expended on basal metabolism and locomotion was also influenced by the intake of juniper. Finally, juniper intake impacted the energy budget of specialists to a

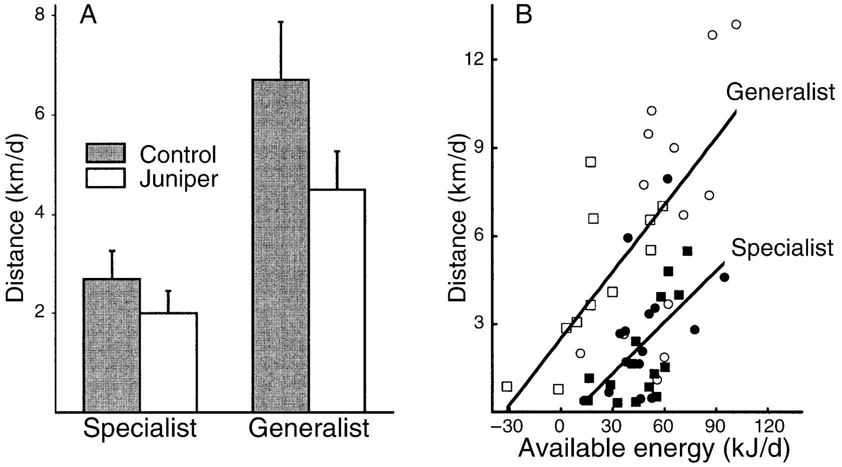

FIG. 3. (A) Locomotor activity of specialists and generalist woodrats on control and juniper diet treatments and (B) relationship between distance run and available energy in specialist and generalist woodrats. Circles indicate control diet, and squares indicate juniper diet. Open symbols represent specialist woodrats, and filled symbols represent generalist woodrats. Because there was no significant diet effect for locomotor activity ANCOVA, control and juniper diets were combined for each of the specialist and generalist regressions. Linear regression results: generalist, $y = 2.5 + 0.08x$, $P = 0.0004$, $R^2 = 0.44$; specialist, $y = -0.45 + 0.06x$, $P = 0.002$, $R^2 = 0.30$.

lesser extent than generalists. In the subsequent paragraphs, we compare the energetic consequences of processing juniper in specialist and generalist woodrats and identify potential explanations and implications of a compromised energy budget in woodrats.

Apparent metabolizable energy intake

The amount of energy excreted in the urine and feces while consuming a juniper diet is substantial and has negative consequences on efficiency of AMEI in woodrats. Both specialists and generalists decreased efficiency of metabolizable energy ([AMEI/(energy intake)] × 100) from 60% on control diet to 38% on a juniper diet. Low-energy metabolism of diets containing PSMs is consistent with other findings (Foley 1987, 1992, Guglielmo and Karasov 1996, Iason and Murray 1996) and suggests that processing PSMs on a daily basis impinges on energy budgets. The energy excreted by woodrats on juniper diet above that on control is similar or greater than the energetic demands of reproduction and thermoregulation in mammals. For example, urinary energy excreted on a juniper diet above that on control equaled 45–50% of BMR, or maintenance expenditure, in woodrats. In comparison, reproductive demands of the female cotton rat during pregnancy were 16% of maintenance expenditure (Randolph et al. 1977). The annual reproductive and thermoregulatory costs in female golden-mantled ground squirrels were 10% and 37% of resting metabolism during torpor, respectively (Kenagy et al. 1989). Finally, the cost of growth in juvenile cotton rats was 36% of BMR (Derting 1989).

To better understand the true cost of excreted energy, we partitioned the total energy excreted by woodrats on juniper diet above that excreted on control into several quantifiable components. The increased energy excreted on the juniper diet is referred to as the ‘‘excretory cost of toxins (ECT)’’ for simplicity (Fig. 4). ECT (kJ/d) was calculated as (energy excreted on juniper diet) − (energy excreted on control diet). ECT was 83.5 kJ/d for specialist and 55.8 kJ/d for generalist woodrats. We identified the proportion of energy loss derived from PSMs and fiber. Energy from PSMs and fiber dilutes the total amount of energy that can be harvested from the diet and therefore inflates true excretory costs of processing PSMs (Jakubas et al. 1993, Foley and McArthur 1994, Guglielmo and Karasov 1996). We also identified the proportion of energy lost in the urine and feces that could be attributed to endogenous energy sources and/or metabolizable energy from the diet that was not absorbed. Although the majority of endogenous energy could not be quantified, we quantified glucuronic acid as a single source of endogenous energy that contributes to ECT. Calculations for quantifying each component of ECT are presented in Appendix B.

PSMs that are not absorbed and/or are excreted as unconjugated detoxification metabolites and fiber represent sources of energy from the diet that may contribute to ECT. We estimated that the excretion of energy in the form of ingested PSMs from juniper (specialist, 30.8 kJ/d; generalist, 28.4 kJ/d) would explain a maximum of 37% and 51% of ECT in specialists and generalists, respectively (Fig. 4; see Appendix B). Fiber can explain up to an additional 21% of ECT (specialist, 17.7 kJ/d; generalist, 4.8 kJ/d). Although energy from PSMs and fiber explained a large proportion of the ECT in specialist and generalist woodrats, they cannot entirely explain the elevated energy excreted by woodrats on juniper diets above that on control (Fig. 4).

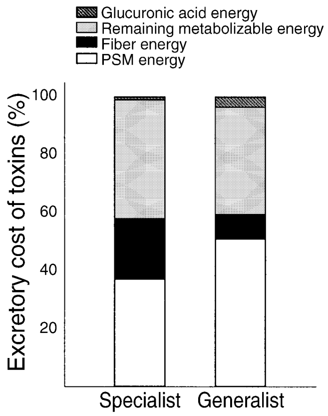

FIG. 4. Partitioning of excretory cost of toxins (ECT) for specialist and generalist woodrats fed the juniper diet. ECT is partitioned into the percentage of energy derived from plant secondary metabolites in juniper diet (PSM energy), fiber in juniper diet (fiber energy), and total apparent metabolizable energy (ECI − PSM − fiber). Total apparent metabolizable energy is further partitioned into energy derived from glucuronic acid (glucuronic acid energy) using 13.5 kJ/g glucuronic acid (Guglielmo 1996), and remaining metabolizable energy (total apparent metabolizable energy − glucuronic acid energy). Calculations for each component of ECT are found in Appendix B.

We propose that the ECT not accounted for by PSMs or fiber is attributed to the excretion of metabolizable energy that would normally be available for expenditure and thus represents a true cost of consuming PSMs. The contribution of remaining metabolizable energy was estimated for specialist (35.0 kJ/d) and generalist (22.6 kJ/d) woodrats and comprised 40% of ECT (Fig. 4). One component of metabolizable energy that was quantified and can contribute to ECT is the excretion of glucuronic acid. Glucuronidation is a detoxification pathway that conjugates ingested PSMs to glucuronic acid, a derivative of endogenous glucose. Although glucuronidation is one of many pathways employed by herbivores to eliminate PSMs, it explained 3% of the remaining metabolizable energy excreted on juniper diets in specialists (1.01 kJ/d) and 9% in generalists (2.02 kJ/d, Fig. 4). These data suggest that specialists may rely on glucuronic acid conjugation less extensively than generalists and may therefore retain more metabolizable energy. Our data also suggest that a larger proportion of metabolizable energy excreted in specialist woodrats consuming juniper is derived from sources other than glucuronic acid compared to generalists. The remaining metabolizable energy not accounted for by glucuronic acid may include the byproducts of protein and fat catabolism and/or excretion of microbes, gut cells, and digestive and salivary enzymes. Future research is needed to determine how the intake of PSMs might influence each of these variables and the extent to which these variables might contribute to the loss of energy in urine and feces.

Energy expenditure

The high excretory costs associated with consuming juniper foliage compromised energy budgets and altered energy expenditure in woodrats. Mammals can compensate for a reduction in AMEI by (1) increasing gross energy intake, (2) reducing basal metabolic rate, and/or (3) minimizing energy expended on energy dependent activities. We investigated how adult, nonreproductive woodrats, within their thermoneutral zone, compensated for the energetic costs of processing juniper. Specialists responded to the energetic costs of processing juniper through increased intake, reduced BMR, and decreased locomotor activity. Generalist woodrats also increased energy intake, but to a lower extent than specialists. In addition, generalists maintained BMR and decreased locomotor activity on a juniper diet compared to control. The following sections propose mechanisms for changes in intake and energy expenditure and emphasize differences between specialists and generalist woodrats. Although these comparisons are limited in the extent to which they apply to specialist and generalist herbivores in general, they provide initial evidence that dietary specialists have the capacity to alleviate the energetic costs of processing PSMs to a greater extent than generalists.

Energy intake.—All herbivores are faced with the challenge of compensating for the energy reducing effects of consuming plant material (Hammond and Wunder 1991, Foley 1992, Guglielmo and Karasov 1996, Young Owl and Batzli 1998, Mangione et al. 2001). Increased energy intake is a common response to energy limitations in mammals (Hammond and Wunder 1991, Speakman and McQueenie 1996, Young Owl and Batzli 1998). For example, specialist and generalist woodrats had greater energy intake on a juniper diet than control for a given body mass (Table 1). Greater energy intake by generalists is primarily due to the higher energy content of juniper diet compared to control (Table 1), as dry matter intake was similar on control and juniper diets (least squares means, $P = 0.74$). However, specialists increased energy intake through greater dry matter intake (least squares means, $P < 0.0001$). Generalist herbivores typically cannot compensate for reduced metabolized energy by increasing dry matter intake of plants containing PSMs due to physiological limitations of processing greater quantities of PSMs (Lawler et al. 1998, Dearing et al. 2000, Mangione et al. 2000, Marsh et al. 2003). For example, generalists lost body mass on juniper due to low metabolizability of juniper diet and an inability to increase energy intake on a juniper diet. Dietary specialists can consume greater quantities of the PSMs present in their preferred diet than generalists and are thus not limited by intake (Atsatt and Ingram 1983, Lawler et al. 1998, Dearing et al. 2000, Marsh et al. 2003). Juniper specialists are therefore able to mitigate the low metabolizability of juniper through compensatory energy intake.

Theory suggests that greater intake of PSMs by specialist herbivores is due to more efficient detoxification mechanisms compared to generalists (Freeland and Janzen 1974, Foley et al. 1999). Our data, combined with literature data, suggest that specialists are both more energy efficient and have a greater capacity to eliminate juniper PSMs compared to generalists. For example, specialists excreted less than half the glucuronic acid per unit juniper consumed as generalists. Lower reliance on glucuronidation by specialists compared to generalists is consistent with other specialist–generalist comparisons (Boyle et al. 1999) and results in lower glucose loss per unit PSM ingested. Recent studies also demonstrated that specialist woodrats have a higher capacity to eliminate PSMs than generalists have (Sorensen and Dearing 2003). For example, the juniper specialist (*N. stephensi*) has been documented to absorb fewer juniper PSMs than the generalist (*N. albigula*; Sorensen et al. 2004). We argue that compensatory feeding on toxic diets is restricted to herbivores, such as specialists, that possess energy-efficient detoxification pathways and enhanced capacity to eliminate PSMs.

BMR.—Reduced metabolic rate is an additional strategy that animals may employ to cope with energy limitations (Hill et al. 1985, Munch et al. 1993, Deerenberg et al. 1998). BMR is positively correlated with food intake (Lindstrom and Kvist 1995), digestibility of diet (Veloso and Bozinovic 1993), metabolizable energy intake (Song and Wang 2002), and body mass and composition (McNab 2002). Therefore, reductions in any or all of these parameters are predicted to reduce BMR in mammals and conserve energy available for other activities. The changes in BMR observed in woodrats fed juniper foliage are in contrast to these predictions. Specialists decreased BMR on a juniper diet compared to control, despite increased dry matter and energy intake, constant DEI and AMEI, and increased body mass. Generalists did not change BMR on a juniper diet compared to control, despite decreased DEI, AMEI, and body mass. Although the precise mechanisms responsible for decreased BMR in specialists and maintained BMR in generalists on juniper diet are unclear, the high energetic requirements of BMR indicate that even small changes in BMR could influence energy available for activities such as locomotion, reproduction, and thermoregulation. In the subsequent paragraphs, we discuss several dietary factors that may influence BMR in specialist and generalist woodrats.

Changes in concentrations of nutrients may explain changes in BMR in woodrats on control and juniper diets. For example, lower nitrogen intake can result in lower BMR (Ross et al. 1992). However, a recent study in specialist and generalist woodrats found no change in BMR with changes in nitrogen content on control diets (Boyle and Dearing 2003) that are similar to changes in nitrogen between control and juniper diet in this study (see Appendix A). In addition, specialists consumed more nitrogen on juniper diet (0.13 g) than control (0.12 g) due to higher juniper intake, yet they decreased BMR. Fiber may also influence BMR by altering DEI or intestinal morphology (Hammond and Wunder 1991, Bozinovic et al. 1997, Young Owl and Batzli 1998). In contrast to these predictions, specialists maintained DEI on control and juniper diets, yet decreased BMR, whereas generalists decreased DEI, yet maintained BMR.

PSMs in juniper may have pharmacological effects or induce physiological changes that influence BMR. Several terpenes present in juniper are considered neurotoxins (Koppel et al. 1981) and could influence BMR. Regulated hypothermia, which is characterized by reduced metabolic rates and body temperature, can increase tolerance to toxins (Gordon et al. 1995) and/or minimize energy expenditure when energy is limited (Carpenter and Hixon 1988, McNab 2002). Decreased metabolism associated with PSM intake has been observed in insects (Appel and Martin 1992, van Loon 1993) and recently in mammals (Boyle and Dearing 2003) and may reflect a response by herbivores to regulate BMR to increase tolerance to PSMs or conserve energy. However, metabolic depression is not a typical response to the ingestion of PSMs and may be constrained in many herbivores. Several studies have demonstrated that the metabolic costs of processing PSMs increase BMR in mammalian herbivores (Thomas et al. 1988, Iason and Murray 1996, Bozinovic and Novoa 1997). Increased BMR on toxic diets may reflect the metabolic costs associated with syntheses of detoxification enzymes and cofactors, biochemical reactions (Appel and Martin 1992, Batt et al. 1992), and/or increased liver, gut, and viscera mass (Huntington 1990, Bendele et al. 1993). Although specialists and generalists are exposed to the same PSMs, it is possible that disparate intake levels of PSMs or elimination mechanisms alter the pharmacological or physiological response each species has to the PSMs in juniper.

Locomotion.—Decreased locomotor activity is an additional strategy that can reduce energy expenditure when energy is limited by the intake of PSMs in juniper. Reduced locomotor activity is thought to explain why mammals engaged in metabolically expensive activities, such as lactation, do not increase energy intake or metabolic rate (McLean and Speakman 1999). For example, several species of rodents decreased voluntary wheel running during peak reproductive demands (Randolph et al. 1977, Wade and Schneider 1992). Decreased locomotion may be particularly important in mammalian herbivores if BMR is constrained by the energetic costs of detoxification. In support, fish exposed to metals decreased locomotor activity in response to insufficient lipid and glycogen stores that had been utilized by the process of detoxification (Handy et al. 1999). Although these studies suggest that decreased locomotion is an energy conservation strategy that may mitigate the costs of detoxification, this relationship had previously not been investigated in mammalian herbivores.

We propose that reduced locomotion in woodrats consuming juniper most likely represents an energy conservation strategy rather than an effect of juniper toxicity. Depressed activity from toxicity is characterized by rapid onset and recovery of changes following exposure to toxic compounds (Dell'Omo et al. 2003). In contrast, changes in locomotor activity in woodrats consuming juniper diet were gradual over the three-week period (J. S. Sorensen, *personal observation*). In addition, woodrats were well groomed and maintained their running capacity as illustrated by similar maximum speed on control and juniper diets. The strong correlation between energy availability and locomotion (Fig. 3) strengthens the conclusion that reduced locomotor activity is primarily related to energy limitations associated with processing the PSMs in juniper. Regulation of locomotor activity allows woodrats to maintain the proportional amount of AMEI that is expended on locomotion. For example, generalists expend ~17% of AMEI on locomotion on control and juniper diets, because as AMEI decreased, locomotor activity also decreased. If generalists had not reduced locomotor activity, they would have expended 25% of AMEI on locomotion. These findings suggest that locomotion is expensive and may be regulated in response to energy limitations resulting from the intake of PSMs.

Although reduced locomotor activity appears to benefit the energy budget of woodrats, it may also have negative ecological consequences. Reduced locomotion on a juniper diet may decrease the success of activities such as foraging, defending territories, escaping predators, and finding mates (Swingland and Greenwood 1983, Karasov 1986, Weis et al. 2001). In addition, the results suggest that the negative impact of juniper intake is more pronounced in generalists than specialists, as generalists reduced locomotor activity on a juniper diet to a greater extent than specialists (Table 2, Fig. 3). These differences were not due to difference in speed (Table 1), but could reflect differences in how specialist and generalists behave on running wheels and/or reflect differences in the time and energy devoted to other activities. For example, several activities are negatively correlated with locomotor activity (Lynch 1994). Therefore, differences between species might be a function of the time each species invests in alternative activities. Generalist woodrats may require high levels of activity due to the patchy distribution of high quality forage in the field (Dial and Czaplewski 1990). Therefore, compromised activity induced by juniper intake may exacerbate the energetic costs of processing juniper because generalists would be limited by the distance they can travel to find higher quality forage. In contrast, specialist woodrats may be less impacted by reduced activity on a juniper diet because they preferentially forage on juniper, an abundant food source that is in close proximity to nests (Vaughan 1982). Although additional work is needed to identify the ecological implications of compromised locomotion in natural populations of woodrats, these data provide the first evidence that the intake of naturally consumed PSMs may constrain locomotor behavior in mammalian herbivores.

CONCLUSIONS

Determining the energetic costs of detoxification has played an important role in advancing our understanding of the interactions between plant chemical defenses and herbivory (Freeland and Janzen 1974, Lindroth 1988, Cork and Foley 1991, Freeland 1991, McArthur et al. 1991, Foley and McArthur 1994, Foley et al. 1999). Although previous studies demonstrated that the intake of PSMs is an energetically expensive process, until now, a direct link between these costs and energy expenditure had not been identified. We found that (1) consumption of juniper foliage imposed a significant shift in the energy budget of specialist and generalist woodrats by increasing energy excreted in urine and feces, (2) energy costs of juniper intake resulted in compromised energy expenditure, and (3) juniper specialists overcame costs by ingesting more food and minimizing BMR and locomotor activity. Our study demonstrates that the energetic consequences of processing PSMs are considerable and may exact energetic demands similar to reproduction, thermoregulation, and growth in mammals. The energetic costs associated with the intake of PSMs significantly impacted foraging decisions (intake), physiology (BMR), and behavior (locomotor activity) of mammalian herbivores. Our results also suggest that the impact of PSMs on energy budgets is relative to ecological experience with PSMs. We conclude that compensatory feeding, limited glucuronidation, metabolic depression, and low activity are strategies that can mitigate the cost of processing PSMs in specialist woodrats and that these strategies may be constrained in generalist woodrats. Although these conclusions apply to a single plant containing high levels of secondary metabolites and only two species of mammalian herbivores, they emphasize that the interactions between foraging strategy, PSM concentration in diet, and energy expenditure in herbivores deserves further attention. We are currently pursuing the impact of PSMs on energy budgets using additional specialists and generalist species of herbivorous mammals and secondary metabolites from additional species of plants to better understand how results apply to the specialists–generalist paradigm in general.

ACKNOWLEDGMENTS

We thank D. Carrier, W. J. Foley, and F. Goller for comments on the manuscript. We thank B. Hudson and employees of Wupatki National Monument Visitor Center for assistance and accommodations during our trapping sessions. H. Baldwin, R. Boyle, D. Green, C. Heidelberger, and S. O'Grady assisted in collecting woodrats in the field. Y. Al-Sheikh, E. van Dijk, J. Grose, E. Heward, A. Knudson, E. McLachlan, S. Rogers, L. Santos, C. A. Turnbull, A. Walters, and M. Wong assisted in experimental procedures and animal husbandry and were in part supported by the University of Utah Biology Undergraduate Research Program and NSF Research Experience for Undergraduates. Research was supported by NSF IBN-0079865 and 0236402, Sigma Xi Grant-in-Aid of Research, American Society of Mammalogists Grant-in-Aid of Research, and the University of Utah Graduate School Research Fellowship.

LITERATURE CITED

Appel, H. M., and M. M. Martin. 1992. Significance of metabolic load in the evolution of host specificity of *Manduca sexta*. Ecology **73**:216–228.

Atsatt, S. R., and T. Ingram. 1983. Adaptation to oak and other fibrous, phenolic-rich foliage by a small mammal, *Neotoma fuscipes*. Oecologia **60**:135–142.

Batt, A. M., G. Siest, J. Magdalou, and M. M. Galteau. 1992. Enzyme induction by drugs and toxins. Clinica Chimica Acta **209**:109–121.

Bendele, A. M., J. F. Hulman, S. White, C. Brodhecker, and R. A. Bendele. 1993. Hepatocellular proliferation in ibuprofen-treated mice. Toxicologic Pathology **21**:15–20.

Blumenkrantz, N., and G. Asboe-Hansen. 1973. New method for quantificative determination of urinic acids. Analytical Biochemistry **54**:484–489.

Boyle, R., and M. D. Dearing. 2003. Ingestion of juniper foliage reduces metabolic rates in woodrat (*Neotoma*) herbivores. Physiological and Biochemical Zoology **106**:151–158.

Boyle, R., S. McLean, W. J. Foley, and N. W. Davies. 1999. Comparative metabolism of dietary terpene, *p*-cymene, in generalist and specialist folivorous marsupials. Journal of Chemical Ecology **25**:2109–2127.

Bozinovic, F., and F. F. Novoa. 1997. Metabolic costs of rodents feeding on plant chemical defenses: a comparison between an herbivore and an omnivore. Comparative Biochemistry and Physiology **117A**:511–514.

Bozinovic, F., F. F. Novoa, and P. Sabat. 1997. Feeding and digesting fiber and tannins by an herbivorous rodent, *Octodon degus* (Rodentia: Caviomorpha). Comparative Biochemistry and Physiology **118A**:625–630.

Carpenter, F. L., and M. A. Hixon. 1988. A new function for torpor: fat conservation in a wild migrant hummingbird. Condor **90**:373–378.

Cork, S. J., and W. J. Foley. 1991. Digestive and metabolic strategies of arboreal folivores in relation to chemical defenses in temperate and tropical forests. Pages 133–166 *in* R. T. Palo and C. T. Robbins, editors. Plant defenses against mammalian herbivory. CRC Press, Boca Raton, Florida, USA.

Dearing, M. D., A. M. Mangione, and W. H. Karasov. 2000. Diet breadth of mammalian herbivores: tests of the nutrient constraints and detoxification-limitations hypotheses. Oecologia **123**:397–405.

Deerenberg, C., G. J. F. Overkamp, G. H. Visser, and S. Daan. 1998. Compensation in resting metabolism for experimentally increased activity. Journal of Comparative Physiology **168B**:507–512.

Dell'Omo, G., M. G. Pleskacheva, D. P. Wolfer, H. P. Lipp, and R. F. Shore. 2003. Comparative effects of exposure to an organophosphate pesticide on locomotor activity of laboratory mice and five species of wild rodents. Bulletin of Environmental Contamination and Toxicology **70**:138–145.

Derting, T. L. 1989. Metabolism and food availability as regulators of production in juvenile cotton rats. Ecology **70**:587–595.

Dial, K. P. 1988. Three sympatric species of *Neotoma*: dietary specialization and coexistence. Oecologia **76**:531–537.

Dial, K. P., and C. J. Czaplewski. 1990. Do packrat middens accurately represent the animals' environment or diet? The Woodhouse Mesa study. Pages 43–58 *in* J. L. Bentacourt, T. R. Ven Devender, and P. S. Martin, editors. Fossil packrat middins: the last 40 000 years of biotic change in the arid West. The University of Arizona Press, Tuscon, Arizona, USA.

Edwards, C. W., and R. D. Bradley. 2002. Molecular systematics of the genus *Neotoma*. Molecular Phylogenetics and Evolution **25**:489–500.

Foley, W. J. 1987. Digestion and energy metabolism in a small arboreal marsupial, the Greater Glider (*Petauroides volans*), fed high-terpene *Eucalyptus* foliage. Journal of Comparative Physiology **157**:355–362.

Foley, W. J. 1992. Nitrogen and energy retention and acid-base status in the common ringtail possum. Physiological Zoology **65**:403–421.

Foley, W. J., G. R. Iason, and C. McArthur. 1999. Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores: how far have we come in 25 years. Pages 131–209 *in* H. J. G. Jung and G. C. J. Faley, editors. Nutritional ecology of herbivores: Proceedings of the Fifth International Symposium of the Nutrition of Herbivores. American Society of Animal Sciences, Savoy, Illinois, USA.

Foley, W. J., and C. McArthur. 1994. The effects and costs of allelochemicals for mammalian herbivores: an ecological perspective. Pages 370–391 *in* D. J. Chivers and P. Langer, editors. The digestive system in mammals: food, form and function. Cambridge University Press, Cambridge, UK.

Freeland, W. J. 1991. Plant secondary metabolites. Biochemical evolution with herbivores. Pages 61–82 *in* R. Palo and C. T. Robbins, editors. Plant defenses against mammalian herbivory. CRC Press, Boca Raton, Florida, USA.

Freeland, W. J., and D. H. Janzen. 1974. Strategies in herbivory by mammals: the role of plant secondary compounds. American Naturalist **108**:269–289.

Garland, T. 1983. Scaling the ecological cost of transport to body mass in terrestrial mammals. American Naturalist **121**:571–587.

Gordon, C. J., L. E. J. Gray, N. A. Monteiro-Riviere, and D. B. Miller. 1995. Temperature regulation and metabolism in rats exposed perinatally to dioxin: permanent change in regulated body temperature. Toxicology and Applied Pharmacology **133**:172–176.

Guglielmo, C. G., and W. K. Karasov. 1996. Nutritional costs of plant secondary metabolite explain selective foraging by ruffed grouse. Ecology **77**:1103–1115.

Hammond, K. A., and B. A. Wunder. 1991. The role of diet quality and energy need in the nutritional ecology of a small herbivore, *Microtus ochrogaster*. Physiological Zoology **64**:541–567.

Handy, R. D., D. W. Sims, A. Giles, H. A. Campbell, and M. M. Musonda. 1999. Metabolic trade-off between locomotion and detoxification for maintenance of blood chemistry and growth parameters by rainbow trout (*Oncorhynchus mykiss*) during chronic dietary exposure copper. Aquatic Toxicology **47**:23–41.

Hill, J. O., A. Latiff, and M. DiGirolamo. 1985. Effects of variable caloric restriction on utilization of ingested energy in rats. American Journal of Physiology **248**:R549–R559.

Huntington, G. B. 1990. Energy metabolism in the digestive tract and liver of cattle: influence of physiological state and nutrition. Reproduction, Nutrition, Development **30**:35–47.

Iason, G. R., and A. H. Murray. 1996. The energy costs of ingestion of naturally occurring nontannin plant phenolics by sheep. Physiological Zoology **69**:532–546.

Jakubas, W. J., B. C. Wentworth, and W. H. Karasov. 1993. Physiological and behavioral effects of coniferyl benzoate on avian reproduction. Journal of Chemical Ecology **19**: 2353–2377.

Karasov, W. H. 1986. Energetics, physiology and vertebrate ecology. Trends in Ecology and Evolution **1**:101–104.

Kenagy, G. J., S. M. Sharbaugh, and K. A. Nagy. 1989. Annual cycle of energy and time expenditure in a golden-mantled ground squirrel population. Oecologia **78**:269–282.

Koppel, C., J. Tenczer, U. Tonnesmann, T. Schirop, and K. Ibe. 1981. Acute poisoning with pine oil—metabolism of monoterpenes. Archives of Toxicology **49**:73–78.

Lawler, I. R., W. J. Foley, B. M. Eschler, D. M. Pass, and K. Handasyde. 1998. Intraspecific variation in *Eucalyptus* secondary metabolites determines food intake by folivorous marsupials. Oecologia **116**:160–169.

Lindroth, R. L. 1988. Adaptations of mammalian herbivores to plant chemical defenses. Pages 415–446 *in* K. C. Spencer, editor. Chemical mediation of coevolution. Academic Press, San Diego, California, USA.

Lindstrom, A., and A. Kvist. 1995. Maximum energy intake rate is proportional to basal metabolic rate in passerine birds. Proceedings of the Royal Society of London B, Biological Sciences **261**:337–343.

Lynch, C. B. 1994. Evolutionary inferences from genetic analyses of cold adaptation in laboratory and wild populations of the house mouse. Pages 278–301 *in* C. R. B. Boake, editor. Quantitative genetic studies of behavioral evolution. University of Chicago Press, Chicago, Illinois, USA.

Mangione, A. M., M. D. Dearing, and W. H. Karasov. 2001. Detoxification in relation to toxin tolerance in desert woodrats eating creosote bush. Journal of Chemical Ecology **27**: 2559–2578.

Marsh, K. J., W. J. Foley, A. Cowling, and I. R. Wallis. 2003. Differential susceptibility to secondary compounds in *Eucalyptus* explains feeding by the common ringtail (*Pseudocheirus peregrinus*) and the common brushtail possum (*Trichosurus vulpecula*). Journal of Comparative Physiology **173B**:69–76.

McArthur, C., A. Hagerman, and C. T. Robbins. 1991. Physiological strategies of mammalian herbivores against plant defenses. Pages 103–114 *in* R. T. Palo and C. T. Robbins, editors. Plant defenses against mammalian herbivory. CRC Press, Boca Raton, Florida, USA.

McLean, J. A., and J. R. Speakman. 1999. Energy budgets of lactating and non-reproductive brown long-eared bats (*Plecotus auritus*) suggest females use compensation in lactation. Functional Ecology **13**:360–372.

McLister, J. D., J. S. Sorensen, and M. D. Dearing. 2004. The effect of juniper (*Juniperus monosperma*) consumption on the cost of thermoregulation in the woodrats *Neotoma albigula* and *Neotoma stephensi* depends upon acclimation temperature. Physiological and Biochemical Zoology **77**: 305–312.

McNab, B. K. 1986. The influence of food habits on the energetics of eutherian mammals. Ecological Monographs **56**:1–19.

McNab, B. K. 2002. The physiological ecology of vertebrates: a view from energetics. Cornell University Press, Ithaca, New York, USA.

Munch, I. C., N. H. Marckussen, and N. A. Oritsland. 1993. Resting oxygen consumption in rats during food restriction, starvation and refeeding. Acta Physiologica Scandinavica **148**:335–340.

Pianka, E. R. 1981. Resource acquisition and allocation among animals. Pages 300–314 *in* C. R. Townsend and P. Calow, editors. Physiological ecology: an evolutionary approach to resource use. Blackwell Scientific Publishers, Oxford, UK.

Randolph, P. A., J. C. Randolph, K. Mattingly, and M. M. Foster. 1977. Energy costs of reproduction in the cotton rat, *Sigmodon hispidus*. Ecology **58**:31–45.

Robbins, C. T., A. E. Hagerman, P. J. Austin, C. McArthur, and T. A. Hanley. 1991. Variation in mammalian physiological responses to a condensed tannin and its ecological implications. Journal of Mammalogy **72**:480–486.

Ross, L. G., J. M. McKinney, S. K. Cardwell, J. G. Fullarton, S. E. J. Roberts, and B. Ross. 1992. The effects of dietary protein content, lipid content and ration level on oxygen consumption and specific dynamic action in *Oreochromis niloticus*. Comparative Biochemistry and Physiology **103A**:573–578.

SAS Institute. 2003. JMP statistics and graphics guide. Version 5.0.1.2. SAS Institute, Inc., Cary, North Carolina, USA.

Schmidt-Nielsen, K. 1997. Animal physiology: adaptations and environment. Fifth edition. Cambridge University Press, Cambridge, UK.

Silverstein, L., B. G. Swanson, and D. Moffett. 1996. Procyanidin from black beans (*Phaseolus vulgaris*) inhibits nutrient and electrolyte absorption in isolated rat ileum and induces secretion of chloride ion. Journal of Nutrition **126**: 1688–1695.

Song, J., O. Kwon, S. Chen, R. Daruwala, P. Eck, J. B. Park, and M. Levine. 2002. Flavonoid inhibition of sodium-dependent vitamin C transporter 1 (SVCT1) and glucose transporter isoform 2 (GLUT2), intestinal transporters for vitamin C and glucose. Journal of Biological Chemistry **277**:15252–15260.

Song, Z. G., and D. H. Wang. 2002. The maximum metabolizable energy intake and the relationship with basal metabolic rate in the striped hamster *Cricetulus barabensis*. Acta Theriologica **47**:417–423.

Sorensen, J. S., and M. D. Dearing. 2003. Elimination of plant toxins: an explanation for dietary specialization in mammalian herbivores. Oecologia **134**:88–94.

Sorensen, J. S., C. A. Turnbull, and M. D. Dearing. 2004. A specialist herbivore (*Neotoma stephensi*) absorbs fewer plant toxins than a generalist (*Neotoma albigula*). Physiological and Biochemical Zoology **77**:139–148.

Speakman, J. R., and J. McQueenie. 1996. Limits to sustainable metabolic rate: the link between food intake, BMR, and morphology in reproducing mice (*Mus musculus*). Physiological Zoology **69**:746–769.

Stapley, J., W. J. Foley, R. Cunningham, and B. Eschler. 2000. How well can common brushtail possums regulate their intake of *Eucalyptus* toxins? Journal of Comparative Physiology **170B**:211–218.

Swingland, I. R., and P. J. Greenwood. 1983. The ecology of animal movement. Clarendon Press of Oxford University Press, Oxford, UK.

Taylor, C. R., K. Schmidt-Nielsen, and J. L. Raab. 1970. Scaling of energetic cost of running to body size in mammals. American Journal of Physiology **219**:1104–1107.

Thomas, D. W., C. Samson, and J. Bergeron. 1988. Metabolic costs associated with the ingestion of plant phenolics by *Microtus pennsylvanicus*. Journal of Mammalogy **69**:512–515.

van Loon, J. J. A. 1993. Gravimetric vs. respirometric determination of metabolic efficiency in caterpillars of *Pieris brassicae*. Entomologia Experimentalis et Applicata **67**: 135–142.

Vaughan, T. A. 1982. Stephen's woodrat, a dietary specialist. Journal of Mammalogy **63**:53–62.

Veloso, C., and F. Bozinovic. 1993. Dietary and digestive constraints on basal energy metabolism in a small herbivorous rodent. Ecology **74**:2003–2010.

Wade, G. N., and J. E. Schneider. 1992. Metabolic fuels and reproduction in female mammals. Neuroscience and Biobehavioral Reviews **16**:235–272.

Weis, J. S., G. Smith, T. Zhoe, C. Santiago-Bass, and P. Weis. 2001. Effects of contaminants on behavior: biochemical mechanisms and ecological consequences. BioScience **51**: 209–217.

Young Owl, M., and G. O. Batzli. 1998. The integrated processing response of voles to fibre content of natural diets. Functional Ecology **12**:4–13.

APPENDIX A

A table showing nutrient and chemical composition of control and juniper diets is presented in ESA's Electronic Data Archive: *Ecological Archives* E086-008-A1.

APPENDIX B

An explanation of calculations for individual components of the energetic cost of toxins is presented in ESA's Electronic Data Archive: *Ecological Archives* E086-008-A2.