The Journal of Experimental Biology 207, 2351-2360
Published by The Company of Biologists 2004
doi:10.1242/jeb.01020

Morphological plasticity varies with duration of infection: evidence from lactating and virgin wild-derived house mice (*Mus musculus*) infected with an intestinal parasite (*Heligmosomoides polygyrus*; Nematoda)

Deborah M. Kristan* and Kimberly A. Hammond

Department of Biology, University of California, Riverside, CA 92521, USA

*Author for correspondence at present address: Department of Biological Sciences, California State University, San Marcos, CA 92096, USA (e-mail: dkristan@csusm.edu)

Accepted 7 April 2004

Summary

With chronic parasite infection, host response to the parasite may change throughout the duration of the infection as the host progresses from the acute to the chronic phase. We investigated the effects of parasite infection ranging in duration from 30 to 120 days on host morphology both alone and in combination with lactation by using captive wild-derived house mice (*Mus musculus*) experimentally infected with a naturally occurring intestinal nematode (*Heligmosomoides polygyrus*). We found that some changes in host morphology were greatest at 30–60 days post-infection (e.g. spleen mass) followed by a decline towards the control state whereas other morphological changes were greatest at 90–120 days post-infection (e.g. small intestine mass) after a relatively steady increase with infection duration. For all infection durations, the morphological responses to parasite infection were similar for virgin and lactating mice (except for lean body mass). After accounting for changes in body mass with lactation, lactating mice increased organs of the gastrointestinal tract as well as liver and kidney but had less body fat than virgin mice. This is the first study to demonstrate that morphological plasticity of mice parasitized by *H. polygyrus* varies with infection duration and that this variation is generally similar for lactating and virgin mice.

Key words: infection duration, lactation, phenotypic plasticity, Nematoda, *Mus musculus*, *Heligmosomoides polygyrus*.

Introduction

Wild animals are frequently infected by one or more parasite species throughout their lives (Behnke et al., 2001; Cox, 2001). While some parasites exist in the host for a very short time, others can remain for weeks, months or years (Behnke et al., 1992), producing a chronic infection. Hosts do not always develop life-time immunity to parasites that produce a chronic sublethal infection and so repeatedly incur the cost of parasitism (e.g. Fuller, 1996). Therefore, chronic infections may have subtle yet important effects on host ecology and life history. For example, costs associated with host immune response and tissue repair resulting from parasitism can alter energy allocation and, if energy is limited, this will leave less energy available for other uses, such as reproduction (Sheldon and Verhulst, 1996; Demas et al., 1997; Nordling et al., 1998). Alternatively, animals may use resources for reproduction and compromise their ability to respond to parasites (e.g. Allander, 1997; Deerenberg et al., 1997; Ilmonen et al., 1999; Derting and Compton, 2003).

Lactation is an extremely energy-demanding time for mice (Hammond et al., 1994), with an increase in food intake of more than 100% over that consumed by virgin mice (Hammond et al., 1996). Because much of this extra energy consumed by lactating mice is converted to milk and exported to pups, it does not represent the actual levels of increased energy expenditure, in terms of metabolism, for lactating females (Speakman, 2000). However, mice must still ingest and process all the calories needed to synthesize milk and must produce and deliver milk to pups, which may account for part of the true increase in resting metabolism observed in laboratory mice during lactation (Hammond and Diamond, 1992; Hammond et al., 1994; Speakman and McQueenie, 1996). Wild house mice in captivity given a 14 h:10 h L:D light cycle can produce approximately one litter each month (Bronson, 1979; D. Kristan, personal observation) and, in nature, mice are not always limited to a specific reproductive season (Bronson, 1979) as is typical for many other small mammals. Therefore, female house mice are likely to spend much of their lives either pregnant, lactating or both. Importantly, immune function of female mice during pregnancy and lactation is typically diminished compared with non-reproducing mice (Selby and Wakelin, 1975; Ferguson et al., 1982; Sulila and Mattsson, 1990; Medina et al., 1993), causing them to be especially susceptible to a broad range of parasites.

Numerous nematode species infect wild *Mus* (Doran, 1955). Mice respond to the larval stages of the nematode *Heligmosomoides polygyrus* with both a cell-mediated and antibody-mediated immune response (Liu, 1965; Panter, 1969; Monroy and Enriquez, 1992), and laboratory mice are more susceptible to infection with *H. polygyrus* when lactating (Shubber et al., 1981). After adult worms emerge in the mouse small intestine (approximately 9–11 days after ingesting an infective-stage larva; Monroy and Enriquez, 1992), the mature parasites produce an immunosuppressive factor to suppress worm expulsion (Dehlawi and Wakelin, 1988; Monroy et al., 1989; Monroy and Enriquez, 1992; Scott and Koski, 2000) and presumably to enhance the ability of newly emerging adults to survive. At this point, the mouse's initial immune response to the larval stage begins to diminish, and the adult *H. polygyrus* worms can last up to 8 months in the mouse (Ehrenford, 1954).

For many parasites that produce chronic, sometimes lifelong, infections there is often an initial phase with an active immune response by the host followed by a quiescent phase that can be interrupted by periodic reactivation of the parasite (e.g. as seen with *Toxoplasma gondii*; Luft et al., 1983; Bosch-Driessen et al., 2002). Some factors affecting host response to a parasite infection include host age, presence of additional parasites and environmental conditions. For example, responses to infection varied when laboratory mice were subjected to caloric restriction (Carlomagno et al., 1987; Effros et al., 1991; Otesile et al., 1991; Peck et al., 1992; Shi et al., 1998; Kristan and Hammond, 2001), protein deficiency (Slater and Keymer, 1986; Tetzlaff et al., 1988; Peck et al., 1992; Boulay et al., 1998; Cintra et al., 1998; Anstead et al., 2001) or cold exposure (Banerjee et al., 1999; Monroy et al., 1999; Aviles and Monroy, 2001). Many experimental studies of host response to parasites use one infection intensity and measure host responses after a single time period. Our goal was to determine (1) how morphological plasticity in the host varies with infection duration and (2) if host responses to different infection durations change with another simultaneous challenge. Therefore, we examined the effects of simultaneous lactation and parasite infection for captive wild-derived house mice (*Mus musculus* L.) infected with an intestinal nematode (*Heligmosomoides polygyrus* Dujardin 1845) for 30–60 days or for 90–120 days.

First, we hypothesized that morphological changes associated with acute parasite infection would be most pronounced at the shortest infection duration and then would diminish with longer infection durations. Based on our work with laboratory mice, we predicted that adult wild-derived house mice would increase several organ masses (e.g. small intestine, spleen) and lean body mass (Liu, 1965; Kristan and Hammond, 2000, 2001). The mechanistic basis of our prediction is that organ mass changes may be associated either directly or indirectly with the immune-inducing phase of *H. polygyrus* infection that occurs soon after infection and that, as time passes, these organ mass changes will be less evident. For example, splenomegaly occurs with induced antibody production in mice infected with malaria (*Plasmodium berghei*; Hansen et al., 2003). Second, we hypothesized that *H. polygyrus* infection and lactation would interact to affect host responses when the two demands occurred simultaneously. We predicted that morphological changes associated with lactation (e.g. increased small intestine mass; Hammond et al., 1994, 1996; Kristan, 2002) would be diminished in parasitized mice because energy normally used to respond to lactation would be diverted to respond to parasitism. *H. polygyrus* infection in wild-derived mice is associated with a diminished digestive efficiency and decreased rate of glucose transport by the small intestine (Kristan and Hammond, 2003). Therefore, energy demands on the host during *H. polygyrus* infection are measurable and have the potential to be substantial enough to affect energy needed for lactation unless mice can increase energy acquisition enough to meet both demands.

Materials and methods

Our experiment had two independent variables: parasite infection duration and lactation (lactating, virgin) and numerous dependent variables (organ masses, body mass, body composition). Parasitized mice were infected for 30, 60, 90 or 120 days. For purposes of analysis, we grouped parasite infection duration into three categories (uninfected, infected for 30–60 days, infected for 90–120 days). At the beginning of the experiment, all females in the virgin group were 50–104 days old, all females used for the lactation treatment were 71–164 days old and all males were 71–134 days old. All mice were 2–4 generations captive born and males were uninfected and used only for mating purposes. Unparasitized mice were killed at 30–60 or 90–120 days post-sham infection to account for possible age effects. For lactating mice, we infected females on the same day they were paired with males and killed females at 15 days post-partum (when laboratory mice were at their peak lactation; Hammond et al., 1994), as close to 30–60 or 90–120 days post-infection as possible. For lactating females, the actual infection duration and number of litters produced prior to dissection varied depending on when a litter was born relative to the infection/pairing date (Table 1). Because pinworm (*Syphacia obvelata*) is present in our vivarium, we determined the presence or absence of pinworm by examining cecal contents for each mouse on the day of dissection to determine possible confounding effects of pinworm infection on our experiments.

Table 1. *Actual infection duration or duration since control inoculation (# days ± 1 S.E.M.), and parity (# litters produced ± 1 S.E.M.) for lactating mice*

	Parasitized		Unparasitized	
Duration group	Actual duration	Parity	Actual duration	Parity
30–60	57±8	1.5±0.3	44±4	1.3±0.2
90–120	110±6	3.3±0.3	128±28	4.0±1.0

Parasite maintenance and mouse infection procedures

We cultivated *H. polygyrus* infective-stage larvae (L3) from non-experimental animals, and mice in the parasitized group were given 300±3 to 300±24 L3 (# worms ± 1 S.D.; range is because more than one parasite culture was used during the experiment) suspended in tap water by using a 200 µl pipette tip (Fisherbrand Redi-Tip on a Pipetman; Kristan and Hammond, 2001) placed in the back of the mouse's throat. Contents were dispensed into the mouse's throat and the mouse swallowed to complete the infection. All mice in the unparasitized group were given an equal volume of tap water only. Infection status of all mice was checked 14–16 days post-infection using a modified McMaster technique that detects eggs in mouse feces (Bowman, 1995).

Organ morphology and body composition

We gave mice terminal anesthesia between 08.30 and 11.30 h by intraperitoneal injection of 0.04 ml sodium pentobarbital (65 mg ml^{-1}). We removed the small intestine (see below), stomach, cecum, large intestine, heart, liver, spleen, kidneys and lungs. Excess fat and connective tissue were removed from each organ and returned to the mouse carcass. We weighed stomach, cecum and large intestine, with contents and then without contents, after rinsing with mammalian Ringer solution (for composition of Ringer solution, see Karasov and Diamond, 1983).

The small intestine was rinsed *in situ* with cold mammalian Ringer solution prior to removal from the mouse. We divided the small intestine into three regions of equal length (proximal, mid and distal) and measured the wet mass of each region after lightly blotting to remove adherent Ringer solution. Total small intestine mass was the sum of the three regions, corrected for mass of the parasites as described below. To determine mucosal and serosal components of the small intestine, we cut two 1.5-cm sleeves per region and weighed each sleeve. We scraped the mucosal/submucosal tissue (hereafter called 'mucosa') away from the muscularis/serosal tissue (hereafter called 'serosa'; Diamond and Karasov, 1984) and dried the tissue from the scrapes for 2 days at 55–60°C. The dry mass:wet mass ratio was calculated for each sleeve and we used the average of these ratios to calculate mucosal and serosal dry mass for the entire small intestine (Diamond and Karasov, 1984; Hammond and Diamond, 1992). We measured dry mass of other organs and the carcass after drying to a constant mass at 55–60°C for 2 and 14 days, respectively.

To determine body composition, we ground the dried carcass and then extracted lipids for 6.5 h using petroleum ether (Goldfische apparatus; Labconco, Kansas City, MD, USA). The difference in mass between the unextracted and the extracted samples was taken as grams of fat in the carcass. We measured fat content of liver and the combined fat content of spleen, heart, lung, kidney, cecum, stomach and large intestine by soaking organs in 10 ml aliquots of petroleum ether for six 24 h periods (pouring off ether at the end of 24 h and replacing it with fresh ether). We calculated the difference in organ mass before and after extraction to determine amount of fat (g) in liver and other organs. Total fat mass of each mouse was the sum of carcass and organ fat masses, and lean mass was calculated as initial whole body mass minus total fat mass minus contents of stomach, cecum and large intestine.

Parasite infection intensity and corrected small intestine mass

We removed and counted all *H. polygyrus* from small intestine tissue (including sleeves used for mucosal/serosal scrapes) using a dissecting microscope to determine the infection intensity for each parasitized mouse. Total parasite wet mass (number of worms multiplied by average worm mass; Kristan and Hammond, 2001) was subtracted from small intestine wet mass prior to calculation of small intestine dry mass used in analyses. When we examined each intestinal region separately, parasite mass was subtracted only from mass of the proximal region, where adult parasites occur (Bansemir and Sukhdeo, 1996).

Food intake and digestive efficiency

For the three days prior to dissection, food intake rate (I; g day^{-1}) and fecal output rate (O; g day^{-1}) were measured (Hammond and Diamond, 1992). Average percent apparent dry matter digestibility (i.e. digestive efficiency) was calculated as $[(I–O)I–1]\times100$.

Statistics

To examine effects of possible confounding variables, we first used a multivariate analysis of variance (MANOVA) to determine if mouse age or pinworm infection affected dependent variables. We included the variable 'pinworm' because we found pinworm in 18 of 37 virgin mice and 11 of 16 lactating mice. Pinworm infection was not significant ($P>0.05$). Because we found no effects of age ($P>0.05$) on any dependent variable we combined all uninfected mice into a '0' day infection duration group [therefore, infection duration has three levels of 0 (unparasitized mice), 30–60 days and 90–120 days]. For all lactating mice (parasitized and unparasitized) we also used MANOVA to examine any effects of parity (# of litters produced during the experiment) and found that parity did not affect dependent variables ($P>0.05$).

We used MANOVA with factors '*H. polygyrus* infection duration' (0, 30–60, 90–120) and 'reproduction' (virgin, lactating) and found that both factors were significant ($P=0.013$ and $P<0.0001$, respectively). Therefore, we used independent analysis of variance (ANOVA) or analysis of covariance (ANCOVA; with body mass as a covariate when appropriate; see below) to determine which of these two treatments and which dependent variables contributed to the overall multivariate significance (Stevens, 1986). We regressed each dependent variable against body mass to determine if body mass effects were significant. If the regression was significant we used body mass as a covariate in the ANCOVA and present least squares means ± 1 S.E.M. for the dependent variables. If the regression between the dependent variable and mass was not significant, we present the arithmetic mean ± 1 S.E.M. We report F statistics and P values from the *post-hoc* ANOVAs or ANCOVAs.

Table 2. *Sample size for each treatment group where lactating or virgin mice were uninfected or were infected for either 30–60 days or 90–120 days*

Infection duration (days)	Lactating	Virgin
0 (uninfected)	9	20
30–60	4	10
90–120	3	7

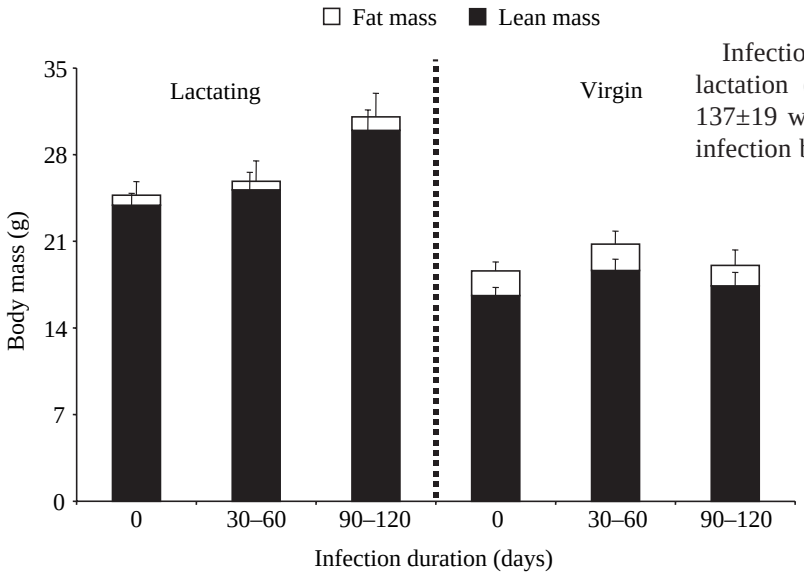

Fig. 1. Average whole body mass and body composition for virgin and lactating mice that were either unparasitized (0 infection duration) or that were parasitized for 30–60 or 90–120 days. Error bars are +1 S.E.M.

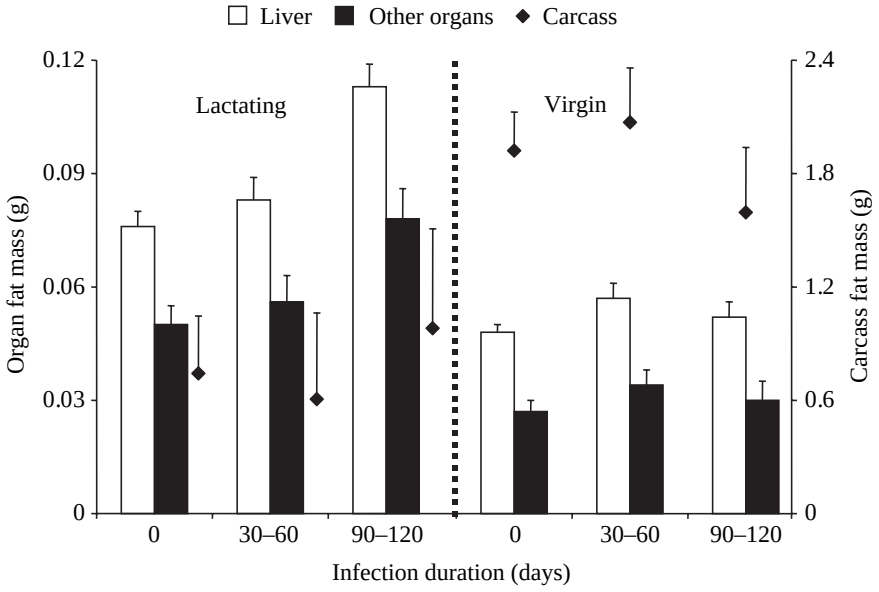

Fig. 2. Fat mass of organs [liver and others combined (heart, lung, kidney, spleen, stomach, large intestine, cecum)] and carcass for virgin and lactating mice that were either unparasitized (0 infection duration) or that were parasitized for 30–60 or 90–120 days. Error bars are +1 S.E.M.

Results

All mice given parasites developed mature infections, and no control mice became infected. Final sample sizes varied among groups (Table 2) because not all mice assigned to the lactation groups produced litters at the necessary times. Regressions of body mass with each dependent variable were significant for all variables except grams of body fat, therefore whole body mass was included in the ANOVA model as a covariate for all variables except grams of body fat.

Parasite infection intensity

Infection intensity did not vary with infection duration or lactation (P>0.05), with an average infection intensity of 137±19 worms per mouse. One lactating mouse cleared her infection by 73 days and one virgin mouse by 126 days post-inoculation and three others had infection intensities more than 2 S.D. below the mean (one lactating mouse with six worms, one virgin mouse with 15 worms, one virgin mouse with 24 worms). If these five mice were excluded, the mean infection intensity was 170±17 worms, ranging from 54 to 282 worms.

Body mass and composition

Body mass was 40% greater for lactating than virgin mice ($F_{1,47}$=54.7, P<0.0001) and was greater for mice at 90–120 days post-infection compared with uninfected mice ($F_{2,47}$=4.0, P=0.025; Fig. 1). Although the interaction between treatments was non-significant ($F_{2,47}$=3.1, P=0.052), it may be biologically important to note that body mass was heavier at 90–120 days of infection for lactating but not virgin mice. Lean mass was 50% greater for lactating than virgin mice ($F_{1,47}$=86.0, P<0.0001) and also increased with infection duration ($F_{2,47}$=4.8, P=0.013; Fig. 1) such that mice infected for 90–120 days had a greater lean mass than uninfected mice, but there were no other differences among groups. There was a significant interaction between treatments ($F_{2,47}$=3.2, P=0.048) because the increase in lean mass with infection duration occurred for lactating but not virgin mice (Fig. 1). Lactating mice had 55% less fat than virgin mice ($F_{1,47}$=12.0, P=0.001) but amount of body fat did not vary with infection duration.

The decrease in body fat with lactation occurred for fat in the carcass (58% decrease; $F_{1,47}$=13.0, P=0.001; Fig. 2) but, in contrast, total organ fat content was 73% greater for lactating mice than

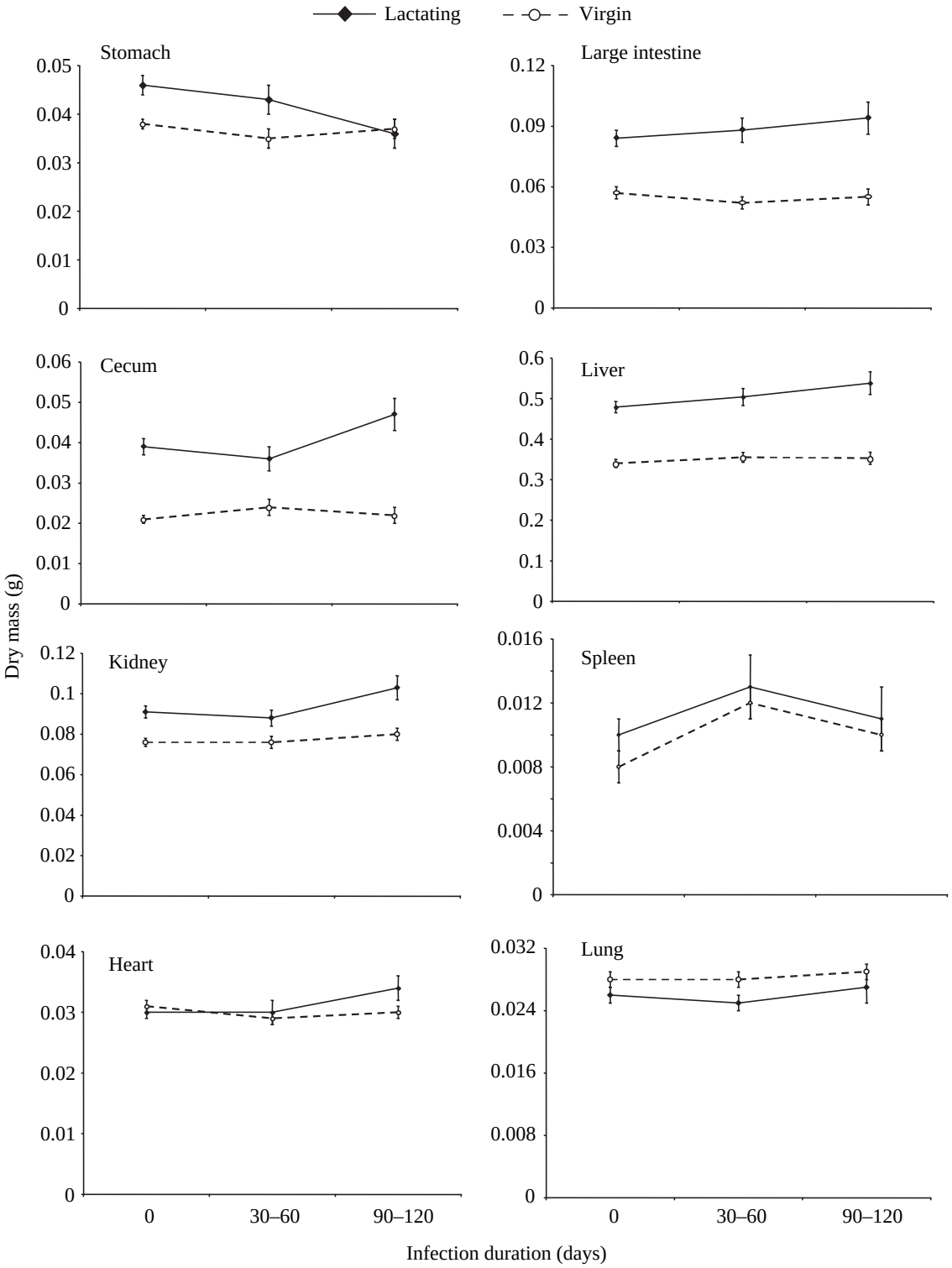

Fig. 3. Organ dry masses for virgin and lactating mice that were either unparasitized (0 infection duration) or that were parasitized for 30–60 or 90–120 days. Error bars are +1 S.E.M.

virgin mice ($F_{1,47}$=66.0, P<0.0001). Total organ fat content also varied with infection duration ($F_{2,47}$=6.4, P=0.003) such that mice infected for 90–120 days had more organ fat than uninfected mice. When we examined the contribution of organ fat associated with liver *vs* all other organs (except the small intestine) we found that lactating mice had twice as much fat in their liver than virgin mice ($F_{1,47}$=71.2, P<0.0001) and 32% more fat in the other organs combined ($F_{1,47}$=25.4, P<0.0001). Mice infected for 90–120 days had 39% more fat in their liver than uninfected mice ($F_{2,47}$=6.2,

P=0.004) and 22% more fat in other organs combined ($F_{2,47}$=3.8, P=0.029; Fig. 2).

Organ morphology

We examined effects of each treatment on total organ mass. Masses of kidneys ($F_{2,46}$=3.4, P=0.042), spleens ($F_{2,46}$=4.0, P=0.025), stomachs ($F_{2,46}$=3.9, P=0.028) and small intestines (see below) varied with infection duration (Fig. 3). Spleen mass was greatest at 30–60 days post-infection and then decreased with longer infection durations to become similar to that in uninfected mice. Stomach mass decreased with longer infection duration, and kidney mass was greatest for mice infected for 90–120 days but was similar between uninfected mice and mice infected for 30–60 days. After accounting for body mass effects, lactating mice had larger livers ($F_{1,46}$= 71.4, P<0.0001), kidneys ($F_{1,46}$=16.4, P<0.0001), stomachs ($F_{1,46}$=4.9, P=0.031), cecae ($F_{1,46}$=55.1, P<0.0001), small intestines (see below) and large intestines than virgin mice ($F_{1,46}$=43.0, P<0.0001; Fig. 3). The decrease in lung mass with lactation approached but did not reach significance ($F_{1,46}$=3.9, P=0.056; Fig. 3). Although the interaction between treatments for cecum mass was non-significant ($F_{2,46}$=3.1, P=0.057), it may be biologically relevant to note that there was a tendency for an increase in cecum mass with parasite infection for lactating but not virgin mice.

Small intestine morphology

Small intestine length was greater for parasitized than unparasitized mice and for mice parasitized for 90–120 days compared with mice parasitized for 30–60 days ($F_{2,46}$=10.8, P<0.0001; Fig. 4). Lactating mice had longer small intestines than virgin mice ($F_{1,46}$=20.8, P<0.0001; Fig. 4). Because qualitative results are the same for absolute small intestine dry mass and analysis of dry mass using length of small intestine as a covariate, we report only absolute dry mass results for all small intestine variables. Small intestine dry mass increased with duration of parasite infection ($F_{2,46}$=13.5, P<0.0001) and was 32% greater for lactating than virgin mice ($F_{1,46}$=11.3, P=0.002; Fig. 4). Parasite infection duration increased the mass of the proximal region of the small intestine ($F_{2,45}$=14.1, P<0.0001; Fig. 5A) but did not affect the mid or distal regions. Lactating mice had increased masses of all regions (proximal, $F_{1,45}$=4.8, P=0.034; mid, $F_{1,46}$=14.4, P<0.0001; distal, $F_{1,46}$=4.5, P=0.040; Fig. 5).

Across the entire small intestine, both infection duration and lactation produced increases in mass of mucosal ($F_{2,46}$=10.5, P<0.0001 and $F_{1,46}$=8.0, P=0.007, respectively) and serosal ($F_{2,46}$=18.4, P<0.0001 and $F_{1,46}$=8.4, P=0.006, respectively; Fig. 4) tissue. Mucosal mass increased by 40% from uninfected mice to mice at 90–120 days post-inoculation and by 27% for lactating compared with virgin mice. Serosal mass increased by 91% from uninfected mice to mice at 30–60 days post-inoculation and then decreased such that mice parasitized for 90–120 days had an average of only 43% greater serosal mass than uninfected mice. Lactating mice averaged 51% more serosal mass than virgin mice.

When we examined each region separately, mucosal mass was greater with parasite infection for only the proximal region ($F_{2,45}$=10.2, P<0.0001; Fig. 5A) and with lactation only for the mid region ($F_{1,46}$=11.2, P=0.002; Fig. 5B). There was a significant interaction between treatments for mucosal mass of the mid region ($F_{2,46}$=3.4, P=0.041) because the increase in mid mucosal mass with lactation was more for uninfected than infected mice (Fig. 5C). Serosal mass increased for all three regions with parasite infection (proximal, $F_{2,45}$=16.3, P<0.0001; mid, $F_{2,46}$=10.3, P<0.0001; distal, $F_{2,46}$=5.9, P=0.005), with the greatest increase at 30–60 days post-inoculation followed by a decrease in serosal mass towards the control condition for mice infected for 90–120 days. Serosal mass also increased during lactation for the proximal ($F_{1,45}$=4.9, P=0.032) and mid ($F_{1,46}$=9.4, P=0.004) regions but not the distal region ($F_{1,46}$=3.8, P=0.056; Fig. 5).

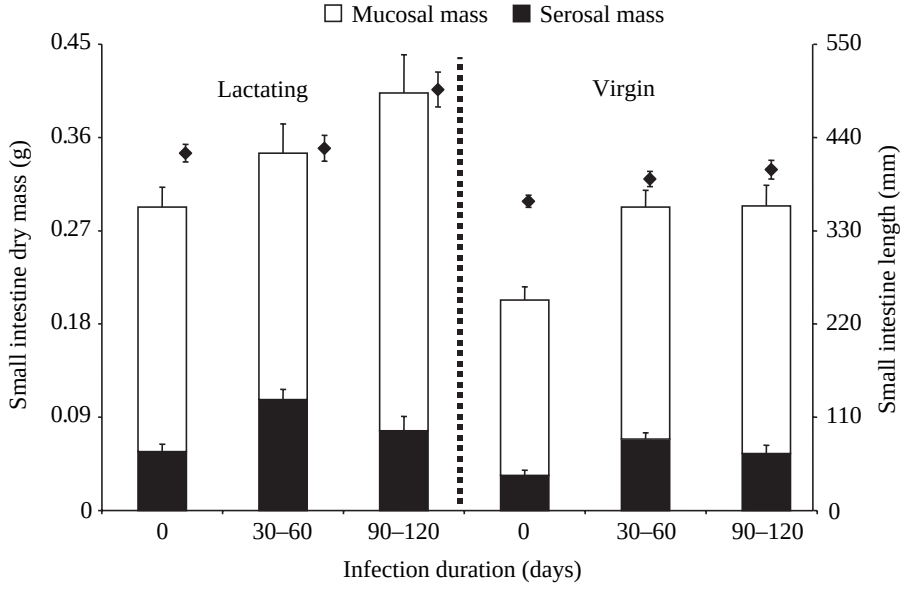

Fig. 4. Small intestine length (shown by diamonds) and dry mass (shown by bars) subdivided by 'serosal' and 'mucosal' components for virgin and lactating mice that were either unparasitized (0 infection duration) or that were parasitized for 30–60 or 90–120 days. Error bars are +1 S.E.M.

Food intake and digestive efficiency

Food intake was 122% greater for lactating than virgin mice ($F_{1,44}$=45.4, P<0.0001; Fig. 6) and increased slightly with infection duration ($F_{2,44}$=3.3, P=0.046). Because digestibility did not differ with either experimental treatment, digestible food intake was similar to absolute food intake (Fig. 6).

Discussion

We demonstrate that phenotypic plasticity of lactating and virgin wild-derived mice varies with infection duration and, except for lean body mass, there is no interaction between lactation and *H. polygyrus* infection for morphological responses of the host. To our knowledge, our research is the first to demonstrate changes in host phenotypic plasticity with infection duration for a sublethal parasite system.

Morphological plasticity and body composition

Lactation

Increased body mass during lactation was due primarily to larger masses of all components of the gastrointestinal tract and central processing organs that are associated with increased food consumption used to meet energy demands of lactation. Increased food intake will increase masses of digestive organs very quickly (as early as 1–2 days; Toloza et al., 1991) to support the increased bulk of ingesta. Of course, in addition to actually ingesting and digesting the extra food, the resulting nutrients must then be processed and transported throughout the body for use in peripheral areas (e.g. milk production by the mammary glands; Hammond et al., 1994), hence lactation is also associated with increased liver mass (present study; Hammond et al., 1994) and blood volume (Suzuki et al., 1993, 2000).

Despite an increased body mass, lactating mice are either maintaining or enhancing fat stores in their organs while using fat stores originating elsewhere in the body (e.g. abdominal, subcutaneous). All organs, especially the liver, had much greater fat for lactating than virgin females. Among its numerous functions, the liver is used to synthesize and export lipids and it is possible that we measured some of these additional lipids prior to them being exported.

Parasitism

As predicted from our work with laboratory mice (Kristan and Hammond, 2000, 2001), lean mass increased with *H. polygyrus* infection primarily because of increased masses of small intestine and spleen. Spleen mass increased from uninfected mice to mice that had been infected for 30–60 days, but by 90–120 days post-infection spleen mass was decreasing towards the size for unparasitized mice. In contrast to the pattern seen with spleen mass, small intestine mass showed a continual increase with infection duration. Infection duration can differentially affect host morphology both among and within organs. Changes in small intestine mass were not uniform throughout the length of the intestine or among tissue layers. For example, serosal mass changed for regions of the small intestine that were unoccupied by *H. polygyrus* as well as for regions that were occupied. It is possible that greater serosal mass of infected mice is due to increased tissue accumulation in the muscularis at sites previously occupied by larvae or from any remaining larvae that did not emerge into the lumen prior to dissection. Alternatively, it is possible that changes in the serosal layer may reflect changes in patterns of peristalsis (dependent on the muscularis layers) with *H. polygyrus* infection. Ingesta may have been passed through the small intestine more quickly (perhaps using more frequent peristalsis) for parasitized than unparasitized mice and thereby contributed to changes in mass of the serosal layer throughout the entire small intestine. Indeed, wild-derived house mice infected with *H. polygyrus* can have a slight (2%) but significantly diminished ability to

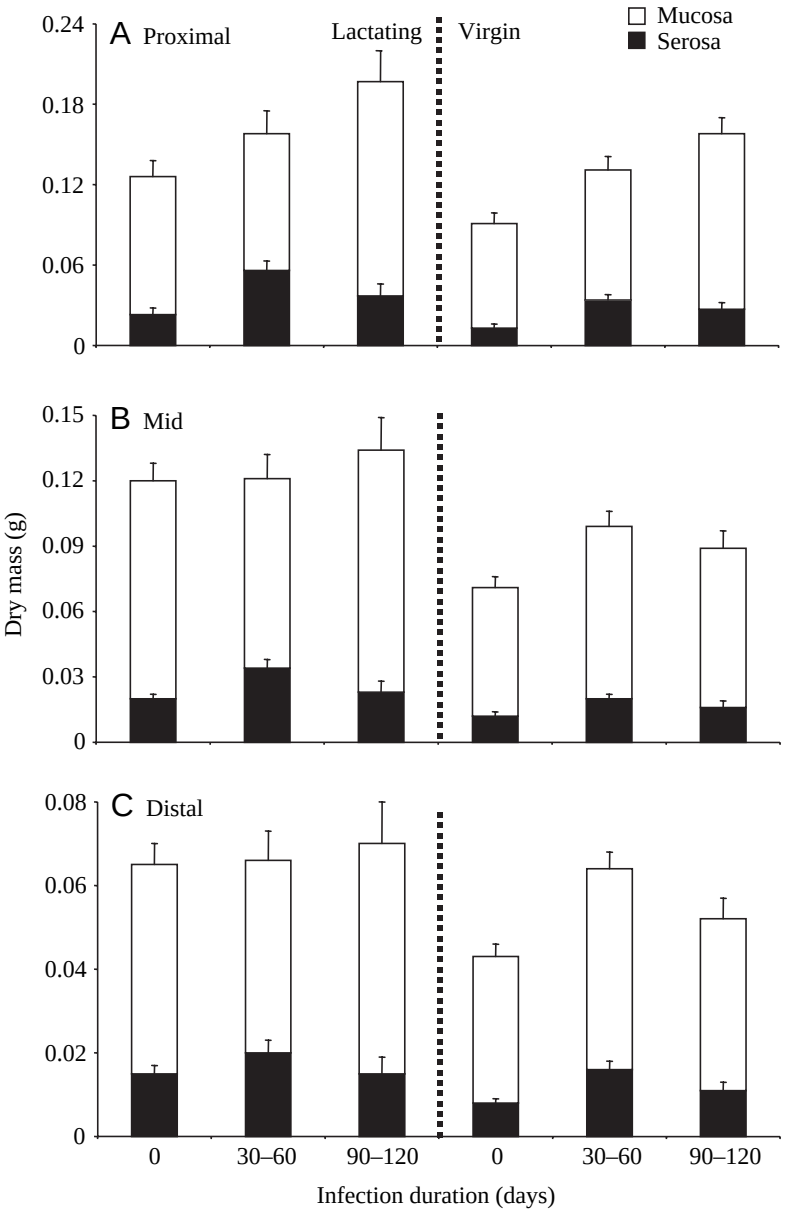

Fig. 5. Small intestine dry mass subdivided by 'serosal' and 'mucosal' components for three regions (proximal, mid, distal) for virgin and lactating mice that were either unparasitized (0 infection duration) or that were parasitized for 30–60 or 90–120 days. Error bars are +1 S.E.M.

digest food (but see present study; Kristan and Hammond, 2003), which is consistent with faster passage of food through the gut. The effect of *H. polygyrus* on host food passage time remains to be tested.

The overall body composition and specific distribution of fat for wild-derived mice used in the present study may more closely represent truly wild mice where access to adequate food during high demand times such as lactation may be critical yet also difficult. Bronson et al. (1991) have shown that young wild-derived house mice deprived of food use up fat stores within approximately 2 days and that at lower temperatures (10°C) fat stores were nearly depleted in only 1 day. The dramatic decreases in fat stores for lactating mice in our study (despite *ad libitum* food availability) further supports the idea that wild mice probably quickly use any fat stores and must subsequently support themselves on continual energy input throughout most of the high demand period. Given that parasite infection is so common in wild rodents it is not altogether surprising that mice respond to infection not by using fat but through other means.

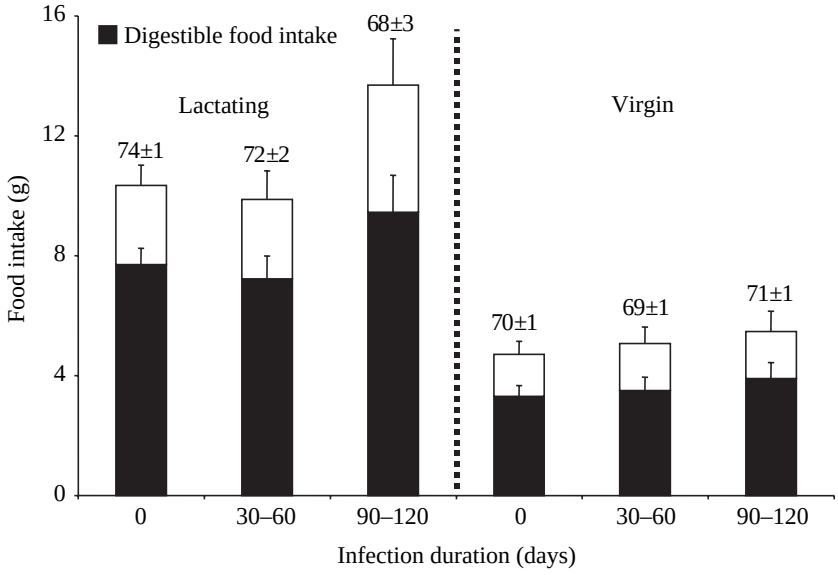

Fig. 6. Total food intake (whole bar) and digestible food intake (black portion of bar) for virgin and lactating mice that were either unparasitized (0 infection duration) or that were parasitized for 30–60 or 90–120 days. Percent digestibility is shown above each bar. Error bars are +1 S.E.M.

Interactive effects of simultaneous demands

We predicted that morphological changes associated with lactation would be diminished in parasitized mice because energy normally used to respond to lactation would be used to respond to parasitism. Only lean mass showed an interaction between parasite infection and lactation, and our prediction was supported for mice infected for 30–60 days (lactating mice had only 34% greater lean mass than virgin mice) but not for mice infected for 90–120 days (lactating mice had 72% greater lean mass than virgin mice) compared with uninfected mice, where lactating mice had 43% greater lean mass than virgin mice. In contrast to our prediction, lactation did not alter response to parasite infection for the other variables. We based our prediction on an expected increase in resting metabolism with *H. polygyrus* infection, which does not occur for wild-derived house mice (Kristan and Hammond, in press). If metabolism does not increase with parasite infection, then there may not be a trade-off between infection and lactation in terms of energy allocation. A comparison of energetic costs of immune responses for laboratory *versus* wild-derived house mice may help elucidate a mechanism for observed morphological and metabolic differences of these mouse strains when infected with *H. polygyrus*.

Interestingly, while responses to the demands of *H. polygyrus* infection and lactation remained largely independent of each other, both demands produced similar results in morphological plasticity of the small intestine, where average mass of lactating mice was similar to mice that had been parasitized for 90–120 days (0.347±0.02 *vs* 0.349±0.02, respectively). Therefore, similar mass changes of the small intestine occurred with two very different demands. Changes in the small intestine mass with these two demands were probably achieved through very different pathways. For example, small intestine mass changes during lactation are probably a response to increased food intake whereas mass changes during *H. polygyrus* infection may be due to both direct parasite effects (especially in the proximal region) and indirect effects such as changes in nutrient movement and processing.

Conclusions

Our results emphasize the importance of examining the qualities of a demand (such as duration or intensity) rather than simply the presence or absence of a demand when measuring phenotypic plasticity in response to environmental demands. We propose that immune response during the initial infection phase with *H. polygyrus* produced morphological responses in the wild-derived mouse that differed from morphological responses during the chronic stages of infection. Chronic parasite infection is a valuable model to examine the relationship between demand duration and phenotypic plasticity of animal responses.

Ali Salehpour assisted with weighing organs and organ fat extractions. Dr Lee Drickamer graciously provided offspring of wild-caught house mice that we used to initiate our house mouse breeding colony. Dr Edward Platzer determined the species of pinworm that occurred in our mice. Helpful comments were given by three anonymous referees. This research was supported by a NSF Doctoral Dissertation Improvement Grant (IBN-0073229) to D.M.K.

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