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COMBINED EFFECTS OF COLD EXPOSURE AND SUB-LETHAL INTESTINAL PARASITES ON HOST MORPHOLOGY AND PHYSIOLOGY

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Summary

Multiple, simultaneous demands elicit physiological and morphological responses that may jeopardize an animal's ability to respond to future challenges, especially when resources are limited. Laboratory mice (*Mus musculus*) experimentally infected with an intestinal nematode (*Heligmosomoides polygyrus*) and then exposed to cold showed phenotypic plasticity of morphological and physiological responses. The parasitized mice maintained a similar body mass to the unparasitized mice but had less body fat and showed changes in some organ masses, a greater resting metabolic rate (RMR) and a diminished glucose uptake capacity both at the site of infection and in regions of the small intestine not occupied by parasites. Cold-exposed mice had a greater RMR, less body fat, a greater glucose transport capacity and showed changes in organ masses compared with mice maintained at room temperature. The responses to cold exposure were not affected by parasitism for any dependent variable. The costs of having parasites during simultaneous cold exposure included decreased energy reserves and greater maintenance requirements, which may then decrease the energy available for future expenditures, such as reproduction.

Key words: *Mus musculus*, *Heligmosomoides polygyrus*, mouse, resting metabolic rate, glucose transport, body composition, phenotypic plasticity.

Introduction

Animals are often faced with variable and unpredictable environments. An animal's response to the challenges or demands of a variable environment must be adjusted to the type, number and intensity of demands present (Hammond et al., 1994). Phenotypic plasticity (changes in the phenotype, including morphology and physiology) is a common response to changing environmental conditions. For example, laboratory mice simultaneously exposed to cold while lactating showed phenotypic plasticity in organ morphology, small intestine function, resting metabolic rate (RMR) and body composition (Hammond et al., 1994). Although there is controversy over aspects of phenotypic plasticity, such as its genetic basis, methods of description and the role of natural selection (Via et al., 1995; Scheiner and Callahan, 1999), it is undeniable that phenotypic plasticity occurs in numerous taxa as a response to variable environments (Piersma and Lindström, 1997; Starck, 1999a).

Sub-lethal parasite infection can be an important, and often unpredictable, demand for small mammals. First, many sub-lethal parasites can reside in their host for weeks, months or years (Behnke et al., 1992), causing a chronic infection. Second, animals do not develop life-time immunity to some sub-lethal parasites and are repeatedly susceptible (e.g. Fuller, 1996). Third, the costs associated with the immune response and the physical damage caused by parasites may alter energy allocation in parasitized animals (Sheldon and Verhulst, 1996) and, if energy is limited, result in a physiological trade-off (Stearns, 1992) between somatic and reproductive expenditures (Sheldon and Verhulst, 1996; Nordling et al., 1998; Ilmonen et al., 1999; Williams et al., 1999; Lochmiller and Deerenberg, 2000). Hosts commonly show morphological and physiological plasticity in their responses to parasite infection, including changes in body composition, organ mass and enzyme production (Liu, 1965; Martinez et al., 1999). Because chronic infection with sub-lethal parasites places a constant, though possibly small, demand on the host (Munger and Karasov, 1989, 1991, 1994), these parasites may be significant especially when infection co-occurs with other demands (Feore et al., 1997).

Many nematodes cause sub-lethal, chronic infections in their hosts (Behnke et al., 1992). Intestinal nematodes can affect food intake (Arneberg et al., 1996; Arneberg and Folstad, 1999) and, potentially, nutrient absorption by altering the neural function of the small intestine (Galeazzi et al., 2000), which may then interfere with overall energy metabolism. To explore this further, we examined the phenotypic plasticity of responses of laboratory mice (*Mus musculus*) simultaneously exposed to cold and given a sub-lethal intestinal nematode

(*Heligmosomoides polygyrus*). Although some changes in organ morphology associated with lactation in *Mus musculus* are partly reversible (Hammond and Diamond, 1992) and are therefore better described as phenotypic flexibility, a subcategory of phenotypic plasticity (Piersma and Lindström, 1997), it is not known whether and to what extent morphological and physiological changes associated with cold-exposure or *H. polygyrus* infection are reversible. Therefore, we use phenotypic plasticity throughout to describe phenotypic changes in morphology and physiology associated with cold exposure and parasite infection, even though these changes may be reversible.

H. polygyrus naturally occurs in wild mouse populations (Spurlock, 1943; Forrester, 1971; Anderson, 1992), produces no clinical symptoms of disease unless high doses are used (Monroy and Enriquez, 1992) and has a direct life-cycle in which mice become parasitized by ingesting infective-stage larvae. Once ingested, larval stages embed in the muscularis of the small intestine and elicit both cellular and humoral immune responses in the mice (Monroy and Enriquez, 1992). After maturation, the adult worms move into the lumen, attach to the villi in the proximal small intestine (Bansemir and Sukhdeo, 1996), consume mucosal tissue (Bansemir and Sukhdeo, 1994) and can persist for approximately 8 months (Ehrenford, 1954) without killing the mouse, after which all the adult worms have died and been shed in the feces, leaving the mouse uninfected. Therefore, this parasite is an excellent model system in which to study the effects of chronic sub-lethal parasites on host energy metabolism.

We hypothesized that parasites, in combination with the additional energy demand of cold exposure, will affect host morphology and physiology. On the basis of previous studies, we predicted that parasitized mice would increase the size of their spleen, liver and small intestine (Liu, 1965) and that cold-exposed mice would increase the size of their kidney, heart and small intestine (Konarzewski and Diamond, 1994). Changes in organ size during cold exposure result primarily from increased food intake and processing (Konarzewski and Diamond, 1994), whereas changes in organ size during *H. polygyrus* infection result, in part, from an immune response (Liu, 1965). Second, we predicted that both cold-exposed and parasitized mice would have a greater RMR and decreased fat stores to meet the demands of cold exposure (thermoregulation) and parasitism (e.g. immune response, tissue repair). Third, we predicted that cold-exposed mice would have an increased glucose transport capacity (Konarzewski and Diamond, 1994) because of a greater food intake and the associated upregulation of nutrient transport capacity of the small intestine (Toloza et al., 1991), but that parasitized mice would have a decreased glucose transport capacity in the portion of the small intestine where the parasites occurred (Symons, 1960) as a result of tissue damage. Lastly, we predicted that parasitized mice would show greater responses to cold exposure (e.g. food intake, RMR, changes in body composition) than unparasitized mice because parasite infection can hinder an animal's ability to cope with other physiological challenges, such as cold (Banerjee et al., 1999; Monroy et al., 1999) and caloric restriction (Carlomagno et al., 1987). We measured host RMR, food intake, whole-body fat content, organ sizes and the ability of the small intestine to transport glucose out of the lumen. We found that the simultaneous demands of parasite infection and cold exposure yielded changes in energy acquisition and allocation as reflected in phenotypic plasticity of metabolism, body composition and organ mass and function.

Materials and methods

Our design had two main effects with two levels each: parasitism (parasitized, unparasitized) and temperature (5 °C, cold-exposed; 23 °C, control). We used 10, individually housed, virgin female Swiss-Webster laboratory mice *Mus musculus* (50–90 days old; Hilltop, Scottdale, PA, USA) in each treatment group. All data from one mouse from the parasitized, cold-exposed group were discarded because of technical problems with the glucose uptake experiment.

Parasite maintenance and mouse infection procedures

Moist feces containing *Heligmosomoides polygyrus* (Dujardin, 1845; = *Nematospidoides dubius*, Baylis, 1926) eggs were mixed with tap water and strained through two layers of cheesecloth. The resulting liquid was centrifuged, the supernatant was discarded and the pellets were spread on moist filter paper. The filter paper was kept moist by placing its edge in a covered Petri dish of tap water. The filter paper with fecal spread was incubated at 23 °C for 9 days, during which time the eggs hatch and mobile larvae move into the tap water. After 9 days, the infective third-stage larvae (L_3) were collected from the tap water. There were 144±16 L_3 per 50 µl (mean ± 1 S.D.) as determined by counting the number of L_3 in four 50 µl samples using a compound microscope.

We anesthetized all mice by intraperitoneal injection of sodium pentobarbital (50 mg kg^{-1}) mixed with sterile saline. Mice recovered from anesthesia within 2–3 h. Mice in the parasitized treatment group were given approximately 300 L_3 suspended in tap water *via* an intra-gastric stainless-steel curved feeding tube. Control mice were gavaged with tap water only (N=10) or were anesthetized but not gavaged (N=10) to examine the effects of the larval administration procedure.

Cold acclimation and digestive efficiency measures

For the first 6 days post-control gavage or post-infection (PI), mice were maintained at 14 h:10 h L:D photoperiod at 23 °C and fed standard laboratory chow (Purina Mills, Inc.) *ad libitum*. On day 7 PI, mice were switched to a high-carbohydrate diet (Custom Karasov Diet, ICN; 55 % sucrose, 15 % casein, 7 % cottonseed oil, 2 % brewer's yeast, 4 % salt mix, 1 % vitamins and 16 % non-nutritive bulk; Diamond and Karasov, 1984) necessary for the glucose uptake measurements. On day 14 PI, mice in the cold-acclimated group were moved to an environmental chamber (5 °C; 14 h:10 h L:D). Cold-exposed mice were kept at 5 °C for 10 days, while control mice remained at 23 °C. Body mass, food

intake rate (I; g wet mass day^{-1}) and fecal output rate (O; g dry mass day^{-1}) were measured on each of the last 3 days of the cold acclimation period. Percentage dry matter digestibility (DMD) was calculated as DMD=[(I–O)/I]100. Daily food intake was converted to digestible energy intake by multiplying food intake by dietary caloric content (15.1 kJ g^{-1} as reported by the manufacturer, ICN Nutritional Biochemicals) by average DMD measured over 3 days.

Resting metabolic rate

Between 08:00–10:00 h on day 23 PI, we measured the RMR of non-postabsorptive mice as the rate of O_2 consumption ($\dot{V}_{O_2}$, to ±0.002 %, ml min^{-1}) using open-flow respirometry. A mouse was placed in a 600 ml Plexiglas chamber in a dark cabinet at 30±1 °C (within the *Mus* thermoneutral zone; Hart, 1971). Dried (Drierite) air entered the chamber at 650–700 ml min^{-1} from mass-flow controllers. Air leaving the chamber was dried, scrubbed of CO_2 (soda lime) and re-dried before entering an Applied Electrochemistry S3A/II O_2 analyzer connected to a Macintosh computer. Oxygen consumption was measured for two animals simultaneously such that reference air was sampled for 2.5 min followed by RMR for 17.5 min. This cycle was repeated for 3 h, and we recorded data every 5 s. We analyzed the output using customized software (WartHog Systems) and calculated the rate of O_2 consumption using Equation 4a of Withers (1977). We calculated RMR as the mean of the three lowest 2 min intervals of $\dot{V}_{O_2}$.

Organ morphology and body fat measures

On day 24 PI, mice were anesthetized between 08:30 and 11:30 h by intraperitoneal injection of 0.07 ml of sodium pentobarbital (65 mg ml^{-1}). After removal of the small intestine (see below), mice were killed with an incision to the diaphragm. The stomach (from cardiac to pyloric sphincter), small intestine (from pyloric sphincter to ileocecal valve), cecum, large intestine (from just distal to the sacculent cecum to the anus), heart, liver, spleen, paired kidneys and paired lungs were dissected out, and excess fat and connective tissue was removed and returned to the carcass. We rinsed away the stomach, cecum and large intestine contents with mammalian Ringer's solution. We measured the dry mass of all organs after drying to a constant mass for 48 h at 55–60 °C. The empty carcass was dried to a constant mass at 55–60 °C for 2 weeks.

We ground the empty, dried carcass (including pelage) and extracted and measured the fat content using petroleum ether in a Goldfische apparatus (Labconco). We extracted 1.5–2.0 g samples until the entire carcass was extracted. We re-dried the samples and calculated the percentage of fat, which was then multiplied by the wet mass of the empty carcass to determine the total fat mass (g) for each mouse. Lean mass was thus calculated as total body mass minus body fat mass. The fat content of the body organs was not measured or included in total body fat content because organ fat is less than 4 % of total body fat mass for this strain of laboratory mice (D. M. Kristan and K. A. Hammond, unpublished data).

Small intestine morphology and glucose uptake measurements

The small intestine was rinsed *in situ* with cold Ringer's solution, excised and kept in cold oxygenated Ringer's solution (bubbled at 3 l min^{-1} using a gas mixture of 5 % CO_2:95 % O_2). We divided the small intestine into three regions (proximal, mid and distal) of equal length. We measured the wet mass of each region after lightly blotting to remove adherent Ringer's solution, and summed the three masses to determine total intestinal mass. We separated mucosal/submucosal tissue (hereafter called 'mucosa') from muscularis/serosal tissue (hereafter called 'serosa'), as described by Diamond and Karasov (1984), from two 2 cm 'sleeves' per region. The 'mucosal' layer is used in nutrient digestion, absorption and transport to the body *via* its vascularization, whereas the 'serosal' layer is primarily supportive tissue. We calculated the dry mass:wet mass ratio for each sleeve and used this ratio to calculate the mucosal and serosal wet mass and the dry mass of the entire small intestine (Diamond and Karasov, 1984; Hammond and Diamond, 1992).

We measured the maximal transport velocity (V_{max}) of the brush-border D-glucose transporter *in vitro* using the everted sleeve technique (Karasov and Diamond, 1983; Diamond and Karasov, 1984). Briefly, we everted each region of the small intestine so that the mucosa faced outwards. From each region, we cut two 1.5 cm long sleeves and two 2 cm long sleeves, immediately adjacent to each other (the 2 cm sleeves for measuring relative mucosal and serosal mass as described above, and the 1.5 cm sleeves for measuring glucose uptake). To measure glucose uptake, we mounted everted sleeves on stainless-steel rods and incubated them for 2 min in Ringer's solution at 36 °C containing 50 mmol l^{-1} D-glucose and trace amounts of D-[^{14}C]glucose. The incubating Ringer's solution contained trace amounts of L-[^{3}H]glucose to correct for glucose in the adherent mucosal fluid and for passive uptake of D-glucose. This correction procedure allows the carrier-mediated uptake of D-glucose to be calculated. We measured the amount of isotope taken up by the tissue using liquid scintillation counting (LS 6500 scintillation system, Beckman). We determined the glucose uptake capacity for each of the three regions of the small intestine by multiplying the measured uptake rate per milligram wet mucosal tissue by the wet mucosal mass of the region. We then summed these values for each region to determine the glucose uptake capacity for the entire small intestine (based on wet mucosal mass).

Statistical analyses

Our data consist of two independent variables [parasitism, parasitized, unparasitized; temperature, 5 °C (cold-exposed), 23 °C (control)] and numerous dependent variables (food intake, DMD, body mass, small intestine morphological variables, organ masses, body fat, glucose uptake capacity and RMR). We first regressed dependent variables against body mass to test for body mass effects, except for organs that were regressed against body mass minus organ mass to remove the effects of changing organ mass on changing body mass (Christians, 1999). If the regression was significant, we used

the residuals of the regression for additional analyses. We used a 2×2 two-factor multivariate analysis of variance (MANOVA), which tested for significant differences between treatments for all dependent variables together. Because this MANOVA was significant (Wilks' lambda, $F_{51,54}$=6.07, P<0.0001), we used independent 2×2 two-factor ANOVAs to determine which treatments and which dependent variables contributed to the significant result of the MANOVA. Differences among intestinal regions were tested with a 2×2 two-factor repeated-measures ANOVA using Wilk's lambda. The two control groups (gavaged *versus* anesthesia only) were compared for all dependent variables with a one-factor MANOVA. Throughout, we used P<0.05 as the level of significance. Values are presented as means ± 1 S.E.M.

Results

There were no differences between the two control groups (gavaged *versus* anesthesia only) for any variable measured (P>0.05), so control animals were pooled for all analyses. We determined that all mice given L_3 developed mature infections by detecting *H. polygyrus* eggs in their feces at 14 days PI using a modified McMaster technique (Bowman, 1995). No control mice became infected.

Body mass and body composition

Body mass did not differ among treatment groups ($F_{2,35}$=0.17, P=0.85; Fig. 1) but heavier mice had more body fat (r^2=0.79, P<0.0001) and greater dry masses of the large intestine (r^2=0.59, P<0.0001), stomach (r^2=0.66, P<0.0001), cecum (r^2=0.27, P=0.001), liver (r^2=0.51, P<0.0001), kidney (r^2=0.42, P<0.0001), lung (r^2=0.29, P<0.0001) and heart (r^2=0.12, P=0.035); therefore, we used residuals of the body mass regressions for these variables in subsequent analyses.

Body composition was significantly affected by both cold and parasite infection such that parasitized mice had less fat than unparasitized mice ($F_{1,35}$=13.57, P=0.001) and cold-exposed mice had less fat than mice at 23 °C ($F_{1,35}$=4.64, P=0.038; Fig. 1). Because overall body mass did not change with parasite or temperature treatment, the absolute lean mass of parasitized mice increased by 8 % at 23 °C and by 13 % at 5 °C ($F_{1,35}$=12.72, P=0.001), and cold exposure resulted in a 4 % increase in lean mass for unparasitized mice and an 8 % increase for parasitized mice ($F_{1,35}$=4.22, P=0.047). Lean mass is made up of organ and non-organ masses. Non-organ lean mass did not change with either cold acclimation or parasite infection ($F_{2,35}$=3.26, P=0.05), and thus increased organ masses (especially of the spleen and small intestine for parasitized mice and of the kidney and heart for cold-exposed mice; Table 1) produced the observed lean mass increase that occurred with parasite infection and cold exposure.

Resting metabolic rate

As expected, RMR was greater for mice exposed to 5 °C (31.7±0.8 kJ day^{-1} and 33.2±0.9 kJ day^{-1} for unparasitized and parasitized mice, respectively) than to 23 °C (26.3±1.0 kJ day^{-1} and 28.8±0.8 kJ day^{-1} for unparasitized and parasitized mice, respectively; $F_{1,35}$=33.72, P<0.0001). Parasitized mice had a greater RMR than unparasitized mice ($F_{1,35}$=5.2, P= 0.029) by 4 % at 5 °C and by 9 % at 23 °C. Parasitized and cold-exposed mice may have a higher RMR in part because they had a greater lean mass than unparasitized, control-temperature mice. Because lean mass increased with both cold exposure and parasite treatments, we examined the relationship between lean mass and RMR. Although there were no significant relationships between lean mass and RMR for any individual treatment group, lean mass was weakly positively correlated with RMR (r^2=0.17, P=0.009) for all mice combined. When we used residuals from this regression, cold-exposed mice still had a significantly greater RMR than control mice ($F_{1,35}$=21.85, P<0.0001), but parasite infection had no effect ($F_{1,35}$=0.3, P=0.609).

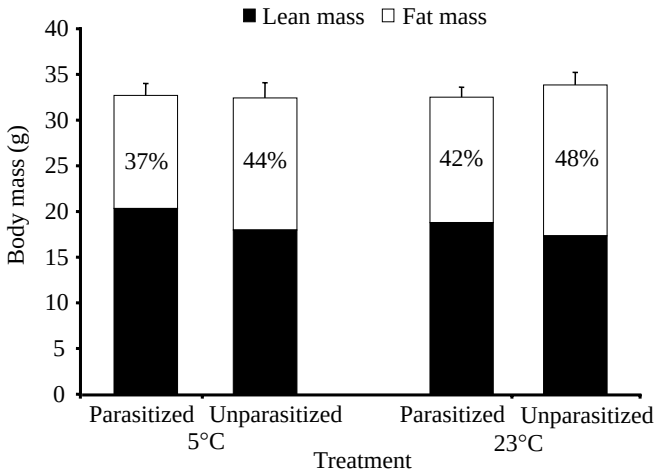

Fig. 1. Body composition (fat *versus* lean mass; g) of parasitized and unparasitized mice at two temperatures. Percentage values are percentage fat mass and error bars are 1 S.E.M. of whole-body mass. N=10 for each group except for parasitized mice at 5 °C, for which N=9.

Cold acclimation and digestive efficiency measures

Cold-acclimated mice ate more than control mice ($F_{1,35}$=142.16, P<0.0001), but parasite treatment had no effect on food intake ($F_{1,35}$=1.30, P=0.262; 5 °C, 7.6±0.2 g day^{-1} for parasitized mice and 7.7±0.2 g day^{-1} for unparasitized mice; 23 °C, 4.8±0.1 g day^{-1} for parasitized mice and 5.1±0.3 g day^{-1} for unparasitized mice). The mean percentage dry matter digestibility was 79±0.2 % for all mice regardless of parasite treatment or acclimation temperature. Therefore, digestible energy intake was greater for mice in the cold ($F_{1,35}$=132.64, P<0.0001) but was not affected by parasite infection ($F_{1,35}$=1.46, P=0.235).

Organ morphology

Cold-exposed mice had greater kidney, liver, lung and heart dry masses than control-temperature mice, and parasitized

Table 1. *Effects of cold exposure and parasite infection on the mean dry mass of the organs of parasitized and unparasitized mice at two temperatures*

	Organ mass (g)							
	Parasitized		Unparasitized		Effects of parasitism		Effects of temperature	
Organ measured	5 °C	23 °C	5 °C	23 °C	F[a]	P	F	P
Small intestine	0.4680±0.020	0.4714±0.026	0.3514±0.011	0.3413±0.017	16.16	<0.0001	0.27	0.605
Mucosal layer	0.2919±0.008	0.2897±0.011	0.2568±0.007	0.2252±0.007	33.84	<0.0001	3.89	0.057
Serosal layer	0.1761±0.014	0.1817±0.015	0.0947±0.006	0.1161±0.016	30.04	<0.0001	1.02	0.320
Stomach[b]	0.0484±0.003	0.0464±0.003	0.0496±0.003	0.0531±0.004	2.37	0.133	0.06	0.809
Cecum[b]	0.0273±0.003	0.0246±0.003	0.0248±0.003	0.0255±0.002	0.43	0.516	0.55	0.463
Large intestine[b]	0.0656±0.007	0.0654±0.004	0.0660±0.008	0.0658±0.005	0.19	0.666	0.45	0.508
Spleen	0.0404±0.003	0.0416±0.003	0.0192±0.001	0.0215±0.001	84.06	<0.0001	0.60	0.442
Kidneys[b]	0.1109±0.004	0.0973±0.004	0.1102±0.004	0.095±0.003	1.36	0.252	54.51	<0.0001
Liver[b]	0.5891±0.028	0.5406±0.019	0.5485±0.034	0.5333±0.018	3.57	0.067	5.95	0.020
Heart[b]	0.0395±0.001	0.0307±0.001	0.0402±0.001	0.0312±0.001	0.40	0.532	171.79	<0.0001
Lung[b]	0.0413±0.001	0.0395±0.001	0.0398±0.002	0.0378±0.001	4.15	0.049	5.87	0.021

Values are means ± 1 S.E.M., N=10 except for parasitized mice at 5 °C, for which N=9.
[a]ANOVA; numerator degrees of freedom (d.f.)=1, denominator d.f.=35, except for lung, for which denominator d.f.=34.
[b]Tested as residuals of organ mass regressed against whole-body mass minus organ mass.

mice had a greater lung, spleen and small intestine dry mass than unparasitized mice (Table 1). The lungs were the only organ that changed in mass with both cold exposure and parasite infection, but there was no significant interaction between treatments. There were no effects of parasitism or cold exposure for any other organs. The greater small intestine dry mass of parasitized mice was due to increases in both the serosal and the mucosal layers (Fig. 2). The serosal layer increased more than the mucosal layer, as demonstrated by the relatively decreased percentage of mucosa in parasitized mice by 10 % at 5 °C and by 5 % at 23 °C ($F_{1,35}$=18.84, P<0.0001; Fig. 2).

Given that *H. polygyrus* resides only in the proximal part of the small intestine, we examined changes in each of the three regions (proximal, mid, distal) to determine whether morphological changes were consistent across the entire small intestine. Mucosal dry mass varied among regions ($F_{2,34}$=138.87, P<0.0001; Fig. 3). There was a significant interaction between parasite infection and small intestine region ($F_{2,34}$=6.33, P=0.005) such that parasitized mice had more mucosa in the mid and distal regions ($F_{1,35}$=20.94, P<0.0001 and $F_{1,35}$=17.77, P<0.0001, respectively), but did not differ from unparasitized mice for the proximal region. Cold-exposed mice had a greater mucosal mass in the mid region ($F_{1,35}$=4.21, P=0.048) but not in the proximal or distal regions (P>0.05) compared with mice at 23 °C. Serosal dry mass varied among regions ($F_{2,34}$=14.7, P<0.0001; Fig. 3), and there was a significant interaction between parasite infection and small intestine region ($F_{2,34}$=23.60, P<0.0001) such that the serosal dry mass of parasitized mice increased more in the proximal region (the site of infection) than in other regions (proximal, $F_{1,35}$=67.52, P<0.0001; mid, $F_{1,35}$=6.52, P=0.015; distal, P>0.05; Fig. 3). Serosal dry mass did not vary with temperature treatment for any region.

Glucose transport

The qualitative results were the same for the rate of glucose uptake standardized either to dry or wet mucosal mass; we present data using wet mucosal mass as this is the *in vivo* condition. Parasitized mice had a 27 % lower glucose uptake rate than unparasitized mice when cold-exposed and a 50 % lower glucose uptake rate at 23 °C (Fig. 4; $F_{1,35}$=32.82. P<0.0001). Despite an overall increase in wet mucosal tissue mass for parasitized mice at 5 °C (unparasitized, 1.48±0.04 g; parasitized, 1.77±0.06 g) and at 23 °C (unparasitized, 1.26±0.05 g; parasitized, 1.68±0.06 g), parasitized mice had a lower total glucose uptake capacity than unparasitized mice ($F_{1,35}$=20.55, P<0.0001; Fig. 5). Cold exposure did not affect

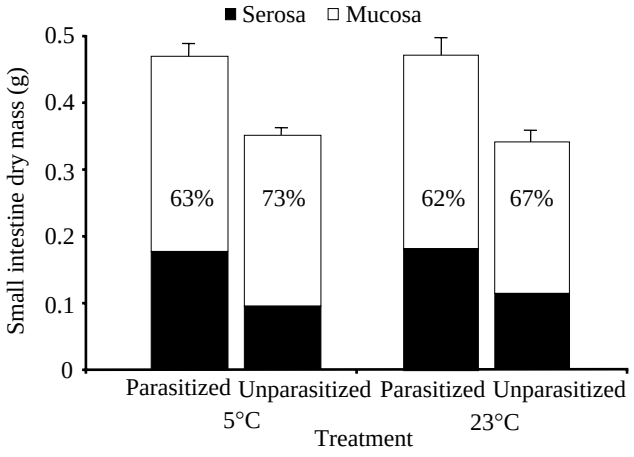

Fig. 2. Total small intestine dry mass (g) partitioned into mucosal and serosal dry mass for parasitized and unparasitized mice at two temperatures. Percentage values are percentage mucosal dry mass and error bars are 1 S.E.M. of total small intestine dry mass. N=10 for each group except for parasitized mice at 5 °C, for which N=9.

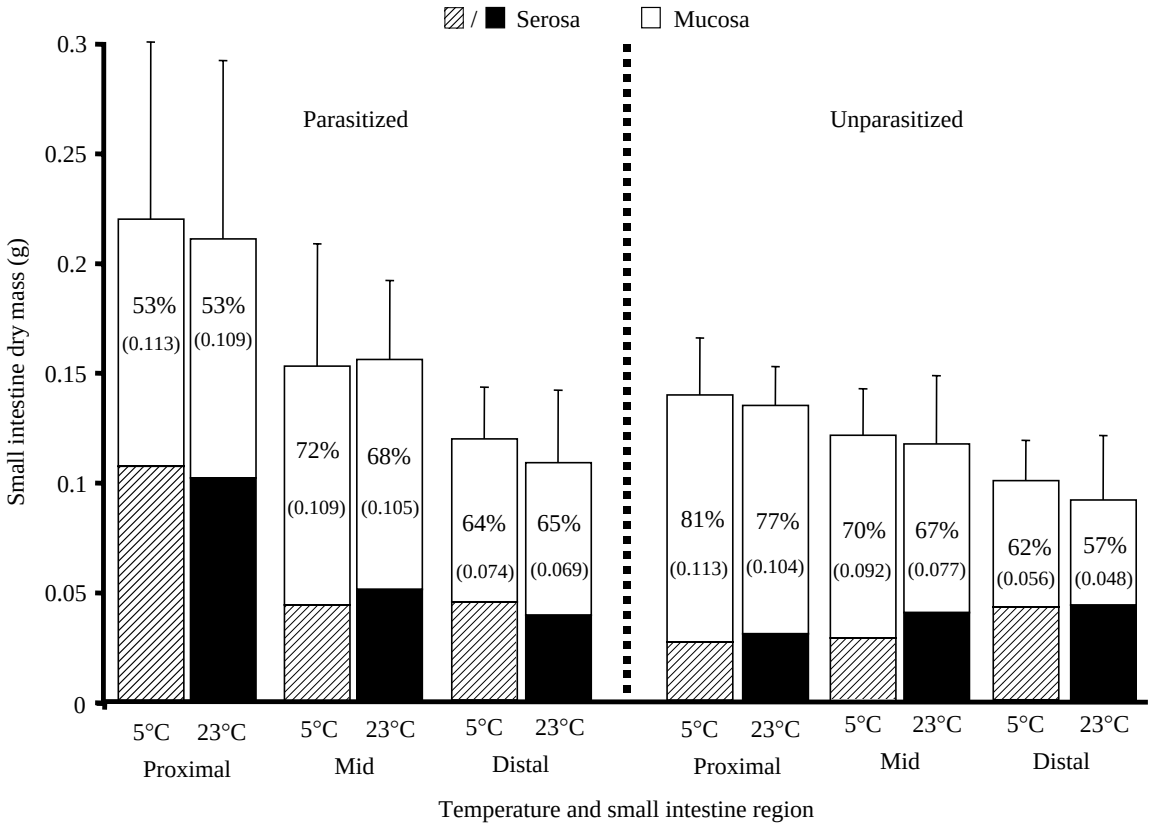

Fig. 3. Dry mass (g) of three small intestine regions, showing serosal dry mass and the percentage and absolute value (g; in parentheses) of mucosal dry mass for parasitized and unparasitized mice at two temperatures. Error bars are 1 S.E.M. of each total small intestine region dry mass. N=10 for each group except for parasitized mice at 5 °C, for which N=9.

glucose uptake rate (P>0.05) but, because cold-exposed mice had more mucosal tissue in the mid region, their total uptake capacity was greater than mice maintained at 23 °C ($F_{1,35}$=9.73, P=0.004; Fig. 5).

We next examined each intestinal region separately. There were differences in glucose uptake capacity and glucose uptake rate among regions ($F_{2,34}$=108.76, P<0.0001 and $F_{2,34}$=33.99, P<0.0001, respectively). Parasitized mice had lower glucose

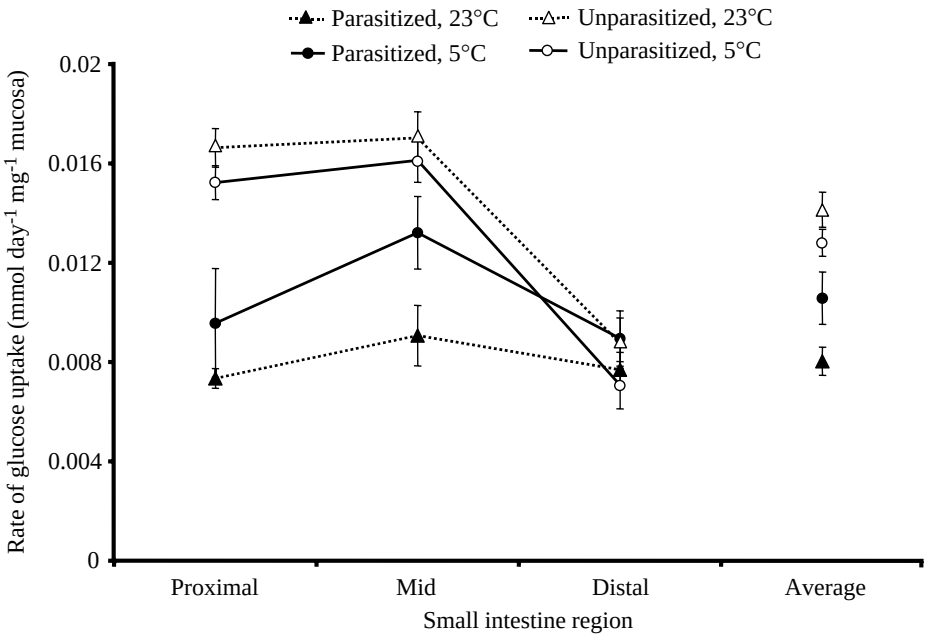

Fig. 4. Rate of glucose uptake (mmol day^{-1} mg^{-1} mucosa) for three small intestine regions and the average rate for the entire small intestine for parasitized and unparasitized mice at two temperatures. Values are means ±1 S.E.M. N=10 for each group except for parasitized mice at 5 °C, for which N=9.

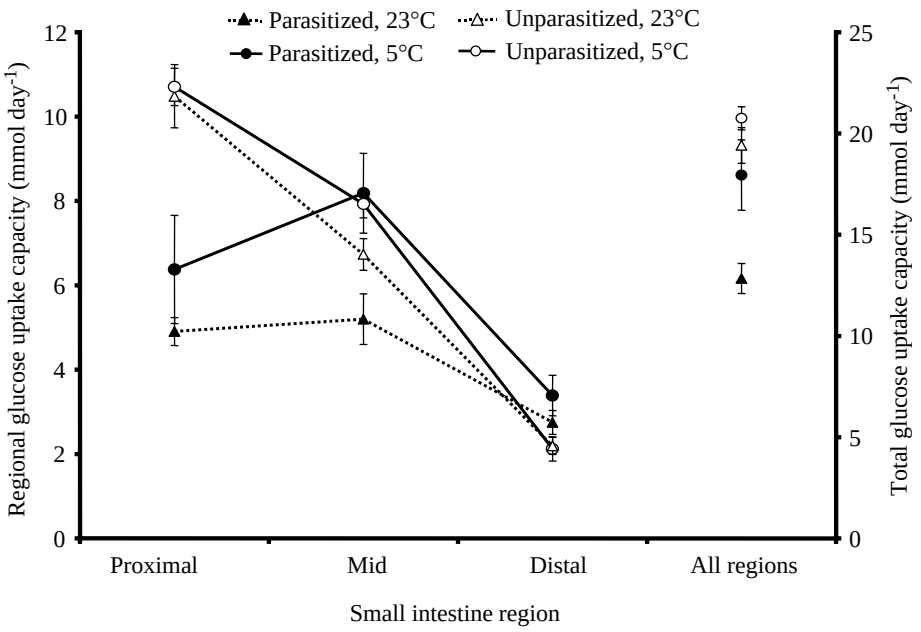

Fig. 5. Glucose uptake capacity (mmol day^{-1}) for each small intestine region (left-hand *y*-axis) and total uptake capacity of the entire small intestine (right-hand *y*-axis) for parasitized and unparasitized mice at two temperatures. Values are means ±1 S.E.M. N=10 for each group except for parasitized mice at 5 °C, for which N=9.

uptake rates in the proximal ($F_{1,35}$=41.46, P<0.0001) and mid ($F_{1,35}$=22.05, P<0.0001) regions, but did not differ from unparasitized mice in the distal region (Fig. 4). Because of differences in mucosal mass among regions, total uptake capacity was lower in the proximal region of parasitized mice ($F_{1,35}$=42.63, P<0.0001), greater in the mid region of cold-exposed mice ($F_{1,35}$=12.50, P=0.001) and greater in the distal region of parasitized mice ($F_{1,35}$=8.18, P<0.007; Fig. 5). There were no other significant effects of either parasite infection or cold exposure on glucose transport in any other small intestine region.

Discussion

Simultaneous demands and phenotypic plasticity

We did not observe all the expected changes in organ masses of parasitized or cold-exposed mice. For example, parasitized mice did not have a greater liver mass than unparasitized mice and cold-exposed mice did not have a greater small intestine mass than mice at 23 °C, as was found in previous studies. We also found a greater lung mass for cold-exposed and parasitized mice compared with mice at 23 °C and unparasitized mice, respectively. Increased lung mass may be associated with increased ventilation responses that correlate with the greater RMR that occurred with cold exposure and parasite infection. Differences in some morphological responses to *H. polygyrus* and cold exposure between our results and previous studies (Liu, 1965; Konarzewski and Diamond, 1994) may be because we used either different strains of laboratory mice or different treatment intensities. Still, as predicted, there were mass changes in numerous organs when mice were given cold exposure and parasite infection. Despite morphological plasticity in lean mass, our mice maintained their overall body mass because of simultaneous decreases in body fat mass. Therefore, studies only measuring body mass as an index of morphological plasticity would not discern the potentially important changes in body composition. Our data contribute to the growing body of literature showing that animals respond to changing environmental demands with phenotypic changes. These phenotypic changes are probably reversible much of the time, although this reversibility (termed phenotypic flexibility) is not often demonstrated in studies of phenotypic plasticity (but see Starck, 1999b).

Simultaneous demands and energy acquisition

Changes in energy allocation, as demonstrated by morphological plasticity, were related to changes in energy acquisition. Although parasitized mice used 4–9 % more energy per day for maintenance, they consumed the same amount of energy as unparasitized mice. Interestingly, parasitized mice could probably have increased their food intake, as indicated by their excess capacity to transport glucose compared with the actual glucose intake in their consumed food (i.e. all the mice had a safety margin: [(maximum glucose uptake capacity minus actual glucose intake)/actual glucose intake]100 (Toloza et al., 1991). Safety margins may indicate the overall status of an animal's ability to cope with additional and unpredictable short-term demands (Diamond and Hammond, 1992). Safety margins for parasitized mice (84.3 % at 5 °C and 115.6 % at 23 °C) were smaller than for unparasitized mice (113.7 % at 5 °C and 202.1 % at 23 °C) regardless of cold exposure. However, because safety margins are calculated using maximal transport capacity and because animals may not function at their maximum capacity, we do not know whether cold exposure or *H. polygyrus* infection actually diminished the safety margins for glucose transport.

As predicted, cold-exposed mice increased both their food intake and their total glucose uptake capacity to fuel the increased cost of living in a cold environment. Also as

predicted, parasitized mice had a decreased glucose uptake capacity, driven by a decreased transport rate despite an overall increase in mucosal tissue available for absorption. One possible explanation is that, as seen with other nematode species (Symons, 1965), *H. polygyrus* infection resulted in an increased cell turnover rate in the small intestine which may produce an accumulation of immature mucosal cells not yet functional for glucose transport (Ferraris, 1994). Alternatively, some parasites change the physicochemical conditions of the small intestine lumen, and this may affect enzyme kinematics associated with glucose transport (e.g. tapeworms *Hymenolepis diminuta* in the rat; Mettrick, 1971; Podesta and Mettrick, 1975).

Because cold-exposed and parasitized mice had decreased fat stores, mice with these challenges may have mobilized their fat rather than increased their nutrient absorption to meet their energy requirements. Of course, this response could not be sustained indefinitely. Instead, mice given these demands for a longer time may achieve a new steady state with less body fat.

Relationship between morphology, energy acquisition and resting metabolism

Changes in body composition were correlated with changes in RMR, such that increased lean mass was correlated with increased RMR. Both parasitized and cold-exposed mice had a greater lean mass and RMR than their respective controls, but only cold-exposed mice increased their food intake. Recall that glucose transport rate was diminished in parasitized mice in the proximal and mid regions but did not differ in the distal region (Fig. 4). However, the total transport capacity of the distal region was actually greater for mice with parasites than for unparasitized mice because of increased mucosal tissue mass (Fig. 5). Therefore, parasite infection not only had effects at the infection site, such as mechanical damage *via* the consumption of mucosal tissue, but also yielded indirect effects on glucose absorption in the rest of the small intestine. Our observed changes in glucose transport may be related to changes in the nutrient density of ingesta. It is well established that increased nutrient transport by the small intestine can be induced by increased nutrient density of the ingesta (Ferraris et al., 1992; Ferraris, 1994) and that absorption of both carbohydrates and proteins normally decreases from the proximal to the distal small intestine (Ferraris et al., 1992). The distal small intestine may compensate for the decreased function of the proximal small intestine by increasing its transport capacity (Reville et al., 1991). Therefore, *H. polygyrus* infection may indirectly increase the glucose transport capacity of the distal small intestine because of the greater nutrient density of ingesta reaching the distal intestine that results from malabsorption of glucose in the proximal region, where parasites have damaged the mucosa. Despite this increased transport capacity of the distal region, parasitized mice still had a lower overall capacity to acquire glucose compared with unparasitized mice. The simultaneous demands of cold exposure and intestinal parasites may be important because of the diminished nutrient acquisition ability of the small intestine and also because of the increase in energy requirements (e.g. the RMR of parasitized, cold-exposed mice was greater than for any demand alone).

Concluding remarks

Many studies link environmental demands to phenotypically plastic responses (Piersma et al., 1993; Piersma, 1998; Piersma and Gill, 1998; Starck, 1999a,b), but few link morphological plasticity to energy metabolism (Piersma et al., 1996; Battley et al., 2000) and ultimately to changes in life-history variables. We suggest that plasticity in the organ mass component of lean body mass is correlated with resting metabolic rate. Unlike some other studies using simultaneous exposure of parasites and a second demand (Carlomagno et al., 1987; Chao et al., 1990; Banerjee et al., 1999; Monroy et al., 1999), we found that mice were not affected more by cold exposure when they were parasitized. However, when challenged with parasites and cold exposure simultaneously, mice do have fewer energy reserves available. Parasitized females, not challenged with cold exposure, produce smaller pups at weaning (D. Kristan, unpublished data). This effect on reproduction may potentially be increased if females were also challenged by an additional demand such as cold exposure or caloric restriction.

Importantly, the effects of sub-lethal parasite infections, in combination with other, simultaneous demands, have not been addressed by current models of host–parasite associations (e.g. Roberts et al., 1995; Cornell et al., 1999) primarily because of a lack of empirical evidence. Understanding how parasite infection affects host performance at the whole-animal level and the mechanisms behind the organism's performance is critical to the development of a more complete and accurate model of host–parasite interactions at the population level and to understand the relationships between morphological plasticity, physiology and life-history traits.

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