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Interplay between metabolic rate and diet quality in the South American fox, *Pseudalopex culpaeus*

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Abstract

We studied the metabolic costs associated with the ingestion of peppertree fruits (*Schinus molle*) in the culpeo fox, *Pseudalopex culpaeus*, the second largest canid in South America. Throughout its range of distribution, this fox feeds on rodents and other small vertebrates, and also on peppertree fruits, which represent 98% of total fruits consumed in semiarid Chile. Peppertree contains a high diversity of phytochemicals. Foxes feeding on diets containing rats and peppertree fruits (mixed diets) exhibited a 98.9% increase in basal rate of metabolism when compared to rat-acclimated foxes. Thus, acute ingestion of chemically defended fruits has an energetic cost for the fox, reflected in higher values of basal metabolism. Increased metabolic rates may be associated with increased protein synthesis for detoxification and for tissue repair, including the production of biotransformation enzymes.

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1. Introduction

Animals cope with seasonal variations in food and energy availability by physiological and/or behavioral mechanisms. They may adjust to reduced dietary quality by regulating their metabolism to reduce energy needs or by altering their gut morphology to extract and assimilate more energy from their diet (McNab, 1986, 1988, 2002; Lovegrove, 2000).

A food item may be selected because of its nutritional value or rejected because of its toxicity. It has been hypothesized that small mammals

lower their basal energy expenditure to survive on a diet of poor quality (Choshniak and Yahav, 1987; Veloso and Bozinovic, 1993; Cork, 1994; Koteja, 1996). Indeed, small mammals maintained chronically on a high-fiber (low quality food) diet have lower basal metabolic rate (BMR) than those consuming a low-fiber diet (Veloso and Bozinovic, 1993; Koteja, 1996). Nevertheless, acute treatments have not detected effects of diet quality on BMR among small mammals (Choshniak and Yahav, 1987; Bozinovic, 1995; Bozinovic and Novoa, 1997; Bozinovic et al., 1997). To the contrary, large mammals exhibit metabolic depression during periods of experimental fasting. In fact, this is the most common response of endotherms to experimental under-nourishment or fasting (Harlow, 1981; Fuglei and Øritsland, 1999; Rosen and Trites, 1999, 2002).

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Vertebrates respond to plant secondary compounds by the formation of inactive complexes, molecular degradation or excretion. All these mechanisms are capable of affecting the rate of metabolism (Thomas et al., 1988; McArthur et al., 1991; Foley and McArthur, 1994; Foley et al., 1995; Mangione et al., 2001). Thus, the amount of plant secondary metabolites that an individual can ingest is related to its capacity to detoxify and eliminate them (Freeland and Janzen, 1974; McNab, 2002). If toxic secondary compounds are not detoxified, they may have a variety of consequences. For example, meadow voles processing secondary compounds increase their rate of metabolism by 14–23% (Thomas et al., 1988). This capability is ecologically important because it constitutes a constraint on how much of a food containing toxins can be ingested, and hence may be used to predict diets (Belovsky and Schmitz, 1991).

We studied the physiological effect of an acute ingestion of peppertree fruits by the large South American fox, *Pseudalopex culpaeus*. The culpeo fox is the second largest (6–8 kg) in South America (Ginsberg and Macdonald, 1990). Throughout its range of distribution, it feeds on rodents and other small vertebrates, but it also feeds on fruits (Medel and Jaksic, 1988; Jaksic, 1997). Of 4488 feces analyzed in semiarid Chile from September 1988 to January 2001, 12.4% contained only fruits, 32% contained fruits and vertebrates and 55.6% contained only vertebrates (Silva, 2001). This author reported that fruits of the peppertree *Schinus molle* (Anacardiaceae) represent 98% of total fruits consumed by the culpeo fox. Previously, Castro et al. (1994) reported for other environments of northern Chile that peppertree fruit represent 82% of total fruits consumed. These two studies report that maximum fruit consumption occurred between April and August, a period of low availability of animal prey. The Phytochemical Database (2001), see also <http://www.raintree-health.co.uk/plants> (peppertree.html), reports that peppertree contains tannins, alkaloids, flavonoids, steroidal saponins, sterols, terpenes, gums and essential oils, while its fruits are rich in triterpenes, sesquiterpenes and monoterpenes (see also Cipollini, 2000).

We investigated the consequences of shifting the diet of culpeo fox from high (rat diet) to low energy items (mixed diet of rat and peppertree fruit). Our specific goal was to study the metabolic

cost associated with the ingestion of peppertree fruits.

2. Material and methods

Between May 1998 and June 2000, 11 non-reproductive adult individuals of culpeo fox (7 males and 4 females), ranging in body mass from 2600 to 7100 g were captured in central Chile at Quebrada de la Plata (33°31'S, 70°50'W, elevation from 400 to 850 m) and in northern Chile at Aucó (31°30'S, 71°06'W, elevation from 400 to 1700 m). Five foxes were trapped during August 1998–December 1998 (three males and two females, assigned to rat diet), three in June 1999 (two males and one female, assigned to mixed diet), two in August 1999 (two males, assigned to mixed diet) and one female in June 2000 (assigned to fruit diet). Treatments were designed according to the field diet of culpeo fox (Castro et al., 1994; Silva, 2001). Foxes were caught using padded leg-hold traps (Victor Soft Catch No. 1.5, Chagnon's Trapping Supply, Manistique, MI). Captured foxes were immobilized with Ketamine hydrochloride (0.1–0.2 ml/kg estimated body mass). Weight, age (estimated by tooth wear) and gender were recorded.

Foxes were transported in iron cages (1.0×1.0×0.8 m³) to the laboratory on the day of capture. Animals were individually housed in iron cages (2.0×1.5×1.0 m³) in an animal room (4.0×10.0×2.5 m³). The 11 foxes were maintained during the first 4 days on ground beef and water ad libitum with a photoperiod of 12L:12D and ambient temperature of 20 °C (control diet), and then BMR was measured. After taking BMR measurements (control BMR), 5 individuals per group were assigned to two experimental diets: live laboratory rats (400 g/day) and a mixed diet (proportion 1:4 of sliced laboratory rats to peppertree fruits, supplied ad libitum) and maintained during 10 days on each treatment. In addition, we measured 1 individual maintained during 10 days with an exclusive diet of peppertree fruits. We did not acclimate more individuals to the peppertree diet because we knew that culpeo foxes do not consume these fruits in isolation of other food items (Silva, 2001). In sum, we had four different diets: (1) control diet, with ground beef and water ad libitum; first 4 days for 11 foxes. (2) Rat diet, with live laboratory rats; 10 days for 5 foxes. (3) Mixed diet, with sliced laboratory rats and fruits;

10 days for 5 foxes. (4) Fruit diet, exclusively of peppertree fruit; 10 days for 1 fox.

BMR was measured in a computerized (Datacan V) open-flow respirometry system (Sable Systems, Henderson, NV) in a metabolic chamber of 588 l ($1.2 \times 0.7 \times 0.7 \text{ m}^3$) at ambient temperatures (T_a) ranging from 20 to 25 °C, within the thermoneutral zone for this species (based on $T_b - T_a = 5.35 m_b^{0.18}$ with $T_b = 37 \text{ °C}$; McNab, 1988). The metabolic chamber received dried air at a rate of 7.2 l/min through custom calibrated flow meters (Gilmont Instruments, Inc.). A small fan inside the chamber enabled adequate mixing of the chamber. After and before the chambers, air was passed through CO_2 absorbent granules of Baralyme [$\text{Ba}(\text{OH})_2$], H_2O absorbent granules of Drierite [CaSO_4] and dust filters, and monitored every 5 s with an Applied Electrochemistry O_2 analyzer model S-3A/I (Ametek, Pittsburgh, PA). Ambient temperature was held constant within $\pm 0.5 \text{ °C}$ by maintaining the metabolic chamber in a room at constant T_a . Oxygen consumption values were calculated with equation 4a of Withers (1977). Each metabolic trial was completed between 09.00 and 16.00 h (7 h), corresponding to the resting period of this species (Silva, 2001). The time for equilibration of the chamber (588 l) was 2–3 h. For analysis, we used measurements obtained at least 3 h after the equilibration period. The selected minimum values of BMR were corrected to standard temperature and pressure and all measurements were taken during the resting phase of foxes and in animals maintained without food for 2 days (48 h), but with water provided ad libitum during this period (postabsorptive). Body mass was measured before and after each metabolic trial. We were unable to measure body temperature immediately after BMR, but we measured T_b before and after each dietary treatment with a Cu–Constantan thermocouple and a Digi-Sense thermometer.

Statistical analyses were performed with STATISTICA 5.0 (1997; Statsoft, OK, USES). Differences between recorded and predicted values were tested using paired *t*-test for dependent samples (Rosen and Trites, 1999, 2002; Ivanović et al., 2002; Harrington et al., 2003). In addition, data were analyzed using ANCOVA with body mass as covariate for analysis of metabolic rates. Results are reported as mean ± 1 S.D. The allometric standard equation of McNab (1988) for carnivores: $\text{BMR} (\text{ml O}_2/\text{h}) = 5.35 \text{ g}^{0.679}$ was used as a standard reference (expected values).

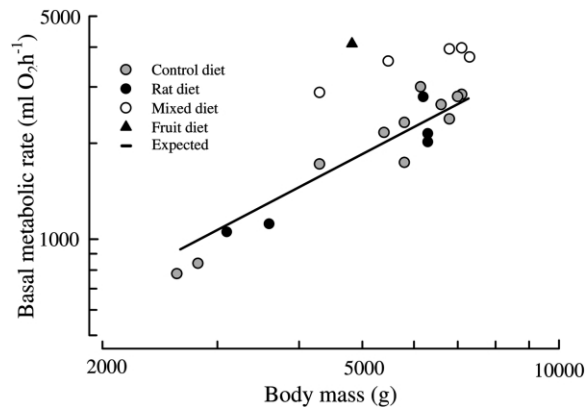


Fig. 1. Relationships between basal rate of metabolism and body mass in culpeo foxes as a function of acclimation to different diets. The allometric standard equation of McNab (1988) for carnivores: $\text{BMR} (\text{ml O}_2/\text{h}) = 5.35 \text{ g}^{0.679}$ was used as a standard reference (expected values). Each data point represents one individual. Log–log scale.

All experiments were conducted according to current Chilean law under permit SAG No. 993 and additionally approved by the Bioethics Committee of the Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. All animals were released at the same site of capture.

3. Results and discussion

BMR of 11 foxes fed on ground beef (control diet) ranged from 780 to 2840 ml O_2/h (mean = $2034.5 \pm 708.5 \text{ ml O}_2/\text{h}$, mean body mass = $5418 \pm 1580 \text{ g}$). These observed BMR-values were not significantly different from expected values based on the ‘carnivore curve’ (paired *t*-test for dependent samples = 1.58, d.f. = 10, $P = 0.14$), though on average they were 6.4% higher than the expected value based on body mass (Fig. 1).

BMR-values of foxes fed on live laboratory rats ranged from 1054 to 2790 ml O_2/h (mean = $1823.6 \pm 735.7 \text{ ml O}_2/\text{h}$, mean body mass = $5100.0 \pm 1607.8 \text{ g}$). These observed BMR-values were not significantly different from those expected from the ‘carnivore curve’ (paired *t*-test for dependent samples = 0.4212, d.f. = 4, $P = 0.695$), nor from initial values obtained for the control diet (same foxes, paired *t*-test for dependent samples matched pairs test = -1.65 , d.f. = 4, $P = 0.17$; Fig. 1). Initial and final m_b were significantly different (paired *t*-test for dependent samples = -8.57 , d.f. = 4, $P = 0.001$).

Foxes fed on mixed diet (sliced laboratory rats mixed with peppertree fruits) doubled their BMR in comparison to foxes fed on pure rat diet (99% increase, from 1823.6 to 3628.2 ml O₂/h, respectively). Values of BMR (mixed diet) ranged from 2881 to 3944 ml O₂/h (mean = 3628.2 ± 443.9 ml O₂/h, mean body mass = 6196.0 ± 1275.6 g). Observed BMR-values were significantly higher (81.1%) than those expected based on the 'carnivore curve' (paired *t*-test for dependent samples = 16.547, d.f. = 4, *P* < 0.00001; Fig. 1) and 46% higher than control values (we only used data from the same five foxes; paired *t*-test for dependent samples = -9.85, d.f. = 4, *P* < 0.0006; Fig. 1). Initial and final body masses were not significantly different (paired *t*-test for dependent samples = -1.39, d.f. = 4, *P* = 0.23).

Mass-specific BMR of the one fox fed exclusively fruit diet increased 74.2% in comparison to the control (same fox; from 0.488 to 0.850 ml O₂/g h). Nevertheless, because its body mass decreased from 6.140 to 4.820 g, total BMR increased just 36.5% (from 3000 to 4097 ml O₂/h), that is, 51.8% higher than the expected value (2697.6 ml O₂/h) based on body mass (Fig. 1). No effect of dietary treatment on *T_b* was observed, with values ranging from 37.5 to 38.7 °C (*t*-test = 0.17, d.f. = 8, *P* = 0.86).

Finally, a one-way ANOVA using body mass as a covariate revealed a significant effect of dietary acclimation on BMR in culpeo fox ($F_{2,16} = 47.878$, *P* < 0.0001). A post hoc Tukey test revealed significantly higher (*P* < 0.001) values of BMR in mixed-diet acclimated foxes in comparison to control and rat-acclimated foxes (Fig. 2).

In our study site, foxes eat fruits most of the year, with maximum consumption during autumn and winter (Jaksic, 1997; Silva, 2001). Fruit ingestion depends on small mammal abundance: as mammal populations decrease, fruit consumption increases (Castro et al., 1994; Silva, 2001). These authors hypothesized that culpeo foxes use fruits only as a supplementary food resource. The value of food can be measured in terms of metabolizable energy. Silva (2001) hypothesized that during periods of low availability of mammalian prey, a mixed diet should yield a positive energy/mass balance. Nevertheless, it appears that acute ingestion of chemically defended fruits such as peppertree impose an energetic cost to the fox, reflected in higher BMR-values.

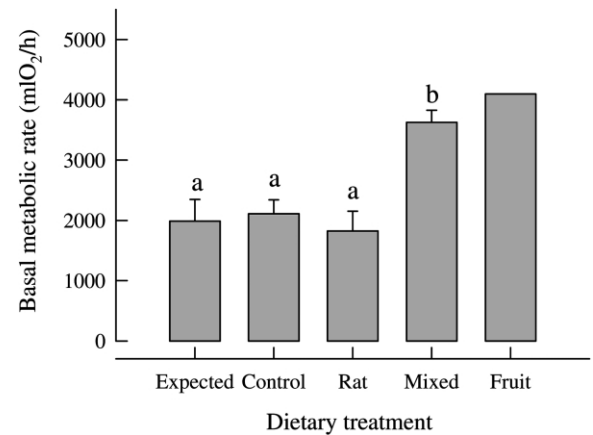


Fig. 2. BMR in culpeo foxes acclimated to different diets. The fruit-diet bar represents one individual. Similar letters indicate non-significant difference between groups after a post hoc Tukey test.

What is the mechanism by which BMR is altered? In general, metabolic depression in response to food with low energy content and/or high digestion cost has been observed in many animals, as a common response to periods of experimentally induced low energy diet (Velo and Bozinovic, 1993; Koteja, 1996; Rosen and Trites, 1999, 2002). In addition, time and space variability in food quality and abundance can reduce metabolic rate (Rosen and Trites, 2002). But this situation is not applicable to foxes in this study; in contrast to acute experiments, foxes fed on a mixed diet of rats and fruit or on a fruit diet showed an increase in BMR (Fig. 1). An increase in BMR after eating a diet with low energy content is characteristic of the increased foraging effort seen in the 'hunger response' or detoxification pathways of plant secondary metabolites (Foley et al., 1995; Mangione et al., 2001; Rosen and Trites, 2002). In our study, the increased metabolic rate may be associated with increased protein synthesis for detoxification and tissue repair, including the production of biotransformation enzymes (Foley and McArthur, 1994). In addition, the exposure to peppertree fruits, which contain phytochemicals with high biological activity, perhaps enlarges the liver, a metabolically active organ responsible for removing many toxins. In fact, extracts of peppertree fruit produce stimulant physiological activity among small mammals (i.e. uterine stimulant, diuretic, purgative), while peppertree fruit consumption causes heartburn, diarrhea and liver grip-

ing among humans (Coimbra, 1994). Thus, pharmacological effects may have influenced BMR of foxes. Future studies ideally should establish the time course of BMR response to the addition of peppertree fruits to the diet.

The effects of phytochemicals to mammals are relatively well known, but not the mechanisms involved. Indeed, the tendency in the literature has been to interpret metabolic changes as adaptive, although there are not enough studies of intraspecific variation in BMR to make generalizations. Further research on the association among BMR, detoxification and diet quality is still needed to understand the complex interplay between physiology and ecology. Our contribution here adds a set of data points, this time from a rather large animal.

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