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Physiology

Warmer ambient temperatures depress liver function in a mammalian herbivore

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Diet selection in mammalian herbivores is thought to be mainly influenced by intrinsic factors such as nutrients and plant secondary compounds, yet extrinsic factors like ambient temperature may also play a role. In particular, warmer ambient temperatures could enhance the toxicity of plant defence compounds through decreased liver metabolism of herbivores. Temperature-dependent toxicity has been documented in pharmacology and agriculture science but not in wild mammalian herbivores. Here, we investigated how ambient temperature affects liver metabolism in the desert woodrat, *Neotoma lepida*. Woodrats ($n = 21$) were acclimated for 30 days to two ambient temperatures (cool = 21°C, warm = 29°C). In a second experiment, the temperature exposure was reduced to 3.5 h. After temperature treatments, animals were given a hypnotic agent and clearance time of the agent was estimated from the duration of the hypnotic state. The average clearance time of the agent in the long acclimation experiment was 45% longer for animals acclimated to 29°C compared with 21°C. Similarly, after the short exposure experiment, woodrats at 29°C had clearance times 26% longer compared with 21°C. Our results are consistent with the hypothesis that liver function is reduced at warmer environmental temperatures and may provide a physiological mechanism through which climate change affects herbivorous mammals.

1. Introduction

Diet selection in mammalian herbivores is well known to be influenced by intrinsic factors such as nutrients and plant secondary compounds as well as by their interactions [1]. However, the influence of environmental factors like ambient temperature has received less attention. There has been growing evidence for a phenomenon called temperature-dependent toxicity (TDT) [2]. Under this paradigm, the toxicity of dietary plant secondary compounds is predicted to increase at warmer temperatures owing to interactions between ambient temperature and mammalian physiology. If plant secondary compounds are more toxic to mammalian herbivores at higher temperatures, then a warming environment could greatly affect the diet selection of mammalian herbivores.

Multiple lines of evidence provide support for TDT. Pharmacologists have reported decreases in the lethal dose of numerous plant-derived compounds when administered to laboratory rodents at higher ambient temperatures [3]. Agricultural scientists have documented that a deleterious condition in cattle known as fescue toxicosis, caused by fungal toxins in infected grasses, was exacerbated at warmer temperatures [4]. Lastly, ecologists have shown that herbivorous rodents experienced temperature-mediated changes in the selection of diets containing plant secondary compounds [5].

TDT is thought to be caused by a decrease in liver function at warmer temperatures. The liver is responsible for drug metabolism, and as the largest visceral organ, also plays a key role in thermoregulation [6]. Previous work in laboratory rodents has documented decreases in hepatic gene expression of xenobiotic metabolism and in hepatic enzyme activity at warmer temperatures [7,8]. Energetic processes like biotransformation may become compromised in mammals at higher temperatures as they reach their maximal capacity to dissipate body heat [9].

Table 1. Body mass (mean ± s.e.) of desert woodrats (*N. lepida*) before and after two temperature experiments. Sample size for each experiment is indicated.

	long, 30 day acclimation ($n = 13$)		short, 3.5 h exposure ($n = 15$)
temperature	before (g)	after (g)	after (g)
cool	113.6 (±6.9)	119.3 (±7.3)	115.8 (±3.1)
warm	120.1 (±7.8)	123.7 (±8.2)	115.6 (±3.1)

The hypothesis that liver function decreases with increasing ambient temperature has not been tested in an ecologically and evolutionarily relevant system. Here, we compared liver function of herbivorous rodents exposed to two ambient temperatures with a non-destructive assay. Two experiments were conducted whereby animals were acclimated to temperature treatments for 30 days or exposed for 3.5 h prior to measuring liver function. We predicted that liver function of herbivorous rodents would decrease at warmer temperatures.

2. Material and methods

Woodrats (genus *Neotoma*) are nocturnal, herbivorous rodents that feed on an array of toxic plants [10,11]. Populations of the desert woodrat (*Neotoma lepida*) inhabiting the Mojave Desert feed mainly on creosote bush (*Larrea tridentata*), the resin of which contains toxic phenolic compounds [12,13]. We collected 21 animals from the Mojave Desert near Beaver Dam, Utah (37°06′ N, 113°58′ W) in October 2010 and July 2011. Animals were brought to the University of Utah (Salt Lake City), maintained at room temperature (23–25°C) on a 12 L : 12 D cycle and fed rabbit chow (Harlan Teklad 2031) for at least three months before experiments.

The two temperature treatments were 'cool' (21°C) and 'warm' (29°C). As in our previous studies [5,14], these temperatures were selected to be within the thermal neutral zone (29°C) or just below this zone (21°C) for *N. lepida* (see electronic supplementary material, figure S1). The temperatures are commonly experienced by animals in the Mojave Desert for up to six months of the year based on monthly mean and daily maximum values (see the electronic supplementary materials). All animals were exposed to cool and warm temperature treatments after which non-destructive liver function assays were conducted. A cross-over design was used to control for temperature order. Two experiments were conducted with different acclimation periods. A longer, 30 day acclimation period permitted physiological changes to occur, similar to responses across seasons (e.g. changes in insulation). A shorter, 3.5 h exposure period removed any acclimation effect and better reflected daily temperature fluctuations in nature. Animals were returned to room temperature for 3 days between temperature treatments during the 30 day experiment and for at least two weeks during the 3.5 h experiment. Room temperatures were controlled with thermostats and space heaters (Delonghi Safe Heat, Model DUH30; electronic supplementary material, table S1).

Hypnotic state assays were conducted to measure liver function after each temperature treatment. These assays are a validated method of whole-organism liver function and are non-destructive, which allow paired *t*-test analyses [8]. Briefly, a hypnotic agent was administered (IP, 100 mg kg^{-1}) 4 h into the light cycle, and animals were returned to home cages. The length of time spent in the hypnotic state, as indicated by the inability of the animal to right itself, was recorded for every animal in each temperature treatment as a proxy for toxin clearance time. Longer clearance times indicated decreased liver function. The hypnotic agent, hexobarbital, was used as a proxy compound for dietary toxins consumed by this species [15,16]. A minimum wash-out period of seven days was observed after each assay. No difference in clearance time between light and dark cycles was expected because toxin concentration has a greater influence on hepatic systems involved with drug metabolism compared with circadian rhythmicity in rodents [17]. Moreover, after the hexobarbital injection, animals were placed on their back, which is not a natural sleeping posture for woodrats.

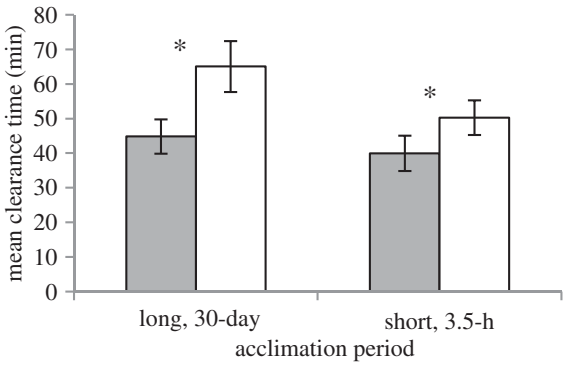

Figure 1. Clearance time (mean ± s.e.) of hexobarbital in desert woodrats (*N. lepida*) at two ambient temperatures (grey = 21°C, white = 29°C) during two acclimation periods. Asterisks indicate $p < 0.05$.

3. Results

Liver function of the desert woodrat was significantly lower at higher ambient temperatures. After the 30 day experiment, average clearance times were 45% longer in the warm temperature group compared with the cool group ($t = -3.31$; d.f. = 12; $p = 0.006$; figure 1). After the 3.5 h experiment, average clearance times were 26% longer in the warm group compared with the cool group ($t = -2.15$; d.f. = 14; $p = 0.049$; figure 1).

We observed qualitative changes in nesting behaviour during the 30 day experiment. Cool-acclimated animals constructed large nests out of paper towels, shavings and cotton batting, and were often found in their nests (figure 2*a*). By contrast, warm-acclimated animals cleared all bedding material from an area of the home cage (figure 2*b*) and were often found sprawled in the cleared area.

Body mass remained relatively unchanged during experiments (table 1). There was no difference in average body mass between cool and warm temperature groups prior to the long acclimation period ($t = -1.67$; d.f. = 12; $p = 0.12$), however, the temperature groups differed slightly after the long acclimation period ($t = -2.17$; d.f. = 12; $p = 0.051$). Average body mass did not differ between temperature groups after the short exposure period ($t = 0.13$; d.f. = 14; $p = 0.898$).

4. Discussion

Evidence from pharmacology and agricultural science has demonstrated that toxicity increases with elevated temperatures,

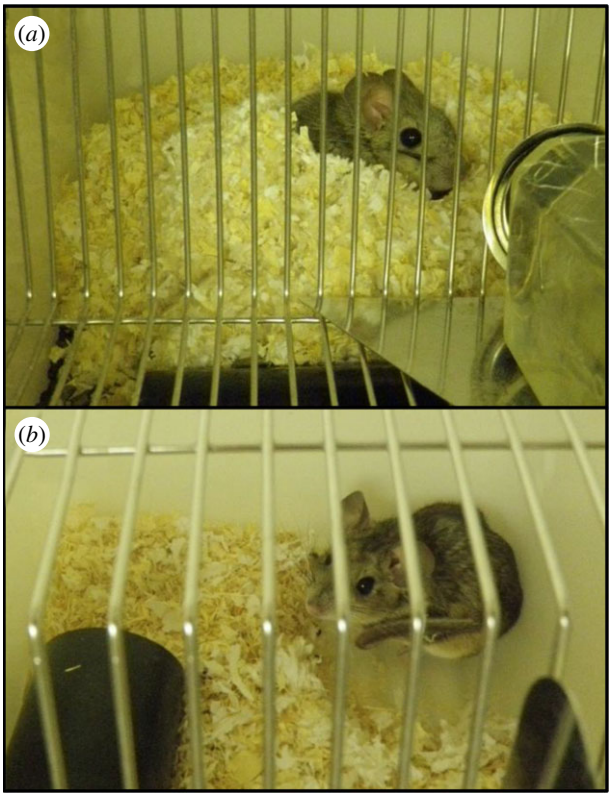

Figure 2. *Neotoma lepida* nesting behaviour at (*a*) cool, 21°C and (*b*) warm, 29°C temperatures. (Online version in colour.)

yet this phenomenon has remained untested in an ecologically and evolutionarily relevant system. Here, we investigated the effect of ambient temperature on mammalian liver function in the desert woodrat. Our results revealed that liver clearance is dependent on ambient temperature, and animals at warmer temperatures showed significantly reduced clearance compared with cooler temperatures. This pattern is apparent regardless of temperature exposure time (figure 1), representing the lack of an acclimation effect. It is worth noting that the warm temperature (29°C) was within *N. lepida*'s thermal neutral zone, which is the range of ambient temperatures were metabolic rates are lowest and considered optimal with respect to energy use. Our data suggest that even acute exposures to warmer temperatures could significantly impair liver function and potentially the detoxification capacity of the desert woodrat. With up to 75% of its diet comprised creosote bush [13], *N. lepida* ingest remarkable doses of toxins at every meal and increased toxicity caused by a warming environment could become an insurmountable challenge.

The physiological mechanism responsible for reduced detoxification capacity in the mammalian liver with increasing temperature is speculative. Laboratory rodents experienced decreases in both hepatic enzyme activity and gene expression of key biotransformation enzymes at warmer temperatures [7,8]. The underlying cause of these decreases is most probably the result of an increase in peripheral circulation under high temperatures to increase heat dissipation and protect the liver [18].

It is possible that small mammals like woodrats may be able to mitigate the reduction in liver function by seeking out cooler microclimates. The desert environment is thermally heterogeneous and cooler ambient temperatures may be present in places like drainages. As detoxification occurs on the scale of minutes to hours, animals using such microclimates may be more exposed to predation during this time. Regardless, the use of behavioural adaptations by herbivores for mitigation remains to be examined.

Although this study investigated a single species, our results may have critical implications for other mammalian herbivores given predicted temperature regimes. As warmer temperatures result in decreased liver function, mammalian herbivores may be forced to change their foraging strategy to reduce intake of toxic plant secondary compounds [2]. However, alternative food sources may be unavailable. If herbivores are forced to decrease toxin intake without an alternative food source, then these mammals would probably experience a reduction in overall energy intake. In a potentially cascading effect, herbivores might move to other environments. Thus, through decreased liver function, TDT may be able to partially explain the observed population shifts of small mammalian herbivores to higher elevations and latitudes [19,20]. In fact, a recent review found food availability to be the most common cause for climate-mediated extinctions [21]. Our results suggest that predictions of increased surface temperature and extreme weather events like heat-waves [22] may pose a yet unrecognized threat to mammalian herbivores, which comprise over 40% of extant mammalian species [23].

Hypnotic state assays offer multiple benefits as a measurement of liver function. Unlike traditional assays that require liver tissue and specialized equipment, hypnotic state assays are non-destructive thereby permitting repeated measurements from a single individual. Additionally, the hepatic enzymes that metabolize hypnotic agents also metabolize plant toxins. Hexobarbital, the agent used in this study, is metabolized by cytochromes P450 2B that are important for the detoxification of plant toxins found in creosote bush [15,16]. Lastly, hypnotic state assays are a tool accessible to physiological ecologists working with non-model species that can aid in elucidating often complex plant–mammal interactions [15]. Thus, further study of TDT in a wide range of mammalian herbivores is achievable.

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