The Journal of Experimental Biology 204, 1991–2000 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
JEB3187

EFFECTS OF ALTITUDE AND TEMPERATURE ON ORGAN PHENOTYPIC PLASTICITY ALONG AN ALTITUDINAL GRADIENT

KIMBERLY A. HAMMOND[1,2,*], JOE SZEWCZAK[2] AND ELŻBIETA KRÓL[1,3]

[1]*Department of Biology, University of California, Riverside, CA 92521, USA,* [2]*University of California White Mountain Research Station, 3000 East Line Street, Bishop, CA 93514, USA* and [3]*Department of Zoology, University of Aberdeen, Aberdeen AB24 2TZ, Scotland, UK*

*e-mail: khammond@ucrac1.ucr.edu

Accepted 13 March 2001

Summary

Small mammals living in high-altitude environments must endure decreased ambient temperatures and hypoxic conditions relative to sea-level environments. Previously, it was noted that heart, lung and digestive tract masses and blood hematocrit increase along an altitudinal gradient in small mammals. Increases in digestive organ mass were attributed to lower ambient temperatures and greater food intake, and increases in lung mass and hematocrit were attributed to hypoxia, but these assumptions were not explicitly tested. In addition, it was not clear whether changes in heart and lung mass were a function of an increase in organ blood content or of an increase in organ tissue mass. We used captive deer mice (*Peromyscus maniculatus sonoriensis*) to determine the relative effects of ambient temperature and oxygen concentration (P_{O_2}) on organ mass and blood hematocrit along an altitudinal gradient. We also exsanguinated hearts and lungs to determine whether changes in mass were associated with the blood content or with increases in tissue mass. We found that small intestine mass was, as expected, correlated positively with energy intake and negatively with ambient temperature. Heart mass was also negatively correlated with temperature. Lung mass and hematocrit were, as expected, positively correlated with altitude (and P_{O_2}). Interestingly, the masses of both small intestine and kidney were negatively correlated with altitude. For kidney mass, this correlation was apparent in cold-exposed mice but not in warm-exposed mice. We also found that changes in both heart and lung mass were mainly a function of changes in tissue mass rather than blood content. These data show that different abiotic variables have different effects on organ masses at high altitude, but also that phenotypic plasticity in response to cold temperatures and low oxygen pressures at altitude is widespread across several different organ systems, suggesting a general elevated whole-body response.

Key words: deer mouse, *Peromyscus maniculatus sonoriensis*, phenotypic plasticity, lung mass, heart mass, small intestine mass, altitude, temperature, hypoxia.

Introduction

Over the last few decades, it has become apparent that phenotypic plasticity (changes in the magnitude of anatomical, morphological or physiological characters) and phenotypic flexibility (reversible phenotypic plasticity) of various morphological and physiological characters are widespread in the animal and plant kingdoms (for general references, see Piersma and Lindström, 1997; Schlichting and Pigliucci, 1998). It has been well documented in laboratory studies on endotherms that organ size and functional capacity are correlated with changes in both short-term aerobic performance (Bech and Østnes, 1999; Chappell et al., 1999; Hammond et al., 2000) and long-term sustainable metabolic rate (see Moss, 1989; Redig, 1989; Daan et al., 1990; Loeb et al., 1991; Hammond and Wunder, 1991; Hammond et al., 1994; Konarzewski and Diamond, 1994; McDevitt and Speakman, 1994; Koteja, 1996; Speakman and McQueenie, 1996; Derting and Austin, 1998; Starck, 1999a; Starck, 1999b; Hammond and Kristan, 2000). It is also important to understand how changes in organ size and function affect changes at the level of organism function in natural settings to determine whether phenotypic plasticity is important for survival and fitness.

One way of examining the efficacy of plastic traits for improving whole-organism performance is to study them in different seasons. Piersma and colleagues used this approach to examine changes in organ and muscle size and function in migratory shorebirds. They documented preflight increases in muscle and heart mass and concomitant decreases in digestive organ mass (Piersma et al., 1993; Weber and Piersma, 1996; Battley and Piersma, 1997; Piersma and Gill, 1998; Piersma, 1998; Piersma et al., 1999). Increases in the mass of flight muscles required to maintain high metabolic output come at the expense of increased tissue maintenance costs (Kersten and Piersma, 1987; Piersma et al., 1996).

Table 1. *Sample sizes and experimental design for temperature* versus *altitude and tissue* versus *blood volume experiments*

Altitude (m)	Temperature treatment	Temperature *versus* altitude				Tissue *versus* blood volume	
		1998		1999		1999	
		Females	Males	Females	Males	Females	Males
3800	Cold	7	3	8	2	5	5
	Warm	6	3	6	1	5	5
370	Cold			4	1	4	3
	Warm			6	1	4	3

Another way to examine the benefits of organ plasticity is to examine these changes along natural environmental gradients. Deer mice (*Peromyscus maniculatus*) represent an ideal model species for this approach. Deer mice inhabit one of the widest altitudinal ranges of any North American mammal, living from below sea level (Death Valley) to more than 4300 m above sea level. They also possess a number of naturally occurring hemoglobin haplotypes that have population gene frequencies that (i) are strikingly correlated with altitude and (ii) affect short-term aerobic performance (Snyder, 1978b; Chappell and Snyder, 1984; Chappell et al., 1988). It has also been shown that there is selection for maximum aerobic performance in free-living deer mice at high altitude (Hayes and O'Connor, 1999).

Deer mice living in semi-natural conditions (enclosed outdoor cages) display variation in the sizes of their digestive organs, heart and lungs across an altitudinal gradient (Hock, 1961; Hock, 1964; Hammond et al., 1999) that may be important in allowing them to live at high altitudes. The digestive organs are larger in high-altitude mice, presumably as a result of higher food intakes brought about by decreased ambient temperatures and higher thermoregulatory costs. The masses of heart and lung tissue are also greater in high-altitude mice, presumably as a result of lower oxygen partial pressures (P_{O_2}) because lung function must be greater to gain enough oxygen and heart muscles must be larger to pump more blood to the tissues. Ambient temperature is closely correlated with P_{O_2} across large altitude ranges, so it is not possible to distinguish between the effects of these environmental factors in the studies on organ phenotypic plasticity mentioned above. In addition, because of the inherent difficulty in distinguishing tissue mass from blood contained in the tissue in simple mass measurements in these studies, it is unclear whether the changes observed in cardiac and respiratory tissues were a function of organ tissue (hyperplasia and/or hypertrophy of alveolar, vascular or interstitial tissues) *per se* or of changes in tissue blood volume or flow (Tucker and Horvath, 1973) or pulmonary edema (Bartlett and Remmers, 1971). Finally, in the previous studies from this laboratory (Hammond et al., 1999), a low-altitude-derived subspecies of deer mouse (*P. maniculatus bairdii*) was used. It is desirable to place this type of study in an evolutionary context by using animals native to the environmental conditions in question to understand how natural selection may have acted upon them.

The aims of this study were (i) to investigate the effects of temperature and altitude, as independent environmental factors, on organ phenotypic plasticity in deer mice (temperature *versus* altitude experiment), and (ii) to identify the nature of increases in organ mass along an altitudinal gradient (tissue *versus* blood content experiment). Following from these goals, we predicted that, in high-altitude mice, the increase in mass of digestive organs would be induced by low ambient temperature, while the increase in heart and lung mass would be a result of low P_{O_2}. We also expected that the increase in mass of the heart and lungs would be a consequence of increased tissue mass and blood content rather than increased blood content alone. We used a species of deer mouse (*P. maniculatus sonoriensis*) derived from a population native to high altitude for this research.

Materials and methods

Experimental design

Animals

For this study, we used 55 female and 27 male *Peromyscus maniculatus sonoriensis* (Wagner) of a similar age (70–120 days at the time of death; Table 1). These mice were born in captivity in a colony that was 3–6 generations removed from the wild (trapped in the vicinity of Barcroft Laboratory). The study was carried out in the summer and autumn of 1998 (13 females, six males) and 1999 (42 females, 21 males) at the University of California's White Mountain Research Station (WMRS) and at the University of California at Riverside Campus (UCR). We performed two separate sets of experiments: 'temperature *versus* altitude' and 'tissue *versus* blood content'.

Temperature versus *altitude*

We acclimated mice at two sites in the summers of 1998 and 1999. The sites were located at either UCR (370 m above sea level; 1999 only), WMRS Barcroft Laboratory (3801 m above sea level; both 1998 and 1999). The mean barometric pressures/oxygen partial pressures at the sites are 760/150 Pa for UCR and 480/101 Pa for Barcroft (for a complete description of the WMRS site, see Hammond et al., 1999).

At each site, we housed mice in either a cold or a warm environment (see Fig. 1 and Table 2 for temperatures). At the 3800 m site, mice were housed in either a 'cold' outdoor enclosure (2.2 m×2 m×2 m) adjacent to buildings or a 'warm'

Table 2. *Dates of measurement and mean daily temperatures for the last 14 days of acclimation for all sites and temperature combinations*

Altitude (m)	Year	Temperature treatment	Dates of organ mass measurements	Mean 24 h temperature (°C)	Mean low temperature (°C)	Mean high temperature (°C)
3800	1998	Cold	4/9–5/9	10.0±0.5	4.3±0.5	15.6±0.8
		Warm	4/9–5/9	24.8±0.3	22.0±0.4	27.6±0.3
3800	1999	Cold	23/8–27/9	5.6±0.3	4.1±0.3	6.7±0.4
		Warm	23/8–27/9	24.5±0.1	23.5±0.1	24.5±0.1
370	1999	Cold	19/10–19/11	6.6±0.2	5.0±0.1	8.0±0.2
		Warm	24/10–21/11	20.3±0.1	20.2±0.1	20.4±0.1

Values are means ± S.E.M. (see Table 1 for values of *N*).

constant-temperature room. The outdoor enclosures were made of galvanized 0.635 cm hardware cloth and were open to the environment except for a plywood roof. At the 370 m site, the cold environment was a constant-temperature cold room on a 14 h:10 h light:dark (L:D) photoperiod, and the warm environment was a constant-temperature room on a photoperiod to match the photoperiod of the field sites (approximately 14 h:10 h L:D when measurements were made). The range of temperatures in the 370 m cold site was 5 °C during the night and 8 °C during the day to reflect the changes in mean day and night temperatures at the high-altitude site. The temperature range in the 370 m warm site was approximately 20 °C throughout the 24 h period, which was restricted by the vivarium room temperatures at UCR. In both of the site and temperature treatments, we recorded ambient temperature at 5 s intervals using an Onset Computer Corporation Stowaway XTI data-logging unit placed within an empty cage filled with bedding.

All individuals were housed separately in plastic cages (27 cm×21 cm×14 cm) on aspen sawdust bedding. They were given *ad libitum* food, water and bedding and approximately 1 g of cotton wool. Mice were allowed to acclimate to experimental conditions for between 4 and 12 weeks (mean 6.7±0.3 weeks).

We measured the food and energy intake of all mice in the 3 days prior to killing them. At the end of the food intake period, mice were injected with sodium pentobarbital (100 mg kg^{-1} body mass), and we measured the masses of the internal organs (heart, lungs, liver, kidney, spleen, stomach, small intestine, cecum and large intestine).

Tissue versus *blood content*

This experiment was performed in 1999 only at 370 m (UCR) and 3800 m (Barcroft). Mice were treated as described above except that they were exsanguinated after anesthesia and prior to dissection (see below for details) and we did not use internal organs except for the heart and lungs.

Experimental measurements

Food and energy intake

In 1998, mice were fed a high-carbohydrate diet (Custom Karasov; ICN Biochemicals; 55 % sucrose, 15 % protein, 7 % fat, 2 % brewer's yeast, 4 % salt, 1 % vitamin mix and 16 % fiber; energy equivalent 15.1 kJ g^{-1}), and in 1999 they were fed mouse chow (LabDiet Rodent Diet 5001; 60 % carbohydrate, 28 % protein and 12 % fat; energy equivalent 16.7 kJ g^{-1}). Because there were differences in the energy content of the two diets, we used energy intake as the important variable. In previous research, it has been shown that energy intake is a better approximation of response to changes in energy demand than the absolute ingested mass of the diet (Hammond and Diamond, 1992; Hammond et al., 1994). Thus, energy intake was calculated as the mass of food eaten multiplied by the energy equivalence of the diet.

Hematocrit

We measured hematocrit in intact mice only, and only in 1999. After mice had been anesthetized (as above), but before they died, a blood sample (approximately 200 µl) was drawn

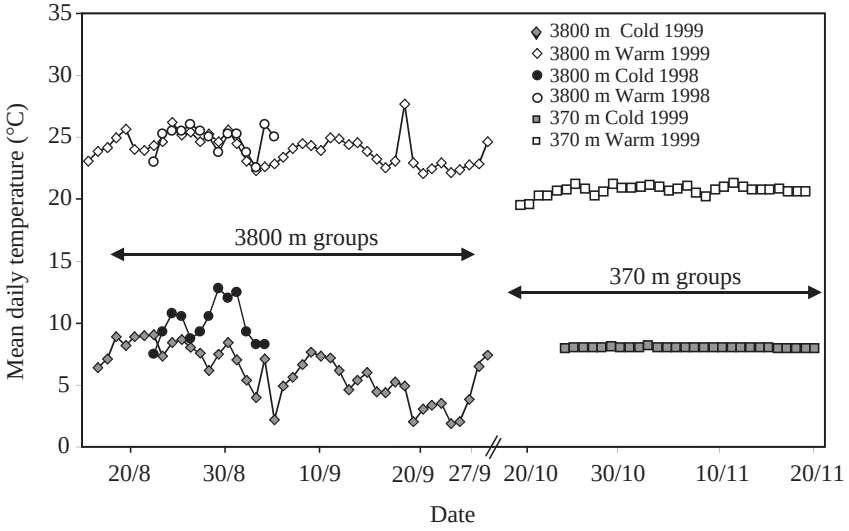

Fig. 1. Mean daily air temperatures (°C) to which the experimental animals were exposed and the six different treatments and dates of these experiments.

with a heparinized capillary tube using a retro-orbital puncture. These samples were centrifuged for 10 min, and hematocrit was calculated as the proportion of packed cells as a percentage of the total volume of blood in the tube.

Dissection and measurement of intact organs

This section refers to the mice used in the temperature *versus* altitude comparisons only. After the induction of anesthesia and blood sampling, an incision was made in the abdominal wall. The small intestine was flushed of contents *in situ* with cold mammalian Ringer's solution and excised. The remainder of the gut was removed, separated into stomach, cecum and large intestine, washed out with Ringer's solution and weighed. The stomach, cecum and large intestine were placed in a drying oven at 60 °C for 48 h and weighed again to obtain organ dry mass.

The liver, kidneys, spleen, heart and lungs were removed, cleaned of fat and connective tissue, blotted dry and weighed. They were dried for at least 48 h at 60 °C and weighed again to obtain dry mass.

Exsanguination

This procedure was performed for the 'tissue *versus* blood content' experiment in 1999 only. Once soundly anesthetized, an incision was made from the lower abdomen to the upper sternum. The viscera were then exposed by reflecting the abdominal musculature. We then exposed the heart and lungs by cutting the diaphragm and sternum. The blood in the pulmonary circulation was exsanguinated *via* a cannula inserted into the right ventricle with the left atrium cut open for outflow, and the right atrium cut open to prevent systemic blood from entering the right ventricle. We then perfused heparinized normal saline through the right ventricle at a non-pulsatile pressure of 20–30 Pa until the lungs changed color to a nearly white shade of pink (approximately 2 min).

Statistical analyses

Initially, our data consisted of two independent variables (temperature and altitude) and many dependent variables (food and energy intake, body mass and gut and vital organ mass). All data were tested for normality and homogeneity of variance. We started with a 2×2×2 three-factor design (two levels of sex, two levels of temperature and two levels of altitude). There were no differences between sexes for any dependent variable, so we combined males and females for each treatment and used a 2×2 two-factor analysis of variance (ANOVA) or analysis of covariance (ANCOVA) (two levels of temperature and two levels of altitude). Unless stated otherwise, cited F and P values are from these statistical tests and we use an alpha of 0.05 for statistical significance. Treatment and error degrees of freedom are used as subscripts for F values. The error degrees of freedom vary because a few measurements were lost. In all cases, we report the mean ±1 standard error of the mean (S.E.M.).

Body masses may have had an effect on organ size, but we removed this effect on the dependent variables by using an ANCOVA. For the 'temperature *versus* altitude' experiment, body mass was a significant covariate for all organ masses except spleen mass, so we present the adjusted means from our ANCOVA for those variables and ANOVA for the spleen. We found no statistically significant body mass covariates for variables in the 'tissue *versus* blood volume' experiment (exsanguinated heart and lung mass). For the combined data set (both intact and exsanguinated mice), in which we measured food and energy intake, body mass was a significant covariate, and we present these data as adjusted means.

We tested all *a posteriori* pairwise comparisons between orthogonal means for main effects for all ANOVAs and ANCOVAs for each dependent variable. For these comparisons, we used a *post-hoc t*-statistic corresponding to the two-sided P values (SAS Institute, 1987). When making comparisons, the root mean square (corrected for sample sizes for the two means in question) is used as the denominator for the total ANOVA or ANCOVA model. Thus, the comparison is in the context of the full model itself.

We used regression analyses to analyze the data in two further ways. First, because ambient temperatures were different for temperature treatments between both altitudes and years, we tested the regression for the mean daily temperature for the final 14 days (prior to the death of the mice) and both mass-corrected energy intake and mass-corrected small intestine mass after they had been corrected for body mass using residuals. Second, we used multiple regression analyses to test the effects of both ambient temperature (14 day means as above) and altitude on food intake, organ masses and hematocrit. For organ masses and food intake, the data were corrected for body mass using residual analysis. We applied a sequential Bonferroni procedure to correct for Type I errors in multiple simultaneous tests (Rice, 1989).

Results

Temperature versus *altitude*

Body mass

There were no differences in whole wet or dry body mass (at the time of death) in mice from different altitudes or temperature treatments (mean wet mass 20.1±0.3 g; mean dry mass 6.2±2 g, N=81).

Hematocrit

The hematocrit (only available for 1999) of high-altitude mice (both warm and cold groups, 47±2 %, N=17) was significantly higher than that of low-altitude mice (both warm and cold groups, 38±1.9 %, N=12, $F_{1,25}$=9.7, P=0.04; Fig. 2).

Energy intake

Energy intake (Fig. 3A) was 75 % higher in animals at cold temperatures than in those at warm temperatures (ANCOVA, $F_{1,77}$=87.2, P=0.0001). Energy intake was also significantly different in animals at different altitudes (ANCOVA, $F_{2,77}$=5.5, P=0.022).

There were almost identical negative regressions between

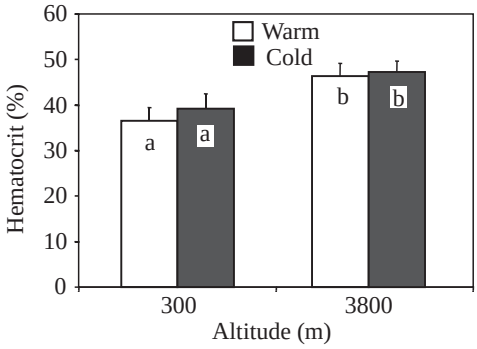

Fig. 2. Hematocrit (%) in deer mice from two different altitudes and either warm (open columns) or cold (filled columns) temperatures. Within a single column color, letters that are different from each other indicate statistically significant differences. Values are means +1 S.E.M. (*N* as in text). Note that these data are for the 1999 season only.

both the mean daily temperature of the last 14 days and the last 5 days of the experiment and energy intake (14-day r^2=0.56; P=0.0001; 5-day r^2=0.56; P=0.0001). There was a significant relationship between energy intake and body mass (r^2=0.206; P=0.0014), and we therefore removed the effect of body mass and tested the residuals of energy intake against 14 day mean temperature. Mass-corrected energy intake was still highly correlated with temperature for the combined data set (r^2=0.57; P=0.0001) and for the two altitudes (370 m, r^2=0.52, P=0.0001; 3800 m, r^2=0.65, P=0.0001) (Fig. 4). There was no significant relationship between altitude and mass-corrected energy intake for either the combined data set or each year independently.

Organ mass

The stomach dry mass (Fig. 3B) of high-altitude mice was 14% lower than that of low-altitude mice (ANCOVA; $F_{1,43}$=5.73; P=0.0211). This effect was apparent (using a mean separation test) in the cold- *versus* the warm-exposed mice. There was no effect of temperature on stomach dry mass in the ANCOVA model.

To assess the effect of the temperature and altitude on stomach mass, we performed a multiple regression analysis. This showed that stomach mass was affected by both altitude and temperature for a total explained variance of 15.6% (r^2=0.156; P=0.027; Table 3). Using sequential Bonferroni tests, however, this difference was not statistically significant.

Small intestine dry mass (Fig. 3C) was 20% higher in cold-acclimated mice than in warm-acclimated mice (ANCOVA, $F_{1,43}$=18.7; P=0.0001). It was also 9% lower in mice from 3800 m than in those from the 370 m site (ANCOVA; $F_{1,43}$=4.4; P=0.042). Note that this difference is not displayed in the mean separation tests (on Fig. 3C) because neither warm- nor cold-acclimated mice possess significantly smaller small intestines at low altitude; only the overall difference (both means together) is significant. Multiple regression

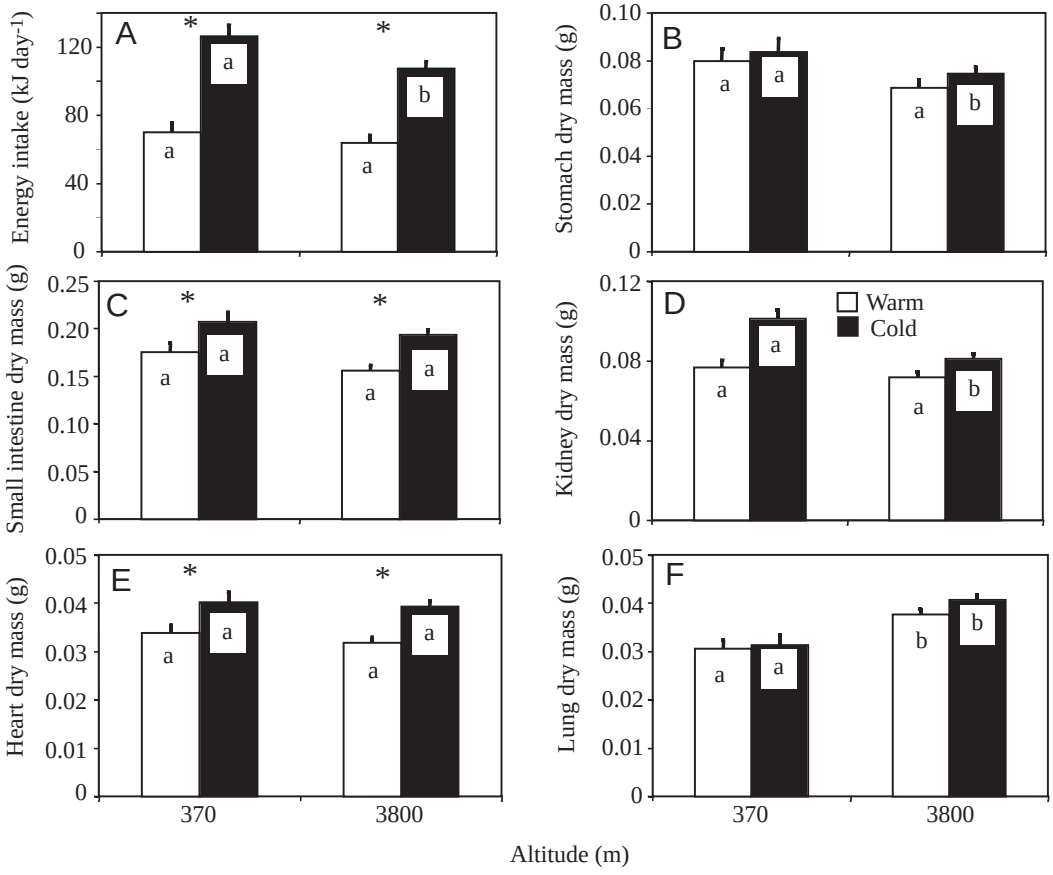

Fig. 3. Energy intake (kJ day^{-1}) and organ masses of deer mice from two different altitudes and either warm (open columns) or cold (filled columns) temperatures. Within a single column color, letters that are different from each other indicate differences if they are statistically significant with respect to altitude. Asterisks indicate statistical differences between temperatures. Values are means +1 S.E.M. (*N* as in text). Note that the data on organ mass are for the intact (with blood) mice only.

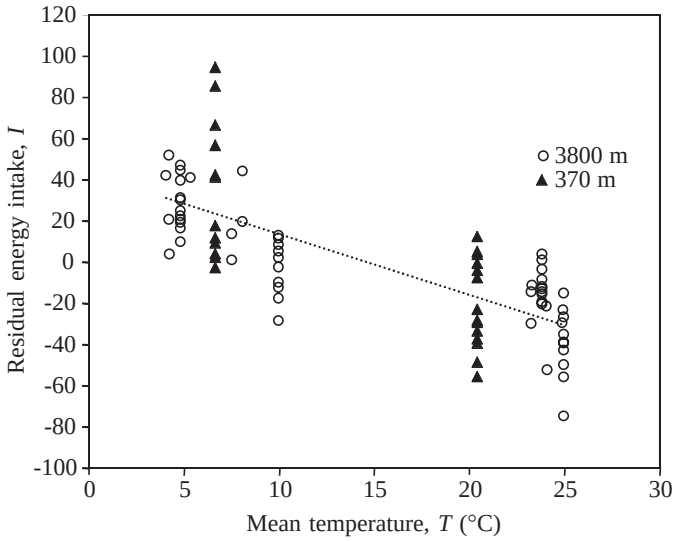

Fig. 4. 14-day mean ambient temperature (*T*) *versus* energy intake (residuals) (*I*) after the effects of body mass had been removed for deer mice at either 3800 m (open circles) or 370 m (filled triangles). The equation for the regression line for the entire data set was $I=-2.95T+43.2$ ($r^2=0.57$; $P=0.0001$).

analyses show that temperature explains a significant amount of the variance in small intestine mass ($r^2=0.37$, $P=0.0001$; Table 3). We also found a significant regression between mass-corrected energy intake and small intestine dry mass ($r^2=0.42$; $P=0.0001$).

Kidney dry mass (Fig. 3D) was 23 % larger in cold-exposed mice than in warm-exposed mice, regardless of altitude (ANCOVA, $F_{1,43}=24.5$, $P=0.0001$). It was also 16 % smaller in high-altitude mice than in low-altitude mice (ANCOVA, $F_{1,43}=13.8$, $P=0.0006$). There was an interaction between altitude and temperature (ANOVA, $F_{1,43}=5.0$, $P=0.031$) because only the cold-exposed mice at low altitude had larger kidney masses relative to the cold-exposed mice at high altitudes. Multiple regression analyses showed that altitude and temperature explained a significant amount of the variance ($r^2=0.33$, $P=0.0016$; Table 3).

Table 3. *Results of multiple regression analyses between dependent organ size and energy intake and altitude and ambient temperatures (averaged on a 24 h basis across 14 days)*

	Partial r^2			
Dependent variable	Altitude	14-day temperature	Total r^2	*P*
Stomach	–0.103	–0.053	0.156	0.0266
Small intestine		–0.366	0.366	0.0001*
Kidney	–0.227	–0.107	0.334	0.0016*
Heart		–0.341	0.341	0.0001*
Lungs	0.403		0.403	0.0001*
Energy intake		–0.584	0.584	0.0001*

For organ masses and energy intake, the residual values, after the removal of body mass, were used in the regressions. Stepwise regressions were used to generate results. Here, we show partial r^2 as well as the total r^2. The sign of the partial r^2 coefficient indicates whether the independent variable has a positive or a negative influence on the dependent variable.

* indicates a statistically significant value for *P* using a sequential Bonferroni test.

Heart dry mass (Fig. 3E) was 21 % larger than in cold- than in warm-exposed mice (ANCOVA, $F_{1,43}=18.9$, $P=0.0001$). There was no significant altitude effect on heart dry mass. The mean 14 day temperature explained a significant amount of the variance in heart mass in multiple regression analyses ($r^2=0.34$, $P=0.0001$; Table 3).

Lung dry mass (Fig. 3F) was up to 27 % larger in high-altitude mice than in low-altitude mice (ANCOVA, $F_{1,43}=29.3$, $P=0.0001$). The regression analysis also showed a significant effect of altitude ($r^2=0.403$, $P=0.0001$; Table 3). There was no statistically significant effect of temperature on lung mass.

There were no changes in the dry masses of the cecum, large intestine, liver or spleen with respect to either temperature or altitude.

Tissue versus *blood content of the heart and lungs*

We were able to test changes in tissue and blood mass only in the heart and lungs because they were the only organs completely exsanguinated by our procedures. Exsanguinated lung dry mass was 29 % larger in high-altitude than in low-

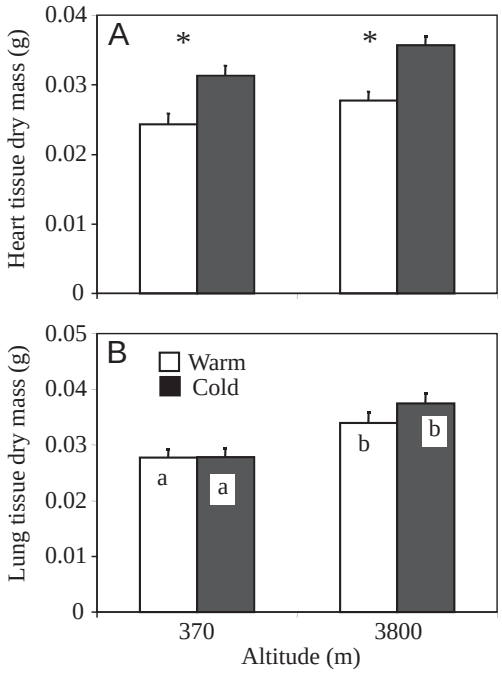

Fig. 5. Histogram showing heart (A) and lung (B) dry masses of exsanguinated deer mice at two different altitudes and acclimated to either warm (open columns) or cold (filled columns) temperatures. Within a single column color, letters that are different from each other indicate statistically significant differences. Asterisks indicate statistically significant differences between temperatures. Values are means +1 S.E.M. (*N* as in text).

altitude mice (ANCOVA, $F_{1,29}$=20.3, P=0.0001; Fig. 5A). There was no significant effect of temperature on exsanguinated lung dry mass. Exsanguinated heart dry mass was 14% greater in high- than in low-altitude mice (ANCOVA, $F_{1,29}$=7.6, P=0.0101; Fig. 5B). Cold-acclimated mice had exsanguinated heart dry masses that were 28% larger than those of warm-acclimated mice.

Discussion

Temperature versus *altitude*

For deer mice (and other endotherms), adaptation to altitude has two main components. First, high altitudes are usually colder than low altitudes, so individuals living at high altitudes generally have increased energy demands and energy intake. Second, the P_{O_2} is reduced at high altitudes, so animals may experience limitations to aerobic activities such as exercise and heat production (Lenfant, 1973; Snyder, 1981; Chappell et al., 1988). This can put animals at high altitudes into double jeopardy: they need to expend energy at higher rates than those at lower altitudes, but must do so in hypoxic conditions. When we started this study, we predicted that changes in digestive organ mass of deer mice living across an altitudinal gradient would be driven by mean daily ambient temperatures (which determine thermoregulatory costs), resulting from higher food intakes, and that changes in lung mass would be driven by differences in P_{O_2} across that gradient. For the small intestine, kidneys and heart, we found that ambient temperature, more than ambient P_{O_2}, drove the phenotypic plasticity we observed. Notably, however, small intestine mass was significantly lower in mice at high altitude. For both lung mass and hematocrit, we found, as expected, that P_{O_2} was the primary determining factor, with high-altitude individuals having larger organs and higher hematocrits than low-altitude individuals. Presumably, the phenotypically plastic changes in organ mass of high-altitude mice help to maintain an adequate oxygen uptake and delivery. Similarly, phenotypic plasticity of mice living in lower ambient temperatures (which includes plasticity in energy intake, small intestine mass and heart mass) is a response to higher energy expenditures.

We did not predict that some organs would be smaller at high than at low altitude (small intestine and kidney). Other authors have noted that organ size often decreases at high altitude on an absolute, but not on a mass-specific, basis (Tucker and Horvath, 1973) and suggest that it is a function of decreased bulk oxygen flow (caused by the lower P_{O_2} at high altitudes). This may be true for the small intestine, but probably is not true for kidney mass because it was only in cold-acclimated mice that the difference in altitude was statistically significant.

A notable difference between the high- and low-altitude temperatures experienced by the animals was that, although the mean daily temperatures were similar at both altitudes, they were more variable at high altitude because the mice were not in environmental chambers at that site. Interestingly, the multiple regression analyses show that both temperature (using the real temperature means rather than categorical 'cold' and 'warm' variables) and altitude are important determinants of kidney mass. Temperature alone was important in determining small intestine mass, heart mass and energy intake. These results confirm and strengthen the ANCOVA results.

This is not the first report of phenotypic plasticity of organs mass or blood characteristics in deer mice associated with altitude. Hock (Hock, 1961; Hock, 1964) demonstrated that native deer mice (*P. maniculatus sonoriensis*) from high altitudes in the White Mountains of California had a larger heart and larger lungs (of similar magnitude to the differences noted here) than *P. maniculatus sonoriensis* from nearby low-altitude populations. We have previously demonstrated a similar degree of plasticity of heart, lungs and digestive organs in the related low-altitude subspecies *P. maniculatus bairdii* (Hammond et al., 1999). Wyckoff and Frase (Wyckoff and Frase, 1990) found that, within the same genus, *P. maniculatus* from high altitudes have a higher hematocrit, hemoglobin content and mean red cell volume than *P. leucopus* from low altitudes. Because the reverse experiments were not run (high-altitude species at low altitude and *vice versa*), it is impossible to determine whether these results are truly a result of acclimation to low P_{O_2} and not of species-specific genetic traits. None of the previous studies differentiated between ambient temperature and P_{O_2} as a determining factor for organ mass plasticity at different altitudes. To our knowledge, this study is the first to demonstrate the differential effects of low ambient temperatures and low P_{O_2} in high-altitude populations.

Deer mice also appear to show Darwinian (genetic) adaptation to a range of altitudes (i.e. oxygen availability) because they have a number of naturally occurring hemoglobin haplotypes that (i) have population gene frequencies strikingly correlated with altitude, (ii) strongly influence blood oxygen-affinity (P_{50}) *in vivo*, and (iii) affect short-term aerobic performance (maximum rate of oxygen consumption, $\dot{V}_{O_2max}$, in exercise and thermogenesis, over periods of several minutes) in laboratory populations (Snyder, 1978a; Snyder, 1978b; Chappell and Snyder, 1984; Chappell et al., 1988). Our mice were also derived from mice caught in the same area as some of the deer mice in the genetic studies. Although we did not haplotype our mice, we know (Chappell et al., 1988) that this population is generally polymorphic for the α-hemoglobins (possessing both low- and high-altitude haplotypes) implicated in altitude adaptation. Nonetheless, the $\dot{V}_{O_2max}$ of the laboratory-reared mice used in the studies on hemoglobin genetics were, on average, lower that those of native wild-caught mice from the same area even after acclimation to test altitudes (Hayes, 1989). The difference is probably because wild mice were exposed to a colder thermal regime and underwent development at high altitudes. Thus, in addition to genetic adaptation, other factors such as phenotypic plasticity may be important in determining the survival of deer mice at high altitudes.

The phenotypic plasticity we observed at high altitude in this study has also been observed in white mice and rats (Timiras et al., 1957; Burri and Weibel, 1971) and, in at least one study,

those changes were related to functional changes. Burri and Weibel (Burri and Weibel, 1971) showed that young that underwent *in utero* development near sea level and were then transferred to altitude (P_{O_2}=100 Pa) as adults developed larger lung volumes than control (normoxic) rats. The increases in volume in the study of Burri and Weibel (Burri and Weibel, 1971) were attributable to increases in the alveolar, capillary and tissue volumes of the lung. The changes in tissue volumes noted in the rats were of a similar magnitude to the changes in tissue mass (20 %) noted in the present study, but since we only measured tissue masses, we cannot assume that they translate to a similar tissue volume.

A different diet was used in the two different years at the high-altitude site and, although the carbohydrate content was similar, the diets had slightly different energy densities (difference 1.6 kJ g^{-1}). While it is a common observation that small mammals eat to satisfy energy demands, rather than to maximize absolute mass intake (Hammond and Diamond, 1992; Hammond et al., 1994; K. A. Hammond and M. Konarzewski, unpublished data), it is also true that the mass of food eaten strongly determines small intestine mass (Hammond et al., 1994; Konarzewski and Diamond, 1994). We have found, however that energy intake is positively correlated with small intestine mass in the present study (as stated above) and that energy intake is highly, and negatively, correlated with ambient temperature. The same correlation exists within the high-altitude site, regardless of diet type. Thus, we suggest that the differences in diet (and energy density) between the two years is not a problem in this particular data set. However, differences in dietary energy density should be taken into consideration.

Tissue versus *blood content of the heart and lungs*

We were interested in determining whether changes in lung and heart mass were a result of increased water content (as in the case of hypoxia-induced pulmonary edema) or were a function of increases in tissue other than blood components of the organs.

Pulmonary edema is a common response to hypoxia in some species (particularly man), so it is important to know whether the responses we observed were potentially maladaptive. In comparisons across both altitude and temperature, we found that dry mass (rather than wet mass alone) is greater in the lungs of high-altitude mice and in the heart of cold-acclimated mice, so we can say with certainty that the increased mass was not the result of edematous tissue or high-altitude pulmonary edema.

Even after discounting the possibility of increased tissue water content, it is not clear whether increases in tissue mass are a functional result of increased tissue mass itself or a result of an increased blood content (which has a density greater than water). The fact that exsanguinated mice, in the present study, show the same pattern as intact mice, however, demonstrates that the changes in tissue mass were not due to changes in tissue blood volume alone. Changes in organ tissue mass may also be accompanied by changes in fractional blood flow when accommodating to hypoxic conditions, as has been noted in rats (Tucker and Horvath, 1973). These changes would not necessarily be measured as increased mass, even in dried tissues, because they are the result of increases in blood flow per gram of tissue. Thus, with larger tissues, it is likely that there is also a greater blood flow if capillary density remains the same as tissue mass increases. Because we did not measure blood flow to the organs, we cannot determine whether there was an increase in fractional heart and lung blood flow in our hypoxic mice.

Another obvious question is whether the greater tissue masses we observed in the lungs of mice at high altitude and in the hearts of cold-acclimated mice were the result of hyperplasia (increased cell number) rather than hypertrophy (increased cell size). We did not measure the DNA content of heart and lung tissues or perform a microscopic examination to determine differences in cell number or size in animals from different treatment groups. Other authors have shown that hypoxia results in hyperplasia rather than hypertrophy of lung tissues in laboratory rodents (Tenney and Remmers, 1966; Bartlett and Remmers, 1971; Burri and Weibel, 1971; Sekhon and Thurlbeck, 1996), but the question has not yet been explicitly addressed in so-called high-altitude-adapted rodents.

Finally, the changes we observed in organ tissue mass are important only if they represent an increase in the functional output or activity of those organs and, thus, an increase in performance of the whole organism. We cannot yet answer this question explicitly because we did not measure either aerobic or sustained metabolic performance, but we can consider results extrapolated from other research. For instance, there is a strong correlation between increases in lung and heart mass (due to hyperplastic increases in tissue mass) and functional output (Burri and Weibel, 1971; Sekhon and Thurlbeck, 1996; Tucker and Horvath, 1973) in hypoxic animals compared with normoxic laboratory animals. Abdelmalki et al. (Abdelmalki et al., 1996) found that, on a mass-specific basis, the soleus and cardiac muscles undergo an increase in size with a resulting increase in aerobic performance and endurance time in laboratory rats. These authors emphasize that the change in aerobic capacity and muscle size are due largely to the imposition of exercise training during exposure to hypoxic conditions and that they are also significant only when considering a loss in body mass partially due to hypoxia-induced anorexia (Gloser et al., 1972; Rose et al., 1988; Sekhon and Thurlbeck, 1996). Thus, we suggest that a significant increase in exsanguinated organ mass is an indication of augmented functional output.

Our study enables us to conclude that, even without additional energy demands (i.e. thermostatic costs in the 'warm' groups, lactation, aerobic activity), cardio-pulmonary and digestive organs respond to changes in both ambient temperature and P_{O_2} at high altitudes. Hayes (Hayes, 1989) suggested that ambient temperature has a greater impact on energetic demands in high-altitude mice than does P_{O_2}. Nonetheless, lung mass does increase in the low-P_{O_2} environment at high altitudes. These changes seem to be

important in allowing individuals to accommodate to the challenging conditions of cold and hypoxia. Thus, we speculate that the capacity for phenotypic plasticity is important in the survival and, potentially, the fitness of deer mice. Because deer mice hemoglobins are genetically adapted to altitude (as described above), it would be of interest to understand how the genetics of hemoglobin and organ phenotypic plasticity interact in determining survival. For instance, does phenotypic plasticity of organ size (particularly lung mass) over-ride the effects of hemoglobin genetics in determining performance? Are individuals that possess high-altitude hemoglobins able to forgo changes in organ mass to accommodate low P_{O_2} and, thereby, spend less energy maintaining tissues at high altitudes?

It is important to emphasize that the phenotypic changes we observed in these mice were widespread across the cardiac, hemotological, respiratory and digestive systems. These systems are closely tied to supporting cellular respiration and all rely on a relatively high bulk transfer of oxygen for efficient aerobic operation. Increases in digestive capacity result from a greater increase in nutrient intake and, thus, an increase in the circulation of oxidizible substrates. An increase in heart and lung function potentially allows for a greater intake and transport of oxygen to drive cellular respiration. These data suggest that there is a much broader-scale system-wide upregulation in response to common abiotic demands than was previously appreciated.

We thank Letty Brown, Noah Hamm, Bryan Qualsey and Simon Hamm for their help in maintaining animals and data collection at White Mountain Research Station in 1998. Debbie Kristan helped with dissections and collected some of the hematocrit data. Mark Chappell kindly read and commented on early drafts of this manuscript. This project was made possible primarily with funds from a University of California Faculty Research Grant for work at White Mountain Research Station, UCR Academic Senate, and NIH-30745-05 funds to K.A.H.

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