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Ingestion of juniper foliage reduces metabolic rates in woodrat *(Neotoma)* herbivores

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Summary

Ingestion of plant secondary compounds by herbivores is predicted to increase resting or basal metabolic rates. We tested this hypothesis with two species of woodrat herbivores, *Neotoma stephensi* and *Neotoma albigula,* consuming diets of juniper (*Juniperus monosperma*), which is rich in plant secondary compounds. In nature, *N. stephensi* specializes on juniper, whereas *N. albigula* consumes a variety of plant species including juniper. We measured resting metabolic rates (RMR) of woodrats on control, 25% juniper and a treatment containing the maximum tolerable dose of juniper (50% juniper for *N. albigula* and 70% juniper for *N. stephensi*). Ingestion of a juniper diet resulted in decreased RMR in both species of woodrats. We propose several potential mechanisms for metabolic depression of *Neotoma* on juniper diets. Our novel results underscore the need for more studies utilizing plant-based diets to determine the general effect of plant secondary compounds on metabolic rates of herbivores.

Key words: detoxification, juniper, metabolic depression, plant secondary compounds, woodrats

Introduction

The unavoidable ingestion of myriad plant secondary compounds by mammalian herbivores should exact a metabolic cost because the detoxification of secondary compounds is an energy driven process (Hardman and Limbird, 1996). Synthesis of numerous detoxification enzymes utilizes energy, and detoxification enzymes require energetic inputs (e.g., ATP and NADPH) to process toxins. The energy budget of a herbivore can be markedly reduced through the excretion of energy-rich detoxification conjugates such as glucuronic acid (Cork, 1981; Mangione, 1999). Furthermore, animals on diets with toxins, either natural or artificial, often exhibit marked increases in liver size and the liver is a major component of metabolic rate (Jean and Bergeron, 1986; Elia, 1992; Handy et al., 1999). Thus, the detoxification of plant secondary compounds is predicted to cause an increase in the resting metabolic rate (RMR) of herbivores (Thomas et al., 1988).

Three studies have investigated metabolic rates of different mammalian herbivores exposed to plant secondary compounds (Thomas et al., 1988; Iason and Murray, 1996; Bozinovic and Novoa, 1997). Voles (*Microtus pennsylvanicus*) consuming diets containing gallic acid (a phenolic) increased basal metabolic rates as much as 22% (Thomas et al., 1988). Sheep (*Ovis aries*) with intravenous administration of oricinol (a phenolic) increased RMR by 5% over controls (Iason and Murray, 1996). Degus (*Octodon degus*) did not exhibit an increase in basal metabolic rate (BMR) on high tannin versus low tannin diets but did show a significant increase in maximum metabolic rate of thermoregulation (MMR) and aerobic scope (Bozinovic and Novoa,

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1997) when consuming high tannin diets. Although all studies were experimentally robust, a potential limitation in extrapolating the results to natural plant-mammal systems is that the treatments contained purified secondary compounds, which represented only a subset of the compounds that herbivores would naturally ingest. Thus, it is possible that metabolic rates of herbivores consuming plant diets with multiple secondary compounds may be different than that of herbivores consuming diets of only a single compound.

We tested the hypothesis that ingestion of secondary compounds from the natural diet of herbivores increases metabolic rate. We studied RMR in two species of woodrats, *Neotoma albigula* and *N. stephensi* consuming diets of juniper. These species of woodrats are sympatric and consume one-seeded juniper (*Juniperus monosperma*) as a natural component of their diet but in differing amounts. *Neotoma albigula* is a generalist that consumes 15–35% juniper in its diet, whereas *N. stephensi* is a specialist that consumes 75–95% juniper year round (Vaughn, 1982).

Juniperus monosperma contains a number of secondary compounds in significant concentrations. Monoterpenes comprise 3–5% of the dry mass of *J. monosperma* (Dearing et al., 2000). Alpha-pinene is the dominant monoterpene, comprising 60% of the total monoterpene content (Adams et al., 1981) and potentially has numerous adverse effects on mammals including neurotoxicity, mucous membrane irritation, diuresis and nephritis (Dearing et al., 2001; Hedenstiena et al., 1983; Koppel et al., 1981). Juniper also produces phenolics (~5% dry weight) but their chemical structures have not been determined (Holechek et al., 1990).

Using the woodrat-juniper study system, we addressed two questions. First, does ingestion of a juniper diet increase metabolic rate? Second, are specialists less affected than generalists?

Materials and methods

Collection of woodrats and juniper

Woodrats and juniper were collected on Woodhouse Mesa, AZ (35°30′ N 111°27′ W). This site was the same one used by Dial (1988) for an extensive diet analysis of *Neotoma stephensi* and *N. albigula*. We trapped *N. stephensi* and *N. albigula* at many of the exact locations as Dial (1988) as indicated by trap markers. We confirmed that *N. stephensi* was consuming more juniper than *N. albigula* by analyzing feces (Williams, 1969) for juniper fragments from captured woodrats. Juniper foliage was collected from several trees at the study site. Foliage was stored on dry ice immediately and until arrival at the University of Utah where it was stored at –20 °C.

Diet and experimental treatments

We used 8 *N. albigula* (3 males; 5 females) and 9 *N. stephensi* (6 males; 3 females) in the experiment. For logistical purposes in measuring metabolic rates, woodrats were assigned to 1 of 2 groups (designated A and B). Group A commenced the experimental regimen one day ahead of Group B. Body mass was recorded immediately before each O_2 consumption measurement.

Animals were maintained on a control diet formulated to be low in nitrogen (~1.25%) and high in fiber (24% acid detergent fiber) to reflect the nitrogen and fiber composition of juniper (1% and 23% respectively; Dearing et al., 2000). Ingredients and percentages were rabbit chow (50%), cornstarch (22%), cellulose (15%), sucrose (9.5%), mineral mix (1.75), vitamin mix (0.055) and corn oil (3%). Rabbit chow was a high fiber formulation from Harland Teklad (2031). Cornstarch, sucrose and corn oil were purchased at a local supermarket; all other ingredients were purchased from Harland Teklad, Wisconsin, USA. The diet was mixed with water to simulate water content of juniper (~55% water). The control diet was used as the base diet to which juniper was added for the diet treatments.

Woodrats were subjected to three sequential diet treatments. Treatment 1 was the control diet described above and was fed to all woodrats for the first 7 days. Treatment 2 was 75% control diet and 25% ground juniper (dry matter) and was fed for 10 days. Because the control diet had similar nitrogen and fiber contents to that of juniper, the addition of juniper to the control diet should have had minimal effects on concentrations of fiber or nitrogen. The dose of juniper in Treatment 2 was designed to provide a substantial acclimation period to juniper such that the detoxification system was fully induced prior to Treatment 3. We designed Treatment 3 to provide the greatest detoxification challenge for each species with no or minimal loss in body mass. Preliminary trials to determine the maximum tolerance to juniper revealed significant differences between the species. As expected from their natural diets, *Neotoma stephensi*, the juniper specialist, could tolerate much greater intakes of juniper than *N albigula*, a generalist forager. Thus, Treatment 3 for *N. stephensi* consisted of a formulation of 70% ground juniper and 30% control diet, whereas Treatment 3 for *N. albigula* was 50% ground juniper and 50% control (dry matter). For simplicity, we refer to Treatment 3 as the “high” juniper treatment. The high juniper treatment was given to both species for 7 days. The N contents of the diet treatments were: Control = 1.25%; Treatment 2 = 1.18; 50% Juniper = 1.13; 70% Juniper =1.08.

For all juniper treatments, *J. monosperma* was homogenized with water using a Polytron homogenizer (Brinkman Ploytron) to produce a paste that was added

to the control diet. Homogenization was necessary to eliminate selective feeding on control diet within the juniper treatment. In all treatments, individual woodrats were offered ~39 g of wet diet (~17.5 g dry matter), which was in excess of intake requirements to maintain body mass. We measured the gross energy contents of each of the treatments using bomb calorimetry. Woodrats on all treatments had ad libitum access to drinking water in water bottles.

We measured digestible and metabolizable energy of woodrats on all three diet treatments. We measured food intake and collected total urine and fecal output for the last 3 days of each treatment. Samples were pooled and analyzed in duplicate for gross energy contents using bomb calorimetry. We calculated digestible energy as:

energy intake (KJ d^{-1}) – energy lost in feces (KJ d^{-1})

and metabolizable energy as:

energy intake (KJ d^{-1}) – energy lost in feces and urine (KJ d^{-1})

Metabolic rate measurements

Resting metabolic rate was measured for all woodrats on the final day of each treatment. Resting metabolic rates were determined by O_2 consumption using flow through respirometry. We placed animals in an 1100 ml dry plastic cylinder with incurrent and excurrent air outlets at opposite ends of the chamber. A mesh floor allowed a comfortable surface for animals to sit and facilitated airflow. The chambers were enclosed in black cardboard with an observation window. The chamber environment was dark and quiet and animals appeared comfortable in the chamber. All animals were acclimated to the chamber on at least three occasions prior to the measurement. Because woodrats are nocturnal, they are less active during the daytime and thus, daytime measurements of metabolic rates provide suitable resting values. Woodrats were never observed sleeping in the chambers, but were motionless during the measurements of oxygen consumption. Even slight motions were detectable as the chamber would move if the animal did, as confirmed by observations through the observation chamber. Measurements were made at ambient temperature (22 °C) and atmospheric pressure was recorded regularly throughout the day. We alternated measurements such that both species were measured throughout the course of the day (08:00–17:00 h). Metabolic rates did not change as a function of time of measurement during the day ($r^2 = 0.007$, $P = 0.55$, $N = 51$).

Animals had not consumed food for several hours prior to RMR measurements. Food was removed at 07:00 h on the day of the measurements. Woodrats consume the majority of their food at the beginning of the dark cycle (19:00 h) and consume very little food during the light cycle (05:00–19:00 h). Thus, most animals probably had not consumed any food for a minimum of 3 hours prior to the measurement. Although little is known about absorption rate of food in these species, mean retention time is estimated to be 3–5 hours (Karasov et al., 1986). We measured metabolic rates in this way rather than fasting animals for 24 hrs, as we were interested in the metabolism of woodrats as it would be in nature when animals are consuming diets with toxins. Because woodrats cache food in nature, it is unlikely that woodrats undergo 24 hr periods of starvation. Iason and Murray (1996) did not fast sheep on toxic diets prior to RMR measurements.

Each woodrat was kept in the respirometry chamber for 2 h at the same time of day for each treatment. The first hour of each period was for acclimation and the second hour for gas exchange measurements. Measurements of O_2 consumption were calculated from the average of the three lowest values of O_2 consumption that lasted for at least 6 min during the second hour that woodrats were in the chamber (Bozinovic and Novoa, 1997). The flow rate through the chamber (500 ml min^{-1}) was controlled by an R2 Flow controller (AEI Technologies, Pittsburgh, Pennsylvania, USA). A subsample of the excurrent air (100 ml min^{-1}) was analyzed for fractional O_2 using a S-3A/II solid oxide cell analyzer and CO_2 using a CD-3A analyzer (AEI Technologies). Data were converted to digital format using a DAQCard1200 (National Instruments) and recorded on a Macintosh laptop with LabHelper software (Warthog Systems, University of California Riverside, California, USA). The excurrent airstream was sampled every 2 s. Analyzers were calibrated regularly throughout the course of the experiment by sampling the incurrent air stream and assuming an atmospheric fractional O_2 concentration of 20.95%. The flow controller was placed downstream of the analyzers and water vapor was scrubbed from the excurrent air upstream of the analyzers. Oxygen concentrations were converted to rates of O_2 consumption ($\dot{V}O_2$ = ml O_2 g^{-1} hr^{-1}) using the software program LabAnalyst (also Warthog Systems). LabAnalyst calculated VO_2 using the formula derived from Vleck (Vleck, 1987):

$$\dot{V}O_2 = STP \cdot FR \cdot ((FiO_2 – FeO_2) + FiO_2 \cdot (FiCO_2 – FeCO_2))/(1 – FiO_2)$$

where STP = correction to standard temperature and pressure, FR = flow rate (ml min^{-1}), FeO_2 and FiO_2 = excurrent and incurrent fractional O_2 concentrations, $FeCO_2$ and $FiCO_2$ = excurrent and incurrent fractional CO_2 concentrations.

Table 1. Food (g dry matter d^{-1}) and gross, digestible and metabolizable energy intakes (KJ d^{-1}) of woodrats on control and juniper diets. The 25% J diet was a mixture of 25% juniper and 75% control; the maximal juniper diet was a mixture of 50% juniper and 50% control for the generalist or 70% juniper and 30% control for the specialist, respectively.

Woodrat	food intake (g d^{-1})			gross energy intake (KJ d^{-1})			digestible energy intake (KJ d^{-1})			metabolizable energy intake (KJ d^{-1})		
	control	25% J	max.	control	25% J	max.	control	25% J	max.	control	25% J	max.
Specialist	11.3 (.76)	11.3 (.79)	10.9 (.90)	191 (10)	221 (13)	220 (14)	124 (6.5)	128 (8.5)	134 (17)	121 (5.9)	120 (7.7)	113 (15.7)
Generalist	12.1 (.53)	12.0 (.70)	10.7 (.72)	212 (11)	223 (13)	210 (15)	140 (6.7)	137 (9.0)	112 (18)	137 (6.6)	120 (8.6)	96 (18.8)

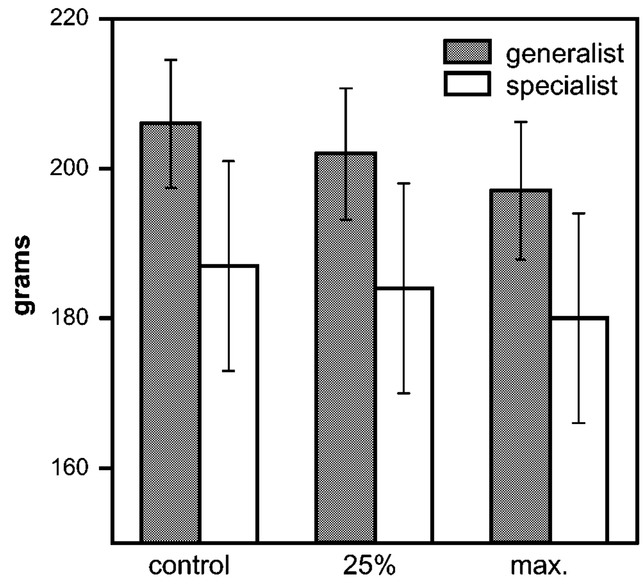
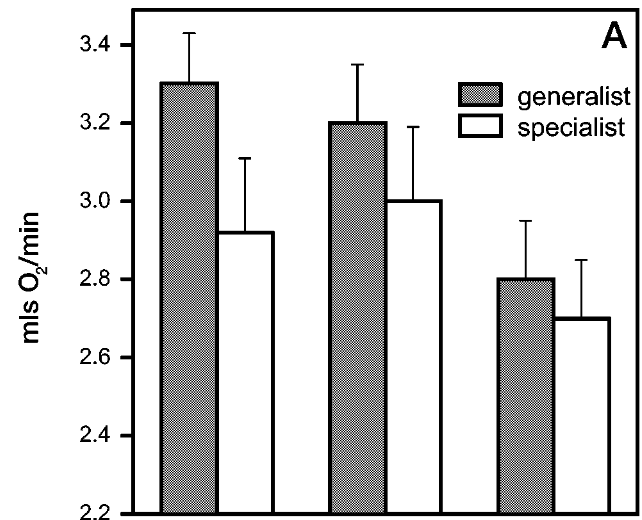


Fig.1. Average body mass of the specialist and generalist on the three diet treatments. The max juniper diet was 50% juniper for the generalist and 70% juniper for the specialist. Error bars are 1 s.e.



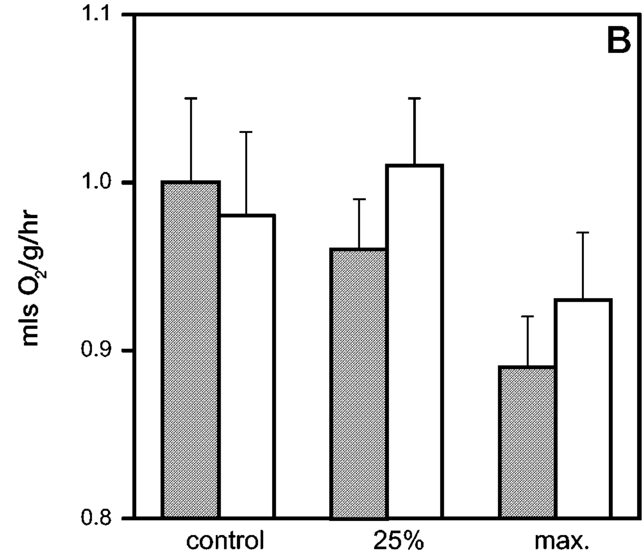


Fig. 2. (A) Whole body and **(B)** mass-specific oxygen consumption of the specialist and generalist on the three diet treatments. The max juniper diet was 50% juniper for the generalist and 70% juniper for the specialist. Error bars are 1 s.e.

Statistical Analyses

Food intake, digestible and metabolizable energy intake, body mass were compared in separate repeated measures analysis of variance (ANOVA) with woodrat species as the between subject factor and diet treatment (control, 25% juniper and max. juniper) as the within species factor and an interaction term (species × treatment). Whole organism oxygen consumption was analyzed with a repeated measures analysis of covariance (ANCOVA) with woodrat species as the between subject factor and diet treatment as the within subject factor, the interaction term: species × treatment, and body mass as the covariate. All analyses were conducted in JMP version 4.

Results

Body mass did not significantly differ between the specialist and generalist during the trial (repeated measures ANOVA, $F_{1.15} = 1.0$, $P = 0.33$; Fig. 1) but did decline with increasing levels of juniper in the diet (repeated measures ANOVA, $F_{2.30} = 7.3$, $P < 0.0001$; Fig. 1). The interaction between species and diet was not significant (repeated measures ANOVA, $F_{2.30} = 0.38$, $P = 0.68$; Fig. 1).

Daily dry matter intake did not differ significantly between species (repeated measures ANOVA, $F_{1.15} = 0.43$, $P = 0.52$; Table 1) or diet treatments (repeated measures ANOVA, $F_{2.30} = 0.91$, $P = 0.41$; Table 1). Both species consumed on average 10.9–12.1 g (dry matter d^{-1}). Daily gross energy intake did not differ significantly between species (repeated measures ANOVA, $F_{1.15} = 0.08$, $P = 0.77$; Table 1) or diet treatments (repeated measures ANOVA $F_{2.30} = 2.24$, $P = 0.14$, Table 1). Woodrats consumed 191–222 KJ d^{-1} regardless of treatment (Table 1). There was no difference in digestible energy between species (repeated measures ANOVA $F_{1.15} = 0.003$, $P = 0.95$, Table 1) or among diet treatments (repeated measures ANOVA $F_{2.30} = 0.59$, $P = 0.50$, Table 1). Similarly, there was no difference in metabolizable energy between species (repeated measures ANOVA, $F_{1.15} = 0.002$, $P = 0.96$; Table 1). Diet treatment had a marginal but insignificant effect on metabolizable energy (repeated measures ANOVA, $F_{2.30} = 3.3$, $P = 0.08$; Table 1). There were no significant interaction terms for any of these analyses ($P > 0.17$, in all cases).

There was no species effect for whole organism oxygen consumption corrected for body mass (repeated measures ANCOVA, species $F_{1.15} = 0.04$, $P = 0.35$ with body mass as covariate, $F_{1.29} = 58.9$, $P < 0.0001$). There was an overall significant effect of diet treatment (repeated measures ANCOVA, $F_{2.29} = 13.6$, $P < 0.0001$). On the maximum juniper diets, whole organism oxygen consumption decreased by 14% for the generalist on the 50% juniper diet and 8% for the specialist on the 70% juniper diet compared with their performance on the control diet (Fig. 2A). While the absolute difference decreased slightly after correcting for body mass, the pattern remained: the generalist decreased mass-specific oxygen consumption by 10% on high juniper treatment and the specialist decreased by 4.8% on the high juniper treatment compared to the control (Fig. 2B). The interaction term (species × diet) was not significant (repeated measures ANCOVA, $F_{2.29} = 1.7$, $P = 0.19$).

Discussion

Detoxification and elimination of plant secondary compounds by herbivores are energy demanding processes predicted to elevate metabolic rates (Thomas et al., 1988). Using a natural plant-herbivore system, we did not find increases in metabolic rates of herbivores consuming diets containing significant quantities of secondary compounds from plant material. Rather both species exhibited significant declines in RMR when consuming a juniper diet. Below we discuss the possible mechanisms underlying these results.

One interpretation of these results is that RMR was not elevated because juniper does not contain great enough quantities of secondary compounds to present a toxic challenge to woodrat herbivores. This scenario seems unlikely. Juniper contains a significant concentrations of a wide variety of plant secondary compounds including terpenes (3–5% dry weight) and phenolics (5% dry weight; Holechek et al., 1990). The predominant terpene in juniper, alpha-pinene, is a documented toxin in mammals (Johnson et al., 1976; Adams et al., 1981; Koppel et al., 1981; Hedenstierna et al., 1983). The dose per g body mass of alpha-pinene ingested daily in nature by the specialist woodrat is half the lethal acute dose in humans, mice and rats (Koppel et al., 1981; Sperling at al., 1967). Ingestion of juniper by woodrats alters acid-base homeostasis and produces diuresis, which may significantly compromise water balance in nature (Dearing et al., 2000; Dearing et al., 2001; Dearing et al., 2002). Lastly, the toxins in juniper are absorbed and metabolized as indicated by excretion of glucuronic acid, a detoxification conjugate, in the urine of woodrats consuming juniper (Dearing et al., 1999; Mangione, 1999). Thus, the compounds in juniper should exact a detoxification cost for herbivores.

We do not believe that preparation of the diet significantly decreased the toxicity of juniper. We were unable to monitor changes in all compounds because juniper has over 35 identified compounds and many unidentified compounds (Adams et al., 1981; Holechek

et al., 1990; Dearing et al., 2000). We did monitor the concentration of alpha-pinene, the predominant monoterpene in the diet. After 24 h, the artificial diet lost ~10% of the initial concentration. Even with this loss of alpha-pinene, the high-juniper treatment clearly represented a toxic hurdle to generalist woodrats because in preliminary tolerance trails further increases in the concentration of juniper (>50%) resulted in decreases in food intake and increases in mass loss of the generalist woodrats. Moreover, the generalist on the high juniper treatment (50% juniper) lost body mass compared to its mass on the control treatment. Since food was provided *ad lib*, the loss of mass suggests that woodrats were unable to increase their food intake to maintain body mass. We interpret this behavior as an indicator that generalists on the 50% juniper treatment reached the threshold for toxin intake and, hence, were unable to ingest more food without severe pharmacological consequences (Meyer and Karasov, 1989; Jakubas et al., 1993; Lawler et al., 1998; Pass et al., 1998; Foley et al., 1999; Pass and Foley, 2000).

Could differences in concentrations of variables other than secondary compounds have resulted in the reduced metabolic rates observed on the juniper diets? By far the greatest difference between the diets was the high concentrations of secondary compounds in the juniper treatments. Slight nutritional differences existed between the control and juniper diets even though they were formulated to be similar. The high juniper treatments contained less nitrogen than the control diet, with the maximum difference being 1.7 mg N/g diet between the control and the 70% juniper treatment. Ingestion of a high protein diet tends to increase metabolic rate due to the increased metabolic costs of digesting nitrogen (Ross et al., 1992; Hailey, 1998). However, the extent of the differences in dietary nitrogen that result in changes in metabolic rates are nearly an order of magnitude greater than the small differences between our diets. Furthermore, additional RMR measurements that we made on woodrats indicate that the small difference in nitrogen between the control and juniper treatments was likely not responsible for the observed differences in metabolic rates. We found no difference in resting metabolic rates of *N. albigula* maintained on 2.5% nitrogen diet (Harland Teklad rabbit chow 2031) compared to that of woodrats on the 1.25% N control diet (T-test; $T = 0.96$, $P = 0.35$, $d.f. = 12$). The difference in nitrogen concentration between rabbit chow and control diet was far greater than the difference between the control and juniper treatments, 12.5 mg N/g vs. 1.7 mg N/g, respectively. The protocol for measuring metabolic rates was identical to that described in this paper and animals did not differ in body mass. Given that these results combined with the extreme differences in protein content that cause changes in metabolic rates in animals in other studies, we suggest that it is highly unlikely that the small differences in protein content of the treatment diets caused the differences in metabolic rates.

Another potential source of variation between the control and juniper diets was fiber type. Both diets had similar concentrations of combined cellulose and lignin fiber (23–24% acid detergent fiber). It is possible that the juniper diet either contained forms of cellulose and lignin more recalcitrant to fermentation, or proportionally more lignin than the control diets. Such differences would be manifest in lower digestible energy (KJ/day) on the juniper diets. Since there was no difference in digestible energy of the any diet treatments, we conclude that the decrease in metabolic rates could not have been the result of differential digestion of fiber.

Surprisingly, the change in RMR of both the specialist and the generalist on toxic diets was opposite in the direction of other studies. In this study, both species decreased RMR when feeding on diets with secondary compounds. In contrast, other studies have documented increased RMR or MMR of herbivores consuming toxins (Thomas et al., 1988; Iason and Murray, 1996; Bozinovic and Novoa, 1997). Below we offer potential causes for metabolic depression of herbivores consuming plant toxins.

Depression of metabolic rate is well documented in animals with significantly reduced energy intakes (Hill et al., 1985; Veloso and Bozinovic, 1993; Koteja, 1996; Rosen and Trites, 1999; Rosen and Trites, 2000). For example, laboratory rats exhibited a 25% decrease in RMR when food intake was reduced for several days by ~25% (Hill et al., 1985). The strategy of reducing metabolic rate is believed to enhance survival during periods of energy limitation. Since the detoxification process results in increased energy losses in the urine, the processing of a toxic diet could reduce available energy (Cork, 1981; Mangione, 1999). In this study, we found no statistical differences in metabolizable energy (ME), but ME was marginally lower ($P = 0.08$) for woodrats consuming the high juniper treatment versus the control. Thus, it is possible that the lower ME, although not statistically significant, represents biologically relevant decreases in available energy. Lastly, energy deficit does not always result in a depression of metabolic rate (Thomas et al., 1988; Weber and O'Connor, 2000). For example, energy deficit was apparent in the study where voles were fed diets of gallic acid, yet voles exhibited an increase in metabolic rate despite a 16% reduction in body mass (Thomas et al., 1988).

The pharmacological effects of juniper toxins may also explain the observed metabolic depression. Many of the terpenes in juniper affect the central nervous system and, thus, could directly decrease metabolism (Koppel et al., 1981). Additionally, it is possible that the reduction in

metabolic rate is a constituent of a regulated hypothermic response generated to reduce the toxicity of juniper compounds. Laboratory rats and mice reduce the effects of a wide variety of toxins by lowering body temperature via reductions in metabolic rates (Gordon, 1993). Thus, juniper toxins may have elicited a similar response in woodrats. We did not measure body temperature in this study, nor did the three other studies examining the effects of secondary compounds on metabolic rate (Thomas et al., 1988; Iason and Murray, 1996; Bozinovic and Novoa, 1997). Thus, it is currently unknown whether changes in metabolic rate of animals on diets with secondary compounds are accompanied by changes in body temperature. Our lab is pursuing the connections between ingestion of secondary compounds, body temperature and metabolism as part of our future research and to convincingly discern among these possible explanations for metabolic depression in woodrats.

We predicted that the resting metabolic rate of the specialist would be less affected than the generalist because the specialist has a greater capacity to ingest and detoxify juniper toxins (Dearing et al., 2000; Dearing et al., 2001; Dearing et al., 2002). Although the magnitude of decrease in RMR for the generalist on control versus high juniper diet was almost double that of the specialist, there was no statistical difference between the species. But while the statistical results imply no difference, the specialist was consuming 40% more juniper than the generalist on the high-juniper treatment. Thus, on a per gram of juniper consumed basis, the specialist seemed to exhibit less of a change in RMR compared to the specialist.

To our knowledge, this is the first study examining metabolic rates of herbivores consuming secondary compounds in plant material. The results are novel in that they differ from those predicted by results from other systems (Thomas et al., 1988; Iason and Murray, 1996). It is possible that the differences among studies are a function of the disparate species examined (woodrats, voles, sheep, degus). However, it is also plausible that the disparate results stem from the use of secondary compounds in foliage versus purified compounds. Our results emphasize the necessity for more studies on the effect of secondary compounds in plant based diets on the metabolic rates of mammalian herbivores.

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References

Adams, R.P., T.A. Zanoni, E.V. Rudloff and L. Hogge. 1981. The south-western USA and northern Mexico one-seeded junipers: their volatile oils and evolution. Biochem. Syst. Ecol. 9: 93–96.

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

Cork, S.J. 1981. Digestion and metabolism in the koala (*Phascolarctos cinereus* Goldfuss): an arboreal folivore. Ph.D thesis. University of New South Wales, Sydney.

Dearing M.D., A.M. Mangione and W.H. Karasov. 2000. Diet breadth of mammalian herbivores: nutrient versus detoxification constraints. Oecologia 123: 397–405.

Dearing, M.D., A.M. Mangione and W.K. Karasov. 2001. Plant secondary compounds as diuretics: an overlooked consequence. Amer. Zool. 41: 890–901.

Dearing, M.D., A.M. Mangione and W.K. Karasov. 2002. Ingestion of plant secondary compounds causes diuresis in desert herbivores. Oecologia 130: 576–584.

Dearing, M.D., J.S. Sorensen, A.M. Mangione and W.H. Karasov. 1999. Detoxification differences of specialist and generalist herbivores. Amer. Zool. 39: 31–32A.

Elia, M. 1992. Organ and tissue contribution to metabolic rate. In: Energy Metabolism:Tissue Determinants and Cellular Corollaries (J.M. McKinney and H.N. Tucker eds) Raven, New York, pp. 19–60.

Foley, J.W., 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? In: Nutritional Ecology of Herbivores. (J.H. Jung and G. C. Fahey, eds.) American Society of Animal Science, Savoy, IL, pp 130–209.

Gordon, C.J. 1993. Temperature Regulation in Laboratory Rodents. Press Syndicate of the Unviersity of Cambridge, Cambridge.

Hailey, A. 1998. The specific dynamic action of the omnivorous tortoise *Kinixys spekii* in relation to diet. Physiol. Zool. 71: 57–66.

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 during chronic dietary exposure to copper. Aquatic Toxicol. 47: 23–41.

Hardman, J.G. and L.E. Limbird (eds) 1996. Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th edn. McGraw Hill, New York.

Hedenstierna, G., R. Alexanderson, K. Wimander and G. Rosen. 1983. Exposure to terpenes: effects on pulmonary function. Inter. Arch. Occupat. Environ. Health 51: 191–198.

Hill, J.O., A. Latiff and M. DiGirolamo. 1985. Effects of variable caloric restriction on utlilization of ingested energy in rats. Amer. J. Physiol. 248: R549–R559.

Holechek, J.L., A.V. Munshikpu, G. Nunez-Hernandez, R. Valdez, J.D. Wallace and M. Cardenas. 1990. Influences of six shrub diets varying in phenol content on intake and nitrogen retention by goats. Trop. Grasslands 24: 93–98.

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

Jakubas , W.J., W.H. Karasov and C.G. Guglielmo. 1993. Ruffed grouse tolerance and biotransformation of the plant secondary metabolite. Condor 95: 625–640.

Jean, Y. and J.M. Bergeron. 1986. Can voles (*Microtus pennsylvanicus)* be poisoned by secondary metabolites of commonly eaten foods. Canad. J. Zool. 64: 158–162.

Johnson, A.E., L.F. James and J. Spillet. 1976. The abortifacient and toxic effects of big sagebrush (*Artemisia tridentata*) and juniper (*Juniperus osteosperma*) on domestic sheep. J. Range Manage. 29: 278–280.

Karasov, W.K., E. Petrossian, L. Rosenberg and J.M. Diamond. 1986. How do food passage rates differ between herbivorous lizards and nonruminant herbivores? Journal of Comparative Physiology B 156: 599–609.

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

Koteja, P. 1996. Limits to the energy budget in a rodent, *Peromyscus maniculatus*: does gut capacity set the limit? Physiol. Zool. 69: 994–1020.

Lawler, I.R., W.J. Foley and B.M. Eschler. 1998. Administration of 5HT3 receptor antagonist increases the intake of diets containing Eucalyptus secondary metabolites by marsupials. Comp. J. Physiol. 168: 611–618.

Mangione, A.M. 1999. Effects of creosote bush phenolic resin on the nutritional ecology of desert woodrats: resin tolerance, detoxification, and impacts on water and energy balance. Ph.D. thesis. University of Wisconsin, Madision, p. 128.

Meyer, M.W. and W.H. Karasov. 1989. Antiherbivore chemistry of *Larrea tridentata*: effects on woodrat (*Neotoma lepida*) feeding and nutrition. Ecology 70: 953–961.

Pass, G.J. and W.J. Foley. 2000. Plant secondary compounds as mammalian feeding deterrents: separating the effects of the taste of salicin from its post-ingestive consequences in the common brushtail possum, *Trichosurus vulpecula*. J. Comp. Physiol. B 170: 185–192.

Pass, G.J., W.J. Foley and B. Bowden. 1998. Vertebrate herbivory on *Eucalyptus* -identification of specific feeding deterrents for common ringtail possums *(Pseudocheirus peregrinus)* by bioassay guided fractionation. J. Chem. Ecol. 24: 1513–1527.

Rosen, D.A. and A.W. Trites. 1999. Metabolic effects of low-energy diet on Stellar sea lions, *Eumetopias jubatus*. Physiol. Biochem. Zool. 72: 723–731.

Rosen, D.A. and A.W. Trites. 2000. Pollock and the decline of Stellar sea lions: testing the junk-food hypothesis. Canad. J. Zool. 78: 1243–1250.

Ross, L.G., R.W. 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*. Comp. Biochem. Physiol. 103A: 573–578.

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

Vaughn, T.A. 1982. Stephen's woodrat, a dietary specialist. J. Mammal. 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.

Vleck, D. 1987. Measurement of oxygen consumption carbon dioxide production and water vapor in a closed system. J. Applied Physiol. 62: 2103–2106.

Weber, J. and T. O'Connor. 2000. Energy metabolism of the Virgina opossum during fasting and excercise. J. Exp. Biol. 203: 1365–1371.

Williams, O.S. 1969. An improved technique for identification of plant fragments in herbivore feces. J. Range Manage. 22: 51–52.