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Original Article

Hylonome

Voluntary exercise in pregnant rats improves post-lactation maternal bone parameters but does not affect offspring outcomes in early life

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Abstract

Objectives: The objectives of this study were to examine the effects of voluntary exercise during pregnancy on maternal post-lactation bone parameters and offspring growth. **Methods:** Pregnant Wistar rats were housed in conventional cages (control), or were housed in raised cages requiring them to rise to an erect, bipedal stance to obtain food/water, throughout pregnancy. Dual energy X-ray absorptiometry and peripheral quantitative computed tomography scans were performed pre-mating and post-weaning. Maternal stress was assessed by fecal corticosterone measurement. Offspring weights were assessed at postnatal days 1 and 25 (weaning). **Results:** Changes in bone mineral over the pregnancy/lactation period were site-specific. Exercise did not affect loss of bone mineral from the lumbar spine, but did attenuate the loss of trabecular bone mineral from the tibial metaphysis and enhance the strength strain index and cross-sectional moment of inertia at the tibial diaphysis ($P \leq 0.05$) in dams in the exercised group. Fecal corticosterone did not differ between dam groups. There were no significant differences in offspring weight between the exercised and control group at either time point. **Conclusions:** Voluntary exercise in the pregnant rat can improve some post-lactation bone parameters and does not adversely affect early postnatal outcomes of the offspring.

Keywords: Gestation, Stress, Dual-energy X-ray Absorptiometry, peripheral Quantitative Computed Tomography, Developmental Origins of Health and Disease

Introduction

Exercise during pregnancy may affect both the mother and offspring[1,2]. In mothers, exercise reduces the risk of pregnancy-associated disorders such as gestational diabetes and pre-eclampsia[1], and may have effects on the maternal skeleton. Pregnancy and lactation are times of increased bone turnover in both humans and rodents[3]. The fetal demand for calcium in late pregnancy and the postnatal demand for calcium during lactation are met primarily from the trabecular rather than the cortical component of the skeleton[4-6]. This loss of maternal bone mineral is usually recovered after lactation has ended, but low bone mass in the puerperal period may be predictive of later osteoporosis risk in women[7]. It is unclear how maternal loss of bone mineral during pregnancy and lactation may affect attainment of peak bone mass and subsequent risk of osteoporosis in later life.

Mechanical loading increases bone mineral in a strain-dependent and site-specific manner[8-10]. Thus exercise during pregnancy may have the potential to attenuate loss of maternal bone mineral over the pregnancy and lactation period, but little work has been done to examine this. A study of women who played tennis during pregnancy showed that exercise eliminated the loss in an ultrasound-assessed bone stiffness index that occurred at the calcaneus during the pregnancy and early lactation period, but not the decrease in bone mineral density

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measured by dual energy X-ray absorptiometry (DXA)[11]. When examining the effects of exercise on bone it is very important to control for effects of stress (mediated by the hypothalamic-pituitary-adrenal axis) as this can influence osteoblast activity and bone formation[12]. We previously demonstrated that voluntary exercise causes bone modeling in non-pregnant female rats without elevating the stress hormone corticosterone[13]. When we applied those same exercises to pregnant rats they still did not induce a stress response. However, there were no differences between exercised and control rats in any of the assessed parameters of bone mineral or area at day 19 of gestation when expressed per gram of body weight[14]. Pregnant rats store calcium in early pregnancy in preparation for the high calcium demands of the pups during late gestation and lactation[3]. The bone mineral increases associated with the physiological state of pregnancy through day 19 appeared to supersede the effects of our mild, voluntary exercise regime. In this study, we examined whether effects of exercise during pregnancy would be evident at the end of the lactation period, or whether the increased calcium demands in late pregnancy and lactation[15] would also mask the effects of exercise on bone.

Exercise during pregnancy can affect the offspring, but comparison of studies is complicated by differing types, intensities, and timing of exercise interventions. The focus of recent work has been on birth weight. Studies in animals and humans have yielded varied results including increases[16] and decreases[17-20], as well as no change[21,22], in birth weight following maternal exercise during pregnancy. A recent review of the work in humans suggests that the long-term impact of pregnancy exercise on the offspring is likely to vary with maternal fitness, health and diet, as well as type and timing of the exercise during pregnancy[23]. Our previous work showed that voluntary exercise during pregnancy in rats increased fetal growth to day 19 of pregnancy, with the fetuses of exercised dams being longer and heavier than those of controls[14]. As birth weight is considered a reflection of the intrauterine environment, these results may indicate that voluntary exercise during pregnancy enhanced the intrauterine environment for the offspring; this may have long-term health effects in later life.

In this study we utilized the bipedal stance ("squat") exercise first described by Yao et al.[24], which positively influences fetal growth without inducing a maternal stress response[14], to test the hypothesis that voluntary exercise during pregnancy in rats will not affect litter size, will attenuate the loss of maternal bone mineral throughout pregnancy and lactation, and will result in heavier offspring relative to those of non-exercised controls.

Materials and Methods

Animals

Twenty virgin female Wistar rats were housed in individual cages in a climate-controlled room in a dedicated animal research facility with a 12:12 hour light:dark cycle. All rats were allowed a minimum of two weeks to habituate to their surroundings before beginning exercise. Feed and water were provided *ad libitum* and initial and residual feed was weighed daily from gestational day (GD) 0 until parturition. Body weight was also measured daily. Body length (nose-tail, nose-ischiatic tuberosity, and nose-atlanto-occipital junction) was measured once under anesthesia (for imaging) and once immediately following euthanasia at study termination. Maternal feed efficiency over the pregnancy period was calculated as total body weight gain from GD0 to GD21 divided by total feed intake over the same period. The Massey University Animal Ethics Committee approved the study protocol and all animal procedures.

Exercise

After the period of habituation to the environment, when they were 92 to 96 days old, the rats were randomly assigned to one of two age- and weight-matched groups. Rats in the exercise group were housed in raised cages from GD0 to lactation day (L) 1 so that they had to achieve an erect bipedal stance to obtain food and water as described previously[13]. Rats in the control group were housed in cages of conventional height for the duration of the trial. Rats in the exercise group were gradually introduced to exercise in the five days immediately prior to mating as described previously[14].

Reproduction

Rats were mated between 98 and 104 days of age. Each sire mated with one exercised and one control dam. Mating was confirmed by visualization of a semen plug and the rats were then returned to their exercise cages for the duration of pregnancy. The day the plug was visualized was designated GD0. All rats that became pregnant gave birth on GD22. The day after parturition was designated L1.

On L1 all offspring were weighed and litter sizes were reduced to 8 per litter. When possible, four male and four female pups close to the mean pup weight were allowed to remain with the mother, but in two litters 6 males and 2 females were selected as there was an insufficient number of female pups. Exercise was stopped on L1, and all mothers and pups were housed in control housing throughout the lactation period. Food intake of the mothers and pups combined, and individual body weights of all animals, were recorded daily. Pups were weaned at L25 and dam body composition and bone parameters were assessed on the day following weaning. We chose to stop exercise on the first day of lactation in order to limit our study to the effects of exercise during pregnancy, as opposed to the combined pregnancy/lactation period. Dam body composition and bone parameters were assessed on the day following weaning to examine whether exercise during pregnancy resulted in physical changes to the dams that persisted throughout the lactation period.

Imaging

Peripheral quantitative computed tomography (pQCT) and dual-energy X-ray absorptiometry (DXA) were performed twice during the trial, once 6 days prior to the beginning of the gradual introduction to exercise (age 86-90 days) and once im-

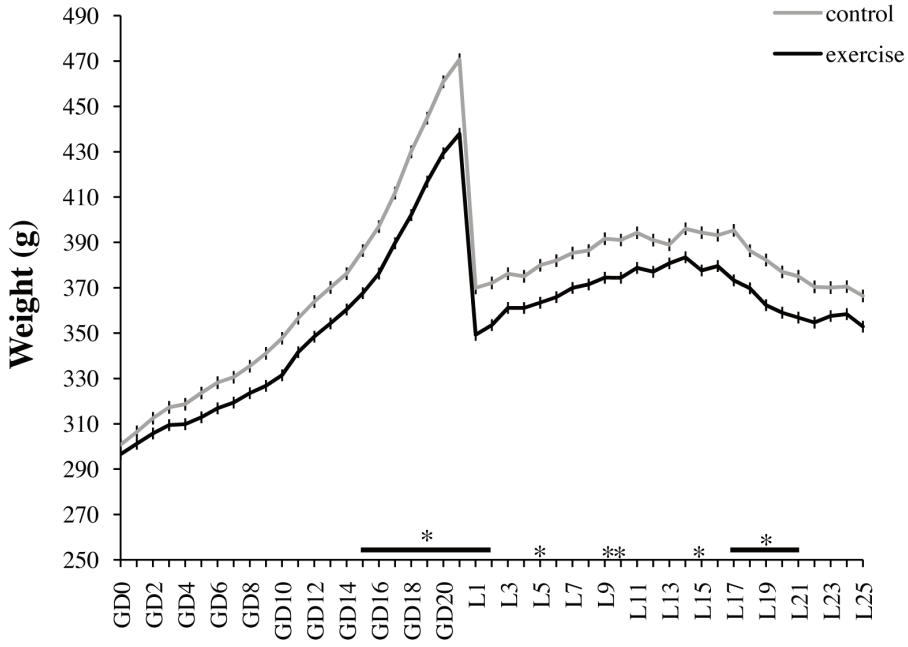

Figure 1. Dam body weight throughout pregnancy and lactation. A * above the horizontal axis indicates that the mean body weight of exercised dams was significantly different from that of control dams on that day, *P≤0.05*. Body weight of the exercised dams differed significantly from that of controls on days GD15-L2, and L5, 9, 10, 15, and 17-21.

mediately prior to euthanasia on the day following weaning (age 146-152 days). Anesthesia, DXA and the baseline pQCT scans were performed as described previously, except that DXA whole body scans included the skull in the ROI in both the baseline and post-lactation scan analyses[13]. Post-weaning pQCT scans were performed *ex vivo*. The right tibias were fixed in 4% paraformaldehyde for one week and then stored in 70% ethanol until scanning. The CV for *ex vivo* pQCT bone parameters ranged from 0.5-2.6%.

Fecal corticosterone

Fecal samples were collected from all rats over three collection periods of four consecutive days each: baseline (pre-exercise/pre-scanning/pre-mating), GD3-GD6, and GD17-GD20. The method for fecal collection and processing was as previously described[13]. The mean recovery of corticosterone from spiked control samples was 76.6±1.9% (n=20) with a CV of 11.3%. The mean percentage recovery was used to calculate results for all the samples.

Statistical analysis

Statistical analysis was performed with SAS 9.2 using PROC GLM. Significance of difference of offspring weights was tested using a nested model, with dam exercise group and offspring sex as fixed effects and dam nested within exercise group as a random effect. Offspring weights reported at L1 are mean weights for all live pups, offspring weights reported at L25 are for 8 pups per litter. Between-time point and between-group differences in dam imaging parameters, feed intake, body weight, and fecal corticosterone levels were determined using repeat measures analysis with dam exercise group, time point and their interaction as fixed effects, dam nested within exercise group as a random effect, and Tukey-Kramer post-hoc adjustments. Fecal corticosterone data were logarithmically transformed prior to analysis to achieve normal distribution. All data are expressed as lsmeans ± SE unless otherwise indicated. Differences are considered significant at *P≤0.05*.

Results

Animals

All animals successfully performed the exercise required by their group. Fifteen of the twenty animals were included in the final analysis. Of the 5 excluded animals, 3 (1 control and 2 exercised) failed to become pregnant, and two (both controls) were excluded for failing to have eight or more live offspring; final group sizes were control n=7, exercised n=8. There were no significant between-group differences in litter size or the male: female ratio of the live pups. Litter size, defined as the number of live offspring on L1, ranged from 9-17 with a mean of 13.5±2.2 pups/litter. Mean litter size was 14.3±0.8 for the control group and 12.9±0.8 for the exercised group (*P=0.22*). As the animals were not under constant observation and the mothers may (often) eat any dead pups immediately, the number of pups born dead could not be accurately assessed; however, there were no differences between groups in the number

	Weight at L1 (g)	*P*	Weight at L25 (g)	*P*
Male	6.5±0.04	0.0007	82.0±0.6	<0.0001
Female	6.2±0.05		78.0±0.6	
Control	6.2±0.1	n.s.	78.4±1.6	n.s.
Exercised	6.4±0.1		81.6±1.4	

Values are least square means ± standard error. n.s. = not significant.

Table 1. Offspring weights at 1 and 25 days after birth.

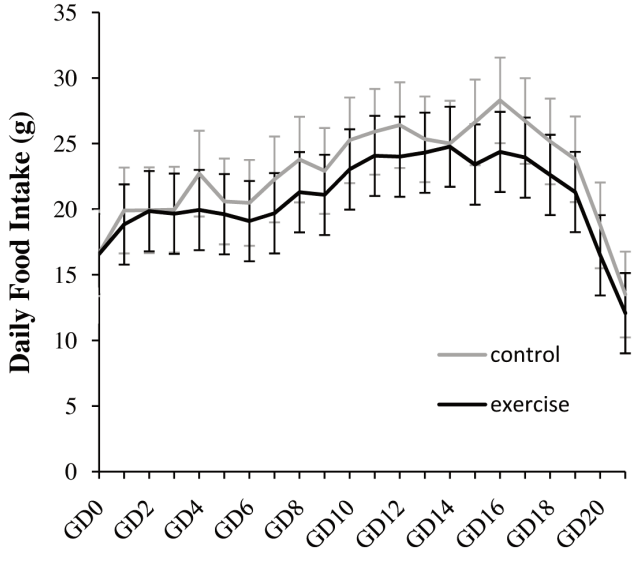

Figure 2. Daily food intake of exercised and control dams during pregnancy. There were no significant differences in daily food intake between groups on any day of pregnancy.

of uneaten dead pups found in the cages.

Rats in the exercised group gained significantly (*P=0.01*) less weight over pregnancy than did control rats. Weight gain in both groups followed a similar trajectory, but body weights, which include the weight of the uterus and its contents until the end of pregnancy, differed significantly from day 15 of pregnancy onwards as shown in Figure 1. Daily food intake during pregnancy did not significantly differ between groups (Figure 2). Total offspring weight also was not different between groups (*P=0.35*). The difference in dam weight between GD21 and L1 was taken as an approximation of the weight of the uterine contents, which includes the fetuses, placentas, and associated fluid. Mean body weight change during this time was -100.7±5.4 g and -88.6±5.0 g for the control and exercised rats, respectively (*P=0.12*). On L1, dams in the control group weighed 370±2.8 g and dams in the exercised group weighed 349.3±2.6 g (*P<0.0001*). During lactation, exercised dams recovered some, but not all, of their body weight deficit relative to controls so that at weaning the groups did not significantly differ but the exercised group was still numerically lighter than control rats. Feed efficiency of the dams over the pregnancy period was 0.35±0.01 and 0.32±0.01 for the control and exercised groups respectively; this difference was not statistically significant (*P=0.08*). Measures of dam length did not differ between groups prior to, or following, the pregnancy/lactation period.

Male offspring were heavier than female offspring at lactation day 1 and at weaning regardless of exercise group (*P<0.001*; shown in Table 1). However, there were no significant differences in offspring weight between the exercised and control groups at either time point (Table 1), and the interaction between sex and exercise group was also not significant (*P=0.47* and *P=0.77* at L1 and L25, respectively).

DXA-assessed changes in dam body composition and bone parameters

Both groups gained whole body BMC and bone area over the pregnancy/lactation period, with a proportionally greater increase in area than BMC resulting in a decrease in whole body areal bone mineral density (BMD_a). Although total fat, lean and body mass increased over pregnancy and lactation, the percentages of fat and lean mass did not change from baseline in either group (Table 2). Thus, when their offspring were weaned, the dams were larger than at mating but did not differ in body composition. When corrected for body weight, both whole body BMC and bone area were significantly lower at the time of weaning than at mating in both exercised and control rats. The decrease in bone area per gram of body weight was significantly less in the exercised rats relative to the controls (*P=0.02*).

Table 3 shows the results of high resolution DXA scans of the first four vertebrae of the lumbar spine. Bone area in both groups increased over the study period, but BMC did not change, resulting in a lower BMD_a at weaning than at baseline. When corrected for body weight, bone area and BMC were significantly lower post-lactation than at baseline in both groups, and this reduction was not affected by exercise.

In the right femur both groups gained bone area and BMC, resulting in no net change in BMD_a. As seen at the other sites, when bone area and BMC are corrected for body weight both groups were lower post-lactation than at baseline (Table 4).

pQCT-assessed changes in dam bone parameters

When changes in the right tibia over the study period were examined using pQCT, both pregnancy/lactation and exercise affected bone parameters in a site-specific fashion. The results

	Baseline		*Post-lactation*		*P*		
	Control	**Exercise**	**Control**	**Exercise**	**Ex**	**Time**	**Ex*T**
Fat Mass (g)	46.38±3.28	52.82±3.07	60.92±3.28	66.20±3.07	n.s.	0.0007	n.s.
Lean Mass (g)	215.91±3.98	208.39±3.72	291.59±3.98	277.84±3.72	n.s.	<0.0001	n.s.
Total Mass (g)	271.85±3.60	270.32±3.37	363.12±3.60	354.27±3.37	n.s.	<0.0001	n.s.
% Fat	17.06±0.92	19.63±0.86	16.78±0.92	18.63±0.86	n.s.	n.s.	n.s.
% Lean	79.42±0.92	77.00±0.86	80.30±0.92	78.48±0.86	n.s.	n.s.	n.s.
Bone Area (cm^2)	60.41±0.45	57.85±0.42	70.71±0.45	69.63±0.42	n.s.	<0.0001	n.s.
BMC (mg)	9.56±0.11	9.11±0.11	10.6±0.11	10.23±0.11	n.s.	<0.0001	n.s.
BMD_a (mg/cm^2)	0.16±0.001	0.16±0.001	0.15±0.001	0.15±0.001	n.s.	<0.0001	n.s.
Bone Area/BW	0.22±0.002	0.21±0.002	0.19±0.002	0.20±0.002	n.s.	<0.0001	0.02
BMC/BW	0.035±0.0004	0.034±0.0004	0.029±0.0004	0.029±0.0003	n.s.	<0.0001	n.s.

*Values are least square means ± standard error. Baseline is 6 days prior to starting exercise training. Post-lactation is 26 days after parturition. DXA, dual-energy X-ray absorptiometry; Ex, exercise group; Time, time of measurement (baseline or post-lactation); Ex*T, interaction between exercise group and time of measurement; BMC, bone mineral content; BMD_a, areal bone mineral density; BW, body weight; n.s., not significant.*

Table 2. Results of *in vivo* DXA whole body scans.

	Baseline		*Post-lactation*		*P*		
	Control	**Exercise**	**Control**	**Exercise**	**Ex**	**Time**	**Ex*T**
Bone Area (cm^2)	1.90±0.02	1.86±0.02	2.06±0.02	2.00±0.02	n.s.	<0.0001	n.s.
BMC (mg)	0.41±0.01	0.39±0.01	0.39±0.01	0.38±0.01	n.s.	n.s.	n.s.
BMD_a (mg/cm^2)	0.21±0.004	0.21±0.003	0.19±0.004	0.19±0.003	n.s.	<0.0001	n.s.
Area/BW	0.0070±0.00006	0.0069±0.00005	0.0057±0.00006	0.0057±0.00005	n.s.	<0.0001	n.s.
BMC/BW	0.0015±0.00003	0.0014±0.00003	0.0011±0.00003	0.0011±0.00003	n.s.	<0.0001	n.s.

*Values are least square means ± standard error. Baseline is 6 days prior to starting exercise training. Post-lactation is 26 days after parturition. DXA, dual-energy X-ray absorptiometry; Ex, exercise group; Time, time of measurement (baseline or post-lactation); Ex*T, interaction between exercise group and time of measurement; BMC, bone mineral content; BMD_a, areal bone mineral density; BW, body weight; n.s., not significant.*

Table 3. Results of high resolution DXA scans of the lumbar spine.

	Baseline		*Post-lactation*		*P*		
	Control	**Exercise**	**Control**	**Exercise**	**Ex**	**Time**	**Ex*T**
Area (cm^2)	1.24±0.02	1.20±0.02	1.41±0.02	1.38±0.02	n.s.	<0.0001	n.s.
BMC (mg)	0.32±0.01	0.29±0.01	0.34±0.01	0.34±0.01	n.s.	0.0039	n.s.
BMD_a (mg/cm^2)	0.26±0.01	0.24±0.01	0.24±0.01	0.24±0.01	n.s.	n.s.	n.s.
Area/BW	0.0045±0.0006	0.0045±0.0006	0.0039±0.0006	0.0039±0.0006	n.s.	<0.0001	n.s.
BMC/BW	0.0012±0.00003	0.0011±0.00003	0.0009±0.00003	0.0009±0.00003	n.s.	<0.0001	n.s.

*Values are least square means ± standard error. Baseline is 6 days prior to starting exercise training. Post-lactation is 26 days after parturition. DXA, dual-energy X-ray absorptiometry; Ex, exercise group; Time, time of measurement (baseline or post-lactation); Ex*T, interaction between exercise group and time of measurement; BMC, bone mineral content; BMD_a, areal bone mineral density; BW, body weight; n.s., not significant.*

Table 4. Results of high resolution DXA scans of the right femur.

of pQCT scans at the right proximal tibial metaphysis are shown in Table 5, and at the right mid-tibial diaphysis are shown in Table 6. At the proximal tibial metaphysis exercise attenuated the loss of bone mineral. The change in trabecular BMC from baseline to post-lactation was significantly different between groups. The decrease in total BMC at this site was also greater in the controls than in the exercised animals, and this difference approached significance (*P=0.07*). Both groups increased in total and trabecular bone area; with the exercised animals gaining more total bone area than the controls. The relative magni-

	Baseline		*Post-lactation*		*P*		
	Control	**Exercise**	**Control**	**Exercise**	**Ex**	**Time**	**Ex*T**
Total BMC (mg)	9.26±0.21	8.64±0.20	8.34±0.21	8.51±0.20	n.s.	0.02	n.s.
Total area (mm²)	13.78±0.27	13.01±0.26	15.79±0.27	16.15±0.26	n.s.	<0.0001	0.05
Total BMD_v (mg/cm³)	672.66±13.61	667.61±12.73	529.61±13.61	529.29±12.73	n.s.	<0.0001	n.s.
Trabecular BMC (mg)	1.68±0.06	1.36±0.06	1.47±0.06	1.48±0.06	n.s.	n.s.	0.02
Trabecular area (mm²)	5.74±0.28	5.51±0.26	7.87±0.28	8.03±0.26	n.s.	<0.0001	n.s.
Trabecular BMD_v (mg/cm³)	294.20±9.81	252.50±9.18	186.56±9.81	184.85±9.18	n.s.	<0.0001	n.s.
Cort/subcort BMC (mg)	7.58±0.22	7.27±0.20	6.86±0.22	7.04±0.20	n.s.	0.04	n.s.
Cort/subcort area (mm²)	8.05±0.23	7.51±0.21	7.93±0.23	8.12±0.21	n.s.	n.s.	n.s.
Cort/subcort BMD_v (mg/cm³)	943.31±8.28	971.30±7.74	865.19±8.28	866.93±7.74	n.s.	<0.0001	n.s.

*Values are least square means ± standard error. All data are from the right proximal tibial metaphysis 5 mm distal to the proximal tibial plateau. Baseline is 6 days prior to starting exercise training. Post-lactation is ex vivo, bones were collected 26 days after parturition. pQCT, peripheral quantitative computed tomography; Ex, exercise group; Time, time of measurement (baseline or post-lactation); Ex*T, interaction between exercise group and time of measurement; BMC, bone mineral content; BMD_v, volumetric bone mineral density; cort/subcort, cortical/subcortical; n.s., not significant.*

Table 5. pQCT results at the right proximal tibial metaphysis (baseline results are *in vivo*, post-lactation results are *ex vivo*).

	Baseline		*Post-lactation*		*P*		
	Control	**Exercise**	**Control**	**Exercise**	**Ex**	**Time**	**Ex*T**
Cortical BMC (mg)	5.57±0.07	5.45±0.07	6.04±0.07	6.17±0.07	n.s.	<0.0001	n.s.
Cortical area (mm²)	4.45±0.07	4.40±0.06	4.63±0.07	4.75±0.06	n.s.	0.001	n.s.
Cortical BMD_v (mg/cm³)	1253.43±9.70	1237.43±9.07	1304.57±9.70	1298.86±9.07	n.s.	<0.0001	n.s.
Endosteal circumference (mm)	4.94±0.10	5.16±0.09	5.03±0.10	5.16±0.09	n.s.	n.s.	n.s.
Periosteal circumference (mm)	8.96±0.07	9.05±0.07	9.14±0.07	9.29±0.07	n.s.	0.01	n.s.
SSI	3.26±0.07	3.20±0.06	3.69±0.07	4.03±0.06	n.s.	<0.0001	0.01
CSMI-x (mm⁴)	2.71±0.08	2.71±0.07	2.46±0.08	2.80±0.07	n.s.	n.s.	0.04
CSMI-y (mm⁴)	2.59±0.15	2.78±0.14	3.79±0.15	4.25±0.14	n.s.	<0.0001	n.s.
CSMI-p (mm⁴)	5.31±0.17	5.48±0.16	6.25±0.17	7.05±0.16	n.s.	<0.0001	n.s.

*Values are least square means ± standard error. All data are from the right mid-tibial diaphysis. Baseline is 6 days prior to starting exercise training. Post-lactation is ex vivo, bones were collected 26 days after parturition. pQCT, peripheral quantitative computed tomography; Ex, exercise group; Time, time of measurement (baseline or post-lactation); Ex*T, interaction between exercise group and time of measurement; BMC, bone mineral content; BMD_v, volumetric bone mineral density; SSI, strength strain index; CSMI-x, cross-sectional moment of inertia in the frontal plane; CSMI-y, cross-sectional moment of inertia in the sagittal plane; CSMI-p, torsional cross-sectional moment of inertia; n.s., not significant.*

Table 6. pQCT values at the right mid-tibial diaphysis (baseline results are *in vivo*, post-lactation results are *ex vivo*).

tude of these changes in bone area and BMC led to lower volumetric bone mineral density (BMD_v) at all sites post-lactation. At the mid-tibial diaphysis, BMC and bone area increased in both groups. Relatively larger changes in both of these parameters resulted in a greater increase in strength strain index (SSI) in exercised animals relative to controls over the pregnancy/lactation period. Similarly, the change in the cross-sectional moment of inertia in the frontal plane (CSMI-x) from baseline to post-lactation also differed significantly between groups.

Fecal corticosterone

Mean daily fecal corticosterone excretion during the first four hours of the dark cycle over each four day collection period is shown in Figure 3. There were no significant between-group differences at any time point or between-time point differences in any group. Although the largest within-group numerical change in fecal corticosterone levels was in the exercised group from the GD3-6 to the GD17-20 collection periods this change was not significant (*P=0.25*). However, when data from both groups were combined mean fecal corticosterone excretion was significantly lower in early than in late gestation.

Discussion

We anticipated that the offspring of exercised dams would be significantly heavier than those of controls during the early postnatal period, but they were not. Exercised dams had heavier fetuses relative to controls at GD19 in our previous study[14].

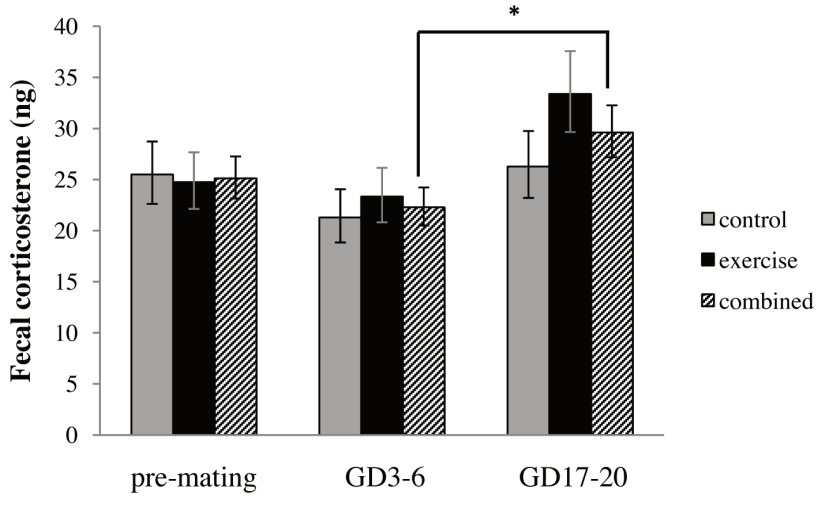

Figure 3. Mean total fecal corticosterone excretion over the first four hours of the dark period for each exercise group and for all groups combined during each 4-day collection period. Data are presented as antilog of lsmeans to facilitate comparison of corticosterone output between studies. The baseline collection period is prior to baseline imaging (pre-mating). There were no significant differences between groups at any time point or between time points for any exercise group. However, when data for all groups were combined, excreted fecal corticosterone at the GD17-GD20 collection period was significantly greater than at GD3-GD6. * Significant difference, $P \leq 0.05$.

That this heavier fetal weight did not persist into early postnatal life may reflect a number of factors. Day 19 of gestation is after the primary period of organogenesis (from implantation to closure of the hard palate) but before the period of greatest total gain of mass in the fetal rat[25,26]. The effects of exercise on fetal growth may differ during the different periods of pregnancy, resulting in different effects on offspring weight. In humans this is known to be the case; exercise in early pregnancy enhances placental function and offspring growth in humans, but continued moderate to high volumes of exercise in later pregnancy is associated with lower birth weight through reduction in fetal fat[2,20]. It is also important to note that in our previous study we found the greatest effects of exercise on fetal weight at GD19 in fetuses located in the mid-uterine horn. In our current study the L1 weights reported are from all live offspring; since the animals had already been born there was no way to be certain of their intra-uterine position. Additionally, as we are interested in the long-term effects of exercise on offspring health, we chose not to disturb the dams and pups on the day of birth so as to allow proper bonding and maternal care, so the early postnatal weights reported are from the day following parturition. At birth rat pups weigh approximately 4.5 g[25], whereas by L1 our pups had grown considerably and weighed approximately 6.5 g. This rapid increase in weight in the immediate postnatal period may also have obscured subtle effects of exercise on pup weight. However, birth or early postnatal weight is only a crude reflection of the intra-uterine environment, and intra-uterine effects on fetal organogenesis during pregnancy can alter organ development without changes in postnatal body weight. For example, it has been reported that pups whose dams underwent exercise during pregnancy had birth weights equivalent to those of control pups, but had increased cell numbers in the hippocampus and better short term memory[27]. Animal models of cardiovascular and renal disease have also shown that later-life disease can result from prenatal influences that do not affect birth weight[28]. In addition, differences in maternal weight during pregnancy, and growth rate and quality in the postnatal period, have also been linked to long-term health effects in both humans and animals[29]. Thus, differences in growth during early fetal life that do not result in differences in postnatal body weight may still have later-life health consequences.

Rats that exercised during pregnancy had a greater post-lactation SSI of the right tibia than non-exercised controls. This was unexpected, as we had previously demonstrated that bipedal stance exercise throughout pregnancy did not significantly alter bone parameters at GD19. The fact that between-group differences were evident after lactation in this study, although exercise was stopped on day 1 of lactation, may indicate that rising to an erect bipedal stance coupled with the rapid weight gain of late gestation increased the strain on the tibia sufficiently to trigger modeling of the bone greater than that induced by pregnancy alone. During pregnancy in the rat, maternal bone meets the demand of the fetuses for calcium by altering bone formation and resorption in a site-specific manner; for example, at the end of pregnancy trabecular bone formation in the lumbar vertebrae has decreased, while cortical bone formation has increased at the periosteal surface of the femur[15]. Since we did not measure bone parameters during and immediately after parturition we do not know if between-group differences were present at that time. However, the increase in the tibial SSI of the dams that exercised during pregnancy clearly shows that there are skeletal benefits of exercise during pregnancy that can persist through lactation.

That the diaphyseal CSMI-x also increased in the exercised dams indicates that the changes reflected by the larger SSI are primarily due to modeling drift, as opposed to changes in bone material. CSMI is a measure of bone geometry, and indicates the bone's ability to resist frontal, sagittal, or torsional bending[30,31]. The increase in CSMI-x indicates that the bone has altered its geometry to resist the bending forces imposed by the caudal muscles of the hindlimb acting to extend the tarsus when the rat rises to a bipedal stance. The torsional CSMI (CSMI-p) also had a greater numerical increase in exercised dams than in controls, although this difference did not reach significance (interaction of exercise group and time, *P=0.08*). In humans, action of the soleus muscle places both bending and torsion forces on the tibia[30]; given the similar anatomic arrangement of this muscle in the rat we may suppose that both bending and torsion stresses are also applied by soleus activity in this species. The CSMI results seen in our study are similar to the findings of other studies in both rats and humans. Yao et al. saw increases in CSMI-x in male rats that performed bipedal stance exercise[32], and Miyagawa et al. found that rats allowed to perform walking exercise had a greater CSMI-x than those that were prevented from walking[33]. In a study examining the relationship between bone structure and distance running in human athletes, Feldman et al. found greater CSMIs in the diaphyseal region of the tibia in runners than in non-runners[30]. Modeling effects, as opposed to material changes, have also been seen in bones at other anatomic sites after exercise. For example, the increased bone strength in the dominant arm of tennis players is primarily due to increases in bone size rather than BMD_v[34].

The differences in bone properties between exercised and control dams are more evident at the tibial diaphysis than at the metaphysis. This site-specificity reflects the forces placed upon the bone by bipedal stance exercise. The forces applied to the bone by this non-impact exercise are primarily those of muscle contraction, which would tend to cause bending forces in the mid-tibia region and not compressive forces at the proximal and distal bone ends. That bones respond to exercise in a site-specific manner is both inherent in Wolff's Law, which holds that bone adapts to the specific loads placed upon it, and has been shown and well-discussed in relation to human runners[30]. The site-specific response of bone to exercise may have implications for the utility of exercise as a therapy for osteoporotic patients.

Dams in our study did not lose whole body bone mineral over pregnancy and lactation. Both groups gained whole body bone mineral content (BMC) and bone area over the study period, in absolute terms, as would be expected in these sexually mature but still growing animals. However, due to a greater increase in bone area than BMC, BMD_a decreased. BMD_a is the ratio of bone mineral to bone area; and can decrease when the BMC decreases or when bone area increases, or both. Increased bone area can increase bone strength (through geometry changes, and also increased total bone mineral content) while leading to a BMD_a decrease. This highlights the fact that decreases in areal bone mineral density do not equal loss of whole body or whole organ bone mineral (reviewed in [35]). It is for this reason that high resolution DXA scans of specific regions, such as the primarily trabecular lumbar vertebrae or the more predominantly cortical femur, are used to make inferences about the state of trabecular or cortical bone. Used in this way DXA can provide useful measures of whole body or regional composition. However, pQCT allows separate analysis of the cortical and trabecular compartments of bone, and can provide a more biologically accurate picture of the bone's response to the physiological states of pregnancy and lactation.

Exercise during pregnancy attenuated the loss of bone mineral over the pregnancy/lactation period from the trabecular bone of the proximal tibial metaphysis. That both groups maintained total, and lost BW-corrected, BMC (measured by DXA) at the primarily trabecular lumbar spine, may be due to the nature of the exercise, which might cause more strain in the bones of the hind limbs than of the spine. Alternatively, it may be due to a physiological tendency to release calcium from the vertebrae rather than the bones of the appendicular skeleton, possibly because of the greater fraction of trabecular bone, which contributes more to meeting the increased calcium demands of gestation and lactation than cortical bone[36]. In humans, exercise during lactation may reduce associated changes in BMD_a of the lumbar spine[37], but little data are available as to whether exercise during gestation, but not lactation, has a similar effect. Although in both humans and rats bone parameters tend to return to "normal" levels after weaning of the offspring and resumption of cycling, this does not occur in all cases[38,39] and low BMD_a in puerperal women has been associated with later osteopenia[7]. The relationship of pregnancy/lactation-associated bone loss with later-life osteoporosis is unclear. Studies have yielded varying results, but little work has been done to examine the long-term effects of pregnancy and lactation on the skeleton of two potentially higher risk groups of women: adolescent mothers and older mothers near the end of their reproductive years[38]. The attenuating effects of exercise during gestation on bone loss that we have seen in this study suggest that even mild exercise during pregnancy could positively influence bone health, perhaps especially in these higher risk populations. Since numerous studies have shown that parental physical activity tends to decrease after having children (reviewed in [40]), and that mothers tend to replace leisure-time exercise with household activities, first pregnancies may present a unique opportunity for targeted exercise interventions at a time when there are fewer perceived barriers to physical activity.

In both the DXA and pQCT scans there were several unexpected between-group differences in imaging parameters at baseline. Whole body bone area (DXA) and trabecular BMC (pQCT) were both significantly less in the exercised group than the controls prior to any experimental intervention. This was surprising, as there were no between-group differences in weight or length. Rats were randomly allocated to the exercise and control groups prior to baseline scanning, and we therefore conclude that these initial differences are due to random chance. Additionally, our repeated measures analysis with dam nested within exercise group accounts for individual dam variability. Therefore, we believe that significant between-group differences in the change in bone parameters from baseline to post-lactation (as indicated by significance of the interaction

between exercise and time as shown in Tables 2-6) reflect exercise-induced differences in the bone response to lactation, rather than accidental pre-treatment between-group differences.

Building on our previous work, we have now shown that bipedal stance exercise in the pregnant rat does not cause a physiological stress response sufficient to chronically elevate fecal corticosterone levels in early, mid or late pregnancy[14]. We used fecal corticosterone to assess the maternal stress response to exercise as it is well-suited to analysis of chronic stressors. We report total corticosterone excreted over the collection period rather than concentration as this provides a more accurate representation of adrenal corticoid production, and our fecal collections were timed to maximize our chances of detecting a stress response, as we have described previously[13]. Maternal stress during gestation can have long-term implications for both humans and rats[41]. Some types of exercise, such as swimming[27] and treadmill running[42], cause a stress response in pregnant rats. However, by choosing a voluntary, moderate exercise we avoided the confounding effects of maternal stress. Although in our model the rats must rise to an erect bipedal stance in order to reach their food, they are able to perform the exercise at their own discretion and without human handling. Additionally, all the rats were monitored to ensure that they were able to reach their food without difficulty. Thus we consider our exercise model to be voluntary in comparison to models such as forced treadmill running or swimming, in which the rat must exercise at a time and intensity not of their own choosing, and which often involve aversive stimuli. An exercise model that does not cause stress in pregnant animals is necessary to examine the effects of exercise during gestation on offspring health without the confounding effects of a stressed uterine environment. The rat model has played a key role in establishing that there are long-term effects of other environmental influences, such as nutrition and stress, during development. The bipedal stance model of exercise that we have utilized does not cause a physiologic stress response in pregnant rats, and thus differences between the offspring of exercised and non-exercised dams can be attributed to the effects of the exercise alone. Thus, this model may be potentially useful for examination of the effects of exercise during pregnancy on the later-life health outcomes of offspring.

Conclusions

Exercise during pregnancy may affect the later-life health of both the mother and her offspring. In this study we demonstrate that very moderate, voluntary exercise in the pregnant rat can improve indices of bone strength measured after lactation and attenuate the loss of trabecular bone mineral, without adversely affecting early postnatal outcomes of the offspring. We have further confirmed that this exercise model does not cause a physiological stress response in pregnant animals, thus allowing its use to examine the effects of exercise during pregnancy on offspring health without the confounding developmental effects of a stressed intrauterine environment. Although we did not see differences in mean early postnatal weights between the offspring of exercised and control dams, previous studies demonstrating differences in fetal growth to GD19 indicate that further evaluation of the long-term effects of this exercise model on offspring health are warranted.

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