Digestive and Metabolic Flexibility Allows Female Degus to Cope with Lactation Costs

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ABSTRACT

Lactation is the most energetically demanding period in the life cycle of female mammals, and its effects on digestive flexibility and the size of internal organs have been extensively studied in laboratory mice and rats since the early 1900s. However, there have been only two studies on this topic for wild rodent species. Here, we analyzed digestive flexibility—that is, changes in gut content, activity of digestive enzymes, and gut morphology—during lactation in the caviomorph rodent *Octodon degus*. In addition, we evaluated changes in the size of other internal organs and analyzed their relationship with the resting metabolic rate. We found that gut content, the dry masses of digestive chambers, the dry mass of liver, and resting metabolic rate were greater in lactating than in nonbreeding control females. In contrast, fat stores were higher in control subjects. Maltase and aminopeptidase-N specific activity did not change with lactation, and both enzymes had greater activity values in the middle portion of the small intestine. Thus, our data indicate that the previously reported increase in food assimilation that occurs during lactation in *O. degus* is related to a mass increase in several central organs, leading, in turn, to higher energetic costs. Fat stores may help to mitigate these costs, but, as expected for small animals, to a limited extent. Our study reveals a complex interplay among energy acquisition, storage, and expenditure processes that ultimately determine an organism's fitness.

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Introduction

Phenotypic flexibility refers to reversible changes in the traits of organisms due to changes in internal or external environmental conditions (Piersma and Drent 2003). Different lines of evidence suggest that phenotypic flexibility is adaptive, evolving through natural selection (see Scheiner 1993, 2002; Roff 1997; Agrawal 2001). Thus, reversible adjustments to changing environmental conditions seem to increase organismal fitness, although this is usually not easy to demonstrate (Schmitt et al. 1999) and may not always be the case (David et al. 2004) .

As an attempt to represent the dynamics of energy budgets, Wiener (1992) proposed the "barrel model," which is very useful for understanding the interplay among environmental variability, phenotypic flexibility, and energy allocation. In Weiner's model, an organism is represented by a barrel, with input energy constraints—for example, foraging, digestion, and absorption—symbolized by funnels connected in tandem and energy outputs—for example, maintenance, growth, reproduction—symbolized by a series of spouts arranged in parallel. When environmental conditions change, organisms are able to respond, at least to some extent, by adjusting the size of the funnels, the output flow through the spouts, or the fluid stored inside the barrel.

One way animals can respond to changes in external conditions is by modifying their digestive attributes. In this sense, digestive flexibility has been suggested as one of the most important and widely used physiological adjustments to changes in both internal and external conditions (Piersma and Lindstrom 1997; Starck 1999; McWilliams and Karasov 2001; Naya and Bozinovic 2004). This is not surprising, given that the digestive tract represents the functional link between energy intake and metabolizable energy (i.e., the energy available to meet all the vital functions, including growth, survival, and reproduction; Karasov 1990; Secor 2001). In addition, gut tissue is one of the most costly tissues to maintain in terms of energy and protein metabolism (McBride and Kelly 1990; Wang et al. 2001), and thus, adjusting the amount of this tissue to functional demands could represent an important energy-saving mechanism. On the other hand, when environmental conditions change, animals can also regulate energy expenditure processes. For example, during periods of nutritional bottlenecks (e.g., when food availability is low or food is of low quality) or enhanced energetic demands (e.g., during cold months of the year or during reproduction), animals can modify their standard metabolic rates (McNab 1986; Cruz-Neto and Bozinovic 2004), change their activity patterns (Costa et al. 1989; Kenagy et al. 2002), use daily or seasonal torpor (Geiser 2004), or ultimately adapt their investment to

growth and reproduction (Calow 1977; Humphries and Boutin 2000; Naya et al. 2007*b*).

The aim of this study was to examine digestive flexibility at different phenotypic levels (i.e., changes in the distribution of digesta throughout the digestive chambers, activity of digestive enzymes, and gut gross morphology) during lactation in the caviomorph rodent *Octodon degus* (Octodontidae). In addition, we evaluated adjustments in the size of other internal organs and their relationship with the resting metabolic rate. While lactation is considered the most energetically demanding period in the life cycle of a female mammal (Thompson and Nicoll 1986; Kenagy 1987), there is little information regarding its effect on digestive flexibility for wild rodent species (but see Derting and Austin 1998; Hammond and Kristan 2000). *Octodon degus* (degu) is a small, diurnal, herbivorous rodent that feeds on grasses, forbs, seeds, and shrub foliage (Meserve 1981; Meserve et al. 1983). These rodents face highly seasonal Mediterranean environments in northern and central Chile, where food quantity and quality change drastically from high during winter and spring months to low during summer and autumn months (Ebensperger and Hurtado 2005). In these environments, *O. degus* live in social groups, and members of groups share the construction and use of underground burrows (Ebensperger et al. 2004). Typically, degus breed once per year (Ebensperger and Hurtado 2005), and, as in other rodents, lactation represents the highest energy cost to breeding females (Veloso and Bozinovic 2000). Degu pups are more dependent than other precocial rodents on maternal milk to complete their postnatal development (Veloso and Kenagy 2005); degu pups do not eat solid food before 6 d of age (Reynolds and Wright 1979; L. A. Ebensperger, personal observations).

Material and Methods

Study Subjects, Animal Housing, and Experimental Design

Study subjects were 1-yr-old female descendants of pregnant females caught during July and August 2002 at Lampa (ca. 500 m altitude, 33°17′S, 70°53′W), near Santiago, Chile. Upon weaning (ca. 30 d of age), degus were kept in same-sex sibling pairs inside 45 × 23 × 21-cm clear polycarbonate rat cages with a bedding of hardwood chips; water and food (commercial rabbit pellets; Champion, Santiago, Chile) were provided ad lib. The chemical composition of the diet was dry matter = 90.6%, ash = 10.8%, crude fiber = 16.5%, neutral detergent fiber = 37.8%, acid detergent fiber = 19.8%, lipids = 3.0%, proteins ($N \times 6.25$) = 20.0%, carbohydrates = 40.3%, total energy content = 18.4 ± 0.5 kJ g^{-1} (Veloso and Bozinovic 1993). Animals were kept in a ventilated room exposed to a simulated natural photoperiod and an ambient temperature of 22°C. At 8 mo of age (i.e., July 2006), sexually naive female pairs were allowed to mate with a same-age unrelated male. Males remained with the females for 60 d, after which pregnant females were easy to recognize by their increased mass and swelling abdomen. The annual rate of females becoming pregnant in our colony ranged from 49% to 75%. On the basis of this variation, we randomly assigned one female from each pair to one of two groups: lactating (females that became pregnant, $n = 10$) and control (females that did not become pregnant, $n = 10$). Approximately 7–10 d before the expected delivery of pups (i.e., October 2006), food was restricted in such a way that females were fed daily with rabbit pellets representing 80%–90% of the energy requirements for lactating and nonlactating adult females (Veloso 1997). We exposed degu subjects to these restricted conditions to create an energetically challenging environment that probably was close to natural conditions without causing mortality of mothers and pups. Eight lactating females reared six pups, while the other two reared seven pups (i.e., mean litter size = 6.2). At the end of lactation, females were separated from their pups and used for experimental determinations; this took place during the last two weeks of November 2006. All research conducted as part of this study conformed to national and institutional guidelines for research on live mammals (permits 893 and 1894 by the Servicio Agrícola y Ganadero, Chile).

Metabolic Rate Determination

Animals were fasted for 36 h before measurements of resting metabolic rate (RMR). Oxygen consumption ($\dot{V}o_2$) was measured in a computerized (Datacan) open-flow respirometry system (Sable Systems, Henderson, NV) in acrylic metabolic chambers of 2 L, at an ambient temperature of 30°C (a temperature within the thermoneutral zone of this species; Rosenmann 1977). The metabolic chamber received dried air at a rate of 800 mL min^{-1} from mass flow controllers (Sierra Instruments, Monterey, CA). The air was passed through CO_2-absorbent granules (Baralyme, St. Louis, MO) before entering the chamber and through H_2O-absorbent granules (Drierite, Xenia, OH) after passing through the chamber and was monitored every second for a period of 2 h by an Applied Electrochemistry O_2 analyzer (model S-3A/I, Ametek, Pittsburgh, PA). Each record was automatically transformed and recorded in ExpeData software (Sable Systems). Resting metabolism was estimated as the continuous range of the lowest 5-min samples during the period of recording. All metabolic trials were completed between 0800 and 1400 hours. Before each metabolic measurement, we recorded body mass (m_b) of animal subjects, using an electronic balance (± 0.1 g; Sartorious, Göttingen, Germany). Oxygen consumption was converted into energetic units using the energy equivalent of 21.14 J mL^{-1} of CO_2 (Hill and Wyse 1989).

Morphological Determination

Degus were anesthetized with methoxyflurane and then immediately killed between 0900 and 1000 hours. The complete gastrointestinal tracts were quickly removed and dissected free of mesenteric attachments without stretching the tissue. The amount of digesta (i.e., the material rinsed out of the intact gut) was weighed separately for each organ in an analytical electronic

balance (±0.0001 g; Chyo JK-180, Kyoto, Japan) and then integrated over the entire gut. Digestive organs were washed with saline solution and their length measured with a plastic ruler (±1 mm). Small intestine width was measured in three sections (proximal, medium, and distal) with a digital caliper (±0.01 mm; Mitutoyo, Aurora, IL), and nominal area was calculated as mean intestinal width × intestinal length × 2. Then, digestive organs were carefully dried with paper towels and weighed (±0.0001 g). Two-centimeter-long portions of the proximal, middle, and distal parts of the small intestine were cut, weighed, and immediately frozen in liquid nitrogen for determination of enzyme activity (see below). Finally, we removed liver, kidneys, heart, lungs, spleen, and abdominal fat and dried them—together with the digestive organs and the animal's carcass—to constant mass in an oven at 60°C for 10 d, after which they were weighed. The small intestine dry mass calculation took into account the tissue samples destined for enzymatic determination.

Enzyme Activity Measurements

For enzyme analysis, the small intestine tissue samples were thawed and homogenized (30 s in an ULTRA TURRAX T25 homogenizer at maximum setting) in 20 vol of 0.9% NaCl solution. Maltase (EC 3.2.1.20) activity was determined according to the method of Dahlqvist (1964), as modified by Martínez del Río (1990). Briefly, tissue homogenates (100 μL) were incubated at 25°C with 100 μL of 56-mmol-L^{-1} maltose solution in 0.1 M maleate/NaOH buffer, pH 6.5. After 10 min of incubation, reactions were stopped by adding 3 mL of a glucose Trinder stop-develop solution (one bottle of glucose Trinder reagent in 125 mL 0.1-mol-L^{-1} TRIS/HCl, pH 7, plus 125 mL of 0.5 mol L^{-1} NaH_2PO_4, pH 7). Absorbance was measured with a spectrophotometer at 505 nm after 18 min at 20°C.

Aminopeptidase-N (EC 3.4.11.2) assays were conducted with L-alanine-*p*-nitroanilide as a substrate. Briefly, 100 μL of homogenate diluted with 0.9% NaCl solution was mixed with 1 mL of assay mix (2.04 mmol L^{-1} L-alanine-*p*-nitroanilide in 0.2 mol L^{-1} NaH_2PO_4/Na_2HPO_4, pH 7). The reaction was incubated at 25°C and stopped after 10 min with 3 mL of ice-cold acetic acid (2 N), and absorbance was measured at 384 nm. The selected pH for measuring the activities was the optimum for each enzyme (Sabat et al. 1999). On the basis of absorbance, the activity of each enzyme was tabulated as enzyme specific activity rate: IU mg^{-1} = μmol hydrolyzed min^{-1} g^{-1} of wet tissue.

Statistical Analysis

Differences in body mass and length between experimental groups were evaluated using one-way ANOVA. Changes in maltase and aminopeptidase-N specific activities were analyzed separately through repeated-measures ANOVA. Differences in all the remaining dependent variables were evaluated separately with the use of one-way ANCOVA. In these analyses, we investigated the following variables as possible covariates: (1) body length for linear measures of digestive organs and small intestine area, (2) carcass dry mass for organ dry masses, and (3) body mass for gut content and RMR. We found significant relationships between carcass dry mass and the dry mass of some organs (i.e., kidneys, lungs, and abdominal fat) and between body mass and RMR. Thus, for all the other cases, we report only results from the one-way ANOVAs. The effect of organ dry masses on RMR was evaluated through Pearson product-moment correlation coefficients. To remove the effect of body size on both variables, we used the residuals of RMR with regard to body mass and the residuals of each organ's mass with regard to carcass dry mass (for those organs where there was a significant correlation for both variables). The assumptions of normality and homogeneity of variance were examined using Kolmogorov-Smirnov and Levene tests, respectively. Interactions between covariates and factors were tested using a parallelism test. Statistical significance was established at the $\alpha = 0.05$ level. Statistical analyses were performed using the statistical package STATISTICA (StatSoft 2001).

Results

Body Size, Organ Morphology, and Gut Content

As expected, females in the second month of pregnancy were heavier than control animals ($F_{1,18} = 19.03$, $P < 0.001$; Table 1). However, there were no differences in body mass or body length between the two groups at the end of lactation (Table 1). Small intestines of control females tended to be longer ($F_{1,18} = 3.66$, $P < 0.07$), but thinner ($F_{1,18} = 11.03$, $P < 0.01$), than those of lactating females (Table 1). Accordingly, no difference was observed in intestinal nominal area between groups. The length of the cecum was greater in lactating than in control females ($F_{1,18} = 4.60$, $P < 0.05$; Table 1). Overall gut content was greater in lactating females ($F_{1,18} = 9.72$, $P < 0.01$), which was related to the larger amounts of digesta in the stomach ($F_{1,18} = 7.55$, $P < 0.02$) and small intestine ($F_{1,18} = 7.79$, $P < 0.02$; Table 1).

With regard to organ dry mass, total gut weight was greater in lactating females ($F_{1,18} = 20.34$, $P < 0.001$; Table 1). This effect was mainly due to heavier small and large intestines ($F_{1,18} = 12.36$, $P < 0.003$ and $F_{1,18} = 11.75$, $P < 0.003$, respectively) and, to a lesser extent, to heavier stomach and cecum ($F_{1,18} = 3.89$, $P < 0.07$ and $F_{1,18} = 3.71$, $P < 0.07$, respectively; Table 1). In addition, the weight of liver and gonads were greater in lactating than in control females ($F_{1,18} = 23.47$, $P < 0.001$ and $F_{1,17} = 6.77$, $P < 0.02$, respectively), while the weight of abdominal fat was greater in control animals ($F_{1,17} = 16.23$, $P < 0.001$; Table 1).

Digestive Enzyme Activities

We did not observe significant changes in the specific activity rate of maltase ($F_{1,18} = 0.04$, $P < 0.84$; Fig. 1*A*) or aminopeptidase-N ($F_{1,18} = 0.98$, $P < 0.33$; Fig. 1*B*) associated with breeding condition. For both enzymes, greater activity values were

Table 1: Body size, gut contents, and organ size variables for each group

Variable	Controls ($n = 10$)	Lactating ($n = 10$)	F (df)	P
Initial body mass (g)	204.1 ± 7.9	252.51 ± 7.8	19.03 (1, 18)	<.001
Final body mass (g)	206.8 ± 6.8	220.1 ± 5.1	2.43 (1, 18)	<.14
Body length (cm)	16.8 ± .3	17.3 ± .5	.67 (1, 18)	<.42
Length (mm):				
Stomach	40.5 ± 2.2	43.5 ± 2.3	.89 (1, 18)	<.36
Small intestine	630.7 ± 41.0	533.7 ± 29.8	3.66 (1, 18)	<.07
Cecum	72.8 ± 2.7	80.8 ± 2.6	4.60 (1, 18)	<.05
Large intestine	504.9 ± 20.3	504.7 ± 14.8	.01 (1, 18)	<.99
Entire gut	1,248.9 ± 47.7	1,162.7 ± 30.5	2.32 (1, 18)	<.15
Small intestine width (mm)	6.4 ± .2	7.8 ± .3	11.03 (1, 18)	<.01
Small intestine area (mm^2)	7,984.1 ± 469.7	8,231.2 ± 456.0	.14 (1, 18)	<.71
Digesta (g):				
Stomach	6.44 ± .48	9.58 ± 1.04	7.55 (1, 18)	<.02
Small intestine	1.39 ± .30	2.54 ± .40	7.79 (1, 18)	<.02
Cecum	8.59 ± .89	9.72 ± .87	.82 (1, 18)	<.38
Large intestine	4.93 ± .77	6.09 ± .62	1.37 (1, 18)	<.26
Entire gut	21.35 ± 1.23	27.92 ± 1.81	9.72 (1, 18)	<.01
Dry mass (g):				
Stomach	.444 ± .017	.507 ± .027	3.89 (1, 18)	<.07
Small intestine	.847 ± .041	1.047 ± .039	12.36 (1, 18)	<.003
Cecum	.492 ± .029	.567 ± .026	3.71 (1, 18)	<.07
Large intestine	.518 ± .035	.664 ± .025	11.75 (1, 18)	<.003
Entire gut	2.302 ± .076	2.785 ± .075	20.34 (1, 18)	<.001
Liver	2.694 ± .144	3.659 ± .150	23.47 (1, 18)	<.001
Kidneys	.547 ± .021	.539 ± .019	.23 (1, 17)	<.64
Heart	.129 ± .004	.136 ± .005	1.81 (1, 18)	<.20
Lungs	.257 ± .024	.259 ± .019	.06 (1, 17)	<.94
Spleen	.103 ± .007	.107 ± .009	1.00 (1, 17)	<.76
Gonads	.079 ± .014	.158 ± .026	6.77 (1, 17)	<.02
Abdominal fat	5.068 ± .917	2.488 ± .855	16.23 (1, 17)	<.001

Note. Data are reported as absolute means ± 1 SEM, except in the case of kidneys, lungs, and abdominal fat, where least squares adjusted means ± 1 SEM (covariate mean value: carcass dry mass = 56.2 g) are reported. Initial body mass = body mass at the second month of pregnancy; final body mass = body mass at the end of lactation.

observed in the middle portion of the small intestine ($F_{2,36} = 36.80$, $P < 0.001$ for maltase; $F_{2,36} = 35.86$, $P < 0.001$ for aminopeptidase-N). We found a positive, highly significant correlation between the specific activities of these two enzymes ($r^2 = 0.59$, $F_{1,58} = 83.11$, $P < 0.001$; Fig. 2). When enzymatic activity was integrated over the entire small intestine (i.e., mean specific activity × small intestine wet mass), the summed activity of maltase and aminopeptidase-N was greater in lactating females than in control animals ($F_{1,18} = 10.54$, $P < 0.005$ and $F_{1,18} = 23.81$, $P < 0.001$, respectively; Fig. 3).

Resting Metabolic Rate

RMR was markedly higher in lactating than in control females ($F_{1,17} = 11.87$; $P < 0.004$). Specifically, at the end of lactation, RMR increased 19.2% in comparison to control animals (control females: 134.6 ± 5.6 mL O_2 h^{-1}; lactating females: 160.4 ± 6.5 mL O_2 h^{-1}; covariate mean value: body mass = 207.2 g). With regard to the relationship between organ dry masses and RMR, we observed a significant, positive correlation for the stomach ($r^2 = 0.20$, $F_{1,18} = 4.54$, $P < 0.05$; Fig. 4*A*), small intestine ($r^2 = 0.27$; $F_{1,18} = 6.69$, $P < 0.02$; Fig. 4*B*), large intestine ($r^2 = 0.22$, $F_{1,18} = 5.14$, $P < 0.04$; Fig. 4*C*), and liver ($r^2 = 0.21$, $F_{1,18} = 4.72$, $P < 0.05$; Fig. 4*D*).

Discussion

Many studies of mammalian reproductive energetics have confirmed the traditional statement that reproduction is the most energetically demanding period of life for a female mammal (e.g., Bronson 1989). In fact, Kenagy (1987) demonstrated that total energy expenditure during reproduction represents ap-

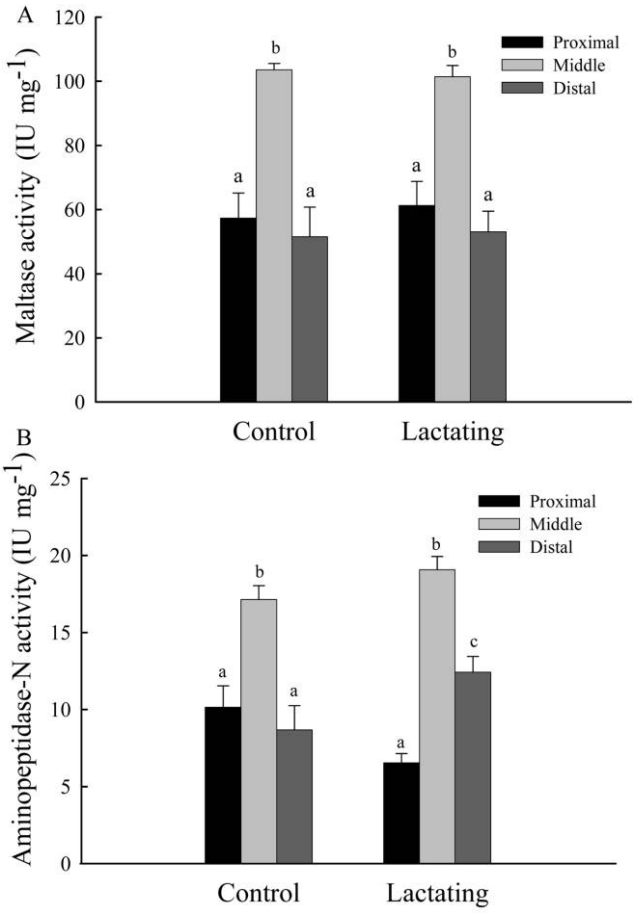

Figure 1. Specific activity rate of maltase (*A*) and aminopeptidase-N (*B*) for each portion of the small intestine in control and lactating females ($n = 10$ for each group). Error bars = 1 SEM. Different letters indicate significant differences ($P < 0.05$) after a Tukey HSD test.

proximately 50% of the annual energy expenditure of squirrels. In addition, energy expenditure in offspring during lactation is considered the most demanding period of the reproductive cycle (Millar 1979; Oftedal 1984*a*, 1984*b*; Sadleir 1984; Thompson and Nicoll 1986; Kenagy 1987; McClure 1987; Kenagy et al. 1989).

The effect of lactation on organ size dynamics has been studied in white laboratory mice and rats since the beginning of the past century (e.g., Herring 1920; Abramson 1934; Poo et al. 1939) and more extensively from the 1950s to the 1980s (e.g., Souders and Morgan 1957; Fell et al. 1963; Campbell and Fell 1964; Craft 1970; Crean and Rumsey 1971; Sigdestad and Osborne 1972; Cripps and Williams 1975; Mainoya 1978; Cañas et al. 1982). In addition, during the past two decades, the study of lactation in laboratory mice has been the main experimental model for testing the energy budget limitation hypothesis (Hammond and Diamond 1992, 1994; Hammond et al. 1994, 1996; Speakman and McQueenie 1996; Johnson and Speakman 2001). However, to date, only two experimental studies have been focused on the effect of lactation on the morphology of digestive organs in wild rodent species (Derting and Austin 1998; Hammond and Kristan 2000). In this sense, our study comprises the first record of organ size adjustments in response to lactation in a wild rodent species from the Neotropical biogeography region.

Both previous experimental and other field studies (e.g., Myrcha 1964, 1965; Gebczynska and Gebczynski 1971; Borkowska 1995; Derting and Hornung 2003) indicate an increase in the size of the digestive organs and the liver during lactation. Moreover, lactation causes greater adjustments in the length and dry mass of the small intestine than do other experimental factors, including diet quality, pregnancy, and environmental temperature (Naya et al. 2007*a*). The results from this study agree with this evidence, where lactating degus presented an important increase in the dry mass of the liver (35.8%), large intestine (28.2%), small intestine (23.6%), cecum (15.2%), and stomach (14.2%). The implications of these structural changes were pointed out many decades ago. For example, Campbell and Fell (1964, p. 96), referring to gut adjustments, stated that "the hypertrophy is caused by the increased food intake and is an adaptive response of the alimentary canal which results in the maintenance of a constant coefficient of digestibility at increased levels of food consumption." Although we were unable to measure food intake and fecal output in this study, a previous study indicated that this idea also holds for our focal species. Specifically, Veloso and Bozinovic (2000) reported that during lactation, female degus increase food consumption without changes in digestibility, which results in higher food assimilation rates.

In contrast to the observed morphological changes, we did not observe any significant differences in the specific activity (i.e., activity normalized by wet tissue mass) of the digestive enzymes maltase and aminopeptidase-N between lactating and control females. This result agrees with an absence of dietary modulation of disaccharidase and aminopeptidase-N in degus

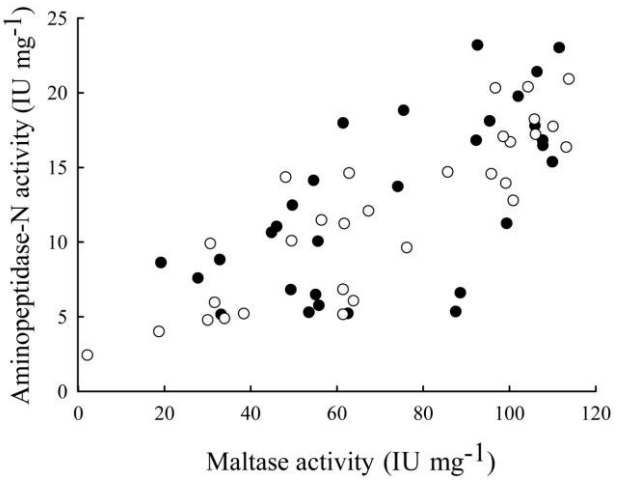

Figure 2. Correlation between maltase and aminopeptidase-N specific activities for control (*open circles*) and lactating (*filled circles*) females.

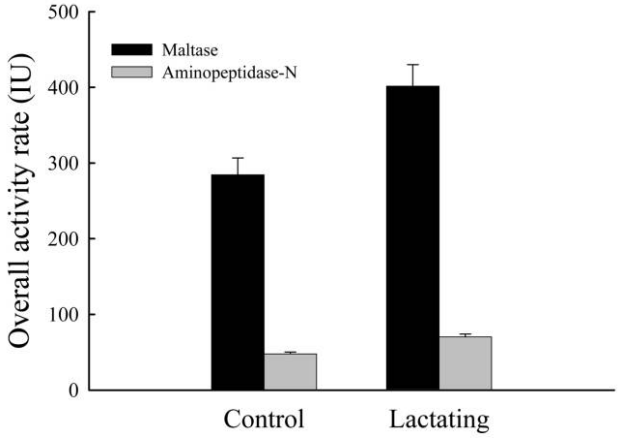

Figure 3. Activity rate of maltase and aminopeptidase-N integrated over the small intestine in control and lactating females ($n = 10$ for each group). Error bars = 1 SEM.

acclimated to diets with contrasting protein and carbohydrate contents (Sabat et al. 1999). Nevertheless, given that the mass of the small intestine was greater in lactating animals, the summed hydrolytic activity was also higher in breeding females. While no previous data on digestive enzymes of lactating rodents were available, a nonspecific increase in the brush-border nutrient transporters during lactation has been repeatedly reported for laboratory and wild species (e.g., Karasov and Diamond 1983; Hammond et al. 1994, 1996; Hammond and Kristan 2000). Interestingly, we found a positive correlation between maltase and aminopeptidase-N specific activity throughout the small intestine. This relationship between both enzymes has been previously reported for other vertebrate species (e.g., birds: Sabat et al. 1998; amphibians: Naya et al. 2005), and, to our knowledge, there is no clear explanation for this phenomenon. We propose that a positive correlation should be the expected neutral pattern under a situation of nonenzymatic modulation. This is because at the time of animals' death, there was random variation in enterocyte development between individuals, and this differential development simultaneously determines the level of several digestive enzymes (i.e., enterocyte development is a latent variable affecting all enzymatic activities). In contrast, when enzymatic specific modulation occurs (e.g., dietary modulation), the regulation in the production of one enzyme is independent of that of other enzymes, which may erase the correlation between their activities.

RMR measurements supported the idea that animals pay an energetic cost linked to the anatomical adjustments that increase food-processing capacities. Specifically, we found that RMR in *Octodon degus* increases by 20% at the end of lactation (day 30) relative to nonbreeding animals. This increase in metabolic rate during lactation was correlated with an increase in the dry mass of several central organs, such as the digestive organs and the liver. This finding agrees with the observation that these organs are very expensive to maintain in terms of

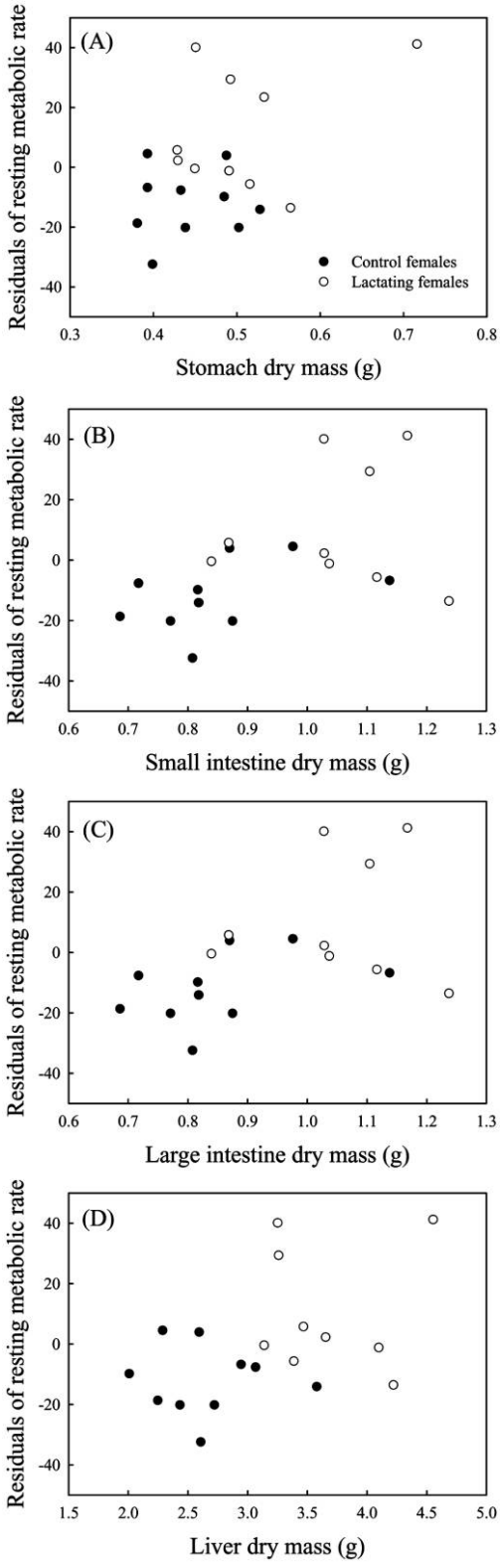

Figure 4. Relationship between residuals of resting metabolic rate (with regard to body mass) and stomach dry mass (*A*), small intestine dry mass (*B*), large intestine dry mass (*C*), and liver dry mass (*D*). Control females are represented by filled circles, and lactating females are represented by open circles.

energy and protein metabolism (McBride and Kelly 1990; Wang et al. 2001). Moreover, data for laboratory rats indicate that, although a rise in specific metabolic activity of different tissues occurs during lactation, a major fraction of the metabolic rate enhancement is due to an increase in the weights of those organs with higher maintenance costs (Cañas et al. 1982). On the other hand, previous data for degus indicate that RMR increases by 52% at day 5 of lactation and 39% at day 18 in relation to nonreproductive animals (Veloso and Bozinovic 2000). Thus, it appears that RMR in *O. degus* decreases linearly during lactation. This is in agreement with the fact that the maximum rate of production in precocial rodents occurs during early lactation, in contrast with altricial rodents, where the maximum energy production occurs during middle to late lactation (Künkele and Trillmich 1997).

Finally, we observed that control animals attained more than twice the amount of fat stores of postlactating females. This finding suggests an important mobilization of body stores during lactation, a fact previously documented for several small-mammal species (e.g., Randolph et al. 1977; Pistole 1989; Michener and Locklear 1990; Virgl and Messier 1992; Rogowitz 1998; Hood et al. 2006). For example, in hispid cotton rats, about 10% of overall metabolizable energy during late lactation comes from the body stores of the mother (Rogowitz 1998), while in big brown bats, females lose approximately two-thirds of their body fat at the peak of lactation, as compared with females collected before or after this stage (Hood et al. 2006). In degus, the difference in fat stores between late-lactating and control females reaches 97 kJ ($=2.58$ g fat $\times$ 37.6 kJ g^{-1}), while the difference in energy expenditures for the overall lactation period ranges from 393.1 kJ ($=13.1$ kJ $d^{-1} \times 30$ d, using the RMR data from this study) to 777.0 kJ ($=25.9$ kJ $d^{-1} \times 30$ d, when our RMR data are combined with those of Veloso and Bozinovic [2000]). Thus, the energy supply from fat stores represents between 24.7% and 12.5% of metabolic rate increase during lactation. Interestingly, these percentages were similar to the extent of restriction in daily energy requirements (10%–20%) of experimental subjects, suggesting that lactating females would not mobilize fat storages if fed ad lib.

As we stated above, lactation comprises the most energetically demanding period in the life cycle of a female mammal (Thompson and Nicoll 1986; Kenagy 1987), and the amount of nutrients and energy that can be supplied from body reserves obtained before lactation is restricted in small-sized species (Oftedal 2000). Accordingly, it is expected that small organisms increase their food ingestion during this highly demanding period (Kenagy et al. 1989; Speakman and Król 2005). Previous studies on *O. degus* showed that lactating females increase food intake and food assimilation (Veloso and Bozinovic 2000). Here, we demonstrate that this increase in digestive processing capacity is linked to an increment in the size of digestive organs and liver, a change that involves a direct energetic cost reflected in higher RMRs. Fat stores may help to mitigate this higher maintenance cost, but, as expected for small animals, only to a limited extent. In summary, this study highlights the complex interplay among energy acquisition, storage, and expenditure processes, which ultimately determine an organism's fitness.

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