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During lactation, women can lose up to 10% of bone mineral density (BMD) at trabecular-rich sites. Previous studies report resistance exercise to slow BMD losses; however, no study has looked at the long-term effects of exercise on BMD during lactation. The purpose of this study was to evaluate the effect of a 16-wk exercise intervention in lactating women from 4-20 wk postpartum on lumbar spine, total body, and hip BMD at 1-y postpartum.

At 4 wk postpartum, women were randomized to the intervention group [IG, n = 18, weight bearing aerobic exercise and resistance exercise] or control group [CG, n = 18, no exercise] for 16 wk. BMD was measured by dual-energy x-ray absorptiometry at lumbar spine, hip, and total body. Repeated-measures ANCOVA was used to test for time and group differences for bone density controlling for covariates: prolactin concentration and calcium intake at 1-y postpartum.

IG lost less lumbar spine BMD from 4 to 20 wk postpartum compared to CG (-3.6 ± 2.1% vs. -5.2 ± 3.3%) and gained similarly from 20 wk to 1-y (IG: 2.7 ± 3.6% vs. CG: 2.7 ± 3.1%). Change in lumbar spine BMD was significantly different over time and between groups from 4 wk to 1-y, when controlling for covariates. No significant differences were seen in total body and hip BMD. These results suggest that resistance exercise may slow bone loss during lactation, resulting in higher BMD levels at 1-y.

THE EFFECTS OF DIET AND EXERCISE ON BONE MINERAL DENSITY DURING THE FIRST YEAR POSTPARTUM

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CHAPTER I

INTRODUCTION

Osteoporosis and osteopenia affects approximately 35 million women in the United States and continues to rise each year (1). Environmental factors such as excessive alcohol intake, poor dietary status, and a sedentary lifestyle increase the risk for the development of this chronic disease (1). Additionally, early bone loss from pregnancy and lactation has been implicated as a risk factor for low bone mass (1). Specifically during lactation, women can lose up to 10% of bone mineral density (BMD) at trabecular-rich sites (lumbar spine, hip, femur, and distal radius) (2). Most women's bone mass is recovered with weaning; however, adolescent mothers, women with short child spacing, and older women who give birth close to menopausal age may not see complete bone recovery, leading to osteoporosis later in life (1).

Exercise strengthens bones by increasing bone formation and decreasing bone resorption (Insert reference). Two studies have examined a 16-week exercise program in breastfeeding mothers: Breastfeeding and Exercise for Healthy Infants and Postpartum Moms (Be Hip Mom) study and Breastfeeding and Exercise for Healthy Infants and Postpartum Moms Too! (Be Hip Mom Too!) (3-4). In addition to the exercise program, the Be Hip Mom Too study included a weight loss component through energy restriction.

Participants in the intervention group of the Be Hip Mom Too study used *MyPyramid Menu Planner for Moms* (5) to record dietary intake daily.

At the end of the 16 weeks in the Be Hip Mom study, the exercise group lost significantly less lumbar spine BMD compared to those who were leading sedentary lifestyles (-4.8 ± 0.6% vs. -7.0 ± 0.3%)(3). In the Be Hip Mom Too! study, lumbar spine BMD losses were similar in each group, (IG: -3.4 ± 2.5% vs. CG: -3.7 ± 3.3%).

The proposed study extends the follow-up time from the two studies to determine the effect of exercise during early lactation on lactation-induced bone loss at one-year postpartum. The specific aim of this study was to evaluate the effect of a 16-week exercise intervention in lactating women from four to 20 weeks postpartum on lumbar spine and hip BMD at one-year postpartum. We hypothesized that women who exercised would lose less lumbar spine and total hip BMD at one-year postpartum compared to those who remained sedentary. We measured dietary intake, hormones, and weight change during this time; these were covariates when analyzing the data. This is the first study to examine the effects of a supervised, randomized exercise intervention on the BMD of lactating women during the first year postpartum.

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CHAPTER II

REVIEW OF THE LITERATURE

Effects of Lactation and Hormonal Response on Bone Mineral Density

By 6 months postpartum, lactating women lose 3-10% of bone mineral density (BMD) at trabecular-rich sites (lumbar spine, hip, femur, and distal radius), equivalent to 2 years of bone loss in women during menopause (1-3). It is known that hormonal changes of prolactin, estrogen, progesterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), and parathyroid hormone-related peptide (PTHrP) effect BMD during lactation (2).

Prolactin, the hormone that stimulates breast milk production in response to infant suckling, rises approximately 10 to 20 times above normal during pregnancy. In lactation, prolactin levels become elevated each time infant suckling takes place. Prolactin is also responsible for inhibiting both LH and FSH, leading to decreased levels of estrogen and progesterone. In addition, prolactin regulates the synthesis of PTHrP in mammary tissue (2). The increase of PTHrP, caused by elevated prolactin levels, combined with the low levels of estrogen upregulates maternal bone resorption and the release of calcium into the bloodstream to be transferred into breast milk for the infant (4).

The exact mechanism by which these hormones actually influences bone resorption is still unclear. To examine specific hormones on BMD, Krebs et al. (5) followed 34 postpartum women (26 fully lactating women and eight non-lactating

women) for 7 months with a follow-up at postweaning (6 months after resumption of menses; about 15 months postpartum). Fifteen of the lactating women completed the postweaning follow-up. Biochemical analysis of hormones, including estradiol and prolactin, and BMD measurements was obtained 0.5, 3, 5, and 7 months postpartum. Repeated-measures analysis of variance was conducted on BMD by time (linear and quadratic), group, and time by group. Stepwise regression with backward elimination was used to predict changes of lumbar spine BMD between 0.5 and 3 months. Independent variables included maternal characteristics: age, parity, height, weight at each cycle, and body mass index (BMI); maternal diet: energy, protein, dietary and total calcium, ratio of calcium to protein, phosphorus, and supplemental vitamin D; infant milk intake; estrogen and prolactin concentrations. With the initiation of lactation, prolactin concentration peaked (140 μg/L) but decreased at 7 months postpartum and 15 months postpartum to levels similar to non-lactating women (50 μg/L and 20 μg/L, respectively). Lumbar spine BMD declined by 0.049 ± 0.072 g/cm^2 during early lactation (0.5 to 3 months) but steadily increased to baseline levels at postweaning measurement. Prolactin was not significantly related to changes in BMD. At 3 and 5 months postpartum, lactating women had significantly lower estradiol concentration (100 pmol/L); however, once lactation had ceased, estradiol concentration was comparable to that of the non-lactating women measured at seven months postpartum (250 pmol/L). Additionally, estradiol concentration was positively associated with change in lumbar spine BMD ($p < 0.001$). Although PTHrP was not measured, intact parathyroid hormone was found to be lower in lactating participants. Research suggests an inverse correlation between serum

parathyroid hormone and PTHrP (6, 7). It is possible that the hormones estradiol, prolactin, PTHrP all work together to affect BMD.

The recent research on PTHrP is limited; however, when evaluating the relationship of elevated PTHrP and decreased concentrations of estrogen on BMD in lactating mice, bone resorption and BMD losses are enhanced (8, 9). Sowers et al. (10) examined the role of PTHrP on BMD by recruiting 115 postpartum women in an 18-month prospective cohort. When controlling for prolactin concentration, breastfeeding status, return of menstruation, estradiol concentration, PTH levels, 1,25-dihydroxyvitmain D levels, dietary calcium, self-reported physical activity, and BMI, elevated PTHrP levels were negatively and significantly associated with BMD change at both the lumbar spine and femoral neck over time ($p < 0.01$). Elevated PTHrP was also associated with elevated prolactin concentration, breastfeeding status, and reduced estradiol concentration ($p < 0.001$), further reinforcing that these hormones are dependent on each other during lactation.

BMD losses usually return to baseline (about 2 to 4 weeks postpartum) measurements once lactation ceases and menses resumes. This is primarily due to the hormones of prolactin and estrogen returning to prepregnant levels (prolactin decreases and estrogen increases). A recent retrospective study conducted by Wiklund et al. (11) found that a longer lactation duration, defined as the total months breastfeeding (exclusively and partial) reported by the mother, was associated with improved bone size and strength 16 to 20 years after lactation ended. Women who breastfed for longer than 33 months were more likely to have larger and stronger bones later in life. One plausible

mechanism the authors suggested is that low estrogen levels during lactation are associated with stimulated periosteal bone formation, which is formed around the outside surface of the bone and contributes to larger and stronger bones. Unfortunately, a limitation to this study was that they did not measure estrogen status to provide evidence. Most research has shown that a chronically low level of estrogen contributes to an increase in bone turnover. While this is the first study to suggest lactation duration being protective of BMD later in life, contrary to others who have only seen the association with parity (12, 13), these findings should be taken with caution.

Paton et al. (12) conducted a study of twins and found that pregnancy and lactation did not have any harmful effects on bone mineral content. The study was divided into three smaller studies. The first studied examined 83 twin pairs of nulliparous and multiparous women. There were no BMD differences observed between parous groups. The second study consisted of 498 twin pairs who were divided into three groups: 1) nulliparous, 2) one to two pregnancies, and 3) three or more pregnancies. Group 3 had significantly higher total body bone mineral content compared to group 1 ($p = 0.03$). The third study was a cross-sectional analysis of 1354 individual women consisting of twin pairs, sister pairs, and their female relatives. The participants were grouped as described above. Adjusted lumbar spine BMD and total bone mineral content was higher in groups 2 and 3 compared to group 1 ($p = 0.001$, $p < 0.001$, respectively). Additionally, adjusted total hip BMD was greater in group 3 compared to group 1 ($p = 0.02$). Z scores of lumbar spine BMD were also higher in groups 2 and 3 than in group 1. Of the 82% who breastfed for more than one month, adjusted bone mineral content and total hip BMD was

significantly increased in these participants; however, lumbar spine BMD was not affected.

Calcium and Vitamin D Intake and Supplementation during Lactation on Bone Mineral Density

Bone is the primary storage site for calcium, storing about 99% of the body's calcium. During lactation, skeletal demineralization is mediated by PTHrP (4). As PTHrP begins to rise and combines with low concentrations of estradiol in the bloodstream, bone resorption is upregulated (4). This results in an increase of calcium and phosphate in the bloodstream, where it travels to the breast (4). Approximately 250 mg of calcium is transferred from mother to breast milk daily (3). To meet the calcium demand, both bone mobilization and renal calcium conservation occurs (4).

Studies determining the effect of calcium intake on BMD have been inconclusive. Krebs et al. (5) reported at postweaning that both dietary and total calcium intake was positively associated with lumbar spine BMD six months after return of menses in well-nourished lactating women ($p = 0.03$). In another study, 10 adolescent (aged 15 to 18) lactating mothers with habitually low dietary calcium intake (444 ± 288 mg Ca/d) were evaluated by Bezerra et al. (15). BMD measurements were conducted at 2 time points: lactation (6-24 weeks postpartum) and postweaning (12-30 months postpartum) with four 24-hour dietary recall questionnaires used to evaluate calcium intake. At postweaning, there was a marginally significant relationship between calcium intake and BMD at the lumbar spine ($p = 0.09$). The small sample size may not have had adequate power to determine a significant difference. Similar to adult lactating women, adolescents had

decreases in BMD during lactation and increases once lactation had ended. Although postweaning bone recovery occurs, the rate may not be sufficient to attain peak bone mass as the adolescent reaches maturity. This may be due to low calcium intake, which can render them prone to the development of osteoporosis later in life.

Supplementation of calcium has also been studied in its relationship with BMD during lactation. Unfortunately, the literature shows that supplementation has little effect on BMD (15, 16, 17). Kalkwarf et al. (16) reported that even though calcium supplementation (1 g calcium/d) did not prevent bone loss during lactation in women with moderate to low calcium diets (< 800 mg per day), it did slightly enhance bone formation in lumbar spine after weaning ($p < 0.05$). When evaluating the effectiveness of calcium supplementation (1 g calcium/d) in lactating Gambian women ($n = 60$) with habitually low calcium intake (< 300 mg/d), Prentice et al. (17) concluded that calcium had no effect on bone mineral content; BMD was not measured. One possible mechanism proposed was that bone and renal adjustments occur independently from calcium status in order to supply calcium to breast milk. The supplementation group had significantly higher urinary calcium output compared to the control group three and 12 months after beginning supplementation ($p \leq 0.005$). Supplemental calcium resulted in a reduction in net fractional calcium absorption and/or an increase in calcium excretion.

In its active form, vitamin D interacts with vitamin-D receptor complexes (VDR-RXR) causing preosteoclasts to mature. Mature osteoclasts help maintain plasma calcium levels by increasing bone resorption if calcium levels are low (18). Fat-soluble vitamin D is essential for promoting intestinal calcium absorption. Plasma calcium levels can be

maintained by increasing absorption in the gastrointestinal tract. In absence of vitamin D, only 10-15% of dietary calcium would be absorbed in the human body (18).

Polymorphisms of the VDR-genotype have been known to alter calcium absorption. A recent study, conducted by Yu et al. (19) evaluated calcium supplementation in lactating Chinese women with habitually low calcium intakes (< 600 mg) with different VDR-genotypes and its effect on BMD. To determine specific VDR-genotypes, a 265-base pair fragment of genomic DNA was extracted, amplified by polymerase chain reaction, and then was digested with Fok1 restriction enzymes. The absence of the Fok1 recognition site in the DNA binding domain demonstrates that cleavage of the fragment will not occur and the allele is defined as "F". The presence of the Fok1 recognition site is defined as "f".

At approximately five weeks postpartum, 120 women were grouped with respect to their VDR-genotype (FF, Ff, ff; n = 40 in each group). Women within each group were then randomly assigned to subgroups where they either received a supplement (600 mg of calcium, n = 20 in each group) or placebo (n = 20 in each group) for one year. Measurements were conducted at baseline, defined as the resumption of menses or termination of lactation (average of 100 ± 46 days postpartum) and one year thereafter. BMD was measured at lumbar spine and left hip at both time points. At baseline, there were no significant differences in BMD between the calcium supplemented and placebo subgroups in any of the three VDR-genotype groups. At one-year postpartum, BMD levels of lumbar spine and left hip significantly increased in the calcium supplemented and placebo subgroups of all three VDR-genotypes groups (FF: $p < 0.05$, Ff: $p < 0.01$, ff:

$p < 0.001$). Women supplemented with calcium in the VDR-genotype "FF" group had a significant increase in percent change of BMD compared to women within the same VDR-genotype given the placebo ($p < 0.05$). Also, there was a significant difference in percent change of lumbar spine BMD between the VDR-genotype "FF" and VDR-genotype "ff" in women who received calcium supplementation ($p < 0.05$). Supplemented women with "ff" VDR-genotype had a lower percent change BMD compared to "Ff" and "FF". The results suggest that women with the VDR-genotype "FF" may have a greater absorption of calcium compared to those with other VDR-genotypes, and when supplementation with calcium occurs, absorption will be further amplified. In addition, serum 25(OH)vitaminD_3 was not significantly different between baseline and one-year follow-up or between groups and ranged approximately 21-30 ng/mL. This was the first study to examine the effects of Fok1 VDR-genotype in postpartum women and suggests that differences within the VDR-genotypes may affect BMD status in women.

Laskey et al. (20) also examined the vitamin D-receptor genotype in breastfeeding women; however, the study was conducted in Caucasian lactating women ($n = 37$) who had moderate to high calcium intakes (1392 ± 528 mg/d), assessed by food frequency questionnaires and a seven-day food diary. DNA was extracted, amplified by polymerase chain reaction, and then digested by using Bsm1 endonuclease. Genotypic polymorphism was defined as BB (absence of restriction site on both alleles), bb (presence of restriction site on both alleles), or Bb (heterozygous). The VDR-genotype frequency of lactating women was: BB, 19%; Bb, 43%; and bb, 38%. Measurements of bone mineral content

and bone area were conducted around two weeks postpartum (baseline) and at three months postpartum. Although there were significant changes in bone mineral composition at the lumbar spine, femoral neck, total hip, and whole body from 0.5 to three months postpartum, results found that VDR-genotype and calcium intake were not related to these changes before or after adjusting for change in bone area. Limitations to this study were 1) a lack of standardizing calcium intake with a supplement to ensure adequate intake, 2) small sample size for each VDR-genotype, 3) women were not equally distributed in each VDR-genotype, and 4) vitamin D status was not reported. This study suggests that the skeletal response during lactation varies from each individual through other mechanisms that need to be further explored.

Currently, the serum vitamin D guidelines are controversial. Historically, vitamin D deficiency has been defined as < 20 ng/mL, insufficiency as 20 to 30 ng/mL, and sufficiency as greater than or equal to 30 ng/mL (21). Laboratory references from Mayo Clinic state that a concentration of 18 to 80 ng/mL of serum vitamin D is considered normal (22). It has also been suggested that for adequate calcium absorption, vitamin D concentrations should be above 32 ng/mL (23, 24, 25). Guidelines from the Institute of Medicine advise that more than or equal to 20 ng/mL is considered sufficient; a recommendation that had been under scrutiny since its suggestion (26). However, a recent study conducted by Kramer et al. (27) exploring serum vitamin D levels in 15,099 adults who participated in the Third National Health and Nutrition Examination Survey, found that mortality rate was similar across the serum vitamin D range of 20 to 40

ng/mL. Additionally, sun exposure, skin pigmentation, and body fat influences serum vitamin D (27).

Serum vitamin D concentration during pregnancy and lactation has not been defined. A recent study, conducted by O'Brien et al. (28) examined serum vitamin D levels during pregnancy and the postpartum period in two cohorts. The Baltimore, Maryland cohort recruited 23 adolescent women (n = 20, African American; n = 3, Caucasian) who intended to breastfeed in the postpartum period. From the San Francisco, California cohort, 13 adult women (n = 11, Caucasian; n = 3, Hispanic) were recruited. Serum vitamin D levels did not differ between pregnancy and the postpartum period or by cohort (San Francisco: 19.3 ± 3.7 ng/mL; Baltimore: 22.4 ± 9.0 ng/mL). Another study by Basile et al. (29) found serum vitamin D status in lactating women (n = 16, Caucasian; n = 9, African American) from South Carolina to be approximately 22.4 ± 8.8 and 28.5 ± 8.6 mg/dL, at baseline depending on the study group. Additionally, Hollis et al. (30) reported a serum vitamin D of 32.9 ng/mL and 27.6 ng/mL at baseline, depending on the study group, in lactating participants from South Carolina.

As stated previously, vitamin D status is important for calcium absorption, and with that bone health. However, the relationship between vitamin D deficiency and BMD remains inconclusive (31, 32). Andiran et al. (31) examined the effects of vitamin D and BMD in 54 lactating mothers in Turkey. People residing in Turkey most often have sufficient vitamin D status from the ultraviolet light; unfortunately, women who dress in the traditional garments may not receive adequate vitamin D from the sun. Low vitamin D status was assessed by evaluating serum 25-hydroxyvitamin D levels; vitamin D

insufficiency was defined if levels fell below 40 nmol/L and deficiency was defined if levels fell below 25 nmol/L. BMD of L1-L4 vertebra and femur neck region was evaluated in 25 mothers who were determined to be vitamin D deficient. Measurements taken showed that 10 of these women were osteopenic. Of these women, all were from the lower socioeconomic class and eight women wore traditional attire. The study did not find any significant relationship between BMD levels and vitamin D levels in deficient mothers ($p = 0.21$, L1-L4 and $p = 0.35$, femur neck). However, since BMD measurements were only conducted in women with low levels of vitamin D, the lack of normal distribution could be a plausible reason why significance was not seen. The lack of recorded calcium intake is also a limitation in this study.

Additionally, Ghannam et al. (32), who studied a lactating population in Saudi Arabia with similar cultural traditions of wearing covered garments, found that 52% of their participants had hypovitaminosis D (24.5 ± 17.2 nmol/L) but the status was not correlated with BMD levels. Vitamin D status did correlate positively with serum calcium levels. BMD in women with severe hypovitaminosis D was measured by DXA and these women had lower z-scores at the spine, femoral neck, Ward's triangle, and trochanter, but BMD did not correlate significantly with vitamin D status. The study reports that the lack of effect on BMD was unexpected. One possible explanation is that the vitamin D levels in the study are below the threshold needed to maintain normal bone mineral density levels.

Effects of Exercise on Bone Mineral Density

There are very few reports on the effects of exercise during lactation on BMD. The first study, conducted by Drinkwater and Chestnut (33), observed changes in bone mineral density in six postpartum female athletes enrolled in a two year longitudinal study. From one month postpartum to six months postpartum, there was a significant decrease in femoral neck BMD by 3.1% and lumbar spine BMD increased 3.4%. Limitations within the study, including small sample size, lack of a non-exercise control group, and absence of defined exercise procedures yielded inconclusive results for determining the effect of exercise on bone mineral density. This study suggests that exercise may be beneficial in preventing losses in lumbar spine BMD and set the foundation for future studies.

Little and Clapp (34) compared 11 fully breastfeeding women who engaged in regular, self-selected recreational exercise for the first three months postpartum with nine (control) non-exercising postpartum women. The exercise group was defined by women reporting exercising more than three days per week longer than 20 minutes per session at more than 50% maximal oxygen consumption. Weight bearing exercise combined with aerobic activity was the primary exercise mode for the exercise group. Total body, lumbar spine, and femur neck BMD was measured at baseline and three months postpartum. At three months postpartum, femoral neck and lumbar spine BMD losses were not statistically significant between the exercise group and control group (lumbar spine: -4.1% and -5.4%, respectively; femoral neck: -2.8% and -2.7%, respectively). The lack of difference may have been due to highly variable exercises (mode, intensity,

frequency, and duration) and the length of study (10 weeks). Lack of random assignment to exercise or control group was another limitation to the study. The duration of the study may not have been long enough and the intensity and type of exercise may not have been appropriate to see the adequate effects of bone remodeling with exercise in this population.

The third study focused on weight bearing aerobic exercise combined with resistance exercise for 16 weeks in 20 fully lactating postpartum women (35). At four weeks postpartum, sedentary women were randomly assigned to an exercise or control group. Aerobic exercise consisted of 45 minutes of walking at 65-85% of predicted maximum heart rate three days per week. Resistance exercises were completed as a split routine three days per week; this focused on strength to the axial skeleton. Lumbar spine BMD loss was significantly less in the exercise group compared to the control (-4.8% vs. -7%, respectively). Hip and total body BMD were not significantly different between groups (total hip: -2.8% exercise vs. -2.2% control; total body: -0.6% exercise vs. -0.8% control). This study was the first to see significant effects of exercise on slowing the loss of BMD, particularly in the lumbar spine, in lactating women.

A recent study conducted by Colleran et al. (36) examined the effects of energy restriction and resistance exercise on BMD in overweight and obese postpartum lactating women. At four weeks postpartum, women (n = 27) were randomly assigned to an intervention (diet and exercise; n = 14) or control group (n = 13) for 16 weeks. Energy restriction of 500 calories per day was used to promote a 0.5 kg to 1.0 kg weight loss per week. Strength exercises (3 days/week) were designed to strengthen the core and

included variations of squats, bench press, bent-over row, deadlift, and military press. Additionally, this group was advised to walk 10,000 steps or 3,000 aerobic steps per day for five days a week. Research assistants traveled to the participants' homes to ensure compliance with the strength training and recorded steps walked. At endpoint, total BMD did not change over time in each group. However, lumbar spine and total hip BMD significantly decreased over time ($p < 0.01$); but differences between groups were not significant [lumbar spine BMD; exercise: -3.4 % vs. control: -3.7%; Hip BMD; exercise and control: -3.1%]. One possible reason as to why significance between groups was not observed is that the study population was either overweight or obese compared to normal weight participants where significance was seen in the previous study.

In nonpregnant, nonlactating women with normal estrogen levels, resistance training has been shown to increase BMD in both lumbar spine and femoral neck as a result of mechanical strain or stress on the bone (37, 38). Krustrup et al. (38) examined the effects of a 16 month training program, consisting of either recreational football (FG), running (RG), or control group (CG) on bone mineral density in 28 untrained premenopausal women (19-47 years old). Training was supervised for one hour twice a week for 16 months and DXA measurements were taken at baseline, four and six months. Whole body BMD was greater ($p <0.05$) in FG after 16 months than after baseline and four month; BMD changes within FG were greater than RG ($p < 0.05$).

In the study conducted by Chilibeck et al. (39), premenopausal women who participated in resistance training using only universal weight machines twice a week did not demonstrate improvement in total body, femoral neck, or lumbar spine BMD. Liang

et al. (40) also observed no improvements in these sites with strength training. However, free-weight (dumbbell) resistance training and jumping (impact) have shown marked improvements in BMD (41, 42).

Bassey et al. (43) found that a vertical jumping exercise regime, comprising of 50 vertical jumps for 20 weeks, significantly improved femoral neck and lumbar spine BMD in premenopausal women compared to the control group. Bassey et al. (43) also examined the effects of jumping on postmenopausal women. The postmenopausal women, not using hormone replacement therapy (HRT), followed a regime of 50 vertical jumps everyday for 12 months. Contrary to the results found in the premenopausal women, the vertical jumps had no effect on femoral neck or lumbar spine BMD. This suggests that alternative methods of training should be applied to increase BMD in postmenopausal women.

Another study reported that aerobic exercise did not affect femoral neck and lumbar spine BMD in postmenopausal women (44). However, when combining aerobic exercise (treadmill, 50 minutes per session, 3 d/wk) with stepping 96 beats for 10 minutes in postmenopausal women, significant improvements in femoral neck and lumbar spine BMD were reported (45). In addition, other studies have combined resistance training and aerobic exercise and indicate positive effects on BMD in postmenopausal women (46).

Effects of Weight Loss on Bone Mineral Density

Approximately 68% of the American adult population is either overweight or obese (47). This excess weight is associated with many health risks; however, this population is believed to have a reduced risk for osteoporosis due to greater BMD. This is

primarily due to extra loading on the weight bearing skeleton and possibly higher circulating concentrations of estrogen (48, 49). Due to the other health risks associated with the overweight and obese population, weight reduction is encouraged. Weight loss can be achieved by creating an energy deficit by restricting calorie intake, increasing energy expenditure, or combining the two. Unfortunately, weight loss has been reported to be associated with losses in BMD (50, 51). One possible mechanism suggests that a decrease in fat mass causes lower levels of circulating androgen precursors to occur, thus decreasing the amount of estrogen and promoting bone loss (49, 52, 53). This is particularly a concern for postpartum lactating women with estrogen levels already lower and lactation-induced bone loss is occurring.

The study by Colleran et al. (36), discussed in the previous section, examined the effects of weight loss by energy restriction and resistance exercise on BMD in overweight and obese postpartum lactating women. Women in the intervention group lost significantly more weight compared to the control group at the end of the 16-week intervention (-5.8 kg vs. -1.6 kg, respectively; $p = 0.03$). Total BMD did not change over time; however, lumbar spine and total hip BMD significantly decreased over time ($p < 0.01$). Group differences were not observed, suggesting that weight loss did not intensify lactation-induced bone loss.

During the menopausal transition, women experience menopause-induced bone loss, losing up to 1.35% of BMD in the spine annually (50). Weight gain of one pound per year usually accompanies menopause. Because of this, weight loss is often encouraged; however, it may further attenuate bone losses during this critical period.

Furthermore, it is important to understand which of the two areas of weight loss (energy restriction and increased energy expenditure) promote or prevent bone losses or alternative strategies like combining weight loss with exercise to prevent BMD loss.

Silverman et al. (54) randomly assigned 86 overweight and obese postmenopausal women to a weight loss or combined program of weight loss and walking for six months. All participants met weekly with a registered dietitian and were instructed to restrict calories by 250-350 kcal/day. Nutritional education was also given to promote a healthy lifestyle. In addition to the weight loss instruction, women in the combined group also participated in a walking regime three days per week, 45-60 minutes per session. Target heart rate of 50-75% maximal was encouraged. At the conclusion of the six month intervention, both groups had significant changes in weight (weight loss: -7.6%. combined: -7.7%; $p < 0.001$), BMI (-7.6% in both groups), and fat mass (weight loss: -15.4%; $p < 0.05$. combined: -12.5%; $p < 0.001$). Significant increases in femoral neck BMD were observed only in the combined walking and weight loss group at six months (+2.01% vs. no change in the control group; $p = 0.001$). Changes in lumbar spine femoral neck BMC and lumbar spine BMC were not seen. The combined group also saw improvement by 11% in osteoporosis after the six month intervention compared to only 6% in the weight loss group (55). Unfortunately, 9% of women in the weight loss group saw a worsening in the classification of osteoporosis compared to the 2% in the combined group. The study indicates that aerobic exercise in addition to weight loss can also protect against menopause-induced bone loss. Wolff et al. (56) conducted a meta-analysis of published control trials in pre- and postmenopausal women and found that in almost all

studies, weight loss by calorie restriction resulted in higher losses of BMD compared to those who achieved weight loss through exercise alone.

Contrary to these results, Macdonald et al. (57) found in normal to overweight postmenopausal women a correlation between weight loss and femoral neck BMD ($r = 0.102$, $p = 0.002$) but not in lumbar spine ($r = 0.026$, $p = 0.436$). For postmenopausal women not using hormone replacement therapy, the effects of weight loss on femoral neck BMD follow-up weight loss on lumbar spine were stronger than current HRT users; however, these results were not significant. When looking at physical activity level, women who lost weight and reported greater physical activity showed less decreases in lumbar spine BMD compared to those who were inactive. A limitation is that physical activity was estimated by a questionnaire, with no clear definition of what type of exercise was used.

Additional studies have been conducted on premenopausal overweight and obese women to further evaluate weight loss and BMD. Uusi-Rasi et al. (58) conducted a three month weight loss program in obese (BMI > 30) premenopausal women predominately through diet, with a nine month maintenance period. All participants received the same intervention with the weight loss program consisting of three parts: week one, low energy diet using the meal-exchange system; weeks two through 10, very low energy diet; and weeks 11-12, low energy diet and education on weight maintenance. Women kept a physical activity diary and recorded the type, intensity, and duration of the exercise completed. Weight reduction resulted in an increase bone turnover, but did not compromise bone strength, bone mineral content, or BMD. The study had several

limitations including lack of an untreated control group to compare bone strength. Also, the weight loss intervention occurred for only three months and exercise logs were used for the total duration of the study. It may be plausible that exercise protected the bone loss during the time of weight loss and weight maintenance.

Summary of Literature Review

Lactation-induced bone loss is usually reversed with weaning and resumption of menses. However, BMD may not return to pre-pregnancy levels in all women. There is a concern that these women are at an increased risk of developing osteoporosis later in life. Several hormones regulate bone resorption during lactation, including prolactin, estrogen, and parathyroid-related peptide (PTHrP). Further research is needed to investigate the mechanism of each hormone on BMD during lactation.

Calcium and vitamin D status also play a major role in bone mineral density. During lactation, calcium is transferred out of the bone and into the breast milk for the infant. Insufficient calcium levels may promote further bone resorption. However, some studies have suggested that those who are low in calcium and supplement with calcium do not see prevention of bone loss. Vitamin D is also important for calcium absorption; if levels are deficient, only 10-15% of calcium will be absorbed, leading to low serum calcium levels. Unfortunately, defined vitamin D status ranges remain controversial. Weight bearing aerobic and resistance exercise may protect bone density during lactation and may enhance bone maintenance when weight loss is occurring.

The study conducted by Colleran et al. (36) was the first to look specifically at the effects of a 16-week intervention of a supervised, randomized exercise combined with

energy restriction on lactation-induced bone loss. Unfortunately, the study only reported changes in BMD during the 16-week intervention and did not address BMD at one-year postpartum. This measure is important because at one-year postpartum, breast milk is no longer the primary source of energy for the infant and in most cases lactation has ceased altogether. With the cessation of lactation and the return of menses, BMD usually returns to baseline levels. Furthermore, the initiation of exercise during the 16-week intervention may enhance bone growth and may still be evident at one-year postpartum.

Therefore, the purpose of our study was to determine if early exercise (4 to 20 weeks postpartum) had any effect on lactation-induced bone loss at one-year postpartum, using the participants enrolled in our two previous studies – Be Hip Mom and Be Hip Mom Too! study. This is the first study to evaluate the long-term effects of supervised, randomized exercise during early lactation on BMD at one-year postpartum.

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CHAPTER III

THE EFFECTS OF DIET AND EXERCISE ON BONE MINERAL DENSITY DURING THE FIRST YEAR POSTPARTUM

Publication in The American Journal of Clinical Nutrition

Introduction

Lactation is a critical period of rapid bone turnover. During lactation, infant suckling stimulates prolactin; the hormone responsible for breast milk production, and it initiates the release of parathyroid hormone related peptide from the mammary tissue into the bloodstream (1). The presence of parathyroid hormone related peptide along with low estradiol concentration in the bloodstream upregulates maternal bone resorption (1). Calcium from bone is then released into the bloodstream, which will be transferred into infant breast milk (1). This increase in bone remodeling results in losses up to 10% of maternal bone mineral density (BMD) at the trabecular-rich sites (lumbar spine, hip, femur, and distal radius) (2). Once resumption of menses and weaning occurs, most women return to their baseline BMD levels; however, adolescent mothers, women with short intervals between pregnancies, and older women who give birth close to menopausal age may not see complete bone recovery, increasing the risk osteoporosis later in life (3).

Previous studies in non-pregnant, non-lactating women have shown that weight-bearing exercise promotes BMD by strengthening the bones and preventing bone resorption in the lumbar spine and femoral neck (4-7). Additionally, studies examining exercise in lactating women have shown decreased losses in lumbar spine BMD. We have reported 2 studies examining a 16-wk exercise program in breastfeeding mothers: Breastfeeding and Exercise for Healthy Infants and Postpartum Moms (Be Hip Mom) study and Breastfeeding and Exercise for Healthy Infants and Postpartum Moms Too! (Be Hip Mom Too!) (8,9). In addition to the exercise program, the Be Hip Mom Too! study included a weight loss component through energy restriction. At the end of the 16 wk in the Be Hip Mom study, the exercise group lost significantly less lumbar spine BMD compared to those who were leading sedentary lifestyles (-4.8 ± 0.6% vs. -7.0 ± 0.3%). In the Be Hip Mom Too! study, lumbar spine BMD losses were similar in each group (-3.4 ± 2.5% vs. -3.7 ± 3.3%).

The purpose of this study was to determine the effect of exercise during early lactation on BMD at 1-y postpartum. We hypothesized that women who exercised during early lactation would lose less lumbar spine and total hip BMD at 1-y postpartum compared to those who remained sedentary. Dietary intake and hormonal status were also assessed at 1-y postpartum.

Subjects and Methods

Study participants

Participants for this study were women enrolled in our 2 previous studies - Be Hip Mom and Be Hip Mom Too! (8,9). The Be Hip Mom study examined the effects of physical activity (resistance and aerobic exercise) on BMD in postpartum normal and overweight breastfeeding women for 16 wk. The Be Hip Mom Too! study examined the effects of a 16-wk resistance and aerobic exercise combined with energy restriction on BMD in overweight and obese postpartum breastfeeding women. Measurements for both studies were conducted at baseline (3 ± 2 wk postpartum) and endpoint (21 ± 2 wk postpartum). For this study, participants returned to the lab at 1-y postpartum (52 ± 2 wk). Measurements were: height; weight; blood draws for hormones; cardiovascular fitness and strength testing; 24-hour dietary recalls; and bone area, mineral concentration and density.

Participants were recruited through prenatal and parenting classes offered at the local hospital and at local obstetricians' offices for both studies. Women were eligible if they were exclusively breastfeeding, healthy, non-smoking, sedentary for at least three months, and had a self-reported body mass index (BMI) between 20 to 30 kg/m^2 (Be Hip Mom) or between 25 to 30 kg/m^2 (Be Hip Mom Too!). Women were excluded if they participated in physical activity more than 3 d/wk in the past 3 mo, delivered by cesarean section, or had a pre-existing condition that disrupted hormonal levels or made them unable to participate in an exercise intervention.

A total of 47 women were randomly assigned to IG or CG and completed the 16-wk intervention (**Figure 1**). Thirty-four of the women enrolled were White, non-Hispanic, 3 Black, non-Hispanic (CG = 2, IG = 1), 1 Asian (IG), and 1 Hispanic (IG). All women enrolled had a college education. In the Be Hip Mom study, 2 women in IG became pregnant and 3 women (IG = 1, CG = 2) were unable to schedule 1-y follow-up appointments. Additionally, in the Be Hip Mom Too! study, 5 women were unable to schedule a 1-y follow-up visit (IG = 3, CG = 2) and 1 woman in CG became pregnant. The final sample size for the combined studies at 1-y follow-up was 36 (18 per group). Random assignments to groups were determined after baseline measurements were completed. Randomization was stratified by parity to control for prolactin levels, as prolactin levels are higher in primiparous compared to multiparous women (10).

The study was approved by the University of North Carolina at Greensboro's Institutional Review Board. Written informed consent was obtained from all study participants.

Study Design

Intervention Group

After baseline measurements were conducted, participants were randomly assigned to IG or CG. Participants in both groups were given a year supply of a multivitamin supplement containing 10 mcg of vitamin D. Beginning at 4 wk postpartum; women in IG completed a 16-wk home-based exercise program that focused on resistance training and aerobic exercise 3 d/wk. The 2 studies had similar intervention protocols designed to increase core strength of the body, the area from the gluteal muscles and hip

up to the scapula, with the intent of increasing bone formation at lumbar spine and hip. Research assistants traveled to the home to ensure exercise compliance. The resistance exercises included variations of squats, bench press, standing military press, dead lift, and bent-over rows, used in one-repetition maximum (1-RM) testing. All exercises were completed at home and participants were given handheld weights and stability balls. After the 16-wk intervention, the women in IG were encouraged to continue exercising.

Both studies included an aerobic component in their intervention. In the Be Hip Mom Study, women were instructed to "briskly walk" at an intensity of 65-80% of each participant's predicted maximum heart rate at time of baseline measurements. Heart monitors were used to confirm subjects were exercising at the prescribed intensity. Aerobic exercise initially lasted 15 min. Time spent in target heart rate gradually increased 5 min/d for the first week and 3 min/d thereafter until aerobic exercise reached 45 min. Once participants exceeded 30 min of aerobic activity, they were instructed to continue 3 d/wk independent of a research assistant's supervision.

The aerobic program in the Be Hip Mom Too! study differed in that participants were given pedometers (Omron Healthcare, Inc., Bannockburn, IL) to monitor steps taken per day. At the beginning of the intervention, participants were instructed to achieve a minimum of 4,000 steps/d and to increase steps by 100 to 200 per day until the goal of 10,000 steps were achieved. Alternatively, 3,000 aerobic steps/d were offered to women who had time constraints. The pedometer recorded aerobic steps as walking at least 100 steps/min for at least 10 min continuously. Three thousand aerobic steps have

been determined by previous researchers to be equivalent to walking 30 min at a brisk pace (11).

Participants in the Be Hip Mom study were instructed to not change their dietary intake; however, the Be Hip Mom Too! study had an additional component to the intervention: restricting energy intake (12). The total energy expenditure equation from the Dietary Reference Intakes (DRI) for overweight, non-pregnant, non-lactating women (13) was used to calculate energy needs. An additional 330 kcal was added for breastfeeding based upon the DRI recommendation, then 500 kcal was subtracted to promote weight loss of 1 lb/wk. No participant was prescribed an energy intake less than 1800 kcal. Participants were asked to enter their dietary intake in *MyPyramid Menu Planner for Moms* (14) at least 3 d/wk. By doing so, dietary compliance was able to be measured along with individualized dietary counseling sessions.

Control group

The women in CG were asked not to participate in any structured exercise or make any changes in their diet. They were allowed to walk their infants in strollers at a leisurely pace (no faster than 2 mph).

After intervention

After the 16-wk intervention, participants in the IG were encouraged to participate in regular exercise. Research assistants did not monitor exercise after the intervention had ceased. The CG was offered the intervention program, including all exercise equipment, exercise protocol and instruction. Dietary counseling was also offered to the CG in the Be

Hip Mom Too! study. All women were contacted monthly to inquire about breastfeeding status and physical activity until their 1-y follow-up visit.

Measures of Treatment

Bone mineral density and anthropometrics

Bone area, mineral composition and density were measured using a different dual-energy x-ray absorptiometry machine for each study (DXA; Delphi A Version 12.3; Hologic, Bedford, MA for Be Hip Mom and Lunar Prodigy Adv., Lunar Radiation Corp, Madison, WI; QDR ENCORE software version 11.20.068 for Be Hip Mom Too!). All 3-time points for each participant in each study were done on the same machine. The same trained technician scanned each participant; this ensured accuracy and precision of the scans. Prior to the participants being scanned, quality control was performed with a phantom spine. All participants lay flat in the supine position on the x-ray table while a total of three scans were performed: total body, lumbar spine (L1-L4) and total left hip (femoral neck, trochanter, and Ward's triangle).

Participant's height was measured using a standardized stadiometer (235 Heightronic Digital Stadiometer, Snoqualmie, WA) and weight was measured using a digital scale (Tanita BWB-800S, Arlington Heights, IL). Participants removed shoes and wore light clothing during measurements.

Cardiovascular fitness and strength

Predicted maximal oxygen consumption (VO_2max) was assessed using a submaximal treadmill test using a modified Balke protocol in both studies. Prior to the treadmill test, blood pressure and resting heart rate (RHR) were measured. Predicted

maximal heart rate was calculated using the heart rate reserve formula [(220-age-RHR) x 85% + RHR]. Participants warmed up on the treadmill for 2 to 3 min and then progressed to a "brisk but slightly uncomfortable" speed either by walking (< 3.7 mph) or jogging (> 3.7 mph). This speed remained constant; however, treadmill grade increased by 2.5% every 2 (Be Hip Mom) or 3 (Be Hip Mom Too!) min and heart rate was recorded at this time. The participants wore heart monitors (Polar, Inc, Woodbury NY or Polar, Electro, Oy, Kempele, Finland) for the duration of the treadmill test and the test was terminated once participants achieved 85% of predicted maximal heart rate. Predicted VO_2max was calculated using the following formulas obtained from ACSM (15):

Walking: (3.5 $mL \cdot kg^{-1} \cdot min^{-1}$) + (speed in $m \cdot min^{-1}$ x 0.1 $m \cdot min^{-1}$) + (grade x $m \cdot min^{-1}$ x 1.8)

Jogging: (3.5 $mL \cdot kg^{-1} \cdot min^{-1}$) + (speed in $m \cdot min^{-1}$ x 0.2 $m \cdot min^{-1}$) + (grade x $m \cdot min^{-1}$ x 0.9)

Predicted maximal oxygen consumption (VO_2max) was determined using a linear regression equation; heart rate (HR) was defined as the independent variable and the dependent variable was the predicted VO_2max.

1-RM tests were used by both studies to assess muscular strength (15). Both studies included squats, bench press, seated or standing military press, stiff-leg deadlifts, and bent-over dumbbell row. Handheld adjustable weights were used for the 1-RM and participants were instructed on proper form and technique for each exercise. Each exercise began at 40% to 60% (Be Hip Mom) or 50% to 70% (Be Hip Mom Too!) of perceived maximum weight capacity with 5 to 10 repetitions. The adjustable weights

increased in total increments of 5-, 10-, 20-lb weights until the participant could no longer complete the full repetition. The heaviest weight lifted without breaking proper form was recorded as the 1-RM. Endurance was measured in both studies by the number of push-up the participants could complete.

Dietary Intake

Dietary intake was assessed by 24-hour dietary recalls at baseline, endpoint, and 1-y postpartum. Recalls were collected in person or by telephone using Nutrition Data System for Research (NDSR, University of Minnesota) software, using a multiple pass system. The Be Hip Mom study conducted recalls on two separate days within the same week and the Be Hip Mom Too! study conducted 3 recalls. All participants were given a food amounts booklet to help determine portion sizes of each food item consumed. Results of the analyses were then averaged for each measurement period.

Hormones and vitamin D status

Serum samples were collected at baseline, endpoint, and 1-y postpartum for analysis of hormones. A trained phlebotomist drew blood samples at the same time each morning, after an overnight fast to control for diurnal variation. Serum samples were then frozen at -70ºC until analyzed. Serum prolactin and estradiol were quantified by enzyme-linked immunosorbent assays (ELISA) (Alpco Diagnostics, Salem, NH). All samples used were thawed once and analyzed in triplicate. Samples for baseline and endpoint for each participant were analyzed in the same assay to eliminate inter-assay variability; however, 1-y samples were analyzed separately. Serum samples were also analyzed, in

duplicate, to determine total 25-OH Vitamin D status using ELISA (DRG International, USA, 450 nm absorbency).

Statistical Analysis

Data were analyzed with JMP software (Version 9.0.0; SAS, Cary, NC). Results are reported as means (SD). Characteristics of each group were compared using ANOVA and Pearson's chi-squared test. Repeated-measures ANOVA was used to test for time and time by group differences in body weight, BMI, strength (1-RM), cardiovascular fitness (VO_2max), dietary intake (energy, protein, calcium, vitamin D), serum vitamin D concentration, and hormone concentrations (prolactin and estradiol).

The main outcome of interest in this study was the effect of exercise on BMD at 1-y postpartum. **Figure 2** is our conceptual model showing what other factors may influence BMD at 1-y postpartum. Those covariates identified were: serum concentrations of vitamin D, prolactin, and estradiol; calcium intake; and weight change from baseline to 1-y postpartum. Although estradiol was included in our model, it is not included in our data analysis because we did not record the phase of the participant's menstrual cycle when serum samples were collected. Repeated-measures ANCOVA was used to test for time and time by group differences for bone (density, mineral content, and area) controlling for these covariates: percent weight change from baseline to 1-y postpartum; and prolactin concentration, calcium intake and vitamin D concentration at 1-y postpartum. Statistical significance was set at $p \leq 0.05$.

Results

Baseline characteristics of participants who completed measurements at all 3 time points in each study are shown in **Table 1**. Prepregnant weight ($p = 0.01$), baseline weight ($p = 0.01$), and baseline BMI ($p = 0.001$) were significantly different between studies, because of the difference in eligibility criteria. However, there were no significant differences in baseline characteristics of the IG and CG of combined data from the 2 studies, used in this analysis (**Table 2**). At 1-y postpartum, the percent of participants still breastfeeding was higher in CG compared to IG; however, this difference was not significant ($p = 0.14$).

Forty one percent of women in CG and 44% in IG resumed their menses at 1-y postpartum with the mean return of menses at 28 ± 14 in CG and 34 ± 13 wk postpartum in IG. Forty four percent of the women in IG and 67% of the women in CG were still breastfeeding at 1-y postpartum; frequency was no recorded. The average weeks postpartum women terminated breastfeeding was 46 ± 11 wk and 42 ± 11 wk postpartum in CG and IG, respectively. Ten women in CG and 9 women in IG began hormonal birth control during the 1-y study period. In both groups, women reported beginning hormonal birth control around 14 wk postpartum. Types of birth control included intrauterine devices, progesterone only pill, and a combination of progesterone and estrogen pill. Of the 19 women on birth control, 3 terminated birth control usage by 1-y postpartum (CG, n = 1; IG, n = 2).

Compliance for aerobic activity was assessed for the Be Hip Mom study (n = 6) by how many aerobic training days were completed (total of 48 d). Women were able to

complete an average 41 aerobic training days (86%). In the Be Hip Mom Too! study (n = 11), average daily steps were recorded to monitor compliance. The average daily steps were 5,385, only meeting 54% of our goal of 10,000 steps. Both groups experienced significant increases in predicted VO_2max over time (**Table 3**). CG had higher measurements of predicted relative VO_2max (ml/kg/min) compared to IG; however, absolute VO_2 (L/min) did not differ between groups or by time.

Women in IG significantly increased muscular strength in all exercises compared to CG. Compliance for resistance exercises was assessed by how many resistance-training sessions were completed (total of 48 d). Women (n = 17) were able to complete an average of 44 resistance-training sessions (92%). Research assistants also did not monitor exercise after the intervention had ceased. In IG, 10 women reported participating in structured exercises after the 16-wk study period. Five women in CG reported beginning an exercise regime after the 16 wk. The average estimated duration of exercise after intervention to 1-y postpartum was 28 weeks. Types of exercises performed by both groups included running, biking, and resistance exercises. All exercises were at a lower intensity and frequency as the 16-wk exercises. Research assistants also did not monitor exercise after the intervention had ceased.

Energy (kcal) intake in both groups significantly decreased from baseline to 1-y postpartum but was not significantly different between groups ($p = 0.005$) (**Table 4**). Protein, calcium, and vitamin D intakes did not change from baseline to 1-y postpartum or between groups. The percent of energy from carbohydrates significantly decreased from baseline to 1-y postpartum and between groups; both protein and fat did not differ.

The percent of participants who met the Estimated Average Requirement for vitamin D (10 ug) and calcium (800 mg) is shown in **Table 5**; the ranges of intake are shown (16). Additionally, 3 women (8%, CG, n = 2; IG, n = 1) reported taking calcium supplements (amount unknown) at 20 weeks postpartum. At 1-y postpartum, 6 women (17%), 3 in each group, reported supplementing their diets with calcium.

At baseline, 28% of the participants were categorized as having a normal BMI; 47% and 25% were classified as overweight and obese, respectively. At 1-y postpartum, 67% of the participants were classified as having a normal BMI, 14% were overweight, and 19% were obese. Weights from baseline to endpoint were significantly greater in IG compared to CG ($p = 0.026$) (**Table 6**). Weight change was not different between groups or over time. BMI significantly decreased ($p = 0.001$), with the change from baseline to endpoint significantly greater in the IG ($p = 0.038$). At endpoint, more participants in IG returned to prepregnant weight status compared to the participants in CG. At 1-y postpartum, approximately 30% of women returned to prepregnant weight.

From baseline to 1-y postpartum, prolactin and estradiol concentrations in both groups changed significantly, $p < 0.05$ (**Table 7**). Serum vitamin D concentration did not differ by group or at baseline and 1-y postpartum. No correlations were observed between serum and dietary vitamin D; however, serum vitamin D concentrations at baseline correlated with serum vitamin D concentrations at 1-y postpartum ($r = 0.53$). Serum vitamin D concentration at baseline or 1-y did not correlate with BMD (lumbar spine, total hip, or body) or changes in BMD at any time point.

The mean concentrations of serum vitamin D at baseline (n = 34) and 1-y postpartum (n = 35) by group is shown in Table 7. The mean concentration of vitamin D of all study participants at baseline was 26.4 ng/mL and 25.7 ng/mL at 1-y postpartum, with no differences between groups. The ranges of serum vitamin D at baseline was 22.6 – 32.5 ng/mL and 20.9 – 28.7 ng/mL at 1-y postpartum. No correlations were observed between serum and dietary vitamin D; however, serum vitamin D concentrations at baseline correlated with serum vitamin D concentrations at 1-y postpartum ($r = 0.53$).

Both groups lost lumbar spine, total body, and hip BMD from baseline to endpoint (**Table 8**). At 1-y postpartum, lumbar spine and hip BMD changed over time ($p < 0.001$) but not by group. All participants were within the normal ranges of BMD levels. Percent changes in total body, hip, and lumbar spine BMD are shown in **Table 9**. Percent weight change from baseline to 1-y postpartum was not significantly correlated to lumbar spine BMD. Therefore it was not included as a covariate in our data analysis. Additionally, BMI was not significantly correlated with BMD. When controlling for dietary calcium intake and prolactin concentration at 1-y postpartum, time by group changes were observed. IG lost significantly less lumbar spine BMD from baseline to 1-y postpartum compared to CG. Additionally, no significant time or group differences were observed in total body or hip BMD.

At 1-y postpartum, 7 (39%) of the 18 participants in IG had returned to their baseline lumbar spine BMD values (**Table 10**). Only 2 (11%) of the 18 participants in CG returned to their baseline lumbar spine BMD values. Of the 7 women in IG, 5 had continued exercising after intervention; 1 participant in CG who returned to baseline

lumbar spine values began exercising. The participants who returned lost significantly less lumbar spine BMD from baseline to endpoint and gained significantly more lumbar spine BMD from endpoint to 1-y postpartum ($p < 0.05$). Five participants (14%; IG = 2, CG = 3) returned to their baseline hip BMD values at 1-y postpartum (**Table 11**). Only 2 participants (IG = 1, CG = 1) returned to both their baseline lumbar spine and hip BMD at 1-y postpartum.

Discussion

The results of this study suggest that resistance exercise during the first year postpartum slows BMD losses in lactating women. Few studies have examined the effects of exercise on BMD in breastfeeding women. In the Be Hip Mom study, the exercise group lost significantly less lumbar spine BMD compared to those leading sedentary lifestyles (-4.8 ± 0.6% vs. -7.0 ± 0.3%) (8). However, in the Be Hip Mom Too! study, exercise did not have a significant effect on lumbar spine BMD (IG: -3.4 ± 2.5% vs. -3.7 ± 3.3%). One possible explanation as to why the results were different between studies may be due to control participants having a higher average BMI in the Be Hip Mom Too! study compared to the latter (27.9 vs. 24.7 kg/m^2). In our study, mean baseline BMI for total participants was 27.5 kg/m^2; however, we did not have enough power to see a correlation between BMI and changes in BMD. This heavier weight may be a contributing factor preventing bone loss during lactation. Previous studies have shown that BMI is positively correlated with BMD (18); however, more recent animal research suggests that a high BMI is related to inflammatory makers which may negatively affect bone mass (19, 20). Additionally, Tanaka et al. (21) found that overweight/obese

postmenopausal Japanese women were at an equal risk of fractures compared to women who are underweight. Therefore, it is unclear whether a greater BMI provides protection against bone loss.

Little and Clapp (22) compared changes in BMD of breastfeeding women engaging in self-selected exercise (3 d/wk, at least 20 min/d) to non-exercising breastfeeding women. Over the 3-mo study period, both groups lost similar amounts of BMD at the femoral neck (IG: -2.8 vs. CG: -2.9) and lumbar spine (IG: -4.1 vs. CG: -5.4). The authors theorized that the variability in exercises (mode, intensity, frequency, and duration) was not as effective as a standardized, structured exercise program. Additionally, the majority of the women performed aerobic activity; resistance exercises may be more effective at stimulating bone growth.

Drinkwater and Chestnut (23) followed 6 female athletes during lactation and compared their BMD changes to exercising, non-pregnant, non-lactating women. Femoral neck BMD decreased in the breastfeeding athletes; however, decreases in lumbar spine BMD were not observed. Although the study had the limitations of a lack of structured exercise and non-exercise lactating control group, the authors hypothesized that the exercise done by the lactating mothers may have been protective against lumbar spine bone loss.

The usual bone turnover cycle occurs over 4 to 8 mo; however, during lactation, this cycle is shortened to 3 to 4 mo (24). It is possible that the study duration by Little and Clapp and Drinkwater and Chestnut may not have been long enough to see significant changes in BMD. The 16-wk intervention completed in both our studies was appropriate

to allow for multiple cycles of bone turnover. Additionally, the resistance exercise in both our studies targeted the core body and was successful at stimulating bone growth in the lumbar spine during the bone turnover cycle.

At 1-y postpartum, only 9 participants returned to their baseline lumbar spine BMD and only 5 returned to baseline hip BMD. These results are different than previous studies that reported the majority of participants returning to baseline BMD (25, 26). The small number of women returning to baseline BMD may be due to a large percentage of women still breastfeeding in both groups (IG: 44%, CG: 67%, $p = 0.18$). However, mean prolactin and estradiol concentrations were not different between those returning to baseline BMD versus those still lower than baseline levels. The 9 women who returned to baseline BMD values lost significantly less lumbar spine BMD from baseline to endpoint (-2.2% vs. -5.1%) and gained significantly more from endpoint to 1-y (5.2% vs. 1.8%) than those who did not return to baseline lumbar spine BMD values. In addition, there were significantly more women in the IG versus the CG, suggesting that resistance exercise had a significant effect in returning to baseline BMD.

Prolactin and estradiol concentrations were similar to those reported by Krebs et al. (2). From 2 to 20 wk postpartum, their study reported a decrease in prolactin from approximately 140 ug/L to 50 ug/L. Our prolactin concentrations were also elevated at baseline and declined during the 16-wk intervention, continuing to decline until 1-y postpartum. In their study, estradiol concentrations were elevated at 2 wk (300 pmol/L) and at 20 wk postpartum, estradiol concentrations decreased to 100 pmol/L. The estradiol concentrations in our study were also elevated at baseline and declined during the 16-wk

intervention; at 1-y postpartum, estradiol began to rise. Both our study and that of Krebs et al. (2) reported the average lumbar spine BMD loss of all participants at 20 wk postpartum to be approximately 4%. In their study, estradiol was positively associated with change in lumbar spine BMD ($p < 0.001$); we did not see this in our study. However, we did not record the phase of the participant's menstrual cycle when serum samples were collected and this may be a reason why we did not see a relationship.

Calcium and vitamin D play a major role in bone mineral density. However, their effects on BMD during lactation are inconclusive. Additionally, supplementation with calcium may not prevent bone loss during lactation, but may enhance bone formation after weaning (27). The Recommended Dietary Allowances of calcium for lactating and non-lactating women is 1,000 mg (28). Although mean dietary calcium was adequate at all three time points, the range of dietary calcium revealed that not all participants were consuming the recommendation. Calcium was included in our analysis because of the correlation between intake and BMD seen in previous studies. Krebs et al. (2) reported that adequate dietary calcium was positively associated with lumbar spine BMD at 6 mo after the return of menses. The average weeks postpartum that women resumed their menses in our study was 28 ± 14 wk for the control group and 34 ± 13 wk for the exercise group. Our 1-y measurements of calcium intake and BMD are approximately 6 mo after the return of menses for most women, similar to that observed by Krebs et al. (2). It is possible that adequate calcium intake is necessary for bone formation after weaning. However, dietary calcium intake at any time point did not correlate with BMD at any time point, but was a significant covariate.

The Recommended Dietary Allowances for vitamin D during lactation is currently 15 ug/d (28). All participants were given a year supply of a multivitamin containing 10 ug of vitamin D. Based on our results; the mean dietary vitamin D of all study participants was 5.2 ug, significantly lower than the RDA. It is also important to note that there were no significant differences between IG or CG. Dietary vitamin D was not correlated with lumbar spine BMD at any time point. However, vitamin D intake at 1-y postpartum positively correlated with calcium intake at 1-y ($r = 0.54$). This is similar to the correlations found by Ghannam et al. (29).

The serum vitamin D guidelines are controversial. Historically, vitamin D deficiency has been defined as < 20 ng/mL, insufficiency as 20 to 30 ng/mL, and sufficiency ≥ 30 ng/mL (30). Guidelines from the Institute of Medicine advise that more ≥ 20 ng/mL is considered sufficient; a recommendation that had been under scrutiny since its suggestion in 2010 (31). However, a recent study conducted by Kramer et al. (32) exploring serum vitamin D levels in 15,099 adults who participated in the Third National Health and Nutrition Examination Survey, found that mortality was similar across the serum vitamin D range of 20 to 40 ng/mL. Using the newer guidelines from the Institute of Medicine, in our study, 100% of our participants at baseline and 1-yr postpartum were ≥ 20 ng/mL. Vitamin D concentration of lactating women has been previously evaluated, and our mean serum vitamin D is similar to previous studies (33, 34). Basile et al. (33) found serum vitamin D status in lactating women from South Carolina to be approximately 22.4 ± 8.8 mg/dL and 28.5 ± 8.6 mg/dL, depending on the

study group. Additionally, Hollis et al. (34) reported a serum vitamin D of 32.9 ng/mL and 27.6 ng/mL in lactating participants.

Women in IG significantly improved muscular strength over time compared to CG. Unfortunately, neither group saw improvements in cardiorespiratory fitness. This may have been due to many participants not meeting the 10,000-step/d recommendations. Contrary to the resistance exercises, research assistants were not present during the aerobic activities in the Be Hip Mom Too! study. Additionally, women in CG improved their cardiorespiratory fitness during the 1-y period. The CG was instructed not to participate in any structured exercise during the 16-wk intervention, but was allowed to walk their babies not faster than 2 mph. Although women in IG lost significantly more weight from 4 to 20 wk postpartum compared to CG, the latter had lower weight at baseline and lost more weight from 20 wk to 1-y postpartum. This weight loss, in both groups, may have contributed to the improvements in aerobic fitness, as shown by predicted relative VO_2max.

Additionally, our study was the first to examine the relationship between weight change and BMD during lactation; previous studies have examined this in the postmenopausal period. Wolffe et al. (35) revealed a correlation between calorie restriction and higher BMD losses; however, weight change in our study did not correlate with BMD.

There are a number of strengths to our study. In both studies, we tested a supervised, randomized exercise intervention as opposed to having participants report exercise completed over time. By doing so, we were able to confirm compliance. The

exercise program also targeted the core body, essential in stimulating bone growth at the lumbar spine. BMD was also measured longitudinally at 3 time points to better understand the changes in bone during lactation using state of the art methods, including statistical analysis by repeated measures over time. Covariates were also addressed and controlled for during statistical analysis. A limitation is the number of dropouts due to pregnancy and moving away from the area. After adjusting for those who did not have BMD measurements at 1-y postpartum, our sample size decreased from 47 to 36. BMD measurements were also conducted on two different machines; however, all 3-time points for each participant were done on the same machine. Although the number of participants who took calcium supplementation were reported, it may have been more beneficial to record amount of calcium consumed in the supplement by these individuals. Multivitamin supplements containing 10 ug of vitamin D was given to each participant; however, compliance was difficult to measure. Additionally, most of the women resumed their menses at 1-y postpartum; however, we did not record the phase of the participant's menstrual cycle when serum hormone levels were collected.

In conclusion, women who participated in a 16-wk resistance and aerobic exercise intervention lost significantly less lumbar spine BMD during the first 20 wk postpartum, resulting in higher lumbar spine BMD levels at 1-y postpartum as compared to women who did not exercise during the postpartum period. Additional research is needed to determine the effects of exercise and diet on BMD during the postpartum period, given the low percent of women who did return to their baseline BMD levels at 1-y postpartum.

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CHAPTER IV

EPILOGUE

The two studies presented in this thesis would not have been successful without the help and dedication of past researchers, research assistants, research staff, and participants. I myself have seen the study evolve in the past three years. The study participants and nutrition professionals that I have met while apart of the study afforded me the experience and confidence to continue on to improving maternal and infant health through nutrition. I have enjoyed my time spent on the study, especially meeting and working with the study participants. I began as an undergraduate research assistant in the Be Hip Mom Too! study. As a research assistant, I had the opportunity to interact with the study participants by facilitating exercise protocols, conducting laboratory assessments, scheduling laboratory meetings, and managing monthly follow-up call logs. My commitment to the study continued as I began the master's program where I evaluated the two studies at one year postpartum.

The presented research was the first to describe and compare changes in bone mineral density, diet, and hormone concentrations in exercising and sedentary women during lactation through one-year postpartum. It also examined the effects of an exercise intervention on the lessening of lactation-induced bone loss in mothers at one-year postpartum.

Our original sample size of the combined two studies was 47; however, we lost many of these women at one-year follow-up due to pregnancy and the lack of availability by the participants. Because of this, our sample size dropped to 36 at one-year postpartum. A larger sample of postpartum lactating women in future studies may be necessary to ensure adequate power at one year.

Another problem that we encountered was that we measured breastfeeding status by call logs. Every month, we asked mothers if they were still breastfeeding; however, knowledge of how many times per day the women were breastfeeding was unknown. Calcium supplementation was also asked during these call logs, but amounts of the supplements were not recorded. Phase of menstrual cycle was not accounted for when the mother's came into the lab for serum hormone concentration collection; therefore, our estradiol concentration was not a strong predictor of bone health. Another possibility for future research would be to account for phases in the menstrual cycles when women come in for their laboratory measurements to ensure accuracy of our findings.

After the 16-week intervention, all participants were recommended to continue exercising. The participants in the control group also had the opportunity to receive the same equipment and exercise protocol, if desired. The monthly call logs included a question about exercise; however, duration, type, and frequency of the exercise were not recorded. In a future study, the development and implementation of a different call log to include the items left out from the previous one would be beneficial.

During the study period, most participants were unable to achieve the aerobic activity recommendations. Many participants from the Be Hip Mom Too! study stated that lack of time, bad weather, illness, and lack of childcare or spousal support were the main barriers affecting meeting their goal. For future studies, the incorporation of walking groups, encouraging family walking programs, providing walking goal incentives, and incorporating indoor aerobic activities may help participants meet their aerobic goals.

There are a few areas for future research that I would like to see explored. The first would be to understand the effects of calcium and vitamin D on bone during lactation. Both adequate calcium and vitamin D status are essential for bone health. Although the majority of women met the Estimated Average Requirement for calcium, some participants' intakes were far below this. Additionally, many women did not meet the Estimated Average Requirement for vitamin D intake. I would recruit postpartum lactating women, and first determine their VDR-genotype. From there, I would then randomize the women in each genotype into four study subgroups (subgroup 1: control, subgroup 2: calcium and vitamin D supplementation, subgroup 3: resistance exercise, and subgroup 4: resistance exercise combined with calcium and vitamin D supplementation). By doing this, the role of VDR-genotype will be further explored along with its role during exercise on BMD. It would also be important to study the concentration of serum vitamin D in breast milk to determine if maternal status correlates with infant vitamin D status.

Another area of research would be to understand why so few women did not return to baseline BMD levels. As mentioned earlier, only 7 women returned to their baseline lumbar spine BMD at 1-y postpartum. Developing a longitudinal study examining women prior to pregnancy and following them to 2-y postpartum would be beneficial to understand changes in BMD.

APPENDIX A

TABLES

Table 1

Baseline characteristics of participants who completed the 1 y measurements in the intervention group and control group of each study

	Be Hip Mom			Be Hip Mom Too!		
	Total Sample n = 15	Intervention Group n = 7	Control Group n = 8	Total Sample n = 21	Intervention Group n = 11	Control Group n = 10
Characteristic						
Age (y)	31.5 (2.6)	31.4 (2.2)	31.5 (3.0)	31.3 (3.7)	32.4 (3.2)	30.2 (3.9)
Parity (n)						
Primiparous	6	2	4	7	3	4
Multiparous	9	5	4	14	8	6
Prepregnancy weight (kg)[1]	61.5 (11.9)	62.4 (14.2)	60.7 (10.3)	72.6 (13.1)	76.0 (8.8)	68.9 (16.3)
Weight (kg)[1]	67.9 (12.2)	67.0 (11.6)	68.7 (13.4)	78.9 (11.6)	83.4 (8.6)	73.9 (12.8)
Height (cm)	164.1 (7.8)	161.9 (6.5)	166.0 (8.8)	164.3 (6.2)	165.9 (5.3)	162.6 (7.0)
BMI ($kg \cdot m^{-2}$)[1]	25.1 (3.3)	25.5 (3.7)	24.7 (3.1)	29.1 (3.4)	30.4 (3.5)	27.8 (2.8)
Weeks postpartum at 1 y measurement	52.5 (7.5)	53.0 (7.7)	52.1 (7.9)	53.5 (2.5)	54.1 (3.0)	52.9 (1.9)

Values are means (SD)

[1] Significantly different between studies, ANOVA, $p \leq 0.01$.

Table 2

Baseline characteristics of participants who completed 1 y measurements in the intervention group and control group with data combined from two studies

	Intervention Group n = 18	Control Group n = 18
Characteristic		
Age (y)	32.0 (2.8)	30.8 (3.5)
Parity (n)		
Primiparous	5	8
Multiparous	13	10
Prepregnancy weight (kg)	70.7 (12.8)	65.2 (14.2)
Weight (kg)	77.0 (12.6)	71.6 (13.0)
Height (cm)	164.3 (5.9)	164.1 (7.8)
BMI ($kg \cdot m^{-2}$)	28.5 (4.3)	26.4 (3.3)
Weeks postpartum at 1 y measurement	53.6 (5.1)	52.6 (5.3)
% Still breastfeeding at 1 y measurement	44.4%	66.7%

Values are means (SD)
No significant differences between groups.

Table 3

Cardiovascular fitness and muscular strength of the participants in the intervention and control groups

	Intervention Group			Control Group		
	Baseline	Endpoint	1 y	Baseline	Endpoint	1 y
VO_2 (mL/kg·min) [1,2] (CG; n = 14) (EG; n = 14)	32.5 (4.5)	36.0 (5.5)	35.7 (6.4)	32.3 (4.6)	34.6 (6.0)	37.1 (6.1)
VO_2 (L/min) (CG; n = 14) (EG; n = 14)	2.5 (0.5)	2.6 (0.6)	2.5 (0.7)	2.3 (0.5)	2.3 (0.5)	2.4 (0.5)
Squats (lbs) [1,2] (CG; n = 15) (EG; n = 13)	78 (21)	108 (24)	98 (24)	75 (19)	86 (23)	94 (24)
Bench press (lbs) [1,2] (CG; n = 15) (EG; n = 13)	47 (12)	63 (13)	60 (13)	50 (16)	52 (17)	58 (17)
Bent-over row (lbs) [1,2] (CG; n = 15) (EG; n = 13)	48 (18)	70 (14)	62 (17)	46 (13)	50 (13)	57 (15)
Deadlift (lbs) [1,2] (CG; n = 15) (EG; n = 13)	77 (19)	109 (19)	100 (26)	73 (21)	81 (24)	89 (25)
Military press (lbs) [1,2] (CG; n = 15) (EG; n = 13)	39 (10)	48 (8)	47 (10)	39 (10)	39 (10)	42 (10)

Values are means (SD)

[1] Significantly different over time, RMANOVA, $p < 0.05$.

[2] Significantly different over time between groups, RMANOVA $p < 0.05$.

Table 4

Reported dietary intake of the participants in the intervention and control groups

	Intervention Group			Control Group		
	Baseline (n = 18)	Endpoint (n = 18)	1 y (n = 18)	Baseline (n = 18)	Endpoint (n = 18)	1 y (n = 16)
Energy						
kcal[1]	2220 (503)	1842 (399)	1706 (360)	2131 (571)	1829 (556)	1750 (498)
kcal/kg	29.4 (7.3)	26.8 (8.9)	25.7 (8.3)	30.2 (8.2)	26.4 (4.5)	27.8 (10.4)
% Energy from:						
CHO[2,3]	52.3 (4.6)	54.7 (5.7)	53.3 (5.5)	51.8 (6.2)	48.7 (6.7)	49.5 (9.1)
Protein	16.2 (2.6)	16.7 (3.7)	18.1 (4.1)	15.0 (3.1)	17.7 (3.7)	19.2 (5.5)
Fat	33.0 (4.3)	30.2 (5.4)	30.1 (6.5)	34.9 (5.4)	35.3 (4.8)	33.0 (7.5)
Protein						
g	88.7 (19.0)	76.0 (21.3)	76.0 (18.1)	78.9 (22.7)	79.4 (21.7)	80.2 (22.4)
g/kg	1.2 (0.3)	1.1 (0.4)	1.1 (0.4)	1.1 (0.3)	1.2 (0.3)	1.3 (0.4)
Calcium (mg)	1366 (477)	1124 (423)	1031 (500)	1091 (374)	990 (292)	1111 (526)
Vitamin D (mcg)	5.3 (2.6)	4.9 (3.1)	4.8 (3.5)	4.4 (2.5)	4.5 (2.2)	5.6 (3.7)

Values are means (SD)

[1] Significantly different over time, RMANOVA, $p < 0.05$.

[2] Significantly different over time between groups, RMANOVA, $p < 0.05$.

[2] CHO: Carbohydrates

Table 5

Dietary calcium and vitamin D intake of all participants

	Baseline	Endpoint	1 y
Calcium			
% of participants that met the EAR of calcium (800 mg)	81%	75%	71%
Range (mg)	483-2068	209-1834	224-2343
% of participants that met the EAR by group			
Intervention	89% (16/18)	83% (15/18)	67% (12/16)
Control	72% (13/18)	67% (12/18)	75% (12/18)
Vitamin D			
% of participants met the EAR of vitamin D (400 IU or 10 mcg)	8%	5.5%	8.8%
Range (mcg) Range (IU)	0.995-10.6 40-424	0.44-11.1 18-444	0.617-15.78 25-631
% of participants met the EAR by group			
Intervention	11% (2/18)	11% (2/18)	11% (2/18)
Control	5% (1/18)	0% (0/18)	6% (1/16)

EAR Estimated Average Requirement

Table 6

Weight and weight changes of participants in the intervention and control groups

	Intervention Group (n = 18)			Control Group (n = 18)		
	Baseline	Endpoint	1 y	Baseline	Endpoint	1 y
Weight (kg)[1]	77.0 (12.6)	71.6 (12.6)	69.8 (14.6)	71.6 (13.0)	69.1 (15.5)	65.8 (16.2)
Weight change						
Base to end[2]		-5.5 (3.3)			-2.5 (4.2)	
End to 1 y			-1.8 (5.6)			-3.3 (3.1)
Base to 1 y			-7.3 (7.8)			-5.8 (4.5)
% Weight change						
Base to end		-7.2 (4.2)			-4.0 (6.0)	
End to 1 y			-2.7 (7.1)			-5.0 (4.3)
Base to 1 y			-9.6 (9.4)			-8.9 (6.4)
BMI ($kg \cdot m^{-2}$)[3]	28.5 (4.3)	26.5 (4.4)	25.9 (5.5)	26.4 (3.3)	25.5 (4.1)	24.2 (4.3)
BMI change						
Base to end[3]		-2.0 (1.2)			-1.0 (1.6)	
End to 1 y			-0.6 (2.0)			-1.2 (1.2)
Base to 1 y			-2.6 (2.8)			-2.2 (1.7)
% BMI change						
Base to end		-7.2 (4.2)			-4.0 (6.0)	
End to 1 y			-2.7 (7.1)			-5.0 (4.3)
Base to 1 y			-9.6 (9.4)			-8.9 (6.4)
% Returning to prepregnant weight		25.0%	30.6%		16.7%	33.3 %

Values are means (SD)

[1] Significantly different between groups, RMANOVA, $p < 0.05$.

[2] Significantly different between groups, ANOVA, $p < 0.05$.

[3] Significantly different over time, RMANOVA, $p < 0.05$.

Table 7

Hormone concentrations of participants in the intervention and control groups

	Intervention Group (n = 18)			Control Group (n = 17)		
	Baseline	Endpoint	1 y	Baseline	Endpoint	1 y
Prolactin (ug/L)[1]	142 (72)	61 (24)	17 (15)	156 (99)	75 (44)	23 (17)
Estradiol (pmol/L)[1]	139 (89)	108 (63)	176 (156)	195 (105)	125 (82)	161 (122)
Serum vitamin D (ng/mL)	26.6 (1.97)		26.1 (1.51)	26.1 (2.26)		25.3 (1.94)

Values are means (SD)

[1] Significantly different over time, RMANOVA, $p < 0.05$.

Table 8

Bone mineral density of participants in the intervention and control groups

	Intervention Group (n = 18)			Control Group (n = 18)		
	Baseline	Endpoint	1 y	Baseline	Endpoint	1 y
Total body						
BMD ($g \cdot cm^{-2}$)	1.179 (0.093)	1.164 (0.094)	1.168 (0.088)	1.129 (0.109)	1.122 (0.118)	1.127 (0.119)
BMC (g)	2554 (483)	2494 (455)	2529 (459)	2402 (574)	2274 (393)	2289 (494)
Area (cm^{-2})	2154 (294)	2123 (268)	2160 (278)	2106 (317)	2007 (167)	2025 (283)
Lumbar spine						
BMD ($g \cdot cm^{-2}$) [1, 2]	1.172 (0.126)	1.130 (0.128)	1.159 (0.128)	1.134 (0.156)	1.075 (0.149)	1.102 (0.149)
BMC (g)	64 (9)	61 (9)	64 (9)	60 (10)	56 (9)	59 (9)
Area (cm^{-2})	55 (5)	54 (6)	55 (5)	53 (7)	52 (7)	53 (7)
Total hip						
BMD ($g \cdot cm^{-2}$) [1]	1.022 (0.133)	0.995 (0.131)	1.007 (0.131)	1.019 (0.146)	0.992 (0.137)	0.988 (0.143)
BMC (g)	32 (5)	31 (4)	32 (4)	31 (5)	30 (5)	30 (5)
Area (cm^{-2})	31 (2)	31 (2)	32 (3)	30 (3)	31 (3)	31 (2)

Values are means (SD)

[1] Significantly different over time, $p < 0.05$, RMANOVA.

[2] Significantly different between groups, when controlling for covariates, $p < 0.05$, RMANCOVA.

Table 9

Percent changes in total body, hip, and lumbar spine BMD of participants in the intervention and control groups

	Intervention Group	Control Group
% change from Baseline to Endpoint		
Total body	-0.93 (1.04)	-0.29 (1.95)
Hip	-2.58 (1.96)	-2.57 (2.22)
Lumbar spine	-3.59 (2.11)	-5.17 (3.28)
% change from Endpoint to 1-y Postpartum		
Total body	-0.30 (2.12)	-0.39 (1.91)
Hip	1.22 (2.99)	-0.43 (2.72)
Lumbar spine	2.66 (3.58)	2.67 (3.05)
% change from baseline to 1-y postpartum		
Total body	-1.22 (2.53)	-0.70 (1.95)
Hip	-1.40 (3.14)	-3.00 (3.11)
Lumbar spine	-1.05 (3.15)	-2.64 (4.41)

Values are means (SD)

Table 10

Characteristics of participants who returned to baseline lumbar spine BMD at 1 y

	Lumbar Spine BMD Returned to Baseline (n = 9)	Lumbar Spine BMD Did Not Return to Baseline (n = 27)
Group, n (%)		
Exercise[1]	7 (78%)	11 (41%)
Control	2 (22%)	15 (59%)
Age (y)	30 (3.7)	32 (3.0)
Weight (kg)		
BMI at baseline (kg/m^2)	29.2 (4.5)	26.9 (3.6)
% change in weight from baseline to 1 y	-10.4 (7.2)	-8.8 (8.3)
Calcium intake at 1 y (mg/d)	914 (450)	1116 (521)
Hormone concentration at 1 y		
Prolactin (ug/L)	23.7 (21.2)	18.8 (14.4)
Estradiol (pmol/L)	148.8 (125.2)	175.1 (144.7)
Menses returned (Wk postpartum)	27.7 (12.4)	32.8 (14.1)
% change in lumbar spine BMD baseline to endpoint[1]	-2.2 (2.2)	-5.1 (2.6)
% change in lumbar spine BMD endpoint to 1-y[1]	5.2 (2.4)	1.8 (3.1)
Breastfeeding status at 1 y, n (%)		
Yes	3 (33%)	17 (63%)
No	6 (67%)	10 (37%)
Parity, n (%)		
Primiparous	5 (56%)	8 (30%)
Multiparous	4 (44%)	19 (70%)

Values are means (SD), unless otherwise specified

[1]Significantly different between groups, ANOVA, $p < 0.05$.

Table 11

Characteristics of participants who returned to baseline total hip BMD at 1 y

	Hip BMD Returned to Baseline (n = 5)	Hip BMD Did Not Return to Baseline (n = 31)
Group, n (%)		
Exercise	2 (40%)	16 (52%)
Control	3 (60%)	15 (48%)
Age (y)	30.0 (4.5)	31.6 (3.0)
Weight (kg)		
BMI at baseline (kg/m^2)	26.0 (3.7)	27.7 (3.9)
% change in weight from baseline to 1 y	-6.6 (5.3)	-9.6 (8.3)
Calcium intake at 1 y (mg/d)[1]	637 (392)	1143 (491)
Hormone concentration at 1 y		
Prolactin (ug/L)	10.1 (10.0)	21.7 (16.6)
Estradiol (pmol/L)	169.0 (129.9)	168.3 (142.1)
Menses returned (Wk postpartum)	32.6 (11.0)	31.2 (14.4)
% change in hip BMD baseline to endpoint[1]	-0.4 (2.1)	-2.9 (1.9)
% change in hip BMD endpoint to 1 y[1]	3.4 (3.8)	-0.1 (2.5)
Breastfeeding status at 1 y, n (%)		
Yes	1 (20%)	19 (61%)
No	4 (80%)	12 (39%)
Parity, n (%)		
Primiparous	1 (20%)	12 (39%)
Multiparous	4 (80%)	19 (61%)

Values are means (SD), unless otherwise specified

[1]Significantly different between groups, ANOVA, $p < 0.05$.

APPENDIX B

FIGURES

Figure 1. Study Design of Participants

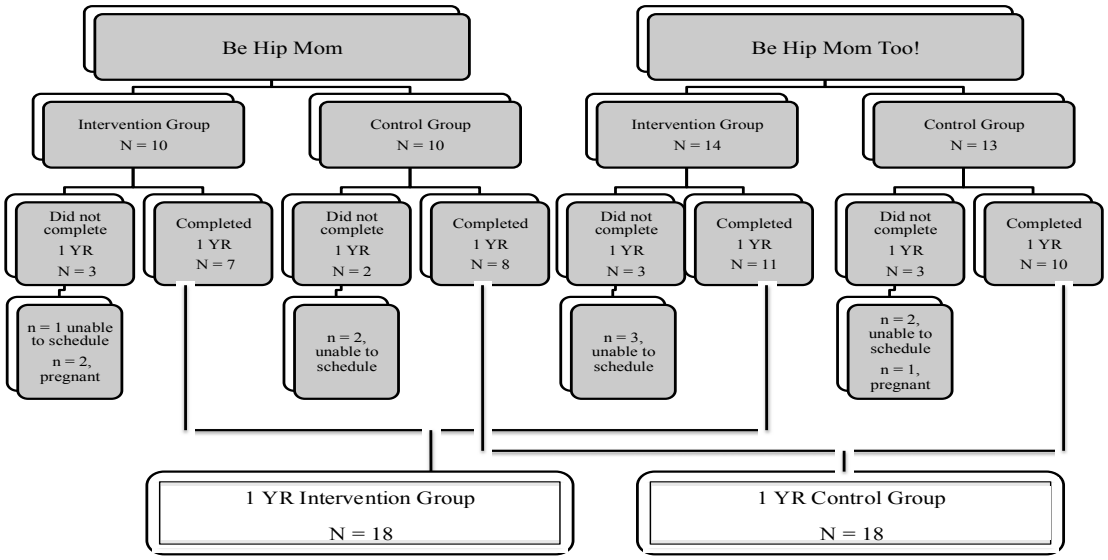

Figure 2. Conceptual Model