
VOLUME 154C, NUMBER 4
NOVEMBER 2011
ISSN: 1532-0456
ELSEVIER
COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY
CBP
Toxicology & Pharmacology

Comparative Biochemistry and Physiology, Part C 154 (2011) 383–390

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Differential expression and activity of catechol-O-methyl transferase (COMT) in a generalist (*Neotoma albigula*) and juniper specialist (*Neotoma stephensi*) woodrat

Michele M. Skopec [a],*, M. Denise Dearing [b]

[a] *Department of Zoology, Weber State University, Ogden, UT 84408, USA*
[b] *Department of Biology, University of Utah, Salt Lake City, UT 84112, USA*

ARTICLE INFO

Article history:
Received 14 June 2011
Received in revised form 15 July 2011
Accepted 15 July 2011
Available online 23 July 2011

Keywords:
Catechol-O-methyltransferase
COMT
Neotoma
Juniper
Dietary specialization
Nutritional ecology

ABSTRACT

Mammalian herbivores, particularly dietary specialists must have an efficient means to metabolize the high doses of plant secondary compounds they consume. We found previously that *Neotoma stephensi*, a juniper specialist, upregulated catechol-O-methyl transferase (COMT) mRNA almost seven fold in response to an ecologically relevant diet (70% juniper). To further investigate the relevance of this enzyme with respect to juniper metabolism, we compared the protein expression, activity and kinetics of the two forms of COMT, soluble (S-COMT) and membrane bound (MB-COMT), in the blood, kidneys and liver of *N. stephensi* on its natural juniper diet to that of *N. stephensi* fed an experimental diet of 70% juniper as well as a non-toxic control diet under laboratory conditions. In addition, we compared these results to that of *Neotoma albigula*, a generalist species, which consumes a diet of 25% juniper in the wild. The specialist consuming juniper under both field and laboratory conditions had increased S-COMT expression and activity in their livers and kidneys, and increased S-COMT activity in their blood compared to the specialist and generalist fed the control diet. The specialist showed expression and activity of S-COMT in their kidneys that was as high as or higher than that in their livers. The generalist had an elevated V_{max} for MB-COMT compared to the specialist that resulted in higher activity for MB-COMT than the specialist despite lower expression of MB-COMT in the generalist's livers and kidneys. This high activity MB-COMT may be in part responsible for differences in the behaviors of the generalist compared to the specialist. We conclude that S-COMT is important in the specialist's ability to consume high levels of juniper.

1. Introduction

Less than 1% of mammalian herbivores are considered to be dietary specialists, where a single species of plant represents 60% or more of their diet (Freeland, 1991; Shipley et al., 2009). The detoxification limitation hypothesis proposes that dietary specialization is rare in mammalian herbivores because of limitations of their detoxification system in metabolizing large doses of a one or a similar suite of plant secondary compounds (PSCs) (Freeland and Janzen, 1974; Marsh et al., 2006). The preponderance of the generalist strategy is thought to be driven by optimizing nutrient needs by incorporating multiple types of plants with different nutrient compositions (Westoby, 1974, 1978) and/or by the physiological requirement of not overloading the detoxification system by alternating the ingestion of multiple types of plants containing PSCs metabolized through different detoxification pathways (Freeland and Janzen, 1974; Torregrossa and Dearing, 2009b).

The mammalian detoxification system is separated into two phases that can work in tandem or independently (Klaassen and Watkins, 2003). Phase I or functionalization involves enzymes that oxidize or reduce their substrates to form reactive metabolites that then can be conjugated by phase II enzymes. Phase II or conjugation involves enzymes that glucuronidate, sulfate, methylate, or conjugate glutathione or other amino acids to their substrates to increase the hydrophilicity of the metabolites for excretion in urine or bile. Phase I is considered to be energetically inexpensive yet physiologically risky as the products of functionalization can often be more reactive than the substrates. Phase II is considered to be safer but more energetically demanding since it requires cofactors that need to be replenished through the diet. The loss of glucuronic acid, a conjugate, can account for 2–9% metabolizable energy intake (Mangione et al., 2004; Sorensen et al., 2005b). Since mammalian herbivores often consume diets low in nutrients that are potentially low in phase II cofactors, but high in PSCs, it has been hypothesized that dietary specialists may rely more heavily on phase I than phase II pathways for metabolism of PSCs (McLean et al., 1993; McLean and Foley, 1997; Boyle et al., 1999; McLean et al., 2001; Dearing et al., 2005a; Sorensen et al., 2005a, 2005b, 2006). While decreasing the use of conjugation may decrease the energetic costs of PSC consumption, it may increase the likelihood of the production of reactive metabolites

---

Abbreviations: COMT, catechol-O-methyltransferase; MB-COMT, membrane COMT; S-COMT, soluble COMT; PSC, plant secondary compound.

* Corresponding author at: 2505 University Circle, Ogden, UT 84408, USA. Tel.: +1 801 626 6167; fax: +1 801 626 7445.

E-mail address: MicheleSkopec@weber.edu (M.M. Skopec).

1532-0456/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2011.07.010

by functionalization enzymes (Ayala and Cutler, 1997; Guengerich, 2006).

The phase II enzyme catechol-O-methyl transferase (COMT; EC 2.1.1.6) may permit herbivores to strike a balance between expensive but safe conjugation and cheap but reactive functionalization. COMT conjugates a methyl group to its substrate that is relatively inexpensive compared to other conjugates e.g., glucuronic acid. COMT is a well studied enzyme in pharmacological and toxicological research because it has a number of endogenous as well as exogenous catechol containing substrates such as catecholamine, catechol estrogens, flavonoids and tea polyphenolics (Männistö and Kaakkola, 1999; Lautala et al., 2001; Zhu, 2002; O'Leary et al., 2003). COMT also metabolizes endogenously produced catecholamines, which are used as neurotransmitters in the central nervous system (CNS) and as hormones in the peripheral circulation. Thus, COMT plays an essential role in CNS function and behavior, as well as the response of many peripheral organs to the sympathetic nervous system (Männistö and Kaakkola, 1999; Wang et al., 2001; Zhu, 2002). COMT also is an important route for the metabolism of exogenous dietary phenolics such as flavonoids and tea polyphenolics (Zhu et al., 1994; Oddy et al., 1997; Bravo, 1998; Piskula and Terao, 1998; Männistö and Kaakkola, 1999; Tsuda et al., 1999; Donovan et al., 2001; Heim et al., 2002; Zhu, 2002; O'Leary et al., 2003; Talavéra et al., 2005; Crozier et al., 2009). Despite COMT's importance in behavior, homeostasis and metabolizing dietary phenolics, its role has not been investigated in wild herbivores.

We chose to examine the role of COMT in a dietary specialist, *Neotoma stephensi*, for several reasons. Its ability to specialize on one-seeded juniper (*Juniperus monosperma*) is well documented (Vaughn, 1982) as is the natural products chemistry of this species (Adams et al., 1981, 1983; Adams, 2000; Utsumi et al., 2009; Adams, 2011). Juniper is an evergreen that contains high levels of PSCs (8–10% dry matter) including condensed tannins, other phenolics and terpenes (Adams et al., 1981, 1983; Adams, 2000; Utsumi et al., 2009; Adams, 2011). The *Juniperus* genus is widespread throughout the Northern Hemisphere and the berries serve as an occasional source of food for many vertebrates, but *N. stephensi* is the only known vertebrate to specialize on the foliage of the plant (Vaughn, 1982; Dial, 1988; Adams, 2011). To get a global picture of the regulation of the detoxification system in *N. stephensi*, we previously used microarray technology to compare the expression of hepatic genes of the specialist to that of a sympatric generalist, *Neotoma albigula* that consumes a maximum of 25% juniper in the wild (Dial, 1988; Skopec et al., 2007). We found large between species differences in gene expression (*albigula* versus *stephensi*), as well as large within species differences in gene expression of the specialist on a low juniper diet versus a diet typical of that ingested in nature (Skopec et al., 2007). Of particular interest was the change in gene expression of COMT that was up-regulated almost seven fold in the specialist when on a 70% juniper diet compared to a 25% juniper diet (Skopec et al., 2007). Such induction of enzyme transcripts is indicative of an enzyme's role in detoxification of that substance.

Previous research has not found up or down-regulation of COMT in response to drug or dietary treatments (Männistö and Kaakkola, 1999; Zhu, 2002); thus the results in *N. stephensi* were novel. To further investigate the role of COMT in detoxification of juniper by the specialist, we compared the protein expression and activity of COMT in captive *N. stephensi* fed a nontoxic control diet lacking juniper, or a 70% juniper diet to that of wild *N. stephensi* feeding on a natural diet of juniper. We also compared the protein expression and activity of COMT in the specialist to that of the generalist *N. albigula* fed a control diet.

2. Materials and methods

2.1. Woodrats

N. stephensi (N = 22) were trapped on Woodhouse Mesa, near Wupatki National Park, 45 km northeast of Flagstaff, Arizona, USA (35°30′ N, 111°27′ W). Sufficient numbers of the generalist, *N. albigula*, were not available at the Woodhouse Mesa location during the collection of *N. stephensi*; therefore, we collected *N. albigula* (N = 7) from Castle Valley, UT (38°30′ N, 109°18′ W). We have previously used this population for comparative studies between the specialist and generalist (Dearing et al., 2000). All woodrats were transported to the University of Utah, Department of Biology's Animal Facility. Woodrats were housed in individual cages (48 × 27 × 20 cm) and had ad libitum access to food and water. Environmental conditions were 12:12-h light:dark cycle, 28 °C and 15% humidity. All experimental procedures involving woodrats were approved by the University of Utah's Institutional Animal Care and Use Committee protocol number 0702015.

2.2. Dietary treatments

The *N. stephensi* were separated into three groups, wild, control and 70% juniper. The wild *N. stephensi* (N = 9; 2 male, 7 female) were trapped and maintained on fresh juniper foliage supplemented with rabbit chow (Harlan Teklad formula 2031) for a maximum of 48 h before dispatch. The *N. stephensi* in the control and 70% juniper groups were maintained in captivity for more than 3 months on rabbit chow prior to the experiments. The captive groups were fed either the nontoxic control diet (N = 6; 3 male, 3 female) of ground rabbit chow or a 70% juniper diet (N = 7; 4 male, 3 female) that consisted of 70% ground juniper and 30% ground rabbit chow on a dry matter basis. 70% juniper is the minimum amount of juniper consumed by *N. stephensi* in the wild (Vaughn, 1982; Dial, 1988) and also the amount of juniper fed to *N. stephensi* in our microarray study (Skopec et al., 2007). The control diet was fed for a minimum of 5 days. The specialists in the juniper treatment were fed 5% juniper diet for 1 day, 25% juniper diet for 2 days and 70% juniper diet for 5 days to give adequate time for the induction of detoxification enzymes involved in metabolizing the PSCs in juniper (Hollenberg, 2002; Bock and Köhle, 2004; Lamb et al., 2004; Haley et al., 2007; Skopec et al., 2007). The juniper used in 70% juniper diet was *J. monosperma* collected from several trees at the trapping sites and kept frozen at −20 °C until use. The juniper was ground with dry ice in a Waring blender until it passed through a 1 mm screen and again stored at −20 °C in air-tight containers until incorporated into 70% juniper diet. The 70% juniper diet was made daily to minimize volatilization of terpenes.

The *N. albigula* (N = 7; 3 male, 4 female) were maintained in captivity for more than 3 months on rabbit chow prior to the experiment and were fed only the control diet for a minimum of 5 days. *N. albigula* cannot maintain body mass long term on juniper diets of 50% or greater in the lab (McLister et al., 2004; Dearing et al., 2005b; Sorensen et al., 2005b) and consumes around 25% juniper in the wild (Dial, 1988). In our microarray study, we saw no differences in the expression of COMT mRNA in *N. albigula* on a control compared to a 25% juniper diet (Skopec et al., 2007), therefore we included only generalists fed a control diet.

Food intakes were measured daily for all captive groups i.e., *N. stephensi* fed control or 70% juniper diets and *N. albigula* fed control diet. Body masses were measured daily for all animals. Food intake was not measured for the *N. stephensi* in the wild group; however, they were observed to be consuming the fresh juniper foliage being provided and maintained body mass from date of collection.

2.3. Tissue collection and preparation

At the end of the trials, the animals were euthanized with CO_2 and blood, kidney and liver tissues were harvested. While the liver tends to have the highest levels of COMT in the body, the kidney also shows high expression and activity of COMT (Ellingson et al., 1999; Männistö and Kaakkola, 1999; Tsunoda et al., 2002; Zhu, 2002). The expression and activity of COMT in the blood may mirror that occurring elsewhere in the body (Masuda et al., 2002). Therefore, kidney and blood were analyzed for COMT expression and activity. There are two forms of COMT, soluble and membrane bound (Männistö and Kaakkola, 1999; Zhu, 2002); all tissues were separated into cytosolic fractions and membrane (erythrocytes) or microsomal (liver and kidney) fractions. The cytosolic fractions contained the soluble form or S-COMT whereas the membrane and microsomal fractions contained the membrane bound or MB-COMT.

Blood (≤1 mL) was collected from euthanized animals via heart puncture and processed as in Masuda et al. (2002) to first separate erythrocytes from plasma and other cellular elements and then to separate the cytosolic fraction of the erythrocytes from the membrane fraction (Masuda et al., 2002). Due to a lack of sample volume, the membrane fraction of the blood was not analyzed.

Liver and kidneys were perfused in situ by injecting cold isotonic saline into the hepatic portal vein (for liver) and renal arteries (for kidneys). The liver and kidneys were removed and weighed. Cytosolic and microsomal fractions were prepared by differential ultracentrifugation as described for laboratory rats by Franklin and Estabrook (1971). All samples were stored at −80 °C until assayed for COMT. Protein concentrations for each fraction were determined colorimetrically via the Bio-Rad Protein assay (Bio-Rad) based on the Bradford bye-binding method.

2.4. Western blots

We chose to use western blots to measure differences in the expression of COMT protein between tissues and treatments rather than measuring mRNA levels since mRNA expression does not necessarily correlate to protein expression due to differences in translational control and protein half-life (Gygi et al., 1999). We therefore are referring to protein expression and not gene expression when using the term "expression" from this point forward. Samples were diluted to 5 µg/µL with 1 M Tris–HCl pH 7.4, placed in loading buffer (4% SDS, 20% glycerol, 0.1% bromophenol blue, 250 mM Tris–HCl pH 6.9, 0.2% 2-beta mercaptoethanol) and denatured by heating at 100 °C for 3 min. Protein (100 µg) was loaded into each well of a 4–20% Tris-glycine iGel (ISC bioexpress) and the samples were subjected to SDS-polyacrylamide gel electrophoresis and then transferred onto polyvinylidene difluoride (PVDF) membranes (Thermo Scientific). The membranes were blocked for 1 h using 5% skim milk in Tris-buffered saline with 0.01% Tween and then incubated with the primary antibody, rabbit anti-COMT antibody (1:300 FL-271 Santa Cruz Biotechnology Inc.). The blot was visualized with peroxidase labeled goat anti-rabbit secondary antibody (1:10,000 KPL) and Pierce ECL Western Blotting Substrate (Thermo Scientific). A Typhoon 8600 (Molecular Dynamics 300-2483) imaging system was used to visualize and quantify protein bands on the membranes.

2.5. Enzyme assays

Enzyme assays were used to quantify the activity of COMT using catechol as the substrate and radiolabeled SAM (adenosyl-L-methionine, S-[methyl-^{3}H], specific activity: >50 Ci/mmole, MP Biomedicals) as the cofactor as in Zürcher and Prada (1982). We checked for linearity of the assay by varying protein concentrations (25, 50, 100, 150 µg of sample protein) and time (100 µg protein incubated for 5, 10, 20, and 30 min). A cytosolic liver fraction from one animal of each species on the control diet was run in triplicate using the following reaction mixture (0.5 mM catechol, 0.1 M sodium phosphate buffer, 1 µM DTT, 1 mM $MgCl_2$, 200 µM SAM 1 µCi/mL ^{3}H-SAM). Guaiacol is the ^{3}H-methylated product formed in the reaction and is non-polar and therefore preferentially separates into the scintillation fluid (BetaMax ES liquid scintillation fluid MP Biomedicals). Decays per minute (dpm) were counted on a Liquid Scintillation Analyzer Tri-Carb 2800 TR (Perkin Elmer).

Dinitrocatechol (Sigma-Aldrich), a selective inhibitor of COMT, was used to determine if any of the methylated catechol products were formed by methlytransferases other than COMT (Zürcher and Prada, 1982; Männistö and Kaakkola, 1999). A cytosolic and membrane fraction of both liver and kidney was tested from one control individual of both species. Protein (100 µg) from each sample was incubated for 20 min with the following reaction mixture (0.5 mM catechol, 0.1 M sodium phosphate buffer, 1 µM DTT, 1 mM $MgCl_2$, 200 µM SAM 1 µCi/mL ^{3}H-SAM and 0.2 µM dinitrocatechol).

To determine if α-pinene, a terpene, and the most abundant PSC in one-seeded juniper, acts as a substrate for COMT, cytosolic and microsomal liver fractions from three *N. stephensi* on control diet were incubated with a reaction mixture that did not contain catechol and instead contained varying concentrations of α-pinene (Sigma-Aldrich) that had been dissolved in ethanol (0.01 mM, 0.05 mM, 0.1 mM, 0.5 mM, 1 mM α-pinene).

Kinetics of S and MB-COMT in *N. stephensi* and *N. albigula* were done on cytosolic and microsomal liver fractions from 3 animals of each species on the control diet using 100 µg of protein and the above reaction mixture except that catechol concentration was varied (0.01 mM, 0.05 mM, 0.1 mM, 0.5 mM, 1 mM catechol) and the reaction was allowed to proceed for 20 min. Lineweaver–Burk double reciprocal plots were used to calculate K_m and V_{max} for each individual. The kinetic data revealed that MB-COMT had a significantly lower K_m for catechol than S-COMT in both species, therefore all further reactions for microsomal fractions were run with a concentration of 0.05 mM catechol and the cytosolic fractions were run with a concentration of 0.5 mM catechol. All other reaction mixture reagents remained the same and the reaction was allowed to proceed for 20 min.

2.6. Statistical analysis

Body mass was analyzed using analysis of variance (ANOVA) with species and diet coded as a single factor. Dry matter intake was analyzed using analysis of covariance (ANCOVA) with species and diet as a single factor and body weight as the covariate. Liver and kidney weights were also analyzed using ANCOVA with species and diet as a single factor but with body mass minus organ mass as the covariate (Christians, 1999). Because there were no significant differences in expression and activity of COMT between the wild *N. stephensi* and *N. stephensi* fed the 70% juniper diet, we combined them into a single group to which we refer to as "*N. stephensi* juniper" in further analyses. Expression and activity were analyzed using ANOVA with species and diet as a single factor. Differences between individual means were determined by post hoc Bonferroni adjusted pairwise comparisons. SYSTAT 10 was used for all analyses (Wilkinson and Coward, 2000). All data are expressed as mean ± SE and $p \leq 0.05$ was used to establish significance.

3. Results

3.1. Body mass, dry matter intake and organ weights

There was a significant difference in body mass of woodrats ($F_{3,\ 25} = 4.770$, $p = 0.009$, Table 1), with wild *N. stephensi* being significantly smaller than *N. stephensi* on control diet ($p = 0.011$). There were no differences among the body masses of the captive groups (*N. stephensi* on control diet and 70% juniper, and *N. albigula* on control diet). Dry matter intake did not differ among woodrats in captivity ($F_{2,\ 16} = 1.289$, $p = 0.303$, Table 1) and body mass was not a significant covariate ($F_{1,\ 16} = 1.479$, $p = 0.242$).

There was a trend for liver mass to be different among groups ($F_{3,\ 24} = 2.568$, $p = 0.078$, Table 1) and body mass minus liver mass was a significant covariate ($F_{1,\ 24} = 60.697$, $p < 0.01$). Post hoc comparison of liver masses to body mass ratios showed that wild *N. stephensi* had higher liver masses to body mass ratios than *N. albigula* fed a control diet ($p = 0.024$). Kidney mass significantly

Table 1
Means ± SE of body mass, food intake, liver mass and kidney mass from woodrats.

Variable	*Neotoma stephensi*			*Neotoma albigula*
	Control diet ($n = 6$)	70% juniper diet ($n = 7$)	Wild ($n = 9$)	Control diet ($n = 7$)
Body mass (g)	196.8 ± 12.4^{a}	$182.1 \pm 16.0^{a,b}$	142.5 ± 7.3^{b}	$176.7 \pm 7.5^{a,b}$
Dry matter intake (g/day)	13.6 ± 0.7	12.3 ± 0.6	NA	14.2 ± 1.1
Liver mass per g body mass (g/g)	$0.046 \pm 0.001^{a,b}$	$0.045 \pm 0.003^{a,b}$	0.053 ± 0.004^{a}	0.039 ± 0.003^{b}
Kidney mass per g body mass (g/g)	$0.012 \pm 0.001^{a,b}$	$0.013 \pm 0.001^{a,b}$	0.015 ± 0.001^{a}	0.011 ± 0.001^{b}

[a,b] Different letters (a, b) denote means significantly different ($p \leq 0.05$) as determined by Bonferroni adjusted pairwise comparisons within the same row.

differed among the groups ($F_{3,\ 23} = 2.993$, $p = 0.05$) and body mass minus kidney mass was a significant covariate ($F_{1,\ 23} = 25.03$, $p < 0.01$). Post hoc comparison of kidney mass to body mass ratios showed that wild *N. stephensi* had a significantly larger kidney mass per gram body mass than the *N. albigula* on control diet ($p = 0.005$).

3.2. COMT expression

The expression of COMT significantly differed among groups in all tissues tested except for blood (see Table 2 for summary of ANOVAs and Fig. 1). In general, albeit with a few exceptions, woodrats on the control diets had lower expression of COMT in the tissues. In the kidney, *N. albigula* fed the control diet had significantly lower expression of both S and MB-COMT compared to both *N. stephensi* groups ($p < 0.001$). The *N. stephensi* fed the control diet had similar expression of MB-COMT but significantly lower expression of S-COMT in their kidneys compared to *N. stephensi* consuming juniper ($p = 0.026$). In the liver, *N. albigula* had lower expression of MB-COMT than both *N. stephensi* groups ($p \leq 0.03$). Expression of S-COMT in the liver of *N. albigula* was significantly lower than only the *N. stephensi* consuming juniper ($p = 0.05$). The *N. stephensi* fed the control diet had lower expression of both MB-COMT and S-COMT in their livers compared to the *N. stephensi* consuming juniper ($p \leq 0.05$).

Table 2
Summary of ANOVAs for differences in the expression of COMT in the various tissues of woodrats tested.

Source of COMT	F	Df	P
Blood S-COMT	0.085	2,10	0.919
Kidney MB-COMT	29.70	2,22	0.000
Kidney S-COMT	51.31	2,23	0.000
Liver MB-COMT	26.54	2,22	0.000
Liver S-COMT	5.33	2,20	0.014

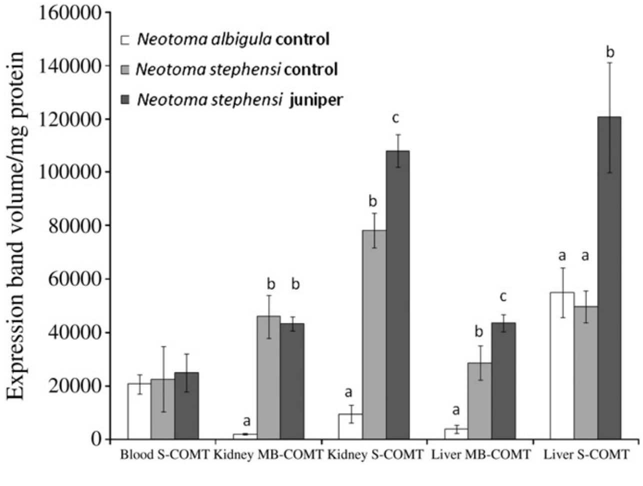

Fig. 1. Expression of the soluble (S-COMT) and membrane bound (MB-COMT) forms of catechol-O-methyl transferase (COMT) in the tissues of *N. stephensi*, a juniper specialist, and *N. albigula*, a generalist, on different diets. Letters a, b and c for each tissue denote means that are significantly different ($p < 0.05$) between species or within species on different diets.

3.3. COMT activity

The COMT activity assay using catechol as a substrate and ^{3}H-SAM was linear for both protein concentration (Fig. 2A) and time (Fig. 2B) in the *N. stephensi* and *N. albigula* cytosolic liver samples ($R^2 \geq 0.96$). When the selective COMT inhibitor dinitrocatechol was present in the reaction mixture, no ^{3}H-methylated products were formed (data not shown). When α-pinene was added to the activity assay as the substrate rather than catechol, no ^{3}H-methylated products were detected (data not shown).

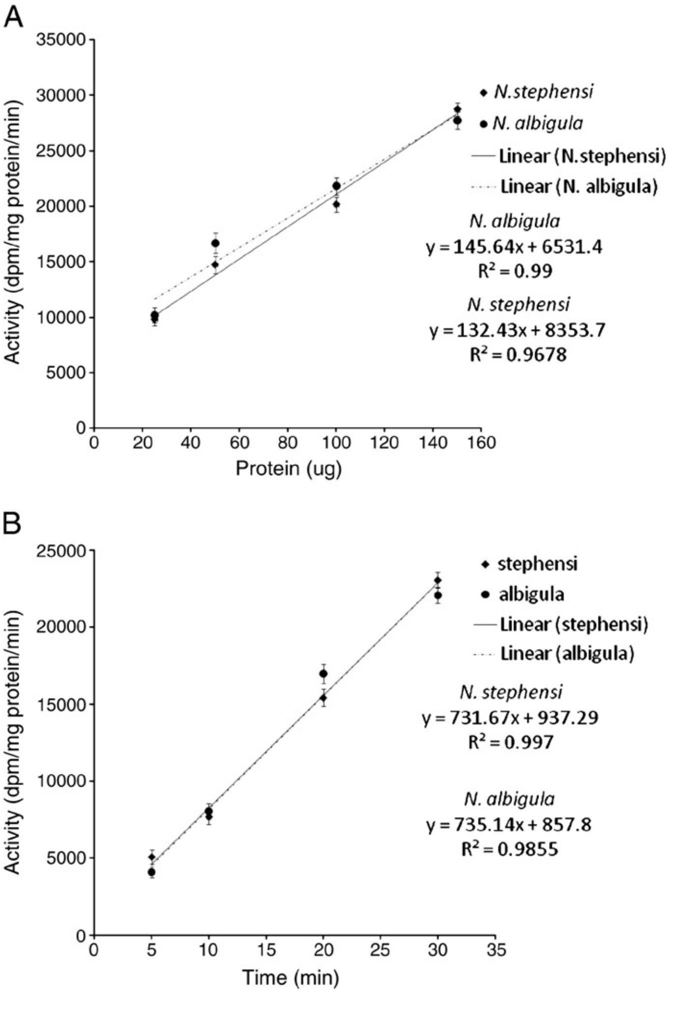

Fig. 2. Activity of liver S-COMT in control *N. stephensi* ($n = 3$) and *N. albigula* ($n = 3$). Panel A shows the linear relationship between the protein concentration of the cytosolic liver fractions of both species and activity. Panel B shows the linear relationship between time and activity of 100 μg of protein from cytosolic fractions of liver extracted from both species of woodrats.

Table 3
Kinetics of COMT in the livers of control woodrats.

Variable	*Neotoma stephensi* (n = 3)		*Neotoma albigula* (n = 3)	
	S-COMT	MB-COMT	S-COMT	MB-COMT
Km (mM catechol)	0.725 ± 0.05[a]	0.145 ± 0.07[b]	0.833 ± 0.34[a]	0.100 ± 0.01[b]
Vmax (mM catechol/mg protein/min)	19167 ± 3632[a,b]	9761 ± 2683[b]	17857 ± 3571[a,b]	20000 ± 1102[a]

[a,b] Different letters (a, b) denote means significantly different ($p \leq 0.05$) as determined by Bonferroni adjusted pairwise comparisons within the same row.

The kinetics of COMT for the substrate catechol differed among the liver fractions and species in the animals fed the control diet tested (Table 3). The K_m for catechol was higher in S-COMT than MB-COMT in the liver fraction in both species ($F_{3,\ 8} = 4.834$ $p = 0.033$). The V_{max} of MB-COMT in *N. albigula* was higher than *N. stephensi* ($F_{3,\ 8} = 4.07$ $p = 0.05$). While our sample size was limited to 3 animals per species, we nonetheless found significant differences between species. Our sample error is also comparable to other studies that used sample sizes of 5–6 animals (Ellingson et al., 1999; Masuda et al., 2002; Tsunoda et al., 2002; Tsunoda and Imai, 2004).

In general, *N. stephensi* had elevated S-COMT activity that was often connected with juniper ingestion compared to *N. albigula*. In contrast, *N. albigula* had higher activity of MB-COMT (see (Table 4) for summary of ANOVAs and Fig. 3). In the blood, *N. albigula* and *N. stephensi* fed the control diet had similar activities of S-COMT while the *N. stephensi* consuming juniper had significantly higher S-COMT activity ($p \leq 0.005$). In the kidneys, *N. albigula* fed the control diet had significantly higher activity of MB-COMT than both groups of *N. stephensi* ($p \leq 0.001$) but significantly lower activity of S-COMT than both groups of *N. stephensi* ($p \leq 0.001$). The *N. stephensi* fed the control diet had similar activity of MB-COMT compared to the *N. stephensi* consuming juniper but a lower activity of S-COMT than the *N. stephensi* consuming juniper in their kidneys ($p = 0.014$). In the liver, *N. albigula* fed the control diet had significantly higher activity of MB-COMT than both groups of *N. stephensi* ($p \leq 0.001$) but significantly lower activity of S-COMT than only the *N. stephensi* consuming juniper ($p = 0.002$). The *N. stephensi* fed the control diet had similar activity of MB-COMT compared to the *N. stephensi* consuming juniper but a lower activity of S-COMT than the *N. stephensi* consuming juniper in their livers ($p = 0.005$).

4. Discussion

Metabolizing and excreting large doses of PSCs may be energetically and physiologically challenging to mammalian herbivores and likely constrains most from being dietary specialists. Phase II enzymes typically produce non-reactive compounds that can be easily excreted in the urine or feces, yet the loss of an energy rich conjugate may significantly compromise an herbivore's energy budget (McLean et al., 1993; McLean and Foley, 1997; Boyle et al., 1999; McLean et al., 2001; Dearing et al., 2005a; Sorensen et al., 2005a, 2005b, 2006). Catechol-O-methyl transferase (COMT) is a phase II enzyme that conjugates a relatively inexpensive methyl group to the xenobiotic, thus it may be an energetically efficient yet physiologically safe pathway that the juniper specialist, *N. stephensi*, utilizes to metabolize the PSCs present in their diets (Skopec et al., 2007). We compared the juniper specialist, *N. stephensi* to *N. albigula*, a dietary generalist, to determine if there were differences in the expression and activity of COMT. We found a number of differences in COMT expression and activity both within and between species and discuss how these differences may influence the physiology of the two species and the implications for COMT as a novel and important pathway for the specialist to metabolize juniper PSCs.

Ingestion of juniper induced the expression and activity of COMT in a range of tissues of the specialist (Figs. 1 and 3). The specialist consuming juniper either in the wild or in captivity showed similar levels of expression and activity of COMT in all tissues tested and was therefore combined into a single group. The lack of difference between the specialist in captivity consuming 70% juniper and those recently trapped from the wild confirm that our 70% juniper diet is ecologically relevant in terms of detoxification enzyme induction. The specialists consuming juniper showed increased expression and activity of S-COMT in their kidneys and livers and increased activity in the blood compared to the specialist and generalist fed the control diet. While the generalist was not fed a diet containing juniper, the upregulation of COMT in response to juniper appears unique to the specialist. In our previous experiment, the generalist showed no change in the expression of COMT mRNA on its ecologically relevant diet of 25% juniper diet compared to a control diet (Dial, 1988; Skopec et al., 2007).

MB-COMT is likely not instrumental in the metabolism of juniper PSCs. The only difference in the expression or activity of MB-COMT in the specialist was that it had a 50% higher expression of liver MB-COMT when feeding on juniper compared to the control diet (Fig. 1). Although it was a significant increase, it was much smaller than the 140% increase in expression of liver S-COMT in the specialist consuming juniper, compared to the control diet (Fig. 1). In laboratory rats and humans, MB-COMT has higher affinity for its substrates but a much lower V_{max} than S-COMT and seems to play a more important role in the metabolism of catecholamines in the nervous system than

Table 4
Summary of ANOVAs for differences in the activity of COMT in the various tissues of woodrats tested.

Source of COMT	F	Df	P
Blood S-COMT	13.06	2,22	0.010
Kidney MB-COMT	157.47	2,22	0.000
Kidney S-COMT	30.64	2,19	0.000
Liver MB-COMT	68.07	2,22	0.000
Liver S-COMT	11.23	2,20	0.001

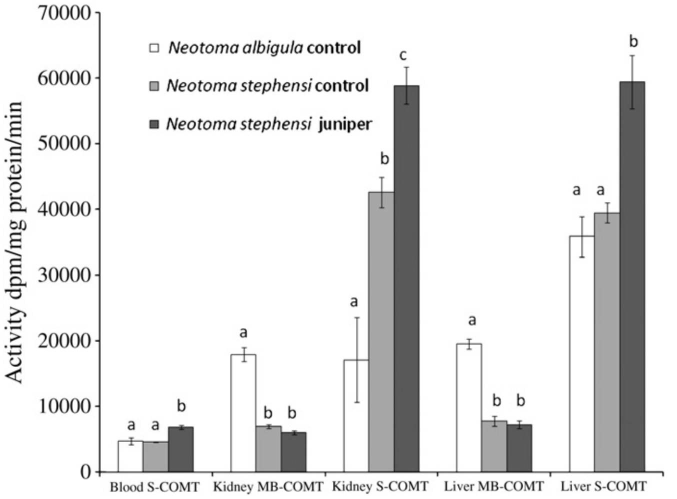

Fig. 3. Activity of the soluble (S-COMT) and membrane bound (MB-COMT) forms of catechol-*O*-methyl transferase (COMT) in the tissues of *N. stephensi*, a juniper specialist, and *N. albigula*, a sympatric generalist, on different diets. Letters a, b and c for each tissue denote means that are significantly different ($p < 0.05$) between species or within species on different diets.

in the metabolism of endogenous or exogenous substrates in peripheral tissues (Männistö and Kaakkola, 1999; Huotari et al., 2002; Zhu, 2002). We found that hepatic S-COMT of the specialist had higher K_m and higher V_{max} values for catechol than MB-COMT. We therefore conclude that the almost seven fold increase in COMT mRNA documented previously (Skopec et al., 2007) was coding for mainly S-COMT translation. Both forms of COMT are transcribed from the same gene with the longer transcript coding for the larger MB-COMT and the shorter transcript coding for S-COMT (Männistö and Kaakkola, 1999; Zhu, 2002).

The COMT enzyme in the specialist consuming juniper most likely metabolizes phenolics in juniper and not terpenes. We found no evidence of a methylated product when α-pinene was substituted for catechol as the substrate in the enzyme assays. As terpenes do not contain any catechol groups for COMT to methylate, it is not surprising that α-pinene was not a substrate. In contrast, it is likely that some of the phenolics in juniper may be substrates for COMT as catechol groups are a common component of plant phenolics (Bravo, 1998; Männistö and Kaakkola, 1999; Heim et al., 2002; Zhu, 2002; Crozier et al., 2009). Total phenolic concentrations in *J. monosperma* vary seasonally from 63 to 79 mg/g foliage but the chemical nature of these phenolics is not well studied (Utsumi et al., 2009). Since it is not possible to order individual phenolics isolated from juniper to test as COMT substrates, looking for differences in methylated metabolites in the urine of the specialists and generalist is the next logical step in understanding how the specialist may use COMT differently than the generalist. Many plant phenolics are known to be methylated by COMT (Zhu et al., 1994; Oddy et al., 1997; Bravo, 1998; Piskula and Terao, 1998; Tsuda et al., 1999; Donovan et al., 2001; Heim et al., 2002; Zhu, 2002; O'Leary et al., 2003; Talavéra et al., 2005; Crozier et al., 2009). Also, the three other major classes of conjugation enzymes known to metabolize phenolics (glutathione-S-transferases, UDP-glucuronosyltransferase, and sulfotransferases) (Oddy et al., 1997; Piskula and Terao, 1998; Donovan et al., 2001; Heim et al., 2002; Lambert et al., 2007; Crozier et al., 2009) are either constitutively low or down-regulated in *N. stephensi* on diets high in juniper (Lamb et al., 2004; Haley et al., 2007; Skopec et al., 2007). The loss of a methyl group as a conjugate may be less costly than glucose or amino acid conjugates used by other phase II enzymes (Klaassen and Watkins, 2003). Therefore the specialist's upregulation of COMT may be an adaptation that allows it to use phase II conjugation and the benefits that go along with it, i.e., broader substrate acceptability and production of less reactive metabolites, while minimizing the loss of a high energy conjugates like glucose or amino acids (McLean et al., 1993; McLean and Foley, 1997; Boyle et al., 1999; McLean et al., 2001; Dearing et al., 2005a; Sorensen et al., 2005a, 2005b, 2006).

The kidneys also appear to play a key role in the processing of PSCs by the specialist based on atypical levels of expression. Both expression and activity of S-COMT were the same if not higher in the kidneys compared to the liver (Figs. 1 and 3). This pattern is unusual compared to that observed in laboratory rats, mice and humans, where S-COMT levels tend to be two to three times higher in the liver than the kidneys (Zhu et al., 1994; Lohr et al., 1998; Ellingson et al., 1999; Männistö and Kaakkola, 1999; Huotari et al., 2002; Zhu, 2002). Therefore, it is not only unusual that *N. stephensi* up-regulates S-COMT in response to juniper in its diet, but also that the specialist has high expression and activity of S-COMT in their kidneys constitutively as well.

There are two possible explanations for the specialist's high kidney S-COMT. First, the high kidney expression and activity of S-COMT may be to augment the liver's metabolism of phenolics (Zhu et al., 1994; Piskula and Terao, 1998; Männistö and Kaakkola, 1999; Tsuda et al., 1999; Talavéra et al., 2005). Second, it is also possible that high expression and activity of S-COMT in the specialist may be an adaptation for living in the desert and feeding on low sodium plants. COMT metabolizes dopamine in the kidney (Wang et al., 2001). Dopamine decreases the reabsorption of sodium in the kidney, causing natriuresis and diuresis (Aperia et al., 1997; Aperia, 2000; Odlind et al., 2000). Both water and sodium are likely limiting to the specialist given that it is a desert dweller (Freeland et al., 1985; Kaspari et al., 2008) and juniper contains less than 0.02% sodium. Moreover, juniper in general, and α-pinene, the major terpene found in one-seeded juniper, are diuretics (Dearing et al., 2001, 2002; Kumar et al., 2010), which may put further stress on the specialist's ability to maintain water balance in its arid environment. By increasing S-COMT expression and activity the specialist may be increasing their kidneys' metabolism of dopamine and therefore increasing sodium and water retention.

As expected, the generalist, *N. albigula,* showed lower expression and activity of S-COMT in their livers and kidneys compared to the specialist consuming juniper. The generalist also had lower MB-COMT expression in kidneys and liver than the specialist, but surprisingly, the generalist had higher hepatic and nephritic MB-COMT activity than the specialist. The difference in the results of the expression versus activity of MB-COMT in the generalist is most likely due to kinetic differences in MB-COMT between the two species. Interestingly, MB-COMT's V_{max} for catechol in *N. albigula* was similar to S-COMT's V_{max} for catechol in both the generalist and specialist. The high V_{max} for MB-COMT is unusual given that in other species tested (humans, mice and rats), MB-COMT tends to have higher affinity but lower V_{max} for substrates than S-COMT (Männistö and Kaakkola, 1999; Zhu, 2002). The MB-COMT K_m for catechol in the generalist was not significantly different from that in the specialist, but the MB-COMT V_{max} for catechol in the generalist was more than double that of the specialist. The much higher V_{max} in the generalist means that it can convert catechol to the methylated product (guaiacol) at a much faster rate than the specialist.

Generally MB-COMT is more highly expressed in the central nervous system and plays a critical role in the metabolism of centrally acting catecholamines and thus behavior (Männistö and Kaakkola, 1999; Huotari et al., 2002; Zhu, 2002). High MB-COMT activity is linked to aberrant human behaviors such as attention deficit disorder, antisocial behavior and polysubstance abuse (Zhu, 2002; Langley et al., 2010). Indeed, a COMT inhibitor cures compulsive kleptomania (Grant, 2011). The high V_{max} of MB-COMT in *N. albigula* may play an important role in governing the behavior of the generalist. Woodrats are prolific cachers and collect and store plants as well as inedible objects they find in their middens (Betancourt et al., 1990; Torregrossa and Dearing, 2009a). Perhaps the MB-COMT's high V_{max} in the generalists leads it to display different behaviors than the specialist, such as the generalist collecting and caching many different types of plants to support their more generalized diet. Dial and Czaplewski (1990) compared the midden contents of *N. stephensi* and *N. albigula* where they occur sympatrically and found that the generalist was a more eclectic cacher than the specialist. In addition, running behavior differs between the species in captivity. The generalist runs on running wheels twice the distance as the specialist (Sorensen et al., 2005b). Further studies are needed to evaluate whether high activity MB-COMT in the generalist alters behavior compared to the specialist.

5. Conclusions

To our knowledge this is the first study to show tissue distribution and enzyme kinetics of COMT in a wild mammalian herbivore. The specialist, *N. stephensi's,* upregulation of S-COMT in the liver and also kidneys is a unique response and may be an integral part of its ability to specialize on juniper. We also found that the generalist, *N. albigula* has an unusually high MB-COMT V_{max} and future studies are necessary to determine if this causes unique behaviors in the generalist.

Acknowledgments

The authors would like to acknowledge the adept technical assistance of the following undergraduate assistants: Mike Coombs, Andrew Hale, Ethan King, Cody Nebeker, Erica Williams, and Mike Young. Funding was provided by NSF IOS 0817527 to M.D. Dearing.

References

Adams, R.P., 2000. The serrate leaf margined *Juniperus* (Section Sabina) of the western hemisphere: systematics and evolution based on leaf essential oils and Random Amplified Polymorphic DNAs (RAPDs). Biochem. Syst. Ecol. 28, 975–990.

Adams, R.P., 2011. Junipers of the world: The genus Juniperus. Trafford Publishing, Bloomington, IN.

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

Adams, R.P., Von Rudloff, E., Hogge, L., 1983. Chemosystematic studies of the western North American junipers based on their volatile oils. Biochem System Ecol 11, 189–193.

Aperia, A.C., 2000. Intrarenal dopamine: a key signal in the interactive regulation of sodium metabolism. Annu. Rev. Physiol. 62, 621–647.

Aperia, A., Eklöf, A.-C., Holtbáck, U., Nowicki, S., Sundelöf, M., Greengard, P., David, S., Goldstein, G.E., Richard, M., 1997. The Renal Dopamine System. Adv. Pharmacol. 42, 870–873.

Ayala, A., Cutler, R.G., 1997. Preferential use of less toxic detoxification pathways by long-lived species. Arch. Gerontol. Geriatr. 24, 87–102.

Betancourt, J.L., Devender, T., Martin, P.S., 1990. Packrat middens: the last 40,000 years of biotic change. University of Arizona Press, Tucson, AZ.

Bock, K.W., Köhle, C., 2004. Coordinate regulation of drug metabolism by xenobiotic nuclear receptors: UGTs acting together with CYPs and glucuronide transporters. Drug Metabol. Revs. 36, 595–615.

Boyle, R., McLean, S., Foley, W.J., Davies, N.W., 1999. Comparative metabolism of dietary terpene, p-cymene, in generalist and specialist folivorous marsupials. J. Chem. Ecol. 25, 2109–2126.

Bravo, L., 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev. 56, 317–333.

Christians, J.K., 1999. Controlling for body mass effects: is part-whole correlation important? Physiol. Biochem. Zool. 72, 250–253.

Crozier, A., Jaganath, I.B., Clifford, M.N., 2009. Dietary phenolics: chemistry, bioavailability and effects on health. Nat. Prod. Rep. 26, 1001–1043.

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

Dearing, M.D., Mangione, A.M., Karasov, W.H., 2001. Plant Secondary Compounds as Diuretics: An Overlooked Consequence. Am. Zool. 41, 890–901.

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

Dearing, M.D., Foley, W.J., McLean, S., 2005a. The influence of plant secondary metabolites on the nutritional ecology of herbivorous terrestrial vertebrates. Annu. Rev. Ecol. Evol. Syst. 36, 169–189.

Dearing, M.D., McLister, J.D., Sorensen, J.S., 2005b. Woodrat (*Neotoma*) herbivores maintain nitrogen balance on a low-nitrogen, high-phenolic forage, *Juniperus monosperma*. J. Comp. Physiol. B 175, 349–355.

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

Dial, K., Czaplewski, C., 1990. Do packrat middens accurately represent the animals' environment or diet? The Woodhouse Mesa study. In: Bentecourt, J.L., Devender, T., Martin, P.S. (Eds.), Fossil packrat middens: the last 40,000 years of biotic change. University of Arizona Press, Tucson, AZ, pp. 43–58.

Donovan, J.L., Crespy, V., Manach, C., Morand, C., Besson, C., Scalbert, A., Rémésy, C., 2001. Catechin is metabolized by both the small intestine and liver of rats. J. Nutr. 131, 1753–1757.

Ellingson, T., Duddempudi, S., Greenberg, B.D., Hooper, D., Eisenhofer, G., 1999. Determination of differential activities of soluble and membrane-bound catechol-O-methyltransferase in tissues and erythrocytes. J. Chromatogr. B Biomed. Sci. Applic. 729, 347–353.

Franklin, M.R., Estabrook, R.W., 1971. On the inhibitory action of mersalyl on microsomal drug oxidation: A rigid organization of the electron transport chain. Arch. Biochem. Biophys. 143, 318–329.

Freeland, W., 1991. In: Palo, R.T., Robbins, C.T. (Eds.), Plant secondary metabolites: biochemical coevolution with herbivores. Plant defenses against mammalian herbivores. CRC Press, Boca Raton, FL, pp. 61–81.

Freeland, W.J., Janzen, D.H., 1974. Strategies in Herbivory by Mammals: The Role of Plant Secondary Compounds. Am. Nat. 108, 269–289.

Freeland, W., Calcott, P., Geiss, D., 1985. Allelochemicals, minerals and herbivore population size. Biochem System Ecol 13, 195–206.

Grant, J.E., 2011. Kleptomania treated with tolcapone, a catechol-O-methyl-transferase (COMT) inhibitor. Progr. Neuro-Psychopharmacol. Biol. Psychiatr. 35, 295–296.

Guengerich, F.P., 2006. Cytochrome P450s and other enzymes in drug metabolism and toxicity. AAPS J. 8, 101–111.

Gygi, S.P., Rochon, Y., Franza, B.R., Aebersold, R., 1999. Correlation between protein and mRNA abundance in yeast. Mol. Cell. Biol. 19, 1720.

Haley, S.L., Lamb, J.G., Franklin, M.R., Constance, J.E., Denise Dearing, M., 2007. Xenobiotic metabolism of plant secondary compounds in juniper (*Juniperus monosperma*) by specialist and generalist woodrat herbivores, genus *Neotoma*. Comp. Biochem. Physiol. C 146, 552–560.

Heim, K.E., Tagliaferro, A.R., Bobilya, D.J., 2002. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J. Nutr. Biochem. 13, 572–584.

Hollenberg, P.F., 2002. Characteristics and common properties of inhibitors, inducers, and activators of CYP enzymes. Drug Metabol. Rev. 34, 17–35.

Huotari, M., Gogos, J.A., Karayiorgou, M., Koponen, O., Forsberg, M., Raasmaja, A., Hyttinen, J., Männistö, P.T., 2002. Brain catecholamine metabolism in catechol-O-methyltransferase (COMT) deficient mice. Eur. J. Neurosci. 15, 246–256.

Kaspari, M., Yanoviak, S.P., Dudley, R., 2008. On the biogeography of salt limitation: A study of ant communities. Proc. Natl. Acad. Sci. U S A 105, 17848–17851.

Klaassen, C.D., Watkins, J.B., 2003. Casarett & Doull's essentials of toxicology. McGraw-Hill, New York.

Kumar, P., Bhatt, R., Singh, L., Chandra, H., Prasad, R., 2010. Identification of phytochemical content and antibacterial activity of *Juniperus communis* leaves. Int. J. Biotechnol. Biochem. 6, 87–91.

Lamb, J.G., Marick, P., Sorensen, J., Haley, S., Dearing, M.D., 2004. Liver biotransforming enzymes in woodrats *Neotoma stephensi* (Muridae). Comp. Biochem. Physiol. C 138, 195–201.

Lambert, J.D., Sang, S., Lu, A.Y.H., Yang, C.S., 2007. Metabolism of dietary polyphenols and possible interactions with drugs. Curr. Drug Metabol. 8, 499–507.

Langley, K., Heron, J., O'Donovan, M.C., Owen, M.J., Thapar, A., 2010. Genotype link with extreme antisocial behavior: the contribution of cognitive pathways. Arch. Gen. Psychiatry 67, 1317–1323.

Lautala, P., Ulmanen, I., Taskinen, J., 2001. Molecular mechanisms controlling the rate and specificity of catechol O-methylation by human soluble catechol O-methyltransferase. Mol. Pharmacol. 59, 393–402.

Lohr, J.W., Willsky, G.R., Acara, M.A., 1998. Renal drug metabolism. Pharmacol. Rev. 50, 107–142.

Mangione, A.M., Dearing, M.D., Karasov, W.H., 2004. Creosote bush (*Larrea tridentata*) resin increases water demands and reduces energy availability in desert woodrats (Neotoma *lepida*). J. Chem. Ecol. 30, 1409–1429.

Männistö, P.T., Kaakkola, S., 1999. Catechol-O-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. Pharmacol. Rev. 51, 593–628.

Marsh, K., Wallis, I., Andrew, R., Foley, W., 2006. The detoxification limitation hypothesis: where did it come from and where is it going? J. Chem. Ecol. 32, 1247–1266.

Masuda, M., Tsunoda, M., Yusa, Y., Yamada, S., Imai, K., 2002. Assay of catechol-O-methyltransferase activity in human erythrocytes using norepinephrine as a natural substrate. Ann. Clin. Biochem. 39, 589–594.

McLean, S., Foley, W.J., 1997. Metabolism of *Eucalyptus* terpenes by herbivorous marsupials. Drug Metabol. Rev. 29, 213–218.

McLean, S., Foley, W.J., Davies, N.W., Brandon, S., Duo, L., Blackman, A.J., 1993. Metabolic fate of dietary terpenes from *Eucalyptus radiata* in common ringtail possum (*Pseudocheirus peregrinus*). J. Chem. Ecol. 19, 1625–1643.

McLean, S., Pass, G., Foley, W., Brandon, S., Davies, N., 2001. Does excretion of secondary metabolites always involve a measurable metabolic cost? Fate of plant antifeedant salicin in common brushtail possum, *Trichosurus vulpecula*. J. Chem. Ecol. 27, 1077–1089.

McLister, J.D., Sorensen, J.S., Dearing, M.D., 2004. Effects of consumption of juniper (*Juniperus monosperma*) on cost of thermoregulation in the woodrats *Neotoma albigula* and *Neotoma stephensi* at different acclimation temperatures. Physiol. Biochem. Zool. 77, 305–312.

Oddy, E., Manchee, G., Coughtrie, M., 1997. Assessment of rat liver slices as a suitable model system for studying the simultaneous sulphation and glucuronidation of phenolic xenobiotics. Xenobiotica 27, 369–378.

Odlind, C., Göransson, V., Reenilä, I., Hansell, P., 2000. Regulation of dopamine-induced batriuresis by the dopamine-metabolizing enzyme catechol-O-methyltransferase. Exp. Nephrol. 7, 314–322.

O'Leary, K.A., Day, A.J., Needs, P.W., Mellon, F.A., O'Brien, N.M., Williamson, G., 2003. Metabolism of quercetin-7-and quercetin-3-glucuronides by an in vitro hepatic model: the role of human [beta]-glucuronidase, sulfotransferase, catechol-O-methyltransferase and multi-resistant protein 2 (MRP2) in flavonoid metabolism. Biochem. Pharmacol. 65, 479–491.

Piskula, M.K., Terao, J., 1998. Accumulation of (−)-epicatechin metabolites in rat plasma after oral administration and distribution of conjugation enzymes in rat tissues. J. Nutr. 128, 1172–1178.

Shipley, L.A., Forbey, J.S., Moore, B.D., 2009. Revisiting the dietary niche: When is a mammalian herbivore a specialist? Integr. Comp. Biol. 49, 274–290.

Skopec, M.M., Haley, S., Dearing, M.D., 2007. Differential hepatic gene expression of a dietary specialist (*Neotoma stephensi*) and generalist (*Neotoma albigula*) in response to juniper (*Juniperus monosperma*) ingestion. Comp. Biochem. Physiol. D 2, 34–43.

Sorensen, J.S., McLister, J.D., Dearing, M.D., 2005a. Novel plant secondary metabolites impact dietary specialists more than generalists (*Neotoma spp.*). Ecology 86, 140–154.

Sorensen, J.S., McLister, J.D., Dearing, M.D., 2005b. Plant secondary metabolites compromise the energy budgets of specialist and generalist mammalian herbivores. Ecology 86, 125–139.

Sorensen, J.S., Skopec, M.M., Dearing, M.D., 2006. Application of pharmacological approaches to plant–mammal interactions. J. Chem. Ecol. 32, 1229–1246.

Talavéra, S., Felgines, C., Texier, O., Besson, C., Gil-Izquierdo, A., Lamaison, J.L., Rémésy, C., 2005. Anthocyanin metabolism in rats and their distribution to digestive area, kidney, and brain. J. Agric. Food Chem. 53, 3902–3908.

Torregrossa, A.M., Dearing, M.D., 2009a. Caching as a behavioral mechanism to reduce toxin intake. J. Mammal. 90, 803–810.

Torregrossa, A.M., Dearing, M.D., 2009b. Nutritional toxicology of mammals: regulated intake of plant secondary compounds. Funct. Ecol. 23, 48–56.

Tsuda, T., Horio, F., Osawa, T., 1999. Absorption and metabolism of cyanidin 3-O-[beta]–glucoside in rats. FEBS Lett. 449, 179–182.

Tsunoda, M., Imai, K., 2004. An assay for determination of rat adrenal catechol-O-methyltransferase activity: comparison of spontaneously hypertensive rats and Wistar–Kyoto rats. Anal. Bioanal. Chem. 380, 887–890.

Tsunoda, M., Takezawa, K., Masuda, M., Imai, K., 2002. Rat liver and kidney catechol O methyltransferase activity measured by high performance liquid chromatography with fluorescence detection. Biomed. Chromatogr. 16, 536–541.

Utsumi, S.A., Cibils, A.F., Estell, R.E., Soto-Navarro, S.A., Van Leeuwen, D., 2009. Seasonal changes in one seed juniper intake by sheep and goats in relation to dietary protein and plant secondary metabolites. Small Rumin. Res. 81, 152–162.

Vaughn, T.A., 1982. Stephen's woodrat, a dietary specialist. J. Mammal. 63, 53–62.

Wang, Y., Berndt, T.J., Gross, J.M., Peterson, M.A., So, M.J., Knox, F.G., 2001. Effect of inhibition of MAO and COMT on intrarenal dopamine and serotonin and on renal function. Am. J. Physiol. 280, R248–R254.

Westoby, M., 1974. An analysis of diet selection by large generalist herbivores. Am. Nat. 108, 290–304.

Westoby, M., 1978. What are the biological bases of varied diets? Am. Nat. 112, 627–631.

Wilkinson, L., Coward, M., 2000. SYSTAT 10: Statistics 1. SPSS, Chicago, IL.

Zhu, B.T., 2002. Catechol-O-Methyltransferase (COMT)-mediated methylation metabolism of endogenous bioactive catechols and modulation by endobiotics and xenobiotics: importance in pathophysiology and pathogenesis. Curr. Drug Metabol. 3, 321–349.

Zhu, B.T., Ezell, E.L., Liehr, J.G., 1994. Catechol-O-methyltransferase-catalyzed rapid O-methylation of mutagenic flavonoids. Metabolic inactivation as a possible reason for their lack of carcinogenicity in vivo. J. Biol. Chem. 269, 292–299.

Zürcher, G., Prada, M., 1982. Rapid and sensitive single step radiochemical assay for catechol-O- methyltransferase. J. Neurochem. 38, 191–195.