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Effect of Nesting Material on Body Weights of Mice Infected with *Toxoplasma gondii*

Jan R Linkenhoker* **and CG Garry Linton**

Toxoplasmosis, a disease caused by the parasitic protozoan *Toxoplasma gondii*, can cause a number of clinical signs in mice, including weight loss. This weight loss likely is related to the host immune response and is important to monitor in *Toxoplasma* studies. Several studies have demonstrated that nesting material can affect body weights of mice. We therefore sought to assess the effects of nesting material on body weights of mice infected with *Toxoplasma*. We housed mice with or without nesting material and weighed and clinically assessed them twice weekly for 30 days prior to and 5 wk after *Toxoplasma* inoculation. Nesting material did not significantly alter the weights of mice after *Toxoplasma* inoculation but did decrease rates of growth prior to inoculation. Nesting material did not affect the clinical outcome of *Toxoplasma* infections, supporting the provision of nesting material in mouse *Toxoplasma* experiments.

Environmental enrichment can enhance the welfare of laboratory mice, and a multitude of enrichment types, such as shredded paper, cotton squares, tissues, tubes, and igloos exist in the laboratory setting. Much research has focused on the effect of environmental enrichment on the behavior and physiology of mice, and nesting material in particular has been demonstrated to have several effects. These effects include changes in corticosterone levels, thymocyte numbers, aggression, weights of spleens and epididymal adipose tissue, food intake, anxiety, learning, and spatial search strategy.[5,7-9] Several studies also have demonstrated that nesting material can alter body weights of normal, naïve mice.[1,2,5-9,11-14]

For many studies, this influence of nesting material on body weight can have important effects on research outcomes, analyses, and conclusions. One example in which affected body weights can affect study results is with studies of toxoplasmosis. Toxoplasmosis, a parasitic disease caused by the protozoan *Toxoplasma gondii*, can cause a number of clinical signs in mice, including rough fur, hunched posture, inactivity, and weight loss.[10] This weight loss has been documented during the second week after experimental infection with *Toxoplasma* and is likely related to initiation of the host immunologic response.[10] Some *Toxoplasma* researchers rely on body weight measurements to predict immune system responses. Therefore, monitoring and analysis of body weights can be important in a mouse *Toxoplasma* study.

Given that nesting material has been shown to affect body weights and that body weights are sometimes important to *Toxoplasma* studies, we wanted to assess the effect of nesting material on the body weights of *Toxoplasma*-infected mice in our facility. The purpose of this study was to determine how nesting material affects the body weights and clinical scores of mice infected with *T. gondii*.

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Department of Health and Human Services, National Institutes of Health, Office of the Director, Office of Research Services, Division of Veterinary Resources, Bethesda, Maryland.
**Corresponding author. Email: linkenhj@od.nih.gov*

Materials and Methods

Animals. All procedures were conducted in compliance with the Animal Welfare Act, other federal regulations, and the *Guide for the Care and Use of Laboratory Animals* (*Guide*) at an AAALAC-accredited facility.[6] *Toxoplasma* inoculations were performed as part of an animal research protocol approved by the National Cancer Institute Animal Care and Use Committee.

Female C57BL/6J mice (age, approximately 1 mo) were housed 4 per cage under routine husbandry practices in the same room, rack, and shelf. A single group of age-matched mice was purchased from The Jackson Laboratory (Bar Harbor, ME), and animals were randomly assigned to each of the 2 groups.

Mice were housed according to temperature, humidity, and lighting standards of the *Guide* with autoclaved bedding (NIH Harwood Bedding, Nepco, Warrensburg, NY), ad libitum food (NIH 31, Harlan, Indianapolis, IN), and ad libitum water. Cages were microisolation caging on a static rack, composed of polycarbonate plastic, approximately 7.25 in. × 11.5 in. in size, and changed weekly.

Sentinel mice exposed to the dirty bedding of these experimental mice tested serologically free of the following agents: Sendai virus, pneumonia virus of mice, mouse hepatitis virus, mouse parvovirus, Theiler murine encephalitis virus, reovirus, rotavirus, lymphocytic choriomeningitis virus, ectromelia virus, parvovirus NS1, polyomavirus, adenovirus types 1 and 2, cytomegalovirus, mouse thymic virus, K virus, cilia-associated respiratory bacillus, *Mycoplasma pulmonis*, and *Encephalitozoon cuniculi*. In addition, mice were free of internal and external parasites.

Experimental design. One group of 12 mice received nesting material (Eco Bedding 100% recycled paper, Fiber Core, Cleveland, OH) and facial tissues (Kimberly Clarke, Neenah, WI) in addition to the standard facility bedding (NIH Harwood Bedding, Nepco). Nesting material was changed every 14 days or more frequently if heavily soiled. The other group of 12 mice received standard bedding only.

Throughout the study, when any single mouse required supportive care (such as DietGel 76A [Clear H2O, Portland, ME] or Transgenic Dough Diet [Bio-Serv, Frenchtown, NJ]), all mice in both groups received the same item. All mice in both groups

received these supplements at approximately 14 days after inoculation with *T. gondii* when clinical signs became apparent.

On day 0, we recorded initial body weights and clinical assessments for all mice. We used the following clinical scoring system: score of 1, mouse was bright, alert, and responsive, with normal gait, attitude, coat, posture, and appetite; 2, mouse was active but slow to move about cage, was slightly hunched, and had a slightly roughened coat; 3, same criteria as for score 2, but mouse also had head tilt, ataxia, or neurologic placement deficits; 4, mouse was quiet, alert, and responsive, but was hunched, had a rough coat, and was reluctant to move until handled; and 5, mouse was unresponsive and in lateral recumbency or moribund state.

All mice were weighed and clinically assessed twice weekly for 30 days before intraperitoneal inoculation with 20 cysts of *T. gondii* strain ME49 (on day 30). For 5 weeks after *Toxoplasma* inoculation, all mice were weighed and clinically assessed twice weekly and were housed and cared for as described earlier.

Statistical analyses. All mice in both groups had the same clinical scores at the same assessment time points, so no analyses were performed on clinical score data. Weights of mice in the 2 groups were analyzed by using 2-way repeated-measures ANOVA (Prism 4.0, GraphPad Software, San Diego, CA), with time and nesting condition as the sources of variance. *P* values less than 0.05 were considered statistically significant.

Results

Effects of nesting material on mice prior to *Toxoplasma* inoculation. The average weight of the mice after assigning them to the 2 treatment groups was 18.0 g for each group. In the 30 day prior to *Toxoplasma* inoculation, mice provided with nesting material had significantly smaller percentage increase in weight and lower individual weights than did mice housed without nesting material ($P \leq 0.0001$ for both comparisons; Figures 1 and 2). Because of their lower rate of weight gain, mice provided with nesting material had lower body weights by the end of the 30-day period prior to inoculation than did mice with standard bedding only. All mice in both groups had clinical scores of 1 at each assessment time point.

Effects of nesting material on mice post-inoculation with *Toxoplasma* In the 5 weeks after *Toxoplasma* inoculation (from day 30 until the end of the study), percent changes in body weights and individual body weights were not significantly affected by nesting material ($P \leq 0.0001$for both percentage and individual changes; Figures 3 and 4). Mice provided with nesting material continued to have lower body weights than did those housed on standard bedding only. However, this result was likely a carry-over effect from the 30 days prior to *Toxoplasma* inoculation, given that mice that received nesting material had lower body weights at the time of inoculation. In addition, all mice in both groups had clinical condition scores of 2 during weeks 2 through 5 of this time period; therefore nesting material did not affect the clinical outcome of *Toxoplasma* infections.

Discussion

Environmental enrichment may enhance the welfare of laboratory mice. One type of environmental enrichment that may benefit mice greatly is nesting material. Mice show strong preferences for environments with nesting material, which allows them to demonstrate natural behaviors and may afford benefits including thermoregulation, enhanced breeding, and shelter.[3]

Despite these benefits, studies have demonstrated much variability in the behavioral and physiologic effects of nesting material on rodents. One of the parameters reported to be variably affected

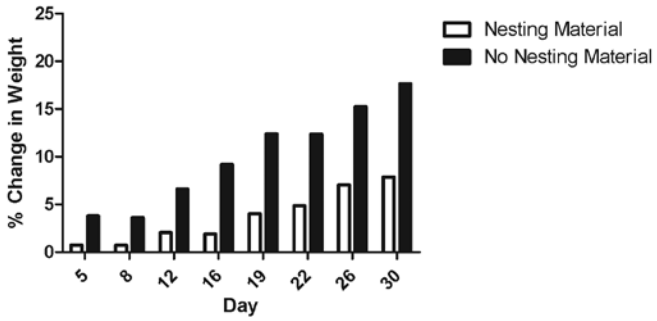

Figure 1. Percentage change in body weight of mice prior to inoculation with *T. gondii*.

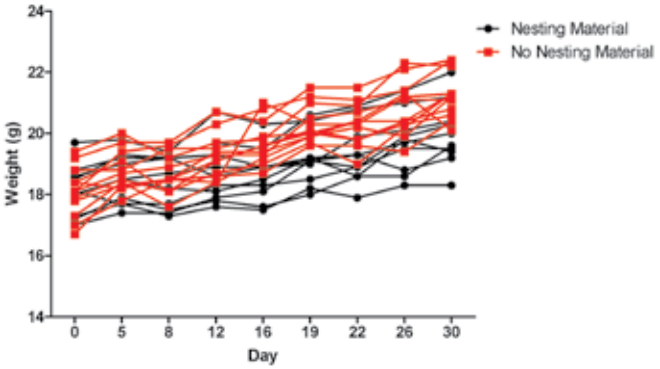

Figure 2. Individual body weights of mice prior to inoculation with *T. gondii*.

by nesting material is body weight. In one study, pups from enriched cages weighed more than did pups from nonenriched cages; in another, nesting material resulted in increased body weight in mice.[13,14] Three additional studies similarly revealed that mice provided with nesting material weighed more than mice housed in standard cages.[1,5,12] In contrast, other authors showed that nesting material led to lower weight gain[7] and that decreased body weight, along with decreased brown adipose tissue, occurred in mice housed with nesting material.[2] Nesting material had no effect on mouse body weight in another work.[8]

Given these variable reports on the effect of nesting material on the body weights of mice, we sought to study the influence of nesting material on *Toxoplasma*-infected mice, whose body weights are an indicator of the immune response to the pathogen.[10] In our current study, nesting material did not have a statistically significant effect on body weights after inoculation of mice with *T. gondii*. This result suggests that nesting material may not have a significant effect on the outcome of *Toxoplasma* studies in mice.

However, prior to *Toxoplasma* inoculation, nesting material resulted in a statistically significant lower rate of weight gain and lower individual weights among our mice. This result is consistent with some but not all previous studies.[1,2,7,8,12-14] These differences may be due to the sex, number, and strain of mice studied and the amount and biologic relevance of the nesting material provided. For example, some authors demonstrated that providing more naturalistic nesting material such as Eco bedding (Fiber Core) allows mice to build more naturalistic, higher quality nests.[3] Those authors also suggested that amount of nesting material provided may affect research focused on the effects of nesting material.[3]

We hypothesize that this decreased rate of weight gain and reduced individual weights prior to *Toxoplasma* inoculation may be due to decreased food consumption, which has been reported to occur in mice housed with nesting material.[1,9,12,13]

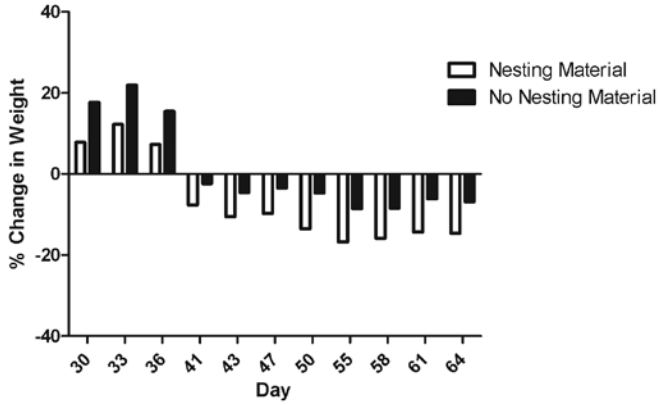

Figure 3. Percentage change in body weight of mice after inoculation with *T. gondii*.

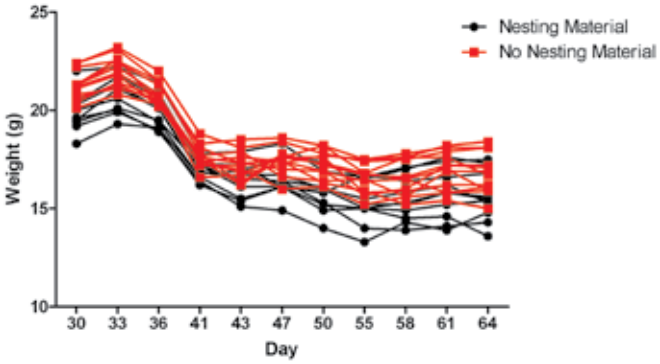

Figure 4. Individual body weights of mice after inoculation with *T. gondii*.

This decreased food consumption may be related to a reduction in caloric needs, given that nesting material reduces heat loss. This reduction in heat loss also may reduce the amount of brown adipose tissue that mice need to maintain, which in turn may lead to decreased body weights. This effect is consistent with the finding of decreased brown adipose tissue in mice housed with nesting material.[2] Additional studies comparing food consumption between mice housed with and without nesting material may confirm this hypothesis.

Although nesting material did alter the body weights of our mice prior to *Toxoplasma* inoculation, it did not significantly affect weights after infection. Therefore, we suggest that nesting material can be used safely in mouse *Toxoplasma* studies, in which body weight may be an important indicator of the immune response. However, our findings are related specifically to the type and amount of nesting material, the strain and sex of mice, and the biologic agent used in this study. Care should be taken in balancing the effect of environmental enrichment on laboratory animal wellbeing and research outcomes.

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