J Comp Physiol B
DOI 10.1007/s00360-012-0726-z

ORIGINAL PAPER

Seasonal plasticity of gut morphology and small intestinal enzymes in free-living Mongolian gerbils

Quan-Sheng Liu · Zhi-Qiang Zhang · Enrique Caviedes-Vidal · De-Hua Wang

Received: 15 July 2012 / Revised: 11 October 2012 / Accepted: 22 November 2012

Abstract The phenotypic plasticity of the digestive system may determine the diversity of animal diets and, thus, their niche width. This study examines the effects of seasonal fluctuations in food quality and temperature on the gut morphology and the activity of sucrase, maltase, and aminopeptidase-N in the small intestinal brush-border membrane of male Mongolian gerbils (*Meriones unguiculatus*). Based on the adaptive modulation hypothesis and the principle of optimal gut function design, we hypothesize that the gut size, tissue-specific activity, and total hydrolytic capacity of intestinal digestive enzyme are upregulated in winter and downregulated in summer in response to diet shifts and energy demand in free-living Mongolian gerbils. Various seasonal modulation patterns in digestive enzyme activity in different regions of the small intestines were observed. The results show that male gerbils have the longest and heaviest small intestines in winter. This mechanism may be adapted to increase their food intake during winter. Male gerbils also exhibit the highest tissue-specific and total sucrase, maltase, and aminopeptidase-N activity in winter and in spring. Seasonal modulations are more distinct in the jejunum than in the duodenum and the ileum of the small intestines. The digestive phenotypic flexibility of male gerbils effectively corresponded with seasonal diet shifts and temperature fluctuations.

Keywords Adaptive modulation hypothesis · Phenotypic plasticity · Mongolian gerbil (*Meriones unguiculatus*) · Aminopeptidase-N · Disaccharidases

Communicated by G. Heldmaier.

Q.-S. Liu · Z.-Q. Zhang · D.-H. Wang (✉)
State Key Laboratory of Integrated Management for Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beichen West Road, Chaoyang, Beijing 100101, China
e-mail: wangdh@ioz.ac.cn

Q.-S. Liu
e-mail: liuqs@gdei.gd.cn

Q.-S. Liu
Guangdong Key Laboratory of Integrated Pest Management in Agriculture, Guangdong Entomological Institute, 105 Xin'gang Xilu, Haizhu, Guangzhou 510260, China

E. Caviedes-Vidal
Departamento de Bioquimica y Ciencias Biologicas, Universidad Nacional de San Luis, 5700 San Luis, Argentina

Introduction

The capacity of the gastrointestinal system of animals to adapt to fluctuating food quantity and quality has been one of the major themes in comparative physiology and nutritional ecology. Digestive phenotype flexibility, which has been well documented in several reviews, is important for regulating diet switching and for very high feeding rates in vertebrates (e.g., Sibly 1981; Stevens and Hume 1995; Karasov 1996; Karasov and Hume 1997; Starck 1999, 2005; McWilliams and Karasov 2001; Karasov and McWilliams 2005; Karasov and Martínez del Rio 2007; Naya et al. 2008; Karasov et al. 2011). The main features of digestive flexibility in animals include modulation of digestive enzymes, changes in nutrient absorption rate, and digesta retention time (Karasov and Hume 1997; Karasov et al. 2011).

The dietary modulation of enzymes and transporters in the intestinal brush-border membranes are widespread in vertebrates, such as intestinal carbohydrases and glucose transporters (Karasov and Hume 1997; Karasov et al. 2011). Some studies suggested that the modulation of these

Published online: 13 December 2012

digestive enzymes is greater in generalist animals than in specialists (e.g., Martínez del Rio 1990; Hernandez and Martínez del Rio 1992; Afik and Karasov 1995; Karasov 1996; Sabat et al. 1998, 1999). The adaptive modulation hypothesis proposed by Karasov and Diamond (1983) effectively explains the dietary modulation of digestive enzymes and transporters. The hypothesis also accounts for the correlation between dietary flexibility and digestive plasticity. Aside from diet, the modulation of energy demands (e.g., lactation, exercise, and thermogenesis) of transporters activity has been systematically investigated in various vertebrates (e.g., Karasov and Diamond 1983; Buddington 1992; Karasov 1992; Karasov and Hume 1997). Considering increased energy demands generally induce hyperphagia and improve nutrient monomer uptake, intestinal hydrolases may be upregulated to match the nutrient transporters (Karasov and Hume 1997; Weiss et al. 1998). Some studies suggested that changes in intestinal hydrolases in response to energy demand variation are modulations in total hydrolytic capacity caused by changes in small intestine tissue mass (Weiss et al. 1998; McWilliams et al. 1999; Nespolo et al. 2002; del Valle et al. 2004), and the modulation in protein-specific activity (del Valle et al. 2004).

This study examines the seasonal changes and the age-related variations in the small intestines (SI) and enzyme activity in free-living Mongolian gerbils (*Meriones unguiculatus*), small winter-active, social rodent. Mongolian gerbils inhabit desert and semi-desert grasslands and occur in the transition zone between grasslands and farmlands. The animal mainly lives in the Inner Mongolian grasslands of China, Mongolia, and the Baikal Region in Russia (Zhang and Wang 1995). Mongolian gerbils are considered pest rodents in grasslands and farmlands (Zhang et al. 2003) because they store an average of 15.5 kg of seeds of grass or crop per family group in its burrow to survive the severely cold, harsh and long (about 170 days) winters. Although they feed mainly on seeds during autumn, winter, and early spring, they also consume a high proportion of green stems and leaves during summer (Xia and Zhong 1966; Chen and Li 2000; Fig. 1). However, high-fiber foods significantly reduce apparent digestibility of Mongolian gerbils (Pei et al. 2001a; Liu and Wang 2007) and downregulate protein-specific hydrolytic enzyme activity in the intestinal brush-border membrane (Liu and Wang 2007). Therefore, the digestive organs and physiology of granivorous rodents have a different seasonal adaptive modulation pattern for seasonal fluctuations in food quality compared with herbivorous rodents, such as voles living in grasslands.

Given the seasonal fluctuations in diet and temperature, and human agriculture disturbance in Inner Mongolia, the Mongolian gerbil is still the dominant rodent species (more than 80 % of all rodents) in local grasslands and farmlands. Aside from behavioral and thermoregulatory flexibility of

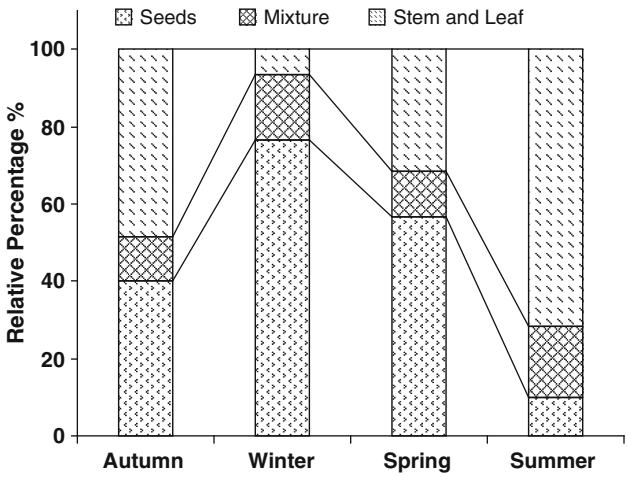

Fig. 1 Compositions of stomach content of wild Mongolian gerbils in varied seasons (Data come from Chen and Li 2000). Values are the percentage of the number of gerbils with different stomach content and the total gerbils captured. Each season involves 3 months

Mongolian gerbils (Liu et al. 2001, 2002; Wang et al. 2003; Li and Wang 2005), their digestive flexibility may be relevant to their outstanding adaptive capacity under local conditions. Based on the adaptive modulation hypothesis and the principle of economic design of gut function (Karasov et al. 2011), we hypothesize the following: (1) The length and mass of the SI are regulated based on seasonal changes in food quality and quantity, and energy demands. (2) The tissue-specific activity of sucrase and maltase in gerbils increases during seasons when they mainly ingest seeds, and is down-regulated during seasons wherein they mainly feed on plant stems and leaves. Furthermore, the tissue-specific amino-peptidase-N activity in gerbils has no apparent seasonality because of the generally poor protein content of the seeds, leaves, and stems eaten by gerbils. (3) The total intestinal hydrolytic capacity of the gerbils is greater in winter than in summer because of hyperphagia, which is needed to meet the higher energy demand. (4) The seasonal modulation patterns of digestive enzymes differ among the regions of the SI because of seasonal changes in food quality and quantity in gerbil habitats. The hypotheses were tested by measuring the size of the SI and the tissue-specific and total activity of sucrase, maltase, and aminopeptidase-N in free-living Mongolian gerbils caught during different seasons.

Materials and methods

Study site and animal capture

The gerbils were captured alive via Sherman traps in the transition zone between grasslands and farmlands in Taipusi Qi, Inner Mongolia (115°17′E and 41°58′N) during the

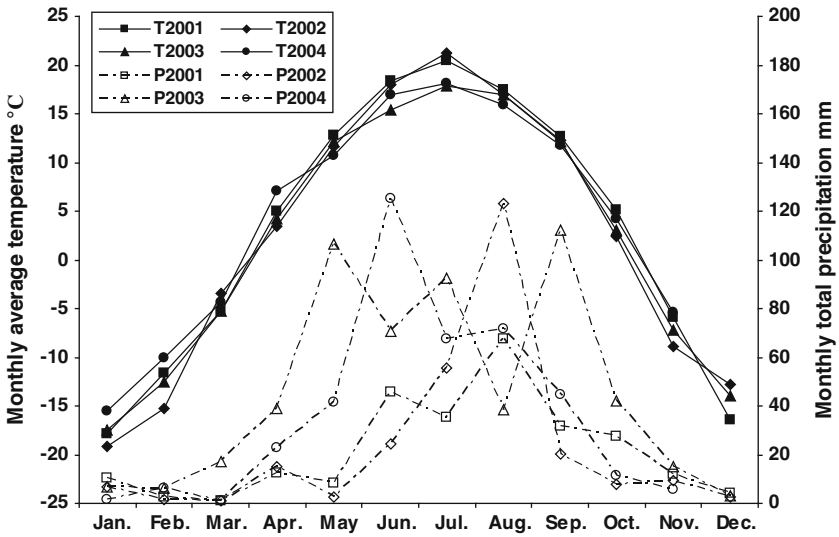

Fig. 2 Monthly average temperature (*T*) and total precipitation (*P*) in Taipusiqi, Inner Mongolia, China during 2001–2004 (data from the Taipusiqi Meteorological Station)

last 10 days of September (autumn), November (winter) 2003, April (spring), and July (summer) 2004. The altitude is 1300–1800 m above sea level and the climate is cool and dry with monthly average temperatures ranging from −20 to 20 °C. The temperatures largely fluctuated between day and night. The annual precipitation varies from 250 to 550 mm (Fig. 2). In this transition zone between crop fields and pasture, the main crops are wheat (*Triticum aestivum*), naked oats (*Avena nuda*), buckwheat (*Fagopyrum esculentum*), rape (*Brassica campestris*) and benne (or til, *Linum ustitatissimum*), and vegetation eaten by gerbils in pasture mainly are *Artemisia sieversiana*, *Aneurolepdium chinense*, *Xanthium sibiricum*, *Artemisia commutata*, *Salsola collina*, and *Setaria viridis*.

All trapped wild gerbils were taken to a local field research station, and housed in plastic cages with dry grass (with some cotton in winter). The animals were checked daily, provided water and seeds of crop in autumn and winter or fresh grass in spring and summer, and were exposed to natural photoperiods and temperatures. All gerbils were killed by decapitation within 3 days after capture to minimize the chance of a rapid change in digestive enzyme activities in response to a diet change. All comparisons were restricted to males to exclude the possible complicating effects of pregnancy and lactation. The age of the trapped animals was estimated based on body length (Xia et al. 1982). In general, body length of gerbils is as follows: young $<$ 90 mm $\leq$ subadult $<$ 110 mm $\leq$ adult. Body length ($\pm$1 mm), i.e., the combined length of the head and the trunk, was measured from the nose tip to the initial caudal vertebra by pressing the animals slightly with a ruler (1 mm).

Gut morphology and intestinal sample collection

The animals were killed after measuring their body mass ($\pm$0.1 g). Their entire gastrointestinal tracts were quickly removed and cleaned of mesenteric attachments on a glass panel on ice. The length of the SI was measured by extending the organ to its unstressed length along a ruler (Pei et al. 2001b; Liu and Wang 2007) and then cut open. The contents were removed, and the SI was rinsed in cold Ringer's solution, blotted dry on tissue paper, and weighed ($\pm$0.001 g). For each gerbil, three 0.8–1.0 cm SI pieces were weighed ($\pm$0.001 g), placed in a plastic tube, and stored in liquid nitrogen for enzyme assays and tissues samples from the most proximal (duodenum), middle (jejunum), and the most distal segments (ileum) of the SI collected (Liu and Wang 2007).

Enzyme activity assays

The effects of seasonal fluctuations in food quality and temperature on the activity of three digestive enzymes sucrase (E.C. 3.2.1.48), maltase (E.C. 3.2.1.20), and aminopeptidase-N (E.C. 3.4.11.2) in the intestinal brush-border membrane were examined. Assays were performed in duplicate, with a mean coefficient of variation of 0.073 % for sucrase, 0.221 % for maltase, and 0.086 % for aminopeptidase-N. Intestinal tissue samples were thawed at 4 °C and homogenized (30 s, using a homogenizer at maximum setting) in 0.9 % NaCl (1:10, w/v) in an ice water bath.

The sucrase and maltase activity was determined using a colorimetric method developed by Dahlqvist (1984) and modified by Martínez del Rio (1990). Liu and Wang (2007) described the disaccharidase assay in detail. Briefly, 100 µl of appropriately diluted tissue homogenate was incubated with 100 µl of 56 mM sugar (sucrose and maltose) solution in 0.1 M maleate/NaOH pH 6.5 at 37 °C. After 10 min of incubation, the reactions were arrested by adding 3 ml of stop/develop reagent. The sample solution was allowed to stand for 20 min. The absorbance was then measured at 505 nm using a

Beckman DU-800 spectrophotometer. The enzyme activity was determined using a glucose standard curve.

Aminopeptidase-N assays were conducted using L-alanine-*p*-nitroanilide as substrate (Maroux et al. 1973). Briefly, 10 µl of appropriately diluted tissue homogenate was incubated with 1 ml of assay solution, consisting of 2.04 mM L-alanine-*p*-nitroanilide in 0.2 M phosphate buffer (NaH_2PO_4/Na_2HPO_4, pH 7.0) at 37 °C for 10 min. The solution was then arrested with 3 ml of chilled 2 N acetic acid. The absorbance was measured at 384 nm, and the activity was determined using a *p*-nitroanilide standard curve.

The protein content of the SI was measured using Folin phenol reagent, with bovine serum albumin as the standard. The absorbance was read at 500 nm (Assay type, Lowry-Low Resolution). Hydrolytic activity, standardized by intestinal wet mass (g), was linearly correlated (Pearson's correlation) with activity standardized per gram of protein (on average $r_p = 0.83 \pm 0.03$ for all regional activity of all enzymes). Each gram of intestinal tissue contained approximately 84 mg of protein (duodenum 84.47 ± 2.07, jejunum 87.10 ± 2.66, and ileum 80.90 ± 2.85). Therefore, the following results were exclusively standardized by wet tissue mass.

The total and standardized intestinal enzyme activity was calculated. Enzyme activity is presented as total hydrolytic activity (µmol min^{-1}), and as activity per unit intestinal wet mass (µmol min^{-1} g wet $tissue^{-1}$). Martínez del Rio et al. (1990) discussed the advantages of our normalization procedures. The total hydrolytic activity of the entire SI was calculated by multiplying the average tissue-specific activity (µmol min^{-1} g wet $tissue^{-1}$) of the three regions by the total wet mass of the SI (Liu and Wang 2007).

Determination of optimum pH and K_m*

The optimum pH of sucrase and aminopeptidase-N was determined in the jejunum or the duodenum of the SI in one gerbil from each season. The assays were performed using the homogenates and a 0.1 M maleate: NaOH buffer system with pH values ranging from 5.0 to 7.5 for sucrase and from 5.5 to 9.0 for aminopeptidase-N. The apparent binding constants (K_m*) were estimated using the substrate concentration at which the hydrolytic rate was half the maximum hydrolytic rate (V_{max}) for sucrase and for aminopeptidase-N. Enzyme activity was assayed at the optimum pH with substrate concentrations ranging from 7 to 224 mM for sucrase and from 0.125 to 8 mM for aminopeptidase-N. The relative activity was calculated to minimize the individual variation (Caviedes-Vidal et al. 2000).

Statistics

All results are shown as mean ± SE. Prior to statistical analyses, all data were tested for normality and homogeneity of variance using Kolmogorov–Smirnov and Levene's tests, respectively. Some data had no normality or homogeneity of variance ($P < 0.05$) and were transformed using log10. A 4 × 3 two-factor analysis of covariance (ANCOVA) was utilized to test for the significant effects of season, age, and their interactions on gut morphology and the total hydrolytic capacity of the entire SI using body mass as covariate. For SI mass, the adjusted body mass (body mass at dissection minus organ mass) was used as a covariate to avoid part-whole correlation in the ANCOVA for organ size (Christians 1999). Repeated measures analysis of variance (RM-ANOVA) was used to examine the effects of season, age, and intestinal region on enzyme activity. A post hoc Tukey's HSD tests were used to isolate seasonal and age differences in each region or in total hydrolytic capacity. Pearson's correlation was used to find the correlations of SI mass with body mass or body length, and of sucrase with maltase or aminopeptidase-N. To test for sucrase-independent maltase-glucoamylase activity, the activity of maltase against sucrase was regressed. The regression coefficients were analyzed using a *t* test. The significance level was set to $P < 0.05$. Kinetic parameters were determined by fitting the kinetic data using nonlinear curve fitting to the following equation: relative activity = (V_{max} × concentration)/(K_m* + concentration). All statistical tests were performed using SPSS.

Results

Gut morphology

Age significantly affected body mass ($F_{2,\ 44} = 186.624$, $P < 0.001$, ANOVA). Age and season interacted with body mass ($F_{6,\ 44} = 5.177$, $P < 0.001$, ANOVA) (Table 1). SI mass ($r_p = 0.760$, $P < 0.001$) with the adjusted body mass and length ($r_p = 0.569$, $P < 0.001$) was significantly correlated with body mass. Therefore, the data were analyzed by ANCOVA. Season significantly affected SI length ($F_{3,\ 43} = 8.974$, $P < 0.001$, ANCOVA) and wet mass ($F_{3,\ 43} = 6.149$, $P = 0.001$, ANCOVA). The male gerbils had the longest and heaviest SI during winter (Fig. 3a, b). The effects of age were not statistically significant for SI length ($F_{2,\ 43} = 0.629$, $P = 0.538$, ANCOVA) and SI mass ($F_{2,\ 43} = 2.489$, $P = 0.095$, ANCOVA).

Regional enzyme activity

Intestinal position highly significantly affected the sucrase activity, maltase activity, and aminopeptidase-N activity (RM-ANOVA, Table 2). However, the specific-mass activity of the digestive enzymes exhibited heterogeneous distributions with the SI, with the highest activity in the jejunum for all enzymes. Significant differences in sucrase

Table 1 Body mass, SI length, and SI mass in the wild male gerbils

Season	Age	*n*	Body mass (g)	SI length (cm)	SI mass (g)	Relative SI L ($*10^2$ cm/kg BM)	Relative SI M (g/kg BM)
Autumn	Young	4	33.3 ± 2.4	33.1 ± 1.5	1.252 ± 0.130	10.18 ± 1.31	34.76 ± 3.78
	Subadult	6	45.6 ± 1.9	32.8 ± 1.0	1.262 ± 0.084	7.21 ± 0.31	27.78 ± 1.41
	Adult	5	53.9 ± 2.1	34.4 ± 1.2	1.542 ± 0.108	6.53 ± 0.24	30.51 ± 1.89
Winter	Young	4	29.1 ± 2.4	34.2 ± 1.7	1.356 ± 0.149	11.53 ± 0.98	40.77 ± 5.13
	Subadult	6	50.0 ± 1.9	37.7 ± 1.0	1.566 ± 0.090	7.63 ± 0.24	32.22 ± 2.35
	Adult	4	55.9 ± 2.4	36.5 ± 1.4	1.540 ± 0.124	6.65 ± 0.25	29.83 ± 3.43
Spring	Young	4	24.8 ± 2.4	30.6 ± 2.0	1.058 ± 0.171	12.17 ± 1.28	34.64 ± 5.23
	Subadult	3	41.6 ± 2.7	31.3 ± 1.4	0.989 ± 0.122	7.49 ± 0.24	22.61 ± 1.00
	Adult	5	63.2 ± 2.1	34.1 ± 1.7	1.468 ± 0.150	5.60 ± 0.19	26.45 ± 0.67
Summer	Young	4	25.7 ± 2.4	30.0 ± 1.9	1.054 ± 0.166	11.28 ± 0.59	32.74 ± 2.76
	Subadult	6	44.0 ± 1.9	32.9 ± 1.0	1.234 ± 0.085	7.51 ± 0.36	27.77 ± 1.65
	Adult	5	63.4 ± 2.1	33.0 ± 1.7	1.383 ± 0.151	5.40 ± 0.38	25.18 ± 1.63
Effects			A***, S × A***[a]	S***[b]	S**[b]	A***[a]	A***, S*[a]

For body mass, values are mean ± SE. For length and mass of small intestine, values are the covariate-adjusted mean ± SE

Effects: *A* age, *S* season, × indicates interactions

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

[a] By two-way ANOVA

[b] By two-way ANCOVA, body mass as covariate

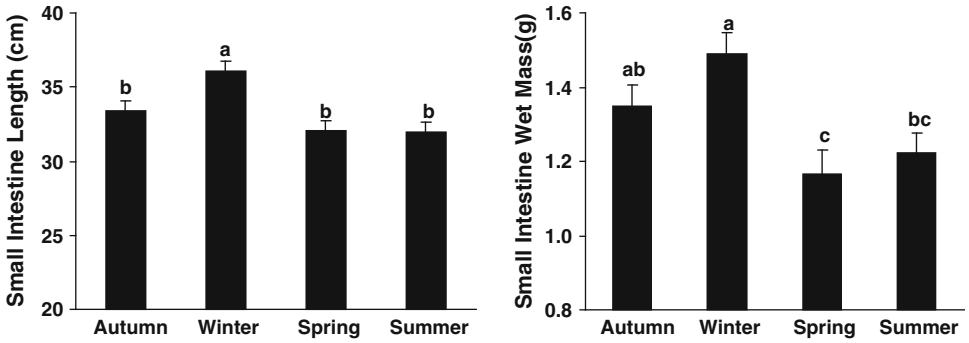

Fig. 3 Small intestine length and wet mass in wild male Mongolian gerbils. *Bars* are the adjusted mean ± SE. *Bars with different letters* differ significantly at $P < 0.05$

activity (jejunum > ileum, duodenum, $P < 0.001$; I = D, $P = 0.067$), maltase activity (J > I > D, $P < 0.001$), and aminopeptidase-N activity (J > I > D, $P < 0.001$) were observed among three SI regions (Fig. 4a-3, b-3, c-3).

Season significantly affected the specific-mass activity of sucrase and maltase. For aminopeptidase-N, the difference was not significant ($P > 0.05$, Table 2). Sucrase had higher activity in winter ($P = 0.007$) and in spring ($P = 0.005$) than in summer (Fig. 4a-2). Maltase had higher activity in spring than in summer ($P = 0.004$) and in autumn ($P < 0.001$) (Fig. 4b-2). Comparison of aminopeptidase-N activity among the seasons indicated a significant difference only between spring and autumn (Spring > Autumn, $P = 0.010$; Fig. 4c-2). Moreover, the interaction between season and position significantly affected the expression of sucrase, which reveals that the modulation pattern of sucrase in the duodenum with that of the seasons differs from that in other regions (Fig. 4a-2).

Age did not significantly affect sucrase and maltase activiy (Table 2). However, the interaction of age with season and intestinal position significantly affected maltase activity, which suggests that the relative distribution of maltase along the intestines varied with season and age. The non-significant interaction between season and age suggests that the sucrase modulation pattern in gerbils with different ages differed with the season (Fig. 4a-1). Age potentially affected aminopeptidase-N activity. Comparisons of aminopeptidase-N activity among gerbils with different ages only indicated a significant difference between young and subadult gerbils (young > subadult, $P = 0.030$; Fig. 4c-3) and no difference was observed between young and adult gerbils ($P = 0.084$). The interaction of season with age significantly affected aminopeptidase-N activity, which indicated that gerbils with different ages have different aminopeptidase-N modulation pattern with different season (Fig. 4c-1).

Table 2 Effects of season, age, region along the intestine, and their interaction on small intestinal enzyme activity in wild male Mongolian gerbils

Factors[a]	*df*	Enzyme activity (μmol/min/g)					
		Sucrase		Maltase		Aminopeptidase-N	
		F	*P*	*F*	*P*	*F*	*P*
Season (S)	3,44	3.934	**0.014**	5.004	**0.005**	2.478	0.074
Age (A)	2,44	0.551	0.580	1.183	0.316	2.733	0.076
Position (P)	2,88	74.614	**<0.001**	61.358	**<0.001**	34.425	**<0.001**
S × A	6,44	2.068	0.077	1.396	0.238	3.881	**0.003**
S × P	6,88	3.139	**0.008**	1.440	0.209	0.948	0.465
A × P	4,88	1.645	0.170	1.896	0.118	1.279	0.284
S × A × P	12,88	0.916	0.535	2.222	**0.017**	0.891	0.559

Bold values indicate the significance of $P < 0.05$

[a] By two-way RM-ANOVA

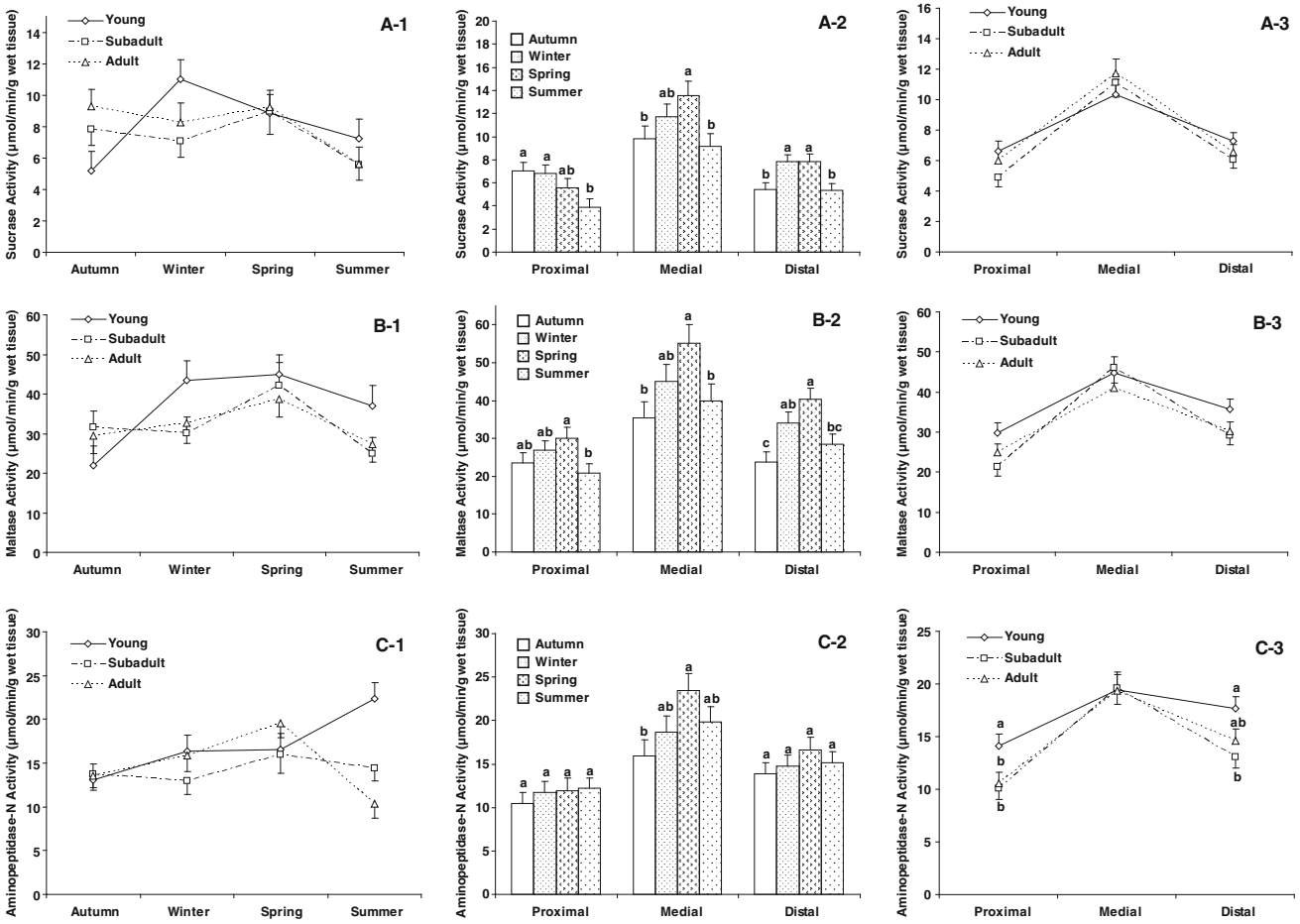

Fig. 4 Tissue mass-specific enzyme activity in small intestines of wild male Mongolian gerbils. *Bars with different letter* differ significantly at $P < 0.05$. Statistical comparisons are in Table 2

Total activity of hydrolases

Seasonal changes had significant effect on the total hydrolytic rates for sucrase ($F_{3,\ 44} = 7.004$, $P < 0.001$, ANOVA) and maltase ($F_{3,\ 44} = 5.824$, $P = 0.002$, ANOVA), and had no significant effect on the total hydrolytic rate of aminopeptidase-N ($F_{3,\ 44} = 2.319$, $P = 0.088$, ANOVA). Age had a significant effect in all

Table 3 Effects of season, age, and their interaction on small intestinal total hydrolytic capacity of wild male Mongolian gerbils

Factors[a]	*df*	Total hydrolytic capacity (μmol/min)					
		Sucrase		Maltase		Aminopeptidase-N	
		F	*P*	*F*	*P*	*F*	*P*
Season (S)	3,43	7.409	**0.0004**	6.369	**0.0011**	2.289	0.092
Age (A)	2,43	2.279	0.115	1.501	0.234	2.075	0.138
S × A	6,43	2.978	**0.016**	1.536	0.189	3.482	**0.007**

Bold values indicate the significance of $P < 0.05$

[a] By two-way ANCOVA using body mass as covariate

cases ($F_{2,\ 44} > 9.75$, $P < 0.001$, ANOVA). Season and age showed a significant interaction on aminopeptidase-N ($F_{6,\ 44} = 3.561$, $P = 0.006$, ANOVA) and a potential interaction on sucrase ($F_{6,\ 44} = 2.275$, $P = 0.053$, ANOVA).

Significant linear correlations were observed between body mass and the total hydrolytic capacity of sucrase ($r_p = 0.503$, $P < 0.001$), maltase ($r_p = 0.501$, $P < 0.001$), and aminopeptidase-N ($r_p = 0.434$, $P < 0.001$). After controlling for the effects of body mass using an ANCOVA (Table 3), the effects of season on sucrase and maltase, which were the same as above, were significant. No significant effect of season on aminopetidase-N was observed. The interaction between season and age significantly affected sucrase and aminopeptidase-N. The effects of age disappeared in all cases, which indicated that the effects of age on total hydrolytic capacity of digestive enzymes are mainly attributed to body mass. The relationship between the total hydrolytic capacity of all enzymes and body mass was better described by a linear rather than an exponential relationship ($r_p = 0.461$, 0.412, and 0.348 for sucrase, maltase, and aminopeptidase-N, respectively).

After removing the effect of body mass, the multiple comparison tests showed that total sucrase activity during summer is significantly lower than in autumn (40.5 %, $P = 0.009$), winter (70.4 %, $P < 0.001$), and spring (45.3 %, $P = 0.007$). The total sucrase activity in winter was slightly higher than that in autumn (21.3 %, $P = 0.056$) (Fig. 5a). The total maltase hydrolytic capacity in winter and spring were significantly higher than that in summer (44.6 %, $P = 0.001$; 38.2 %, $P = 0.005$) and autumn (34.3 %, $P = 0.004$; 28.3 %, $P = 0.022$) (Fig. 5b). Total aminopeptidase-N activity was significantly higher only in winter than in summer (26.0 %, $P = 0.034$), and slightly higher than that in autumn (22.8 %, $P = 0.056$) (Fig. 5c).

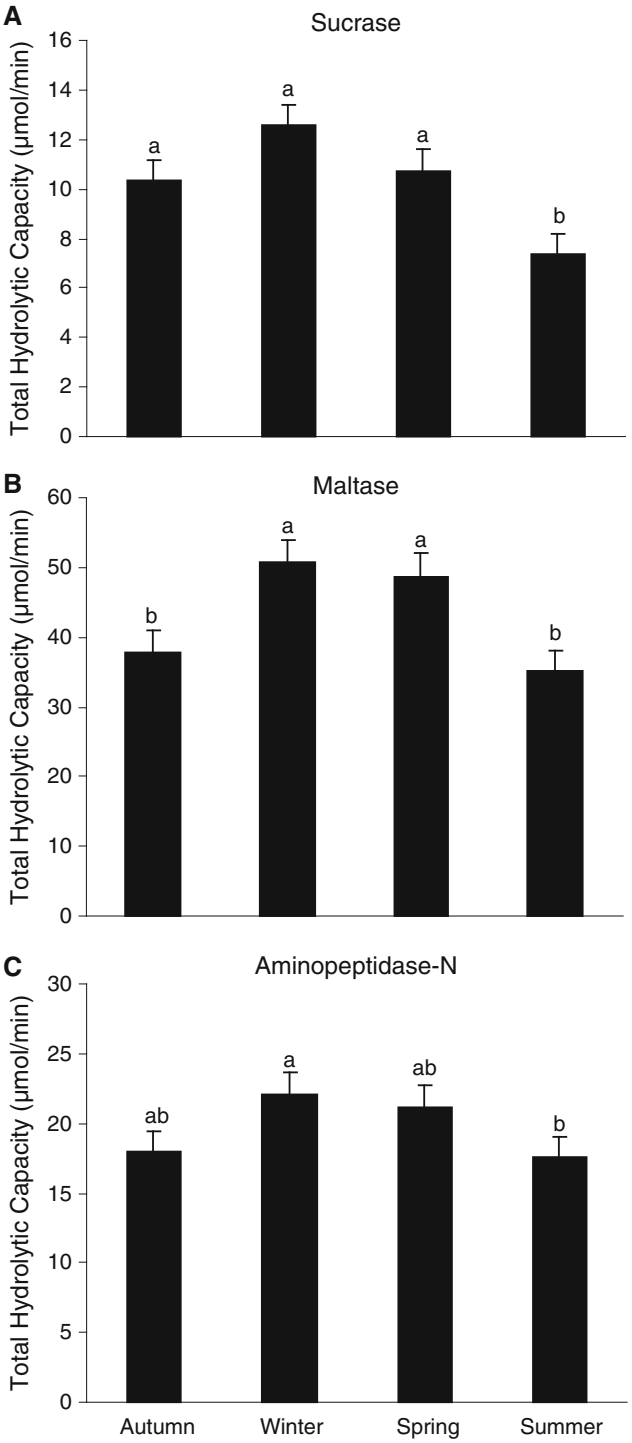

Fig. 5 Total hydrolytic capacity for sucrase (**a**), maltase (**b**) and aminopeptidase-N(c) in wild male Mongolian gerbils. *Bars* are the adjusted mean ± S.E. and within each plot *bars not sharing a common letter at their top* are significantly different ($P < 0.05$)

Relationships among sucrase, maltase, and aminopeptidase-N

Intestinal sucrase activity was positively correlated with maltase activity in the duodenum ($r_p = 0.828$, $P < 0.001$), jejunum ($r_p = 0.861$, $P < 0.001$), and ileum ($r_p = 0.832$, $P < 0.001$) (Fig. 6a). The regression for each region was computed by linear regression (Table 4). The intercepts for the duodenum and ileum were positive and statistically

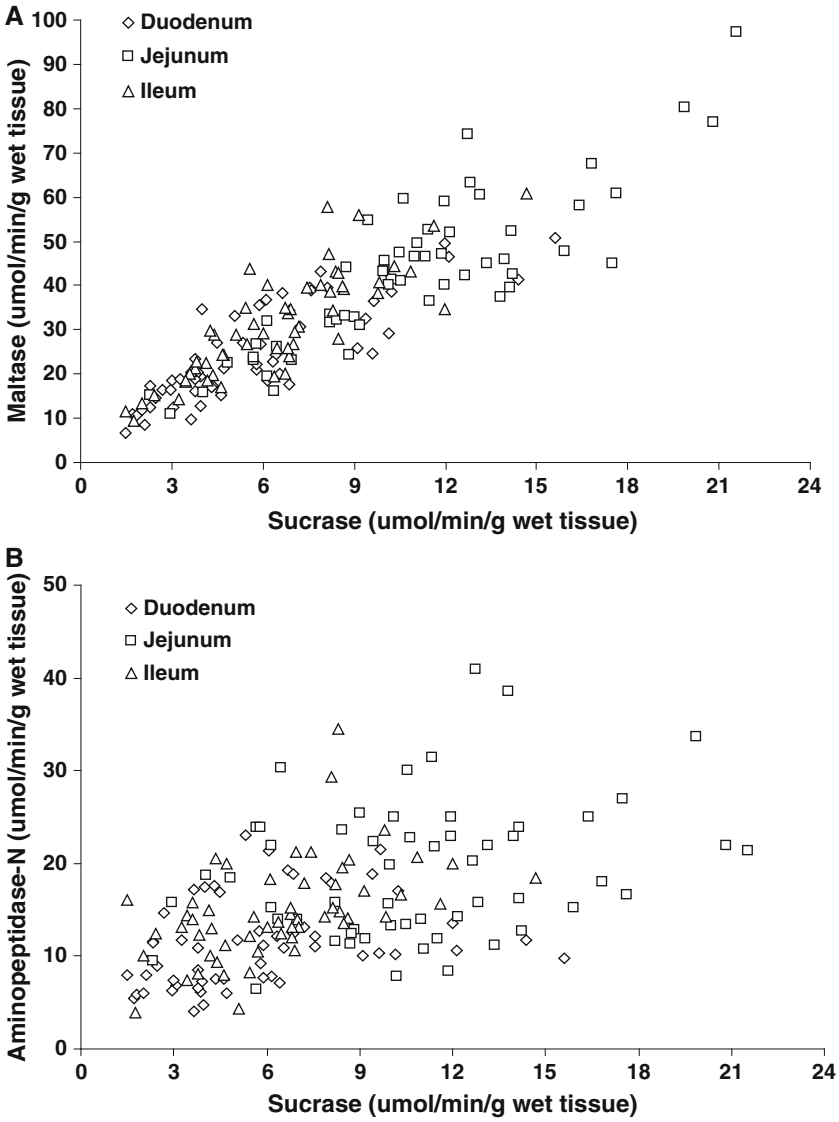

Fig. 6 The relationship between intestinal sucrase activity and maltase (**a**) and aminopeptidase-N (**b**) activity in free-living Mongolian gerbils (*diamond* duodenum, *square* jejunum, *triangle* ileum). Equations for **a**: duodenum: $y = 2.90x + 8.09$, $R^2 = 0.686$, $F = 118.2$, $P < 0.001$; jejunum: $y = 3.51x + 4.58$, $R^2 = 0.741$, $F = 154.8$, $P < 0.001$; ileum: $y = 3.66x + 7.35$, $R^2 = 0.692$, $F = 121.2$, $P < 0.001$, details in Table 4. Equations for **b**: duodenum: $y = 0.50x + 8.57$, $R^2 = 0.111$, $F = 6.73$, $P = 0.012$; jejunum: $y = 0.49x + 13.74$, $R^2 = 0.079$, $F = 4.61$, $P = 0.036$; ileum: $y = 0.98x + 8.59$, $R^2 = 0.246$, $F = 17.66$, $P < 0.001$

Table 4 The relationship between maltase and sucrase activity in three intestinal regions of wild male Mongolian gerbils

Intestinal region	Model I regression				Mean sucrase activity (μmol/min/g)	Mean maltase activity (μmol/min/g)	Maltase activity	
	Slope	t^a	Intercept	t			Sucrase-dependent	Sucrase-independent
Duodenum	2.904 ± 0.267	10.871***	8.809 ± 1.763	4.588***	5.837 ± 0.363	25.349 ± 1.310	66.9	33.1
Jejunum	3.512 ± 0.282	12.441***	4.579 ± 3.298	1.388	11.048 ± 0.566	43.921 ± 2.244	88.3	11.7
Ileum	3.656 ± 0.332	11.007***	7.350 ± 2.323	3.163**	6.610 ± 0.312	31.701 ± 1.393	76.2	23.8

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

[a] t test

significant. However, the intercepts were not significant in the jejunum. The slopes were similar in all three intestinal regions (Table 4). Sucrase activity was weakly positively correlated with aminopeptidase-N activity in the duodenum ($r_p = 0.311$, $P = 0.020$), jejunum ($r_p = 0.281$, $P = 0.036$), and ileum ($r_p = 0.496$, $P < 0.001$) (Fig. 6b).

pH and kinetics

The optimum pH for sucrase and aminopeptidase-N were 5.5 and 7.5 ($n = 4$ for each enzyme, Fig. 7a, b). A slightly different pH was utilized in the assays. However, the measurements provided estimates for 95–99 % (average

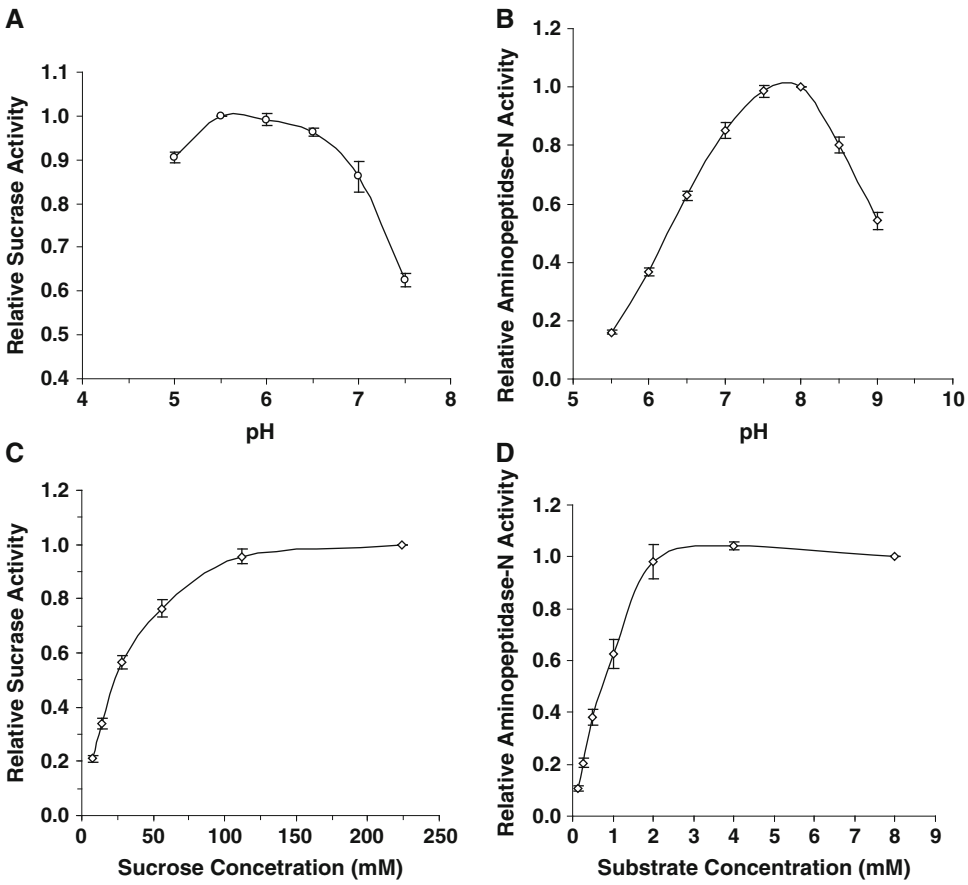

Fig. 7 The effects of pH and substrate concentration on intestinal enzyme activity in wild male Mongolian gerbils. **a** The relationship with solution pH for sucrase activity and **b** for aminopeptidase-N activity. **c** The relationship with substrate concentration for sucrase and **d** for aminopeptidase-N. Values are standardized to the maximum value measured. All points are the mean ± S.E

96 %) and 81–93 % (average 85 %) of the maximal sucrase and aminopeptidase-N activity, respectively. The K_m* for sucrase averaged 30.942 ± 2.517 ($n = 8$) and 0.917 ± 0.139 ($n = 4$) mM for aminopeptidase-N (Fig. 7c, d).

Discussion

The intestinal disaccharidase activity was upregulated in the Mongolian gerbils during the seasons when the animals fed mainly on seeds but downregulated in summer when their diet consisted mainly of grass stems and leaves. The total hydrolytic capacity of the three enzymes was higher in winter than in summer. The seasonal modulation of these digestive enzymes showed different patterns among the three anatomical regions of the SI. Moreover, the effects of age on the total hydrolytic capacity could have been caused by differences in body mass among the studied age groups.

Seasonal modulation of gut size and digestive enzymes

Pattern and magnitude of modulation

Gut size The SI best fits the plug-flow reactor model (Penry and Jumars 1987). The length of SI is often related to difficulty in digesting the usual diet of the animal because greater intestinal length increases retention time in plug-flow reactors, thereby maximizing digestion (Hume 1989; Stevens and Hume 1995). In free-living Mongolian gerbils, the SI was largest in winter and smallest in summer regardless of age. The magnitude of the changes in the SI between winter and summer were 13 % for length and 28 % for wet mass. The changes can be attributed to the acute seasonal fluctuations in local temperature (Fig. 2). Many fields and laboratory studies confirmed that the SI significantly dilates in response to cold (e.g., Gross et al. 1985; Green and Millar 1987; Derting and Bogue 1993; Derting and Noakes 1995; Hammond and Wunder 1995; Derting and Hornung 2003; Naya et al. 2005). Accordingly, the length of the SI increased by 24 % and the mass increased by 25 % after adult male Mongolian gerbils were exposed to 5 °C for 4 weeks in a laboratory (Liu QuanSheng and Wang DeHua, unpublished data). The evident difference in the extent of SI length between gerbils in the field and laboratory could be related to low food quality during summer in the field. The patterns of increase differed in length and mass. Although our previous study failed to test the effects of fiber on small intestinal size (Pei et al. 2001a), it revealed that the length of the SI of Mongolian gerbils remarkably increased whereas the mass

did not when they were fed a diet with higher fiber content than that in previous study (Pei et al. 2001a) for 2 weeks (Liu and Wang 2007).

Tissue-specific enzyme activity Based on the adaptive modulation hypothesis, tissue-specific sucrase activity and maltase activity were upregulated in Mongolian gerbils during winter and early spring, and downregulated in summer. This trend in enzyme activity is consistent with the seed content of their natural diet (Xia and Zhong 1966; Chen and Li 2000; Fig. 1). In laboratory rats, sucrase-isomaltase may be regulated by sucrose (Koldovsky 1981; Cezard et al. 1983), maltose (Koldovsky 1981), and starch (McCarthy et al. 1980; Goda et al. 1983). However, in the two wild rodent species *Phyllotis darwini* (omnivorous) and *Octodon degus* (herbivorous), sucrase and maltase are not regulated by carbohydrate diet (Sabat et al. 1999). By contrast, in free-living Mongolian gerbils (granivorous), tissue-specific sucrase was seasonally regulated by an average of 58 % and maltase by an average of 57 %. Moreover, sucrase activity is downregulated in the jejunum (23 %) and the ileum (40 %), whereas maltase is downregulated in the ileum (26 %) in Mongolian gerbils fed a high-fiber diet (Liu and Wang 2007). Although many species double their carbohydrase activity under high-carbohydrate diets (Karasov and Hume 1997), young gerbils doubled their sucrase and maltase activity from autumn to winter (Fig. 4a-1, b-1). Therefore, the magnitude of the intestinal enzyme modulation in free-living Mongolian gerbils unnecessarily reflects all their ability because the changes in substrate concentration under a natural diet are not all-or-none. This finding merits future study to determine whether diet or age influences the lability of intestinal enzymes, which is important for understanding intraspecific differences in foraging strategies (Khokhlova et al. 1995; Randolph and Cameron 2001).

Seasonal changes in the protein content of natural wild gerbil diets are unknown. However, inducible digestive enzymes may provide information on diet composition and quality (Karasov and Hume 1997). The tissue-specific aminopeptidase-N activity in wild gerbils was significantly higher in the jejunum by 47 % in spring than in autumn, with lowest age-related variance. The tissue-specific aminopeptidase-N activity in adult gerbils was lower by 88 % in summer than in spring. This result shows lability of aminopeptidase-N activity in gerbils, which suggests that the protein content of wild gerbil diet changes seasonally with the diet switching from seeds to grass. In summer, the aminopeptidase-N activity in young gerbils is remarkably higher by 54 % compared with sub-adults and by 115 % compared with adults, which implies that diet preference varies with age. In the wild, the intraspecific difference of diet choice in small mammals is related to varied energy and nutrient demand (Khokhlova et al. 1995; Randolph and Cameron 2001). Under laboratory conditions, a high-protein diet may elicit increasing aminopeptidase-N expression in rat (Raul et al. 1987) and *Phyllotis darwini* (Sabat et al. 1999), but not in *Octodon degus* (Sabat et al. 1999). However, the aminopeptidase-N activity in Mongolian gerbils fed with high-fiber foods was downregulated in the duodenum (24 %) and ileum (43 %) (Liu and Wang 2007).

Total hydrolytic capacity Total hydrolytic capacity depends on both tissue mass-specific activity and intestinal tissue mass. As mentioned above, both of these factors increased in winter with the decline in temperature and the grass content of the diets. Thus, the total hydrolytic capacity of all intestinal enzymes is highest in winter and lowest in summer. The extent of modulation from summer or autumn to winter or spring is 70 % for sucrase, 45 % for maltase, and 26 % for aminopeptidase-N. The extent of modulation seems to be insufficient to deal with the almost twofold increase in food intake caused by the cold temperatures (2.18 at −6 °C, Liu et al. 2002; 1.71 at 5 °C, Li and Wang 2005; 1.79 at 5 °C, Liu and Wang, unpublished data). However, Li and Wang (2005) found that seasonally acclimatized gerbils only increase their food intake by 51 %. Perhaps in the wild, Mongolian gerbils increase their food intake at a similar extent because of the high living costs in summer. The safety margin in food intake may decrease as the energy demands increase (Toloza et al. 1991; Hammond et al. 1994; Karasov and McWilliams 2005). The total hydrolytic capacity reached maximum under instant conditions, thereby including the spare capacity. Even at lowest levels, these three enzymes only hydrolyze and produce about 11 g of glucose and 2.7 g of amino acid. These values exceed the weight of digestible substrates in the normal daily food intake of gerbils (5.8 g, Liu et al. 2002; 6.9 g, Li and Wang 2005; 4.8 g, Liu and Wang 2007). At higher levels, the production of glucose and amino acids reach 16.5 and 3.4 g, respectively. This is even higher than the maximum dry matter intake measured in our laboratory (13.4 g, Liu et al. 2002). Therefore, total hydrolytic capacity was still 'enough but not too much' (Diamond 1991) to load even though the increase in total hydrolytic capacity was less than that in food intake.

Effects of intestinal region on activity

General patterns Mongolian gerbils displayed similar spatial patterns of enzymatic activity for sucrase, maltase, and aminopeptidase-N. The concentration of three enzymes increased from the duodenum to the jejunum and then decreased in the ileum. The pattern in gerbils is similar to most mammals (Vonk and Western 1984; Stevens and Hume 1995; Weiss et al. 1998; Nespolo et al. 2002). However, the pattern is not the same in some birds (Martínez del Rio 1990; Afik et al. 1995; Martínez del Rio

et al. 1990; Levey et al. 1999; McWilliams et al. 1999; Caviedes-Vidal et al. 2000; Witmer and Martínez del Rio 2001; Ciminari et al. 2005; Stein et al. 2005), which declines in enzyme activity from the proximal to the distal regions of SI. The distribution patterns in enzyme activity may reflect the luminal concentration gradient of specific substrates along the SI (Karasov and Hume 1997).

Seasonal modulation patterns Differences in the seasonal change pattern of enzyme activity were observed in varied intestinal regions and brush-border membrane enzymes. These differences may be partly attributed to seasonal diet shifts, such as increases in sucrase and maltase activity during winter and spring. However, the difference may be partly related to temperature and hence with food intake rate. For aminopeptidase-N, the seasonal changes in enzyme activity in the duodenum and ileum suggest that the protein content of food does not vary with the season. However, in the jejunum, the enzyme activity was higher in spring than in autumn. Considering the food in winter and spring are identical, stored seeds, the higher activity in spring may be due to increased food intake. Li and Wang (2005) found that the food intake of gerbils gradually increases from August and peaks in February. With increasing food intake, the luminal substrate concentration in the jejunum increases, which then upregulates the expression of relevant enzymes. This finding explains the highest enzyme activity in the jejunum of gerbils during spring, and the upregulation of sucrase activity in *Akodon azarae* exposed to cold (del Valle et al. 2004). Moreover, these results indicate that the different degrees of modulation of enzyme activity in each intestinal region do not result from difference in sensitivity in the different regions even though the intestinal region affects enzyme activity, but are due to changes in luminal nutrient concentrations (Karasov and Hume 1997).

Relationship of sucrase activity with maltase and aminopeptidase-N activity

Sucrase and maltase A strong positive correlation was observed between sucrase activity and maltase activity within the same tissue homogenates. The correlation is due to the result of the regulation of maltase activity by two independent enzymatic systems, the complex sucrase-isomaltase ('sucrase') and one or two maltase-glucoamylases (Semenza and Auricchio 1989). In the vertebrates studied, sucrase-isomaltase and maltase-glucoamylases contribute about 80 and 20 % of the total maltase activity, respectively. The data in wild gerbils are similar to these levels (Table 4). However, the activity varied among the different intestinal regions.

Sucrase and aminopeptidase-N The correlation between disaccharidase activity and aminopeptidase-N activity is perplexing because disaccharidase and aminopeptidase-N utilize different substrates. However, we surprisingly observed a weak positive correlation between sucrase and aminopeptidase-N in free-living Mongolian gerbils. Similar correlations were also observed in the two passerine bird species *Zonotrichia capensis* and *Diuca diuca* (Sabat et al. 1998), and the species sandpiper (*Calidris mauri*, Stein et al. 2005). This correlation is considered a structural rather than a functional relationship (Stein et al. 2005), and may be a result of the coregulation of disaccharidases and aminopeptidase-N expression either through nonspecific modulation, where the substrate of one of the enzymes leads directly or indirectly to changes in the activity of both enzymes (Karasov and Diamond 1983), or by changes in the ultrastructure of the SI that influence both enzymes. A carbohydrate-free diet significantly affected the tissue-specific activity of sucrase, maltase, and aminopeptidase-N (see, Sabat et al. 1998; Caviedes-Vidal et al. 2000). Moreover, the role of endogenous proteins, polysaccharides, and glucose derived from the blood in gut in the modulation of digestive enzymes is unknown.

Ecological implications of adaptive modulation in digestive phenotype

Four strategies have evolved to cope with the seasonal fluctuations in food availability and quality: (1) phenotypic change and diet switching, (2) migration to alternate feeding grounds, (3) hibernation, and (4) fasting tolerance (Starck 2005). In the transition zone between grasslands and farmlands, Mongolian gerbils apply seasonal digestive phenotypic changes, diet switching, and migration between grasslands and farmlands. In addition, the gerbils store seeds in several underground storehouses in autumn to maintain relatively constant food availability and quality for a long time. Nevertheless, most of the storehouses are usually destroyed by agricultural activity in spring. Hence, gerbils leave the farmlands for the surrounding grasslands (Liu et al. 2001). In late spring and summer, enough seeds are difficult to find. Consequently, the gerbils switch their diet to the easily available grass stems and leaves, and the excess gut tissue and hydrolytic capacity are downregulated to reduce maintenance costs, such as the size of the SI, sucrase, and maltase. In autumn and winter, increased gut size and hydrolytic capacity guarantee that Mongolian gerbils adapt to diet shifts from grass to seeds and meet the increasing energy demand of cold temperatures. In early spring, maintaining a high digestive capacity is relevant to the coming peak of reproduction and dispersal from family groups. Briefly, digestive phenotypic flexibility allows Mongolian gerbils to adapt seasonal changes in temperature, as well as food quality and availability in Inner Mongolia.

However, whether the inducible and reversible phenotypic plasticity facilitates adaption depends on whether the response of organisms to the environmental changes are delayed (Padilla and Adolph 1996). Therefore, determining whether a delay in the response of digestive phenotype in Mongolian gerbils to acute changes of food availability and quality in a shorter time would be significant because their storehouses are demolished by human agricultural activity in spring or late autumn. Such a study would be relevant to controlling gerbil pests by changing agriculture activities (Zhong et al. 1985; Liu et al. 2001).

Acknowledgments We thank three anonymous reviewers for their constructive comments and suggestions for improving the manuscript. We thank Prof. Zhong Wen-Qin, Dr. Liu Wei and Liu Wen-Dong for their logistic support in the field. The work was supported by Grants from the National Natural Science Foundation of China (No. 30625009, 31071930 and 31100297) and partly by projects from Guangdong Provincial Department of Science and Technology (No. 2011B090300039). All experiments were conducted according to current China law.

References

Afik D, Karasov WH (1995) The trade-offs between digestion rate and efficiency in warblers and their ecological implications. Ecology 76:2247–2257

Afik D, Caviedes-Vidal E, Martínez del Rio C, Karasov WH (1995) Dietary modulation of intestinal hydrolytic enzymes in yellow-rumped warblers. Am J Physiol 269:R413–R420

Buddington RK (1992) Intestinal nutrient transport during ontogeny of vertebrates. Am J Physiol 263:R503–R509

Caviedes-Vidal E, Afik D, Martínez del Rio C, Karasov WH (2000) Dietary modulation of intestinal enzymes of the house sparrow (*Passer domesticus*): testing an adaptive hypothesis. Comp Biochem Physiol A 125:11–24

Cezard JP, Broyart JP, Cuisinier-Gleizes P, Mathieu H (1983) Sucrase–isomaltase regulation by dietary sucrose in the rat. Gastroenterol 84:18–25

Chen D, Li YP (2000) Dietary study of Mongolian gerbils population in the Tumete Plain, Inner Mongolia. Inn Mong Prev Med 25:76–77

Christians JK (1999) Controlling for body mass effects: is part-whole correlation important? Physiol Biochem Zool 72:250–253

Ciminari E, del Valle Moyano G, Chediack JG, Caviedes-Vidal E (2005) Feral pigeons in urban environments: dietary flexibility and enzymatic digestion? Rev Chil Hist Nat 78:267–279

Dahlqvist A (1984) Assay of intestinal disaccharidases. Scan J Clin Lab Invest 44:169–172

del Valle JC, López Mañanes AA, Busch C (2004) Phenotypic flexibility of digestive morphology and physiology of the South American omnivorous rodent *Akodon azarae* (Rodentia: Sigmodontinae). Comp Biochem Physiol A 139:503–512

Derting TL, Bogue BA (1993) Morphological responses of the gut to moderate energy demands in a small herbivore, *Microtus pennsylvanicus*. J Mammal 74:59–68

Derting TL, Hornung CA (2003) Energy demand, diet quality, and central processing organs in wild white-footed mice (*Peromyscus leucopus*). J Mammal 84:1381–1398

Derting TL, Noakes EB (1995) Seasonal changes in gut capacity in the white-footed mouse (*Peromyscus leucopus*) and the meadow vole (*Microtus pennsylvanicus*). Can J Zool 73:243–252

Diamond JM (1991) Evolutionary design of intestinal nutrient absorption: enough but not too much. News Physiol Sci 6:92–96

Goda T, Yamada K, Bustamante S, Koldovsky O (1983) Dietary-induced rapid decrease of microvillar carbohydrase activity in rat jejuno ileum. Am J Physiol 245:G418–G423

Green DA, Millar JS (1987) Changes in gut dimensions and capacity of *Peromyscus maniculatus* relative to diet quality and energy needs. Can J Zool 65:2159–2162

Gross JE, Wang Z, Wunder BA (1985) Effects of food quality and energy needs: changes in gut morphology and capacity of *Microtus ochrogaster*. J Mammal 66:661–667

Hammond KA, Wunder BA (1995) Effect of cold temperatures on the morphology of gastrointestinal tracts of two microtine rodents. J Mammal 76:232–239

Hammond KA, Konauewski M, Torres RM, Diamond JM (1994) Metabolic ceilings under a combination of peak energy demands. Physiol Zool 67:1479–1506

Hernandez A, Martínez del Rio C (1992) Intestinal disaccharidases in five species of phyllostomid bats with contrasting feeding habits. Comp Biochem Physiol B 103:105–111

Hume ID (1989) Optimal digestive strategies in mammalian herbivores. Physiol Zool 62:1145–1163

Karasov WH (1992) Tests of the adaptive modulation hypothesis for dietary control of intestinal nutrient transport. Am J Physiol 263(3):R496–R502

Karasov WH (1996) Digestive plasticity in avian energetics and feeding ecology. In: Carey C (ed) Avian energetics and nutritional ecology. Chapman and Hall, New York, pp 61–84

Karasov WH, Diamond JM (1983) Adaptive regulation of sugar and amino acid transport by vertebrate intestine. Am J Physiol 245:G443–G462

Karasov WH, Hume ID (1997) Vertebrate gastrointestinal system. In: Dantzler W (ed) Handbook of comparative physiology. American Physiological Society, Bethesda, pp 409–480

Karasov WH, Martínez del Rio C (2007) Physiological ecology: how animals process energy, nutrients, and toxins. Princeton University Press, Princeton

Karasov WH, McWilliams SR (2005) Digestive constraints in mammalian and avian ecology. In: Starck JM, Wang T (eds) Physiological and ecological adaptations to feeding in vertebrates. Science Publishers, Enfield, pp 87–112

Karasov WH, Martínez del Rio C, Caviedes-Vidal E (2011) Ecological physiology of diet and digestive systems. Annu Rev Physiol 73:69–93

Khokhlova IS, Degen AA, Kam M (1995) Body size, gender, and seed husking and energy requirements in two species of desert gerbilline rodents, *Meriones crassue* and *Gerbillus henleyi*. Funct Ecol 9:720–724

Koldovsky O (1981) Developmental, dietary and hormonal control of intestinal disaccharidases in mammals (including man). In: Randle JP, Steiner DF, Shelan WJ, Whelan WP (eds) Carbohydrate metebolism and its disorders. Academic, London, pp 418–522

Levey DJ, Place AR, Rey PJ, Martínez del Rio C (1999) An experimental test of dietary enzyme modulation in pine warblers *Dendroica pinus*. Physiol Biochem Zool 72:576–587

Li XS, Wang DH (2005) Seasonal adjustments in body mass and thermogenesis in Mongolian gerbils (*Meriones unguiculatus*): the roles of short photoperiod and cold. J Comp Physiol B 175:593–600

Liu QS, Wang DH (2007) Effects of diluted diet on phenotypic flexibility of organs morphology and digestive function in Mongolian gerbils (*Meriones unguiculatus*). J Comp Physiol B 177:509–518

Liu W, Zhong WQ, Wan XR, Wang GH (2001) The behavioral characteristics of Mongolian gerbils, *Meriones unguiculatus*, and

the ecological strategies of controlling during autumn harvest. Acta Theriol Sin 21:107–115 (in Chinese with English abstract)
Liu H, Wang DH, Wang ZW (2002) Maximum metabolizable energy intake in the Mongolian gerbil (*Meriones unguiculatus*). J Arid Environ 52:405–411
Maroux S, Louvard D, Baratii J (1973) The aminopeptidase from hog intestinal brush-border. Biochim Biophys Acta 321:282–295
Martínez del Rio C (1990) Dietary, phylogenetic, and ecological correlates of intestinal sucrase and maltase activity in birds. Physiol Zool 63:987–1011
Martínez del Rio C, Brugger KE, Rios JL, Vergara ME, Witmer M (1990) An experimental and comparative study of dietary modulation of intestinal enzymes in European starlings (*Sturnus vulgaris*). Physiol Zool 68:490–511
McCarthy DM, Nicholson JA, Kim YS (1980) Intestinal enzyme adaptation to normal diets of different composition. Am J Physiol 239:G445–G451
McWilliams SR, Karasov WH (2001) Phenotypic flexibility in digestive system structure and function in migratory birds and its ecological significance. Comp Biochem Physiol A 128:579–593
McWilliams SR, Caviedes-Vidal E, Karasov WH (1999) Digestive adjustments in Cedar Waxwings to high feeding rate. J Exp Zool 283:394–407
Naya DE, Bacigalupe LD, Bustamante DM, Bozinovic F (2005) Dynamic digestive responses to increased energy demands in the leaf-eared mouse (*Phyllotis darwini*). J Comp Physiol B 175:31–36
Naya DE, Bozinovic F, Karasov WH (2008) Latitudinal trends in digestive flexibility: testing the climatic variability hypothesis with data on the intestinal length of rodents. Am Nat 173(4): 122–134
Nespolo RF, Bacigalupe LD, Sabat P, Bozinovic F (2002) Interplay among energy metabolism, organ mass and digestive enzyme activity in the mouse-opossum *Thylamys elegans*: the role of thermal acclimation. J Exp Biol 205:2697–2703
Padilla DK, Adolph SC (1996) Plastic inducible morphologies are not always adaptive: the importance of time delays in a stochastic environment. Evol Ecol 10(1):105–117
Pei YX, Wang DH, Hume ID (2001a) Effects of dietary fiber on digesta passage, nutrient digestibility, and gastrointestinal tract morphology in the granivorous Mongolian gerbil (*Meriones unguiculatus*). Physiol Biochem Zool 74:742–749
Pei YX, Wang DH, Hume ID (2001b) Selective digesta retention and coprophagy in Brandt's vole (*Microtus brandti*). J Comp Physiol B 171:457–464
Penry DL, Jumars PA (1987) Modeling animal guts as chemical reactors. Am Nat 129:69–96
Randolph JC, Cameron GN (2001) Consequences of diet choice by a small generalist herbivore. Ecol Monogr 71:117–136
Raul F, Goda T, Gosse F, Koldovsky O (1987) Short-term effect of high-protein/low-carbohydrate diet on aminopeptidase in adult rat jejuno ileum. J Biochem 247:401–405
Sabat P, Novoa F, Bozinovic F, Martínez del Rio C (1998) Dietary flexibility and intestinal plasticity in birds: a field and laboratory study. Physiol Zool 71:226–236
Sabat P, Lagos JA, Bozinovic F (1999) Test of the adaptive modulation hypothesis in rodents: dietary flexibility and enzyme plasticity. Comp Biochem Physiol A 123:83–87
Semenza G, Auricchio S (1989) Small-intestine disaccharidases. In: Scribner CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease (II). McGraw Hill, New York, pp 2975–2997
Sibly RM (1981) Strategies in digestion and defecation. In: Townsend CR, Calow P (eds) Physiological ecology: an evolutionary approach to resource use. Blackwell Scientific Publications, Oxford, pp 109–139
Starck JM (1999) Structural flexibility of the gastro-intestinal tract of vertebrates—implications for evolutionary morphology. Zool Anz 238:87–101
Starck JM (2005) Structural flexibility of the digestive system of tetrapods—patterns and processes at the cellular and tissue level. In: Starck JM, Wang T (eds) Physiological and ecological adaptations to feeding in vertebrates. Science Publishers, Enfield, pp 175–200
Stein RW, Place AR, Lacourse T, Gulielmo CG, Williams TD (2005) Digestive organ sizes and enzyme activity of refueling western sandpipers (*Calidris mauri*): contrasting effects of season and age. Physiol Biochem Zool 78:434–446
Stevens CE, Hume ID (1995) Comparative physiology of the vertebrate digestive system. Cambridge University Press, New York
Toloza EM, Lam M, Diamond JM (1991) Nutrient extraction by cold-exposed mice: a test of digestive safety margins. Am J Physiol 261:G608–G620
Vonk HJ, Western RH (1984) Comparative biochemistry and physiology of enzymatic digestion. Academic Press, London
Wang DH, Wang ZW, Wang YS, Yang JC (2003) Seasonal changes of thermogenesis in Mongolian gerbils (*Meriones unguiculatus*) and Brandt's voles (*Microtus brandti*). Comp Biochem Physiol A 134:S96 (Abstract)
Weiss SL, Lee EA, Diamond JM (1998) Evolutionary matches of enzyme and transporter capacities to dietary substrate loads in the intestinal brush border. Proc Natl Acad Sci 95:2117–2121
Witmer MC, Martínez del Rio C (2001) The membrane-bound intestinal enzymes of waxwings and thrushes: adaptive and functional implications of patterns of enzyme activity. Physiol Biochem Zool 74(4):584–593
Xia WP, Zhong WQ (1966) The successions and interactions of the rodent and plant communities of abandoned fields in desert steppe at Chagan-AoBao, Inner Mongolia. Acta Zool Sin 18:199–208 (in Chinese with English abstract)
Xia WP, Liao CH, Zhong WQ, Sun CL, Tian Y (1982) On the population dynamics and regulation of *Meriones unguiculatus* in agricultural region north to Yin Mountains, Inner Mongolia. Acta Theriol Sin 2:51–71 (in Chinese with English abstract)
Zhang ZB, Wang ZW (1998) Ecology and management of rodent pests in agriculture. Ocean Press, Beijing
Zhang ZB, Zhong WQ, Fan NC (2003) Rodent problems and management in the grasslands of China. In: Singleton GR, Hinds LA, Krebs CJ, Spratt DM (eds) Rats, mice and people: rodent biology and management. ACIAR Monograph, No. 96, 5649, Canberra, pp 316–319
Zhong WQ, Zhou QQ, Sun CL (1985) The basic characteristics of the rodent pests on the pasture in Inner Mongolia and the ecological strategies of controlling. Acta Theriol Sin 5:241–249 (in Chinese with English abstract)