Voluntary exercise in pregnant rats positively influences fetal growth without initiating a maternal physiological stress response

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Rosa BV, Firth EC, Blair HT, Vickers MH, Morel PCH. Voluntary exercise in pregnant rats positively influences fetal growth without initiating a maternal physiological stress response. Am J Physiol Regul Integr Comp Physiol 300: R1134–R1141, 2011. The effects of increased physical activity during pregnancy on the health of the offspring in later life are unknown. Research in this field requires an animal model of exercise during pregnancy that is sufficiently strenuous to cause an effect but does not elicit a stress response. Previously, we demonstrated that two models of voluntary exercise in the nonpregnant rat, tower climbing and rising to an erect bipedal stance (squat), cause bone modeling without elevating the stress hormone corticosterone. In this study, these same models were applied to pregnant rats. Gravid Wistar rats were randomly divided into three groups: control, tower climbing, and squat exercise. The rats exercised throughout pregnancy and were killed at day 19. Maternal stress was assessed by fecal corticosterone measurement. Maternal bone and soft tissue responses to exercise were assessed by peripheral quantitative computed tomography and dual-energy X-ray absorptiometry. Maternal weight gain during the first 19 days of pregnancy was less in exercised than in nonexercised pregnant control rats. Fecal corticosterone levels did not differ between the three maternal groups. The fetuses responded to maternal exercise in a uterine position-dependent manner. Mid-uterine horn fetuses from the squat exercise group were heavier (P < 0.0001) and had a greater placental weight (P = 0.001) than those from control rats. Fetuses from tower-climbing dams were longer (P < 0.0001) and had heavier placentas (P = 0.01) than those from control rats, but fetal weight did not differ from controls. These models of voluntary exercise in the rat may be useful for future studies of the effects of exercise during pregnancy on the developmental origins of health and disease.

EVENTS THAT OCCUR DURING FETAL development can have long-lasting effects on the health and later-life outcomes of the developing organism (25). Low birth weight in humans is associated with an increased risk of later-life diseases, such as coronary heart disease, hypertension, and insulin resistance (3). Exercise during pregnancy may significantly impact birth weight and later-life health, but the effects of maternal exercise during gestation on fetal growth are unclear, and studies in humans and animals have yielded varying results. Studies in humans have found that exercise during pregnancy was associated with birth weight that was reduced (4, 21, 27), unaffected (46), or increased (13, 26) in the offspring of exercising women. Comparison between studies is complicated by different exercise regimens, and many used self-reported exercise, rather than a standardized, supervised exercise program. Timing during pregnancy, intensity, and type of the exercise may influence its effects on the fetus. For example, moderate weight-bearing (treadmill, stair stepper, or step aerobics) exercise begun in early pregnancy has been shown to increase fetoplacental growth and birth weight (13), whereas a high volume of the same exercises performed in late gestation reduced fetoplacental growth (14). No data are available on the long-term effects of exercise during pregnancy on adult human offspring health outcomes.

Research in animals has been similarly difficult to interpret, with different types and amounts of exercise complicating comparison of results. For example, birth weight of pups from treadmill-exercised dams was lower than from nonexercised dams in some studies (18, 28, 48), and treadmill exercise had no effect on birth weight in others (33). Birth weight of pups from pregnant rats that swam for 60 min/day was lower than (38), whereas birth weight of pups from pregnant rats that swam for only 10 min/day was not different from (1), that of pups from control animals. In addition, exercise during pregnancy may cause stress in experimental animals, providing a major confounder in the interpretation of results. Swimming (1, 16) and treadmill running (7, 8, 16) increase plasma corticosterone levels in pregnant rats, and the effects of swimming-induced stress during pregnancy on birth weight persist through two subsequent generations (42).

Investigation of the effects of maternal exercise during pregnancy in the rat on later-life health of the pups requires a model of exercise that neither elicits a stress response nor negatively affects fetal growth. Tower climbing and rising to an erect bipedal stance (squat) are voluntary resistance-type exercises that have been shown to cause bone modeling in rats (37, 43, 51, 52). Both of these exercises require muscle contraction against gravity, although the dynamic and repeated muscular contractions of all four limbs during tower climbing may give it a greater aerobic component than the more isometric bipedal “squat” exercise, which requires the rats to attain a “standing” position on extended hindlimbs and then maintain that position while they eat and drink. The squat exercise we describe is essentially the reverse of the squat exercise performed by bodybuilders: for humans, maintaining limb flexion while weight-bearing requires more muscular work than maintaining limb extension; for rats, standing on extended hindlimbs demands more effort than maintaining their normal, quadripedal, flexed-hindlimb posture. Additionally, neither exercise requires a nox-
ious stimulus to the rats. This is in contrast to the treadmill running and swimming extensively described in pregnant rats (31, 35, 38, 40); these exercises often involve noxious stimuli to the animals and are likely to require greater energy use than squat exercise or tower climbing. Previously, we demonstrated that voluntary tower climbing and squat exercise to obtain food are sufficiently strenuous to cause modeling of the tibia in nonpregnant rats without a concomitant rise in fecal corticosterone levels (43), a measure of stress in the rat. In this study, we used these same exercises in pregnant rats to test the hypothesis that neither tower climbing nor squat exercise would increase maternal fecal corticosterone levels or adversely affect fetal outcomes at day 19 of pregnancy. We concurrently tested the hypothesis that pregnant rats that performed tower climbing or squat exercise would respond with greater bone changes than pregnant control animals.

**METHODS**

**Animals.** Twenty-four female Wistar rats were habituated to their surroundings and to a low phytoestrogen control diet (AIN-93G, Research Diets) for 2 wk prior to commencing the exercise protocols. All rats were housed in the same room in a climate-controlled dedicated animal research facility with a 12:12-h light-dark cycle. Rats were bedded on kiln-dried wood shavings, and all were housed in pairs during the study period. Feed and water were provided ad libitum, and initial and residual feed and initial and residual water volume were measured three times weekly throughout the exercise trial to monitor intake. Body weight was also measured three times weekly. Body length (from the occipital crest to the base of the tail: crown-rump length) was measured twice: once under anesthesia for imaging and again immediately following study termination. Maternal feed efficiency was calculated as the ratio of weight gain to feed intake on a per-cage (2 rats per cage) basis, as described previously (43). The study protocol and all animal procedures were approved by the Massey University Animal Ethics Committee.

**Exercise.** After the habituation period, at 88–95 days of age, the rats were randomly assigned to one of three age- and weight-matched groups: control, squat exercise, and tower climbing. We previously showed that these exercise models induce bone modeling in nonpregnant rats without initiating a physiological stress response (43). Briefly, control rats were housed conventionally for the duration of the trial in a 419 × 279 × 152 mm cage, with their food and water at a height of ~90 mm from the cage floor. Rats in the squat-exercise group were housed in a modified cage, the sides of which were gradually raised over a 5-day period; at full height, the rats had to obtain an erect bipedal stance to reach their food and water, which was ~220 mm above the cage floor. For the tower-climbing group, the cage lid was replaced with a 2-m-high tower made of wire mesh (12 × 12 mm welded stainless steel, 1.6 mm), and the water bottle was gradually raised over a 5-day period to a final height of 2 m.

During their 5-day gradual introduction to exercise, the rats were observed daily to ensure that they were able to reach the feed and water and that they were maintaining intake and body weight. After this orientation period, all rats were mated; their age at successful mating was 97 ± 4 days. On confirmation of mating by visualization of a plug, the rats were returned to their exercise cages until day 19 of pregnancy. The day the plug was visualized was designated day 0 of pregnancy.

**Imaging.** Peripheral quantitative computed tomography (pQCT) and dual-energy X-ray absorptiometry (DXA) were performed twice during the trial: at 4 days prior to beginning the orientation period (baseline) and following terminal sampling on day 19 of pregnancy. Prior to the initial imaging, the rats were anesthetized with 0.5 ml of ketamine (100 mg/ml) + 0.2 ml of acepromazine (2 mg/ml) + 0.1 ml of xylazine (100 mg/ml) + 0.2 ml of sterile water injected intraperitoneally at a dose rate of 0.06 ml/100 g via a 25-gauge needle. A plane of anesthesia suitable for noninvasive imaging procedures was obtained within 5–10 min and was maintained for ~1 h.

pQCT ( XCT2000 pQCT scanner, Stratec, Pforzheim, Germany) of the right tibia of each rat was performed as described previously (43). Scans were made 5 mm distal to the tibial plateau (proximal metaphysis) and at 50% of tibial length (middiaphysis) (24), with a voxel size of 0.1 mm and scan speed of 5 mm/s. Scans were analyzed using the manufacturer’s software. Metaphyseal bone was analyzed by contour mode 3, peel mode 2, with an outer threshold of 214 g/cm³ and an inner threshold of 606 g/cm³, and diaphyseal bone was analyzed using contour mode 1 with a threshold of 710 g/cm³. The coefficient of variation (CV) for total density ranged from 0.48 to 1.47% at the middiaphysis and from 1.32 to 2.23% at the proximal tibial metaphysis. The CV for total area ranged from 1.47 to 1.72% at the middiaphysis and from 3.73 to 7.11% at the proximal tibial metaphysis without and with repositioning between scans.

Bone mineral content (BMC), areal bone mineral density (BMD), and whole body composition data were determined with a fan beam densitometer (Hologic Discovery A, Bedford, MA) using the small animal application, as described previously (43). Prior to imaging at day 19 of pregnancy, all rats were killed, and their uterus, left fore- and hindlimbs, and brain were removed. Whole body scans were analyzed with the skull and associated tissues excluded from the region of interest. Quality control scans were performed daily to ensure that the precision met the required DXA manufacturer’s CV, which was 0.98 ± 0.10%. High-resolution scans of the femurs had a CV of 0.60% and 1.20% without and with repositioning between scans.

**Fecal sample collection and corticosterone measurement.** To measure the stress response to voluntary exercise, fecal samples were collected from all groups during three periods: before exercise, during the exercise introduction period (immediately before mating), and during the exercise period (days 10–16 of pregnancy). Feces were collected as described previously (43). Samples were freeze-dried and ground, and ethanol extraction was performed by the nonboiling method (23) with the following slight modifications. The dried ethanol extracts were reconstituted in 2 ml of 0.1 M PBS with 0.1% gelatin, pH 7.0 (PBSG), and the final supernatant was used without further dilution. The recovery of corticosterone following extraction was measured as previously described (23). The mean recovery of corticosterone from pooled control samples was 76.6 ± 0.7% (n = 20). The CV for the mean percent recovery was low (4.3%), and the mean percent recovery was used to calculate results for all the samples. All samples were assayed in duplicate by a commercially available RIA kit (double-antibody corticosterone 125-I-RIA kit for rats and mice, MP Biomedicals), and radiation counts were determined in a gamma counter (1261 Multigamma, LKB Wallac) for 5 min each. All the sample and reagent volumes used in the assay were 1/10th of those of the kit protocol. After addition of the precipitant and vortexing, 20 μl of starch [25 g/l starch (Sigma) + 0.05 g/l neutral red (BDH) in PBSG] were added to increase adhesion of the pellet to the tube. The intra-assay CV for corticosterone were 8.4, 6.0, and 7.2%, and interassay CV for corticosterone were 7.8, 8.4, and 11.5% for low-, medium-, and high-concentration solutions, respectively. Fecal samples were weighed after drying and before extraction. Corticosterone is expressed as total nanograms excreted over the collection period, calculated as the fecal corticosterone concentration (ng/g) multiplied by the weight of the fecal sample.

**Sample collection.** On day 19 of pregnancy, the rats were killed by cardiac puncture and terminal blood draw while under anesthesia. The uterus and its contents were removed immediately using a standard-ized procedure as follows: a ventral midline incision was made, and the proper ligaments of both ovaries were transected; the mesometrium was dissected so that all fat remained within the abdomen; then the cervix was transected, and the gravid uterus was removed from the abdomen and weighed. The fetuses were removed from the uterus, weighed, and measured, their sex was determined, and their position within the uterus was recorded. The discoid placentas were freed of...
amniotic membranes and umbilical cord and then weighed. The brain and liver were removed from three male and three female mid-uterine horn fetuses per litter, and the individual weights of these organs were obtained; if three fetuses of each sex were not available from the mid-uterine horn region, then two were used. Fetal sex was initially determined by assessment of anogenital distance (45) and, when questionable, confirmed by visualization of the gonad under a dissecting microscope after sample collection. The single fetus closest to the cervix was classified as having the position adjacent to the cervix, the single fetus nearest the ovary was classified as located at the ovarian end of the horn, and all other fetuses were considered to be in a mid-uterine horn position. Prior to imaging, the left fore- and hindlimbs of the dams were removed at the shoulder and hip, respectively, and the bones were harvested and stored.

Statistical analysis. All statistical analysis was performed with SAS 9.1 using PROC GLM and Tukey-Kramer post hoc analysis. Significance of differences in fetal outcome parameters was determined using a general linear model, with exercise group, fetal sex, dam nested within exercise group, and position of the fetus within the uterus as fixed effects. The interaction between exercise group and position of the fetus within the uterus was also included in the model. Fecal corticosterone was assessed using repeated-measures ANOVA, with the effects exercise group, cage nested within exercise group, collection period, and the interaction of exercise group and collection period. To achieve normal distribution, the fecal corticosterone data were logarithmically transformed prior to analysis. Baseline differences in imaging parameters were assessed by simple ANOVA. \( \text{Day 19} \) values for imaging parameters were analyzed using covariate analysis, with exercise group, baseline parameter value, and total weight of the fetuses carried by the dam as covariates. All data are expressed as least-square means ± SE unless otherwise indicated. Differences are considered significant at \( P \leq 0.05 \).

RESULTS

Animals. All rats successfully performed the exercise required by their group without injury or compromised health. Two animals (1 control and 1 squat exercise) failed to become pregnant and were excluded from the analysis. The 22 gravid dams \( (n = 7 \text{ control}, n = 7 \text{ squat exercise}, n = 8 \text{ tower climbing}) \) collectively contained 294 live fetuses. Mean litter size was 13.3 fetuses per litter, and there were no significant between-group differences in the number of fetuses per litter, the male-to-female ratio, or the number of resorptions at \( \text{day 19} \) of pregnancy.

There were no significant differences in feed intake between groups, and rats in all groups gained weight throughout pregnancy. However, rats in the exercised groups gained significantly less weight (excluding the weight of the uterus and its contents) than rats in the control group (Fig. 1). Maternal crown-rump length at \( \text{day 19} \) of pregnancy did not significantly differ between groups \( (17.40 ± 0.39, 16.57 ± 0.33, \text{ and } 16.86 ± 0.31 \text{ cm for control, squat-exercise, and tower-climbing groups, respectively}) \). Mean overall feed efficiencies \( (43) \) for the control, squat-exercise, and tower-climbing groups over the pregnancy period did not significantly differ at 0.27 ± 0.02, 0.24 ± 0.02, and 0.25 ± 0.01 g body wt gained per gram of feed intake, respectively.

Imaging. \( \text{pQCT of the right proximal tibial metaphysis and midtibial diaphysis of the dams revealed no significant differences in any parameters between groups before, or at completion of, the exercise period (Tables 1 and 2).} \)

The results of whole body DXA imaging, excluding the skull and associated tissues, are shown in Table 3. There were no pre pregnancy differences in body composition. All animals gained fat, increasing their body fat percentage and decreasing their percent lean mass, over the trial period. Total lean mass was significantly lower in squat-exercise than control rats on \( \text{day 19} \) of pregnancy; however, percent lean mass of these two groups did not differ. Whole body BMC was significantly lower in both exercised groups than in controls \( (P = 0.02–0.03) \), and bone area was significantly lower in the tower-climbing than the control group \( (P = 0.02) \), with a similar trend \( (P = 0.06) \) in the squat-exercise group. However, when expressed per gram of body weight, these differences were no longer significant.

The results of DXA imaging of the first four lumbar vertebrae \( (\text{L}1–\text{L}_4) \) are shown in Table 4. There were no preexercise/prepregnancy differences in BMC, bone area, or \( \text{BMD}_a \). The bone area of \( \text{L}_1–\text{L}_4 \) was significantly lower in squat-exercise rats at \( \text{day 19} \) of pregnancy than in control or tower-climbing animals. There was a trend toward a lower BMC of \( \text{L}_1–\text{L}_4 \) at \( \text{day 19} \) in squat-exercise than control rats \( (P = 0.06) \). As with whole body BMC and bone area, when expressed per gram of body weight, these differences were no longer significant. There were no between-group differences in \( \text{BMD}_a \) following the pregnancy/exercise period.

Regional scans of the right hindlimb did not reveal any significant differences between groups in bone or soft tissue parameters.

Fecal corticosterone. Mean daily fecal corticosterone excretion on a per-cage basis (2 rats/cage) during the first 4 h of the dark cycle over each 4-day collection period is shown in Fig. 2. There were no significant differences between groups during any collection period, and there were no significant differences across collection periods in any group. When data from all groups were pooled, fecal corticosterone was significantly lower during midpregnancy than during the preexercise or the exercise adjustment period.

Fetal outcomes. Fetal weight and length and placental weight are shown in Table 5. Fetuses of squat-exercise rats were heavier than those of the other groups (effect of exercise group on fetal weight, \( P = 0.05 \)); however, the interaction between exercise group and position of the fetus within the
Tables 1 and 2: *In vivo* pQCT values at the right proximal tibial metaphysis and right midtibial diaphysis.

**Table 1. In vivo pQCT values at the right proximal tibial metaphysis**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Squat</th>
<th>Tower</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total BMC, mg</td>
<td>9.48 ± 0.30</td>
<td>9.31 ± 0.30</td>
<td>9.41 ± 0.28</td>
</tr>
<tr>
<td>Total area, mm²</td>
<td>13.91 ± 0.51</td>
<td>13.86 ± 0.51</td>
<td>14.04 ± 0.48</td>
</tr>
<tr>
<td>Total BMDv, mg/cm³</td>
<td>682.80 ± 18.38</td>
<td>673.51 ± 18.38</td>
<td>673.25 ± 17.19</td>
</tr>
<tr>
<td>Trabecular BMC, mg</td>
<td>1.67 ± 0.13</td>
<td>1.71 ± 0.13</td>
<td>1.79 ± 0.12</td>
</tr>
<tr>
<td>Trabecular area, mm²</td>
<td>5.52 ± 0.36</td>
<td>5.65 ± 0.36</td>
<td>5.66 ± 0.34</td>
</tr>
<tr>
<td>Trabecular BMDv, mg/cm³</td>
<td>300.16 ± 18.61</td>
<td>302.47 ± 18.61</td>
<td>321.25 ± 17.41</td>
</tr>
<tr>
<td>Cort/subcort BMC, mg</td>
<td>6.67 ± 0.19</td>
<td>6.49 ± 0.19</td>
<td>6.43 ± 0.17</td>
</tr>
<tr>
<td>Cort/subcort area, mm²</td>
<td>8.39 ± 0.29</td>
<td>8.21 ± 0.29</td>
<td>8.38 ± 0.28</td>
</tr>
<tr>
<td>Cort/subcort BMDv, mg/cm³</td>
<td>933.99 ± 16.76</td>
<td>929.04 ± 16.76</td>
<td>910.08 ± 15.68</td>
</tr>
<tr>
<td>Periosteal circumference, mm</td>
<td>13.20 ± 0.24</td>
<td>13.19 ± 0.24</td>
<td>13.27 ± 0.23</td>
</tr>
</tbody>
</table>

**Table 2. In vivo pQCT values at the right midtibial diaphysis**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Squat</th>
<th>Tower</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total BMC, mg</td>
<td>9.59 ± 0.30</td>
<td>9.31 ± 0.30</td>
<td>9.41 ± 0.28</td>
</tr>
<tr>
<td>Total area, mm²</td>
<td>13.91 ± 0.51</td>
<td>13.86 ± 0.51</td>
<td>14.04 ± 0.48</td>
</tr>
<tr>
<td>Total BMDv, mg/cm³</td>
<td>682.80 ± 18.38</td>
<td>673.51 ± 18.38</td>
<td>673.25 ± 17.19</td>
</tr>
<tr>
<td>Trabecular BMC, mg</td>
<td>1.67 ± 0.13</td>
<td>1.71 ± 0.13</td>
<td>1.79 ± 0.12</td>
</tr>
<tr>
<td>Trabecular area, mm²</td>
<td>5.52 ± 0.36</td>
<td>5.65 ± 0.36</td>
<td>5.66 ± 0.34</td>
</tr>
<tr>
<td>Trabecular BMDv, mg/cm³</td>
<td>300.16 ± 18.61</td>
<td>302.47 ± 18.61</td>
<td>321.25 ± 17.41</td>
</tr>
<tr>
<td>Cort/subcort BMC, mg</td>
<td>6.67 ± 0.19</td>
<td>6.49 ± 0.19</td>
<td>6.43 ± 0.17</td>
</tr>
<tr>
<td>Cort/subcort area, mm²</td>
<td>8.39 ± 0.29</td>
<td>8.21 ± 0.29</td>
<td>8.38 ± 0.28</td>
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<tr>
<td>Cort/subcort BMDv, mg/cm³</td>
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<tr>
<td>Periosteal circumference, mm</td>
<td>13.20 ± 0.24</td>
<td>13.19 ± 0.24</td>
<td>13.27 ± 0.23</td>
</tr>
</tbody>
</table>

Values are least-square means ± SE (n = 7 control, 7 squat, and 8 tower). All data are from the right proximal tibial metaphysis 5 mm distal to the proximal tibial plateau. pQCT, peripheral quantitative computed tomography; BMC, bone mineral content; BMDv, volumetric bone mineral density; cort/subcort, cortical/subcortical. There were no significant between-group differences in baseline or day 19 values.
mid-uterine horn region. This may be due to regional differences in fetoplacental blood flow and concomitant regional differences in blood flow alteration with exercise. In rats, fetuses at the ovarian ends of the uterine horns are lighter than those in the mid-horn region (36). However, the smaller mass of fetuses located at the ovarian ends of the uterine horns does not correlate with decreased blood flow; fetuses in the mid-uterine horn region receive less blood flow than fetuses at the ovarian or the cervical end of the horns. This difference in blood flow is most pronounced at day 15 of pregnancy, and blood flow becomes more similar between regions of the uterus by day 21 (during the period of rapid fetal growth) (22). Placental blood flow in the rat is constrained by the necessity of maintaining intraplacental pressure at a level low enough to avoid compression of the labyrinthine vessels of the placenta (39). Fetuses receiving less blood flow under control conditions (the fetuses in the mid-uterine horn) may experience a greater adaptive increase in blood flow with maternal exercise. Acute strenuous exercise causes a reduction in placental blood flow in rats (20), but regular exercise may lead to enhanced placental blood flow at rest and, thus, an increase in overall delivery of oxygen and nutrients to the fetus (11). This would suggest that the different responses to exercise of the fetuses from the squat-exercise and tower-climbing dams may reflect different effects on blood flow of the two exercises.

The lower maternal weight gain over pregnancy in both exercised groups is unsurprising: in humans and rats, exercise has been shown to reduce weight gained by the exercising mother (15, 34). However, the lesser weight gain of rats in the exercised groups did not reflect a lower fat gain, as might be expected. Instead, whole body DXA imaging revealed lower lean mass in the squat-exercise group than controls, a lower BMC in both exercised groups, and less bone area in the tower-climbing group than control animals. When expressed per gram of body weight, these differences in BMC and bone area became nonsignificant, suggesting that the exercised rats had appropriate BMC and bone area for their body weight, which was less than that of the control rats. Rats at 100 days of age are sexually mature but still growing in size; these results suggest that the exercised young adult rats grew less than control rats over the course of their pregnancies. These findings are similar to those of Mottola et al. (34), who found that pregnant rats that performed exercise weighed less, even when all skin and subcutaneous fat had been removed. Thus perhaps placental adaptations to exercise result in improved nutrient delivery to the fetuses at the expense of maternal growth.

We anticipated that squat exercise and tower climbing would cause modeling of the tibia of pregnant rats, as we previously reported in nonpregnant animals (43), but this did not occur. Analysis of pQCT results showed no differences in BMC, bone area, or volumetric bone mineral density between control and exercised groups at the proximal tibial metaphysis or the midtibial diaphysis on day 19 of pregnancy. The lack of a tibial bone response to exercise in these pregnant animals suggests that the physiological state of pregnancy has superseded the bone’s response to the external forces imposed by the exercises we utilized. Rats store calcium during early pregnancy in the maternal femur (49). It may be that the enhancement of periosteal bone formation in the appendicular skeleton that occurs with pregnancy overwhelmed any increase that would

<table>
<thead>
<tr>
<th>BMC, g</th>
<th>Lean mass, g</th>
<th>Fat mass, g</th>
<th>BMC/BW</th>
<th>Bone area, cm²</th>
<th>Bone area/BW</th>
<th>log BMDa, g/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>189.05 ± 3.15</td>
<td>62.76 ± 5.96</td>
<td>73.12 ± 1.66</td>
<td>0.03 ± 0.0008</td>
<td>0.18 ± 0.005</td>
<td>-0.85 ± 0.005</td>
</tr>
<tr>
<td>Squat</td>
<td>187.50 ± 3.15</td>
<td>68.81 ± 5.96</td>
<td>69.83 ± 1.66</td>
<td>0.03 ± 0.0008</td>
<td>0.18 ± 0.005</td>
<td>-0.85 ± 0.005</td>
</tr>
<tr>
<td>Tower</td>
<td>192.70 ± 2.76</td>
<td>105.13 ± 5.20</td>
<td>63.41 ± 1.33</td>
<td>0.019 ± 0.0004</td>
<td>0.13 ± 0.002</td>
<td>-0.83 ± 0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.09 ± 0.004</td>
<td>0.13 ± 0.002</td>
<td></td>
</tr>
</tbody>
</table>

Values are least-square means ± SE (n = 7 control, 7 squat, and 8 tower). Prior to scan at day 19, dams were euthanized and their uteri and left fore- and hindlimbs were removed. DXA, dual-energy absorptiometry; BW, live weight on the day of scanning. *Significant difference from control day 19 value (P ≤ 0.05).

Table 3. Dam whole body (excluding skull and associated tissues) DXA imaging results

<table>
<thead>
<tr>
<th></th>
<th>Baseline (nonpregnant)</th>
<th>Postexercise (day 19 of pregnancy)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
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</tr>
<tr>
<td>Lean mass, g</td>
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</tr>
<tr>
<td>Fat mass, g</td>
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<td>68.81 ± 5.96</td>
</tr>
<tr>
<td>%Lean</td>
<td>73.12 ± 1.66</td>
<td>71.34 ± 1.66</td>
</tr>
<tr>
<td>%Fat</td>
<td>24.07 ± 1.70</td>
<td>25.89 ± 1.70</td>
</tr>
<tr>
<td>BMC, g</td>
<td>7.20 ± 0.20</td>
<td>7.24 ± 0.19</td>
</tr>
<tr>
<td>BMC/BW</td>
<td>0.03 ± 0.0008</td>
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</tr>
<tr>
<td>Bone area, cm²</td>
<td>50.54 ± 1.15</td>
<td>51.39 ± 1.15</td>
</tr>
<tr>
<td>Bone area/BW</td>
<td>0.18 ± 0.005</td>
<td>0.18 ± 0.005</td>
</tr>
<tr>
<td>log BMDa, g/cm²</td>
<td>-0.85 ± 0.005</td>
<td>-0.85 ± 0.005</td>
</tr>
</tbody>
</table>

mid-uterine horn region. This may be due to regional differences in fetoplacental blood flow and concomitant regional differences in blood flow alteration with exercise. In rats, fetuses at the ovarian ends of the uterine horns are lighter than those in the mid-horn region (36). However, the smaller mass of fetuses located at the ovarian ends of the uterine horns does not correlate with decreased blood flow; fetuses in the mid-uterine horn region receive less blood flow than fetuses at the ovarian or the cervical end of the horns. This difference in blood flow is most pronounced at day 15 of pregnancy, and blood flow becomes more similar between regions of the uterus by day 21 (during the period of rapid fetal growth) (22). Placental blood flow in the rat is constrained by the necessity of maintaining intraplacenta
have resulted from the physical strains of our moderate exercises. Women normally lose bone mineral during pregnancy (5); however, there are few published data on the effects of certain moderate exercises during pregnancy without an increase in stress hormone levels. In pregnant women, plasma cortisol was elevated immediately after 40 min of treadmill walking but not after 40 min of aerobic dance (32). A study in men found that subjects needed to exceed ~60% of their maximal O2 consumption to induce an acute rise in cortisol levels (17); although care must be taken when extrapolating data from human to rodent studies, it is unlikely that tower climbing or squat exercise would have utilized a significant portion of the rats’ aerobic capacity.

When data from all groups were pooled, fecal corticosterone output was significantly lower during pregnancy (days 10–16) than prior to pregnancy. Similar reductions of plasma corticosterone concentration have been shown in early pregnancy and midpregnancy in rats, with corticosterone decreasing sharply in early pregnancy and then slowly increasing from day 10 until they are significantly greater than nonpregnant levels by day 22 of pregnancy (2). We chose to assess corticosterone in our pregnant rats in midgestation, when corticosterone levels are least in flux; however, it is important to note that our experimental design may have caused us to miss between-group differences in corticosterone output in early or late pregnancy. The reduction in corticosterone that occurs in midpregnancy in rats differs from the situation in humans, in which pregnancy is a period of “normal” hypercortisolemia, with blood cortisol concentrations rising gradually throughout pregnancy to a peak in the third trimester (30). However, regardless of differences in normal levels of stress hormones during pregnancy, chronic maternal stress has been shown to have long-term deleterious effects on human and rat offspring (50).

Events that occur during fetal development can have long-lasting effects on the health and later-life outcomes of the developing organism (25). Few studies have examined the effects of maternal exercise during pregnancy on the offspring beyond the neonatal period. To the authors’ knowledge, no study has examined the long-term effects of nonstressful exercise in pregnant rats on offspring health. In one study,

<table>
<thead>
<tr>
<th>Fetal wt, g</th>
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<tbody>
<tr>
<td>Adjacent to cervix</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Squat</td>
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<tr>
<td>Tower</td>
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<table>
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<tr>
<th>Fetal length, mm</th>
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<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Squat</td>
</tr>
<tr>
<td>Tower</td>
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<table>
<thead>
<tr>
<th>Placental wt, g</th>
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<tr>
<td>Control</td>
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<tr>
<td>Squat</td>
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<td>Tower</td>
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Values are least-square means ± SE (n = 7 control, 7 squat, and 8 tower dams). *Significant difference from control at the same uterine position (P < 0.05).
offspring of women who exercised throughout pregnancy were lighter at birth than those of controls and were leaner, but not shorter, than those of controls at 5 yr of age (12). This suggests that physiological changes induced by exercise during pregnancy can persist into childhood, but further study is needed to examine the effects of maternal exercise during gestation on the later-life health of the offspring.

Perspectives and Significance

Exercise during pregnancy may have long-lasting effects on offspring health. Investigation of this issue is complicated by the effects of exercise-induced stress on fetal development. The results of this study demonstrate that tower climbing and rising to an erect bipedal stance may be suitable for examining the effects of exercise during pregnancy on the future health of the offspring in the rat. Both exercises enhance fetoplacental growth without causing a concomitant rise in corticosterone output. Our observation that the maternal exercise effects on fetal growth at day 19 of gestation are dependent on the position of the fetus within the uterus suggests that selection of the appropriate offspring for study of long-term effects may be important. We found that fetuses in the mid-uterine horn region were most likely to respond to maternal exercise with enhanced growth. Fetuses in the mid-uterine horn region were closer to the mean fetal weight of the litter than fetuses at the cervical or ovarian end of the uterine horns, which suggests that offspring of average weight should be selected for later-life evaluation. The different bone response to exercise of the pregnant animals in this study compared with the increased bone modeling in this study compared with the increases in bone modeling in nonpregnant animals reported previously (43) also warrants further investigation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


