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Controlled Exercise Is a Safe Pregnancy Intervention in Mice

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During pregnancy, women often show a willingness to make positive lifestyle changes, such as smoking cessation, initiation of a vitamin regimen, improvement of their diet, and increases in their levels of exercise or physical activity. To study health outcomes in both pregnant mice and their offspring, we developed a model of controlled maternal exercise during mouse pregnancy. Female ICR and C57BL/6 mice underwent controlled wheel walking for 1 h daily, 5 d each week, at a speed of 6 m/min prior to and during pregnancy and nursing. Dam body weight, food consumption, pregnancy rates, litter size, pup weights and litter survival were used as markers of pregnancy success and were not significantly affected by controlled maternal exercise. The proposed exercise paradigm is a safe pregnancy intervention and can be explored further.

Pregnancy is a vitally important time for both the pregnant woman and the developing fetus. Various lifestyle changes can maximize the chances for a successful pregnancy and healthy baby. For example, alcohol consumption during pregnancy is known to have negative consequences for the fetus,[4] and a large majority of women eliminate its use throughout gestation. Reports indicate that 51.5% of nonpregnant women consume alcohol compared with 7.6% of pregnant women.[9] Recent data indicate that only 12% of pregnant women in the United States smoke tobacco, compared with 23% to 25% of nonpregnant women.[15] These statistics suggest that many women are willing to forego pleasurable or addictive behaviors in the hope of having a successful and healthy pregnancy.

Furthermore, women will often take additional steps toward improving their own health during pregnancy for the sake of their growing baby. Women hoping to conceive are more likely to consume a folic acid supplement.[10] In one study in the United Kingdom that analyzed smoking and alcohol cessation, caffeine limitation, and fruit and vegetable consumption, 81% of pregnant women were willing to comply with health recommendations.[16] These factors seem to suggest that women are willing to adopt behaviors during pregnancy that will improve both their own health and that of their unborn children. We therefore posit that women may be willing to initiate an exercise routine during gestation if there are clear benefits for doing so.

Exercise has been reported to improve mood, body composition, and glucose tolerance as well as to decrease cancer incidence.[3,17,33] Exercise during pregnancy has been shown to offer an array of positive outcomes for pregnant women, including decreased maternal weight gain and decreased body fat in the second half of gestation.[14] Exercise during pregnancy also improved oral glucose tolerance and reduced gestational diabetes risk.[5,24] Maternal exercise is becoming a highly studied area, and recently the focus has turned to potential beneficial effects on offspring outcomes. For example, maternal exercise resulted in lighter, leaner human offspring,[13,19] and maternal exercise enhanced oral and cognitive skills in 5-y-old offspring.[12] Exercise during pregnancy clearly offers substantial potential benefits for both the pregnant woman and her baby.

Rodents are a model for exploring the benefits of exercise during pregnancy. Using a mouse model, we previously found that voluntary exercise during pregnancy and nursing improved glucose tolerance and insulin sensitivity in adult offspring.[8] Others have shown that voluntary running during mouse pregnancy increased offspring neural development.[7] In addition, voluntary exercise during mouse pregnancy protects transgenic offspring from an Alzheimer-type pathology.[18] Clearly, exercise during pregnancy has important potential offspring benefits. However, voluntary exercise as a model has some limitations, such as long and variable running distances. We therefore sought to develop a model of maternal exercise to control for the limitations of voluntary running. The aim of the current study was to use a paradigm consisting of 60 min of controlled exercise daily for 5 d each week to assess the safety of the model during mouse pregnancy as an alternative to voluntary wheel running. We also explored differences between the ICR stock and C57BL/6 strain as they related to pregnancy outcomes. Ideally, future experiments will be able to use a similar controlled exercise strategy for investigating both maternal and offspring health implications of this intervention.

Materials and Methods

Animals. All animal experiments were carried out according to an IACUC-approved protocol at the University of Kentucky. The University of Kentucky Division of Laboratory Animal Resources is fully AAALAC-accredited. Female ICR (CD1) and C57BL/6 mice were bred and produced one litter at the vendor (Taconic Farms, Germantown, NY) prior to delivery to the University of Kentucky. On arrival, the 3- to 4-mo-old primiparous female mice were single-housed in individually ventilated cages (ACE, Allentown, NJ) with Sani-Chip bedding (Harlan-Teklad, Madison, WI) and maintained on a standard chow diet (Global 18% Protein Rodent Diet no. 2018, Harlan-Teklad) for the duration of the study. Plastic shelters (Mouse Igloos, Bio-Serv, Frenchtown, NJ) and nesting pads (Nestlets, Ancare, Bellmore, NY) were provided for environmental enrichment. All mice were maintained on a 14:10-h light:dark cycle at temperatures between 21 to 24 °C. Quarterly testing was completed on sentinel mice from related racks. Sentinels were negative for mouse

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hepatitis virus, mouse parvoviruses, Sendai virus, *Mycoplasma pulmonis*, Theiler murine encephalomyelitis virus, epizootic diarrhea of infant mice, pneumonia virus of mice, reovirus, lymphocytic choriomeningitis virus, ectromelia virus, mouse adenovirus 1 and 2, polyomavirus, *Encephalitozoon cuniculi*, cilia-associated respiratory *bacillus*, *Clostridium piliforme*, mouse cytomegalovirus, fur mites, and pinworms.

After a 1-wk acclimation period, ICR and C57BL/6 female mice were assigned to control and exercise groups (ICR control, $n = 20$; ICR exercise, $n = 20$; C57BL/6 control, $n = 20$; C57BL/6 exercise, $n = 19$) so that the cohorts were approximately balanced by body weight. Once daily for 5 consecutive days each week, female mice were removed from their home cages and placed for 60 min into a wheel positioned on a motorized platform (Mouse Forced Exercise–Walking Wheel System 80800A, Lafayette Instrument, Lafayette, IN; Figure 1 A and B). The wheel-bed of the control (sedentary) group was not activated and remained completely stationary. Exposure to the wheel beds occurred during the light cycle, between approximately 1 and 3 h after lights-on. After a 5 d-initial training period (3 m/min for 30 min and 3.5 m/min for 30 min on day 1; 4 m/min for 30 min and 4.5 m/min for 30 min on day 2; 5 m/min for 30 min and 5.5 m/min for 30 min on day 3, 6.0 m/min for 60 min on days 4 and 5) the wheel bed of the exercise group was activated at a speed of 6 m/min for the duration of the study. Dams were not removed from their home cages on the day of or the day after parturition, in an attempt to maximize successful rearing. Dam body weight was recorded twice weekly (once after 5 d of wheel exposure and once after 2 d without wheel exposure). Mice were fed a known amount of food at the beginning of the study. Food remaining was recorded, discarded, and replaced with a fresh, known amount of food on a weekly basis. Weekly intake was divided by 7 to achieve a daily intake value. Daily food intake was divided by 2 when the male mouse was in the cage for mating.

After 2 wk of controlled exercise, including the 5-d training period, one male mouse was housed with one female mouse for 2 wk for breeding. At no point during the study did the male mice exercise. Despite this relatively long breeding window, most mice conceived within the first 4 d of mating, as evidenced by the day of delivery. ICR mice that did not deliver litters within 4 d after the first litter was found were removed from the analyses in Figure 2 A and C, in light of their postponed body weight gain relative to the rest of the cohort. The trajectory of body weight for ICR mice with later litter delivery (beyond the designated 4-d window) was significantly different from the trajectory of body weight for ICR dams delivering earlier, as judged by a linear mixed model (data not shown), thus justifying their removal from the analyses in Figure 2 A and C. The delay was most likely caused by a delay in mating or conception, but we cannot state this explanation with certainty because copulatory plugs were not monitored during the study. The day on which pups were found was designated postnatal day 0. On postnatal day 2, litters were standardized to 8 and 6 pups for ICR and C57BL/6 mice, respectively; the C57BL/6 litters were culled to 6 because of the smaller litter size compared with that of the ICR mice. Pups were cross-fostered from other litters of the same group when they did not have at least 8 or 6 pups for ICR and C57BL/6 dams, respectively. Pups were weighed on postnatal days 7, 14, and 21. Food intake, body weight, and litter weight data were not included in the analyses for female mice that did not successfully wean their litters. One exercise group ICR litter was not weighed on postnatal day 21, but the data were included for days 7 and 14. In addition, one C57BL/6 dam refused to walk in the controlled exercise wheel after delivery. Because she did participate prior to delivery, her litter data were included in Figure 3 B and D, but data regarding her body weight, food intake, and litter weight were not included in Figures 2 and 4.

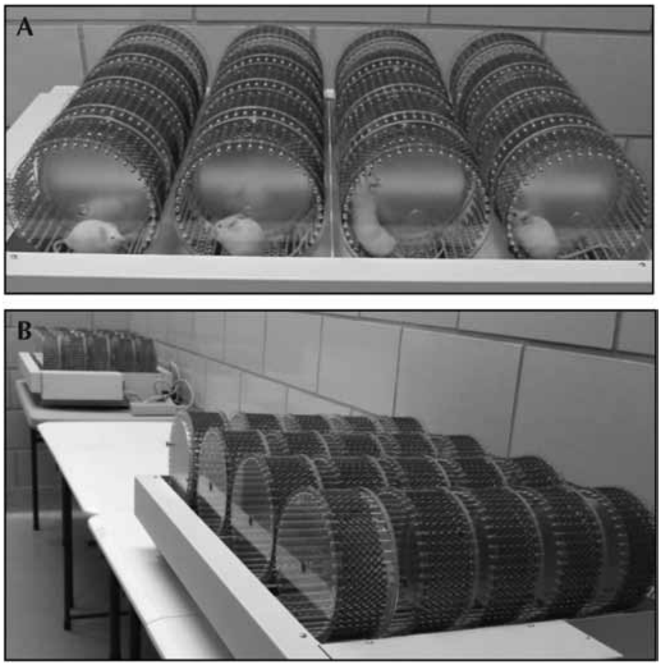

Figure 1. Controlled exercise system. (A) A photograph of the controlled exercise-wheel platform containing ICR mice. (B) Two platforms were set side-by-side so that 20 control mice and 20 exercised mice could be exposed simultaneously to the wheel bed.

Statistics. Analyses were completed by using SAS version 9.3 (SAS Institute, Cary, NC), and figures were made by using SigmaPlot version 11.0 (Systat Software, San Jose, CA) or Prism version 5 (GraphPad Software, La Jolla, CA). Within strata (ICR or C57BL/6), control and exercise groups were compared in regard to proportions of litters born and weaned by using Fisher exact test (Table 1), in regard to repeated measures of body weight and food intake by using linear mixed models with time and group as categorical predictors (Figure 2), in regard to day of birth and pups per litter by using a Mann–Whitney rank sum test (Figure 3), and in regard to repeated measures of pup weight by using linear mixed models with time and group as categorical predictors (Figure 4). In addition, ICR and C57BL/6 mice were compared in regard to proportions of litters born and weaned by using Fisher exact test (Table 1). Significant overall differences in linear mixed modeling would have been followed by Bonferroni-adjusted posthoc tests to compare groups at specific time points, but no significant overall differences were noted. A P value of less than 0.05 was considered significant.

Results

Body weights of ICR and C57BL/6 female mice remained steady over the 2 wk prior to mating (weeks 0 through 2, Figure 2 A and B) and were not significantly affected by the exercise regimen. There was no significant difference between body weights of sedentary and exercise ICR ($F_{17,470} = 0.98$, $P = 0.48$) or sedentary and exercise C57BL/6 ($F_{17,352} = 1.45$, $P = 0.11$) female mice. The body weights began to increase steadily around gestation day 10 (weeks 3.5 through 4 in Figure 2 A and B) and continued to rise as pregnancy progressed. Food consumption followed a similar pattern, although the dams did not begin to

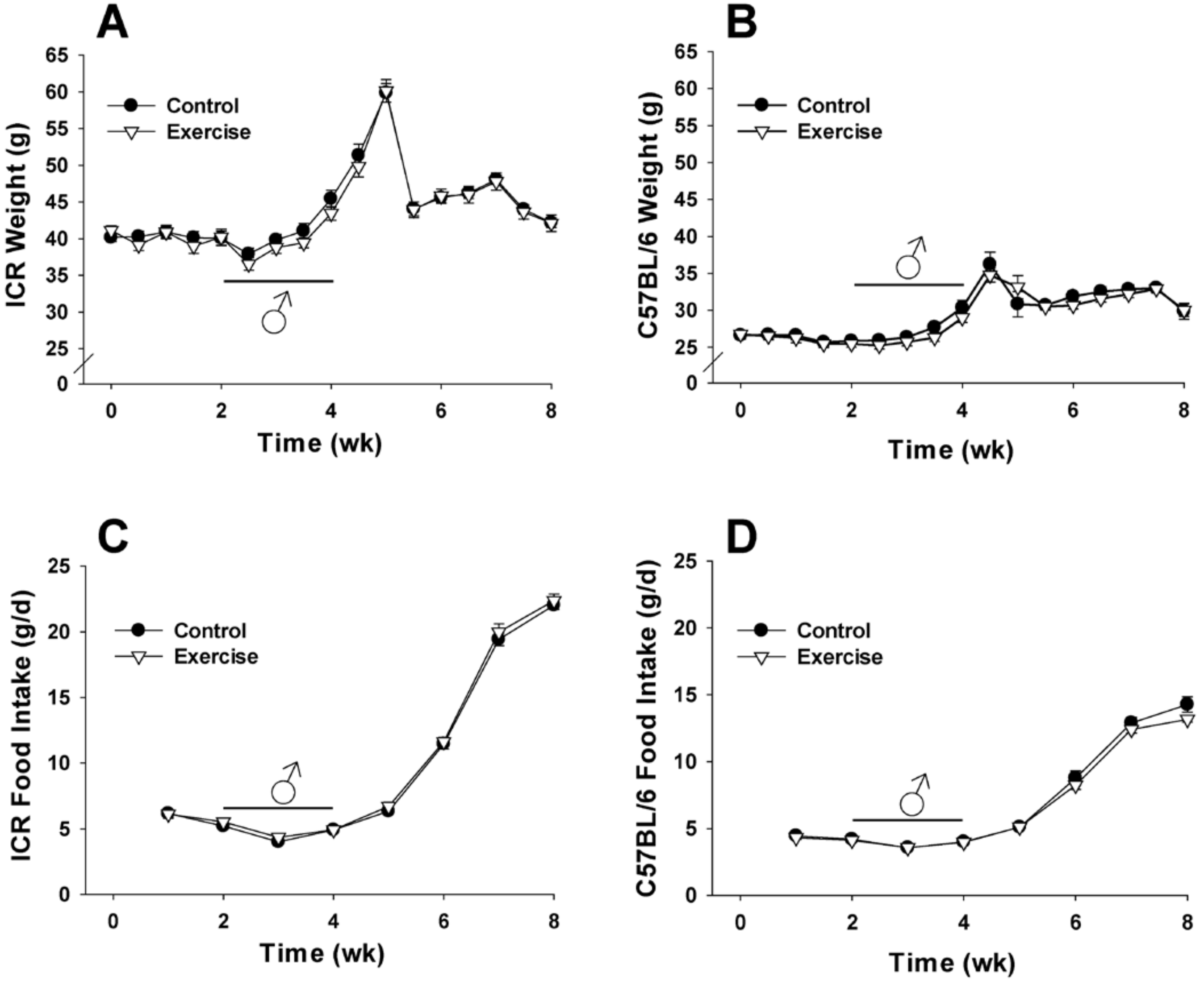

Figure 2. Dam body weight and food consumption in ICR and C57BL/6 mice. Body weights were recorded twice weekly, and food remaining was weighed weekly for the duration of the study. Horizontal lines associated with male symbols show when a male was present in the home cage for mating. There were no significant differences in (A, C) ICR or (B, D) C57BL/6 control or exercising dam (A, B) body weights or (C, D) food consumption at any point during the study. The data from the dams that had litters survive until weaning are included for all groups. Furthermore, only the dams that delivered within the first 4 d are included for the ICR stock. One C57BL/6 dam refused to consistently participate in the exercise intervention after parturition and was excluded. ICR control, *n* = 18; ICR exercise, *n* = 14; C57BL/6 control, *n* = 11; C57BL/6 exercise, *n* = 13; error bars, SEM.

eat substantially more until nursing (weeks 6 through 8, Figure 2 C and D). The exercise intervention did not significantly affect ICR ($F_{8,210} = 0.38$, $P = 0.93$) or C57BL/6 ($F_{8,154} = 1.18$, $P = 0.31$) food consumption. Figure 3 A and B show that the days on which litters were delivered was not significantly delayed due to maternal exercise in either ICR ($\chi^2 = 1.95$, $P = 0.16$) or C57BL/6 mice ($\chi^2 = 1.41$, $P = 0.23$), respectively. Although ICR dams had significantly ($P < 0.001$) larger litters than did C57BL/6 dams, exercise did not significantly alter litter size in either ICR or C57BL/6 mice (ICR: $\chi^2 = 0.12$, $P = 0.73$ for ICR; C57BL6: $\chi^2 = 0.14$, $P = 0.71$; Figure 3 C and D).

Neither the number of litters delivered nor the number of litters that were successfully reared to weaning was significantly different between control and exercise groups in either ICR or C57BL/6 mice (Table 1). All 40 ICR mice (100%) delivered litters, and 90% of each group successfully reared litters to weaning ($P = 1.0$ for both comparisons). Of 20 C57BL/6 control dams, 13 (65%) delivered and 11 successfully reared their pups (55% of total bred and 85% of litters born). In the C57BL/6 exercise group, 15 of 19 (79%) dams delivered and 14 raised pups to weaning (74% of total bred and 93% of litters born). For C57BL/6 dams, the exercise intervention did not cause any significant differences between number of litters born ($P = 0.48$), proportion of those weaned among female mice bred ($P = 0.32$), or those that delivered litters ($P = 0.58$). Regardless of control or exercise designation, the ICR stock had larger proportions of litters born ($P < 0.001$) and weaned ($P = 0.0075$) among female mice bred when compared with C57BL/6 mice. However, the proportion of litters weaned among those born was not significantly different ($P = 1.0$) when the ICR and C57BL/6 mice were compared directly.

Offspring body weight at postnatal days 7, 14, and 21 was recorded, and the average pup weight was calculated for each litter (Figure 4). Neither ICR nor C57BL/6 pup weights were significantly affected by the exercise intervention at any of

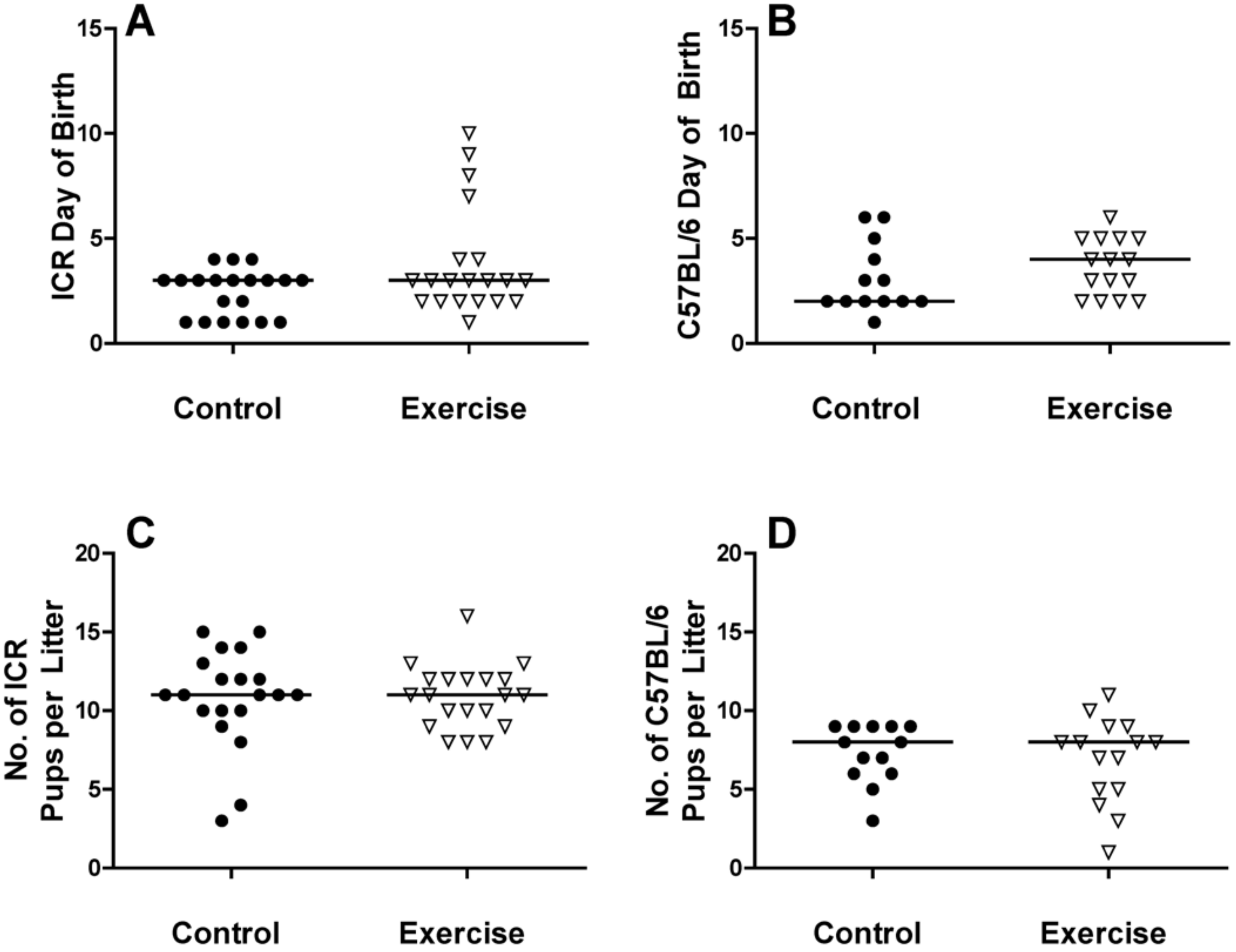

Figure 3. Day of birth and pups per litter. Cages were monitored for delivery, and the first day on which a litter was found was designated as day 1. Exercise intervention did not significantly delay delivery in (A) ICR or (B) C57BL/6 mice. There was no significant difference in the number of (C) ICR or (D) C57BL/6 pups born per litter. ICR control, *n* = 20; ICR exercise, *n* = 20; C57BL/6 control, *n* = 13; C57BL/6 exercise, *n* = 15. Horizontal lines indicate median.

the time points (ICR: $F_{3,552} = 1.34$, $P = 0.26$; C57BL/6: $F_{3,277} = 0.55$, $P = 0.65$).

Discussion

The controlled exercise regimen was completed in both ICR and C57BL/6 mice prior to and during pregnancy and lactation. There were no obvious negative consequences for dams or young litters due to the controlled exercise intervention. There were no significant differences in litter size or weight. The number of litters successfully weaned was not significantly affected by controlled exercise. The intervention was tested in both ICR and C57BL/6 mice, with similar findings.

The differences that we observed between the ICR and C57BL/6 mice were important but not surprising. ICR mice were more likely to conceive and had consistently larger litters than did the C57BL/6 animals; ICR dams and pups also were larger, and ICR dams consumed more food. One study reported that ICR mice are more sensitive to chemically induced diabetes than are C57BL/6J mice.[27] This feature could have potential implications regarding the stock or strain chosen for future studies exploring offspring glucose tolerance and insulin sensitivity.

Exercise improves body composition and cognition and decreases cancer risk.[17,33,34] In addition, exercise during gestation offers a wide array of benefits during pregnancy. In one study, maternal exercise decreased the need for surgical intervention during labor and resulted in earlier delivery, compared with sedentary controls.[11] Implementing an exercise regimen improved oral glucose tolerance in pregnant women.[5] Exercise appears to be a potential positive intervention that can be used during healthy human and rodent pregnancy.

Further exploration of the benefits of exercise during pregnancy has many applications. For example, the antidepressant fluoxetine yielded negative consequences on mouse pregnancy.[6] Because exercise is shown to improve mood,[3] it would be interesting to determine whether exercise during pregnancy may be a viable nonpharmacologic intervention for depression during pregnancy. In one instance, insulin treatment throughout mouse pregnancy resulted in decreased fetal mass.[2] Exercise during pregnancy may be an alternative treatment for hyperglycemia during gestation, given that we already have explored voluntary exercise in such a capacity with positive outcomes (data not shown).

Maternal exercise has been shown to impart offspring benefits in several species. In humans, babies born to exercised

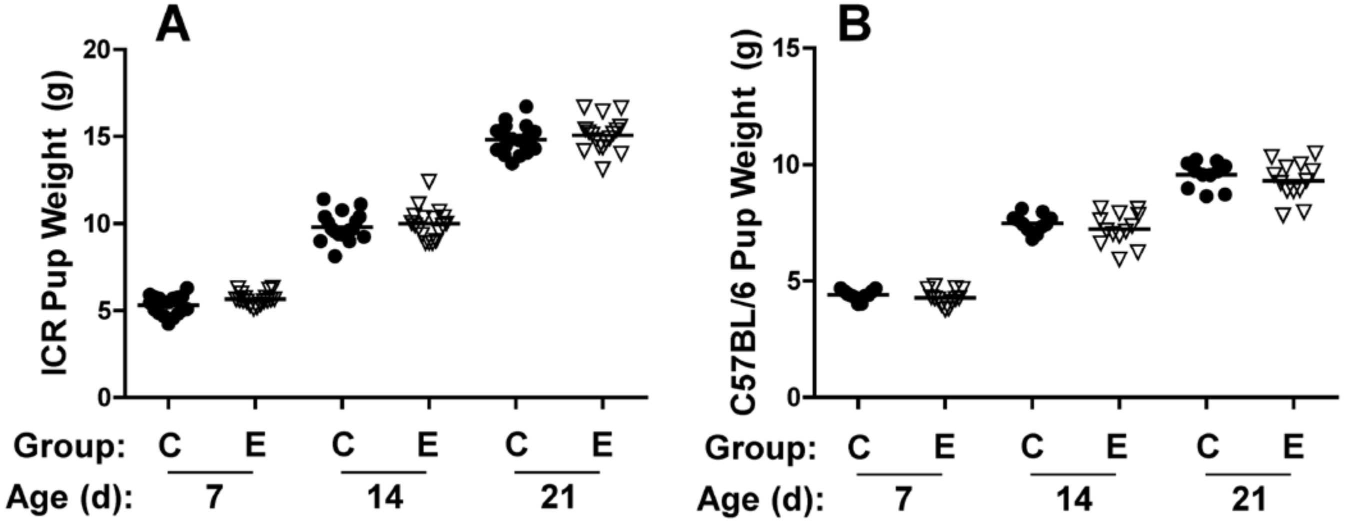

Figure 4. Offspring body weight was not significantly affected by maternal exercise. Pup body weight was recorded 7, 14, and 21 d after birth. Plotted points are averages for the various litters. C, control; E, exercise; ICR control, *n* = 18; ICR exercise, *n* = 18 except for 21 d (*n* = 17); C57BL/6 control, *n* = 11; C57BL/6 exercise, *n* = 13. Horizontal bar indicates mean.

Table 1. No. of pregnancies and no. of litters reared successfully

Group	No. of mice bred	No. of litters born	No. of litters weaned
ICR control	20	20	18
ICR exercise	20	20	18
C57BL/6 control	20	13	11
C57BL/6 exercise	19	15	14[a]

[a]Although 14 litters were weaned successfully, only 13 were included in Figures 2 and 4 because one dam refused to consistently participate in the exercise intervention after parturition.

mothers have decreased fat mass.[13] In fact, these children are still leaner at 5 y of age.[12] If this trend persists as the offspring age, then exercise during pregnancy may hold promise as a means to curtail the obesity epidemic. In addition, voluntary exercise during gestation significantly improved glucose tolerance in aged mouse offspring.[8] When tested in a transgenic mouse model, offspring born to exercising dams had decreased Alzheimer pathology.[18] Thoracic aortas from female offspring born to pigs exposed to exercise during pregnancy displayed greater endothelium-dependent vasorelaxation response than did those isolated from control offspring.[23] Therefore, in addition to the maternal benefits, exercise during pregnancy can provide a number of benefits to the offspring as well.

Voluntary exercise by including a running wheel in the home cages of mice is one intervention that has been tested.[7,8,18] However, this model has some weaknesses that should be acknowledged. Female mice run long and variable distances. In fact, voluntary exercise has been proposed to be a rodent model for obsessive–compulsive-type behavior.[1] In addition, it has been suggested that some animals will postpone or eliminate crucial activities, such as eating and drinking, in favor of running.[26] Although we have seen no indication of dam neglect in the ICR stock,[8] it is still an arguable point in favor of exploring an alternative model.

In addition to eliminating the issues associated with free-choice running, the controlled exercise model also allows for a more precise experimental design. For example, the potential discrepancy in the time that the dam spends away from pup care (nursing) and grooming is eliminated, because control dams are removed from their home cage while the exercise group is subjected to the running paradigm. In addition, running time and distance are equivalent for all mice assigned to the exercise group. Because the dams have only limited and controlled access to the wheel, they do not have an ever-present distraction that arguably may draw them away from nursing and licking their pups.

The speed of the motor driving the wheels is completely programmable. Here, a mild pace (6 m/min) was used to mitigate any potential stress to the dam and her unborn offspring. In our facilities at the University of Kentucky, nonpregnant ICR females use voluntary running wheels at a speed of approximately 15 m/min, whereas C57BL/6 females run an average of approximately 19 m/min (data not shown). Clearly, the speed used for the current study was well below these voluntary speeds, because we did not want to negatively affect pregnancy success. In addition, female mice will dramatically decrease their voluntary running as pregnancy progresses.[8] That being the case, the choice of a low speed was imperative for maintaining a constant pace for the duration of the study. One could argue that the slow running speed used in this experiment may not have elicited a training response. Regardless, these findings are still relevant even if a training effect was absent. For example, studies suggest that climbing as few as 1 to 4 flights of stairs daily may reduce incidence of preeclampsia,[28] and walking may prevent excessive weight gain during gestation.[29] We recently have used a faster pace (10 m/min for 60 min daily, 7 d a week) that caused an increased lean-to-fat mass ratio in exercising ICR female mice compared with control mice and found that this program was a safe pregnancy intervention (data not shown).

Restraint stress during rat pregnancy has been shown to result in decreased term weight,[22] and disruption of the light cycle may decrease pregnancy success in C57BL/6 mice.[30] In humans, stress has been implicated in shortened gestational length.[31] Although we did not directly measure gestation length, the day of delivery was not significantly changed by the exercise intervention. Therefore, we speculate that the controlled exercise paradigm did not significantly stress the pregnant dams more than did disruption of the light cycle and placement in the running wheel bed that occurred within the control groups. Future examination of maternal corticosterone levels and other stress markers is warranted to confirm this suggestion. In addition, subsequent studies should include animals exercising during the dark cycle to reduce stress. Furthermore, an additional group that remains in their home cage during the time of exposure to the Lafayette platform could provide important information about whether removal from the home cage and placement in the wheels is stressful in itself. Stress could certainly play a role in the variability in several parameters, but an important finding is that the exercise paradigm in this study did not appear to cause further stress (or perceptible negative outcomes).

Finally, the motorized wheel system used herein is not unknown in the rodent exercise field. It has been used in mice for studies involving learning, vasoconstriction, and bladder function.[20,21,25,32] To our knowledge, however, no others have explored the same system as a perinatal intervention in mice. We have provided valuable data regarding the use of this particular mouse exercise system before and during pregnancy and lactation in both the ICR stock and C57BL/6 strain.

In conclusion, we have illustrated a model of controlled exercise in the mouse that is safe for use during pregnancy. In addition, we have provided side-by-side data for both ICR and C57BL/6 mice. Future studies using the controlled exercise model will explore the exciting potential for offspring benefits.

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