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Long-chain polyunsaturated fatty acids (LCPUFA) are essential for infant growth and development, and amounts in breast milk depend on maternal diet and body stores. Because exercise increases mobilization and utilization of fatty acids, maternal activity may also influence the amount of LCPUFA in breast milk. In addition, exercise has been shown to improve bone mineral density (BMD) in postmenopausal women. During lactation, bone turnover is elevated to provide calcium for breast milk. This results in decreased BMD.

Therefore, the purpose of these studies was to: 1) investigate the effects of diet and exercise on fatty acid concentrations in maternal plasma and breast milk, and 2) determine the effects of a specific resistance and aerobic training program and dietary intake on BMD and bone turnover during lactation.

In the first study, LCPUFA in plasma and breast milk were measured at 12 weeks postpartum in exercising and sedentary women. Dietary intake was recorded for three days. A subsample of women participated in exercise and rest sessions to examine the acute effects of exercise on breast milk LCPUFA. In the second study, whole body, lumbar spine (LS) and hip BMD and bone mineral content (BMC), and bone turnover markers were measured before and after a 16-wk intervention in breastfeeding women who either participated in a specific resistance and aerobic training program or were sedentary. Dietary intake and body composition were also assessed in all women.

In the first study, there were no differences in essential fatty acid intake between the two groups. Intake of linoleic acid (LA) was 11.05 ± 1.39 and 9.34 ± 0.97 and α-linolenic (LNA) was 0.96 ± 0.12 and 0.82 ± 0.09 grams/day for the sedentary and exercise groups, respectively. No differences were found in LCPUFA in plasma and breast milk between groups. After 30 minutes of exercise, there was a trend for an increase in LA and LNA. In the second study, exercising women lost significantly less LS BMD and LS BMC ($p<0.05$), and had substantial improvements in body composition. Calcium and protein intake were significant predictors of whole body BMD loss with parity being a significant covariate ($p<0.05$). Bone turnover markers were also significant predictors of LS BMD and LS BMC, when adjusted for parity and exercise ($p<0.01$).

These results suggest that women consuming adequate amounts of LCPUFA can exercise moderately without decreasing the LCPUFA in their breast milk. In addition, increased calcium and protein intake lessens BMD loss during lactation. In breastfeeding women, moderate resistance and aerobic exercise was effective in improving body composition and minimizing BMD losses.

EFFECTS OF DIET AND EXERCISE ON MATERNAL BODY COMPOSITION AND BREAST MILK COMPONENTS

by

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APPROVAL PAGE

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INTRODUCTION

Breastfeeding confers many life long health benefits to both mother and child. The following research studies have investigated the effects of moderate exercise during lactation on maternal health status and breast milk composition. Exercise during lactation benefits the mother through increased fitness and improved body composition. Breast milk alone provides the infant with optimal nutrition, and maternal exercise does not negatively impact the quality of breast milk.

The first research study examined the effects of exercise and diet on plasma and breast milk fatty acids. Exercise leads to a mobilization of fatty acids from body stores, and these fatty acids may further be mobilized to breast milk. It is well documented that the n-6 and n-3 fatty acids, specifically docosahexaenoic acid (DHA) and arachidonic acid (AA), accumulate in the brain and retina of infants, and this accumulation is important for the neural and visual development of the infant.

The second study examined the effects of diet and exercise, during lactation, on maternal body composition, bone mineral density, and bone turnover markers. It is well established that lactation leads to loss of bone, specifically in the spine and hip regions. These losses are usually recovered upon weaning; however, recovery does not occur in all women. If bone density remains low, women may be at a higher risk for developing

osteoporosis later in life. Exercise has been shown to be an effective means to improve bone mineral density in postmenopausal women. These research studies were guided by the following specific aims:

Study 1

Specific Aim #1: To determine the effects of moderate exercise on plasma and breast milk fatty acid composition.

Hypothesis: Exercise will have no effect on basal concentrations of plasma and breast milk long-chain polyunsaturated fatty acids (LCPUFA) between sedentary and exercising women. Acutely, concentrations of LCPUFA in breast milk would remain the same after a 30-minute rest session, while increasing after a 30-minute exercise session because of increased mobilization of LCPUFA from maternal fat stores. Chronic, moderate exercise will have no negative effect on plasma and breast milk fatty acids.

Specific Aim #2: To determine the effects of dietary intake on plasma and breast milk fatty acid composition.

Hypothesis: There will be no effect of dietary intake on plasma and breast milk fatty acid composition.

Study 2

Specific Aim #1: To determine if an exercise intervention will minimize BMD losses in lactating women from 3 weeks postpartum to 21 weeks postpartum.

Hypothesis: Lactating women participating in a 16-week exercise program will have less BMD loss in comparison to non-exercising controls, as measured by dual energy x-ray absorptiometry (DXA), specifically in the lumbar spine region.

Specific Aim #2: To determine if an exercise intervention will decrease bone losses due to increased bone formation and decreased bone resorption from 3 weeks postpartum to 21 weeks postpartum.

Hypothesis: Women in the control group will have normal physiological changes in bone markers. In these women, a slow increase in serum bone-specific alkaline phosphatase (BAP) levels (bone formation marker) is expected; however, urinary pyridinium crosslinks (bone resorption marker) will slowly decline during the study period. These changes are normal adaptations after pregnancy, in which the body is in a state of very high bone resorption and low bone formation. Exercise training will result in slower decline, compared to controls, in bone resorption, and greater increase in bone formation, indicating a slowing of overall bone turnover.

Specific Aim #3: To determine if an exercise intervention will improve body composition (lean and fat mass), muscular strength, and cardiovascular fitness levels in lactating women over the 16-week fitness program.

Hypothesis: Lactating women who participate in the 16-week training (including 3 days of resistance training and 3 days of aerobic training per week) program will experience decreases in total body fat percentage and increased lean body mass as compared to sedentary controls, and will have increased muscular strength and cardiovascular capacity as compared to sedentary controls.

Specific Aim #4: To determine the effects of dietary intake on BMD and bone turnover in lactating women.

Hypothesis: Calcium, protein, and energy intake of lactating women will have no significant effect on BMD.

Hypothesis: Higher calcium intake along with exercise will lead to decreased bone mineral loss and a decrease in bone turnover.

CHAPTER I

MATERNAL DIET AND EXERCISE: EFFECTS ON LONG-CHAIN POLYUNSATURATED FATTY ACID CONCENTRATIONS IN BREAST MILK

REVIEW OF LITERATURE

Dietary Sources, Metabolism, and Functions of n-3 and n-6 Fatty Acids

Before the increase in consumption of trans fatty acids and saturated fats in the human diet, n-6 and n-3 fatty acid intakes were at a balance, with a ratio of approximately 1:1. In recent years, the ratio has grown closer to 25:1 (1), leading to a deficiency of n-3 fatty acids in Western diets. According to Simopoulous (1), the optimal intake of linolenic acid (LNA) is 800-1100 mg/day, and the optimal intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is 300-400 mg/day. The latest dietary reference intake (DRI) for linoleic acid (LA) for adult females aged 19-50 is 12 g/d (13 g/d for lactating women), and for LNA the recommendation is 1.1 g/d for this same population (1.3 g/d for lactating women) (2). The current dietary intake recommendation for the ratio of n-6 and n-3 fatty acids is 2-5:1 (3, 4, 5), while the World Health Organization recommends a ratio of 5-10:1 (6).

Fatty acids in the diet come from a variety of sources. LA is found in the seeds of most plants (excluding coconut, cocoa, and palm). LNA can be obtained through the chloroplast of green leafy vegetables, as well as from flax seed and English walnuts. DHA and EPA are mostly found in fatty fish, fish oils, and some algae and ferns, whereas arachidonic acid (AA) can be found in the phospholipids of grain-fed animals (1).

The fatty acids DHA and AA, as well as the ratio of the total n-6 to total n-3 fatty acids, are of the greatest importance to ensure optimal fatty acid status. If the ratio of n-6 to n-3 is too high, as is the case in most western diets, an interference in the conversion of the parent fatty acids into DHA and AA can result. In most cases, an increased level of

LA is seen, which preferentially uses the Δ6 desaturase enzyme to produce AA. This leads to a decreased conversion of DHA from LNA, with a subsequent increase in AA, and potentially a decrease in DHA status.

While fatty acid deficiency is rare, it does occur. Fatty acid deficiency was first discovered in studies using fat-free milk infant formulas and fat-free parenteral adult formulas (7, 8, 9). Deficiency symptoms including skin problems and visual problems were reversed upon addition of LA to the diet. These early studies (7, 8, 9) were groundbreaking in identifying deficiencies and those at risk.

Fatty acids have multiple functions in the human body. Fatty acids can be a source of energy, both for support of physical activity as well as for basal energy needs. Fatty acids are also necessary for growth and for development and maintenance of neural and visual processes. When fatty acids are used for energy, they undergo β-oxidation, a process in which the fatty acid chain is broken down in a stepwise manner to produce acetyl groups, which then move into the mitochondria, through the Krebs cycle, and into the electron transport chain to produce the ultimate energy source, adenosine triphosphate (ATP). This process is constantly taking place, but is especially active during times of fasting, or in the post-absorptive state, in which the carbohydrate status of the individual is low and energy needs to be maintained. In this case, fat oxidation will be upregulated to meet energy needs. In addition to the maintenance of basal energy needs, growth also needs to be supported and maintained.

It is well documented that the n-6 and n-3 fatty acids, specifically DHA and AA, accumulate in the brain and retina of infants (10, 11). This accumulation is important for

the neural and visual development of the infant, as well as for eicosanoid formation. Evidence also suggests that infants are able to convert LNA to DHA and LA to AA (12).

Fatty acids can come from multiple dietary sources, and can be used for many metabolic processes. These processes include energy production, growth, immune health, and brain and retinal development. Deficiency is rare, but has distinct, yet reversible symptoms. Fatty acids need to be present in the body, not only to promote growth and development, but also to prevent illness and disease.

Factors Affecting Concentration of n-3 and n-6 Fatty Acids in Human Milk

Fatty acid levels in human milk are typically studied in order to determine the appropriate amount of the fatty acids needed for infant formula. While human milk is known to be the perfect food for an infant, it is sometimes debated as to what is necessary for those infants who are fed infant formula, or who are premature and may need extra nourishment in those first few months. N-3 fatty acids have been found to play a key role in the development of infants, including both neural and visual development.

The fatty acid content of human milk is influenced to the greatest extent by time postpartum, gestational age, parity, presence of disease, and diet. Maternal diet appears to have the greatest effect (13, 14, 15). Francois *et al.* (16) fed fourteen lactating women six different test fat meals and measured the fatty acid transfer to breast milk over multiple time points. Most of the fatty acids appeared in the breast milk at the first collection (6 hours after feeding), reached the highest levels between 10 and 24 hr, and remained elevated for up to 3 days. Interestingly, EPA and DHA remained elevated

longer than any other fatty acids, and reached their peak at a later time point. The researchers of this study theorize that this may be due to EPA and DHA having smaller pools (except the DHA in the brain) in the body, leading to less availability for energy needs. Similarly, the fatty acids with larger pools appear to be less affected by dietary intake (16). Supplementation of a lactating mother with DHA increases the DHA content of her breast milk. Lactating women were given either 200 mg DHA per day, or a placebo, for two weeks. Supplemented women had a 2-fold increase in DHA in their breast milk on day 14. Infant outcomes, however, were not documented in the study (17). Results of this study by Fidler *et al*. (17), agree with results found by Makrides *et al.* (18) in which 20% of supplemented DHA was transferred to breast milk, independent of amount of supplementation. Del Prado *et al.* (19) fed ten lactating women [^{13}C] linoleic acid based on kilogram body weight, in a controlled diet, at five months postpartum to determine LA and AA concentrations in breast milk. Only about 16% of the labeled LA was recovered at 72 hours, and a minimal amount (0.01%) of labeled AA, product of desaturation of labeled LA, was found in the milk. LA and AA were measured in the milk and were approximately 32.8% and 11.8%, respectively. Del Prado *et al.* conclude that a very small amount of milk AA comes from the conversion of LA or from direct absorption in the intestine. It is suggested that most milk LA and AA come from maternal body stores (19).

Martin *et al.* (20) examined the breast milk (colostrum and mature milk) and white adipose tissue in lactating women at day five and day 30 postpartum. These researchers found that as the duration of lactation increased the proportion of the long

chain polyunsaturated fatty acids (LCPUFA) in white adipose tissue did not change. LA in the adipose was higher, and LNA was lower, in the adipose as compared to colostrum or mature milk. A positive correlation was found in the levels of LA, as well as the LNA: LA ratio, in the adipose tissue as compared to colostrum and mature milk. Even though LNA decreases in human milk as lactation progresses, the infant will actually be receiving a greater amount of the fatty acids in later lactation due to the increased volume and fat content of the milk. These researchers suggest that a greater mobilization of fatty acids from adipose tissue occurs in early lactation (20). The average American diet has more n-6 fatty acids as compared to n-3 fatty acids, as previously discussed. This also directly relates to the ratio in breast milk. The infant will receive much more LA than LNA, which may subsequently result in a deficiency of DHA produced due to the competition of the desaturase enzymes.

The relationship between maternal diet and the lipid content of milk has been somewhat complicated to determine, however, it does appear that there is a connection between maternal body composition and the lipid content of milk (21). Lipids that are incorporated into human milk are derived from lipids in the circulation as well as from the ability of the mammary gland epithelial cells to produce free fatty acids (FFA) and triglycerides (TG). The lipids in the maternal circulation can come from numerous sources, which include the maternal diet leading to intestinal absorption and subsequent release into circulation, endogenous liver synthesis, or mobilization of adipose tissue stores (22, 23, 24). In the post-absorptive state, the lipids are derived mostly from adipose tissue stores, which then undergo lipolysis, forming FFA, which pass through the

circulation bound to albumin, into the mammary gland. The portion of milk lipid that is derived from the liver is in the very low-density lipoproteins (VLDL) form. To determine the sources of the FFA found in the milk produced, it is necessary to separate the FFA by chain length. The medium chain FA (C10:0-C16:0) are those that have been synthesized by the mammary gland (14), and the FA that were originally in the circulation are of longer chain length (C16:0 and longer).

In a recent study, ten lactating women were fed 2.5 mg of [^{13}C] LA per kilogram body weight at five months postpartum. LA was measured in serum, milk, and breath, and the women were separated into groups based on body mass index (BMI; <22.5 or >23.5). Women in the higher BMI group exhaled more labeled CO_2 than the leaner group. While there was no significant difference in total transfer of LA into milk, the leaner group transferred the LA into the milk at a slower rate. Villalpando *et al*. suggest that a large portion of the LA is stored in maternal fat tissue and is released slowly into the maternal circulation (25).

Caloric deficit may be an effective method to increase the mobilization of fatty acids from the adipose tissue to the mammary gland. Researchers have found that when caloric restriction is imposed on the body, the body will mobilize a greater amount of adipose tissue for fuel for the body. This mobilization may lead to increased FFA in the plasma. However, this data is contradictory to the findings by Hagve *et al.* (26). These researchers found that in the serum of male subjects undergoing a 4-week weight loss program, the PUFA (polyunsaturated fatty acid) levels remained constant, except for a significant decrease in LA. If this phenomenon is correct and weight loss leads to a

decrease in LA, this may have beneficial effects in terms of lowering the LA to LNA ratio. A study by Bener *et al.* (27) found that during the observed Muslim holy month of Ramadan, in which participants fasted from sunrise to sunset, no difference was observed in the lipid concentration of breast milk obtained from mothers while fasting and again after their fast was completed. These observed results may have been noted because although fewer meals were consumed each day by the subjects, each individual meal had a higher nutrient and caloric density.

Fatty acids in human milk are affected by multiple factors, with maternal diet, metabolism, and storage being the most influential. Caloric intake may also determine the fatty acid content of human milk. Determining the exact role of these maternal factors, along with other confounding factors, such as maternal exercise, will help to clarify the mechanisms that regulate levels of fatty acids in human milk.

Fatty acids and Infant Development

One of the most critical times for the development of a human is that spent as a fetus and the first few months of life. While the environment in utero is crucial to the survival and development of the fetus, an optimal atmosphere needs to be available to the infant during its first year of life to ensure that the infant develops to the best of its potential. In the first years of life, an infant is exposed to physical, social, and mental stimulation. Among these stimuli, the infant requires proper nutrition in order to grow and develop, as well as to remain free from disease and illness. Breast milk provides this nourishment in a way that no infant formula can duplicate. There are over 200

compounds in breast milk, many of which have a yet unidentified function. However, many of these compounds have been determined to be essential in providing nutrients, immune factors, and developmental aides to the infant. While infant formula does provide adequate nutrients for the health of the infant, it does not contain the immune factors, which provide a defense system for the infant. At this point in time, only a few infant formulas in the US contain the specific fatty acids DHA and AA, which are critical for the neural and visual development of the infant. Other available formulas contain the precursors to the above fatty acids, which are LNA and LA, respectively. Infants are able to convert these precursors to DHA and AA (12, 28), but the extent to which this occurs is not fully known, and may not be sufficient to support the developmental needs of the formula fed infant. Breast milk contains DHA and AA, and provides these nutrients directly to the infant. A debate continues concerning whether infant formula should be supplemented with these fatty acids, which types of fatty acids, and in what amounts they should be added.

The digestion, absorption, and transport of nutrients differ in breast milk as compared to formula. Bile-salt stimulated lipase in breast milk enhances the digestion of LCPUFA. The fatty acids will be digested and absorbed in infants fed supplemented formula, but the difference in absorption of formula and breast milk may be substantial. This may complicate the research, as it may be difficult to determine the proper levels to be added to formula. Simply mimicking breast milk may not be adequate. The lack of bile-salt stimulated lipase in the formula will decrease the absorption of the fatty acids in the formula fed infant.

The two essential fatty acids are LA and LNA, and DHA and AA are known to be necessary for proper neural and visual development in infants. These fatty acids are not produced by the human body; therefore, they must be obtained from the diet. Once ingested, LA can be converted into AA and LNA can be converted into DHA and EPA, most of which occurs in the liver. Specifically, this biosynthesis, as well as the incorporation of the unsaturated fatty acids into the phospholipid membranes occurs in the endoplasmic reticulum of the cells. In a review by Innis *et al*. (29), it was found, from comparing and contrasting multiple studies, that both term and preterm infants are capable of synthesizing AA and DHA from LA and LNA, respectively, dependent on the amounts available in the diet. Interestingly, it has been assumed that preterm infants have a lower conversion of LA and LNA; however, the studies reviewed by Innis *et al*. (29) showed no difference between term and preterm infants in their ability for conversion. Again, dietary intake of the mother was a determining factor.

The nervous system, namely brain tissue, of the infant develops to the greatest extent during the third trimester of pregnancy and in the first few weeks of life. DHA and AA are essential for the development of neural tissue. DHA also plays a key role in the development of the retina. As development of the fetus advances, the amount of DHA in the retina increases (30). Because DHA, AA, and EPA compete for the desaturase enzymes, Δ-5 and Δ-6, the available ratio of these fatty acids needs to be well controlled. High levels of AA in the diet may lead to a reduction in the DHA and EPA in tissues, as well as in plasma and breast milk, while the opposite is true for high levels of DHA and EPA in the reduction of AA (31, 32). Therefore, the recommendation for the ratio of n-6

to n-3 in infant formula has been set at 5:1 to 15:1 by the European Society of Pediatric Gastroenterology and Nutrition (33). No differences were found between visual acuity or rate of growth in infants fed either a ratio of 5:1 or 10:1. This showed that the lower ratio had no negative effect on the infants. The authors also found that while the ratio of n-6 to n-3 fatty acids decreased, no decrease in the plasma AA occurred. However, it is important to note that the reference breast-fed group of infants had higher DHA and AA and a lower plasma LA to LNA ratio, when compared to both formula fed test groups (34).

A group of infants that were supplemented with infant formula containing either DHA only, DHA and AA, or a control formula containing no DHA or AA, from birth until 17 weeks of age, were followed up at 18 months. Compared to the control group of infants, the DHA and AA supplemented group had higher scores on the Mental Development Index (MDI) portion of the Bayley Scales of Infant Development –II (BSID-II), and both supplemented groups scored higher than the control group on the cognitive and motor subtests of the MDI. The infants were also studied at four and twelve months of age, and correlations were seen between red blood cell and plasma DHA and MDI scores at 18 months of age, suggesting a relationship between early dietary intake of DHA and MDI performance (35).

Retinal development is crucial in the first months of life, and DHA is the critical element for proper visual development of the infants. The rod portion of the retina is comprised of about 50% n-3 PUFA, of which DHA is the primary component. While the mechanism through which the n-3 fatty acids aid in the vision process has not been

completely elucidated, when DHA is deficient, vision is impaired. The central nervous system has ways of conserving fatty acids, and in some studies, researchers have been able to induce deficiencies in pregnant and nursing rats. During pregnancy and lactation, the largest changes in DHA in the retina were seen in deficient female rats (36). The eye itself is capable of producing DHA from LNA and 22:5n-3 (37). This conservation and production by the retina are only some of the mechanisms that are necessary to maintain the DHA in the retina.

Uauy *et al*. (38) suggest that the n-3 fatty acids, in addition to DHA, are necessary for the optimal visual development of infants, as well as for normal sleep-wake cycles and heart rate rhythms. Infants fed a diet void of essential fatty acids have major decreases in visual performance, which can be reversed through n-3 fatty acid supplementation. This review looked at numerous studies, and concluded that too many methodological, sampling, supplementation, and analytical differences exist between studies to come to any clear conclusions. It has been suggested that n-3 polyunsaturated fatty acids should be regarded as provisionally essential for infants (38).

Early studies indicated reduced growth in infants fed formula supplemented with DHA only. However, consideration needs to be given to the improper balance of the added fatty acids, and the role they have in hindering infant growth. If the ratio of added fatty acids is inappropriate, infant growth may be adversely affected. This could be remedied by adding both AA and DHA at appropriate levels. High levels of DHA and EPA can compete with AA, and subsequently reduce AA levels, with EPA having a greater effect.

Birch *et al*. (39) studied full-term infants who were breast fed for the first 6 weeks of life, and then randomized to either a control formula or a supplemented formula. The supplemented infants had better VEP (visual evoked potential) acuity at 17, 26, and 52 weeks of age, and had better stereoacuity at 17 weeks of age. The infants experienced no difference in growth between the groups. The results of this study suggest that the need for AA and DHA for the visual development of infants extends beyond the first 6 weeks of life.

When all of the data from the numerous studies are analyzed, and the current knowledge of the fatty acid needs for optimal health and development are combined, adequate information is still lacking. No strong, irrefutable indicators of infant development have been identified. While growth and vision can be measured with relative accuracy, biochemical markers (i.e. plasma level) that definitively relate to the growth or vision have not been discovered, although some correlations between plasma and red blood cell DHA and AA have been related to vision tests in one study (39). Even within the vision tests themselves, there is a lack of complete accuracy, and many researchers disagree about which is the most appropriate test.

The wide variations in developmental results of supplementation studies between breastfed and formula fed diets are due to many factors. These factors include different amounts of LNA, AA, and DHA in the formulas, other differences in formula composition, duration of formula feeding, sample size, outcomes and tests measured, as well as genetic and socioeconomic influence on outcomes (40). Infant intelligence has so many confounding factors that a study would require such specific and limiting criteria,

relating to inclusion/exclusion factors, that a large enough sample could never be obtained, and the study would take too many years to complete. However, a more detailed and prolonged study may be necessary. This question of fatty acid intake and infant development has not yet been answered, but it is known that breastmilk is the best nourishment for the infant. The many components of breast milk that are yet undiscovered, will only shed more light of the subject. For some researchers to say that there is no advantage to breastfeeding (40), even though they were only speaking in terms of fatty acid supplementation, contradicts the national goals to promote breastfeeding. Much more research needs to be conducted to conclusively determine the safety and amounts of supplementation necessary to provide proper nutrition to formula fed infants. Breast milk should be the model for the production of formula. Substances which cannot be mimicked in formula may cause tremendous differences in digestion, absorption, and transport of the fatty acids, and other compounds. If more research is conducted, safe supplementation levels determined, and effectiveness further evaluated, the supplemented infant formula may be a viable alternative to human milk, but it will never be the "perfect" food for the infant.

Fatty Acids and Exercise

The importance of exercise to the overall health of an individual is generally well accepted. However, for women who have recently had a baby and are exclusively breastfeeding their infant, the safety of exercise has been questioned. Studies have shown that not only is it safe for women to exercise moderately while breastfeeding, but

exercise will actually benefit the mother. These benefits include increased cardiovascular fitness levels, as well as a decrease in body fat percentage. Lovelady et al. (40) also found a significant decrease in abdominal skinfold thickness in lactating women who were undergoing a moderate exercise program. Plasma lipids, including TG, total cholesterol (TC), and low-density lipoproteins (LDL) decreased over the study's 12-week period in both the exercise and the control groups. At the same time, high-density lipoproteins (HDL) increased in the exercise group while declining in the sedentary group (41).

Borsheim et al. studied eight healthy males who underwent a 90-minute exercise session and 90-minute rest session. After the acute exercise bout, plasma FFA concentration increased, and remained elevated for an extended period of time. LA increased significantly in the exercise group when compared to the rest group. Exercise decreased AA percentages in the exercise group as compared to the rest group. The data appears to show an increase in FFA mobilization from VLDL and a decreased plasma TG concentration (42). Oscai *et al*. (43) report that exercise training results in an increase in plasma lipoprotein lipase (LPL) activity, leading to greater clearance of TG from the plasma. This mobilization may be important to replace the TG used during exercise. The results of these studies suggest an increase in fatty acid mobilization as a result of exercise.

Exercise leads to the mobilization of fatty acids from the adipose tissue triglycerides. Exercise at intensities between 25% and 65% VO_2 max leads to a 5-10 fold increase in fat oxidation (44). During exercise the release of fatty acids from adipose

increases three fold compared to rest (45). In two studies by Romijn *et al*. (46, 47), it was found that exercise stimulated the liberation of fatty acids from the adipose tissue. The researchers suggest that this liberation is due to the need for fatty acids as fuel for the skeletal muscle. In the earlier study, the researchers found that during endurance exercise fatty acids are both released and oxidized from TG.

Fatty acid mobilization is affected by the intensity of the exercise bout. In a study of male volunteers who were exercised on two separate visits, once at 40% of max, and again at 60%, adipose tissue lipolysis was determined. Lipolysis did not differ in the adipose tissue of the subjects in either session, however, post-exercise fatty acid mobilization was greater after the 60% session. The abdominal subcutaneous adipose tissue did not differ between sessions (48). Romijn *et al*. found that at an exercise intensity of 85% of VO_2 max, plasma fatty acid oxidation was actually lower than the oxidation at 65% of VO_2 max (46, 47). Taken together, these studies suggest that 60-85% of VO_2 max is the optimal intensity for increased fatty acid mobilization and oxidation.

Conclusion

Maintaining an optimal intake and ratio of LA and LNA is important for health of adults and children. Fatty acids perform many functions in the human body, including maintenance of skin and visual health. Exercise mobilizes fatty acids from body stores for energy production, both for basal needs and also to support physical activity. Fatty acids are also taken up by the mammary gland and are incorporated into breast milk. Concentrations of fatty acids in human milk are affected by maternal factors such as time

postpartum, parity, and diet. Improving maternal fatty acid status may, in turn, improve the fatty acid composition of breast milk, providing optimal nutrition for the development, especially visual and neural, of the infant.

REFERENCES

1. Simopoulos AP. Omega-3 Fatty Acids, Part I: Metabolic effects of omega-3 fatty acids and essentiality. Handbook of Lipids in Human Nutrition, CRC Press. 1996: 51-73.

2. Institute of Medicine. *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids*. Food and Nutrition Board. Washington, DC: National Academy Press 2002.

3. Simopoulos A. Essential fatty acids in health and chronic disease. Am J Clin Nutr 2000; 70: 560S-569S.

4. Jequier E. Response to and range of acceptable fat intake in adults. Eur J Clin Nutr 1999; 53: S84-93.

5. Kris-Etherton PM, Shaffer Taylor D, Yu-Poth S, Huth P, Moriarty K, Fishell V, Hargrove RL, Zhao G, Etherton TD. Polyunsaturated fatty acids in the food chain in the United States. Am J Clin Nutr 2000; 71: 179S-188S.

6. World Health Organization. Fats and Oils in human nutrition: report of a joint expert consultation of the Food and Agriculture Organization of the United Nations and the World Health Organization. FAO Food Nutr Paper 1995; 57: 1-147.

7. Hansen AE, Haggard ME, Boelsche AN, et al. Essential fatty acids in infant nutrition. III. Clinical manifestations of linoleic acid deficiency. J Nutr 1958; 66: 564-570.

8. Futterman S, Downer JL, Hendrickson A. Effect of essential fatty acid deficiency on the fatty acid composition, morphology, and electroretinograph response of the retina. Invest Ophthalmology 1971; 10: 151-156.

9. Wheeler TG, Benolken RM, Anderson RE. Visual membranes: specificity of fatty acid precursors for the electrical response to illumination. Science 1975; 188: 1312-1314.

10. Fliesler SJ, and Anderson RE. Chemistry and metabolism of lipids in vertebrae retina. Prog. Lipid Res 1983; 22: 79-131.

11. Sastry PS. Lipids of nervous tissue: composition and metabolism. Prog Lipid Res 1985; 24: 69-176.Salem N Jr., Wegher B, Mena P, Uauy R. Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants. Proc Natl. Acad. Sci. USA 1996; 93: 49-54.

12. Salem N Jr, Wegher B, Mena P, Uauy R. Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants. Proc Natl Acad Sci USA 1996; 93: 49-54.

13. Jensen RG, Bitman J, Carlson SE, Couch SC, Hamosh M, Newburg DS. In The Handbook of Milk Composition, 1995, Ch. 6A, Academic Press, San Diego.

14. Jensen RG. The lipids in human milk. Prog Lipid Res 1996; 35: 53-92.

15. Jensen RG. Lipids in human milk. Lipids 1999; 34: 1243-1271.

16. Francois CA, Connor SL, Wander RC, Connor WE. Acute effects of dietary fatty acids on the fatty acids of human milk. Am J Clin Nutr 1998; 67; 301-308.

17. Fidler N, Sauerwald T, Pohl A, Demmelmair H, Koletzko B. Docosahexaenoic acid transfer into human milk after dietary supplementation : a randomized clinical trial. J Lipid Res 2000; 41: 1376-1383.

18. Makrides M, Neumann MA, Gibson RA. Effect of maternal docosahexaenoic acid (DHA) supplementation on breast milk composition. Eur J Clin Nutr 1996; 50: 352-357.

19. Del Prado M, Villalpando S, Elizondo A, Rodriguez M, Demmelmair H, Koletzko B. Contribution of dietary and newly formed arachidonic acid to human milk lipids in women eating a low-fat diet. Am J Clin Nutr 2001; 74: 242-247.

20. Martin JC, Bougnoux P, Fignon A, Theret V, Antoine JM, Lamisse F, Couet C. Dependence of human milk essential fatty acids on adipose stores during lactation. Am J Clin Nutr 1993; 58: 653-9.

21. Villalpando S, del Prado M. Interrelation among dietary energy and fat intakes, maternal body fatness, and milk total lipid in humans. J Mamm Gland Biol Neoplasia 1999; 4: 285-295.

22. Insull W, Hirsch J, James T, Ahrens EH. The fatty acids of human milk II. Alterations produced by manipulation of caloric balance and exchange of dietary fats. J Clin Invest 1959; 38: 443-450.

23. Hachey DL, Silber GH, Wong WW, Garza C. Human Lactation: Endogenous fatty acid by the mammary gland. Pediatr Res 1989; 25: 63-68.

24. Hachey DL, Thomas MR, Emken EA, Garza C, Brown-Booth L, Adlof RO, Klein PD. Human Lactation: Maternal transfer of dietary triglycerides labeled with stable isotopes. J Lip Res 1987; 28: 1185-1192.

25. Villalpando S, Del Prado M, Lance A, Alfonso E, Rodriguez M, Demmelmair H. [13C] Linoleic acid oxidation and transfer into milk in stunted lactating women with contrasting body mass indexes. Am J Clin Nutr 2001; 74: 827-832.

26. Hagve T, Christopherson B, Hoie K, Johansen Y. Effect of a low fat diet on essential fatty acid metabolism in healthy human subjects. Scand J Clin Lab Invest 1986; 46: 61-66.

27. Bener A, Galadari S, Gillett M, Osman N, Al-Taneiji H, Al-Kuwaiti MHH, Al-Sabosy MMA. Fasting during the holy month of Ramadan does not change the composition of breast milk. Nut Res 2001; 21: 859-864.

28. Carnielli VP, Wattemena DJL, Luijendijk IHT, Boerlage A, Degenhart HJ, Sauer PJJ. The very low birth weight premature infant is capable of synthesizing arachidonic and docosahexaenoic acids from linoleic and linolenic acids. Pediatr Res 1996; 40: 169-174.

29. Innis SM, Sprecher H, Hachey D, Edmond J, Anderson RE. Neonatal polyunsaturated fatty acid metabolism. Lipids 1999; 34: 139-149.

30. Clandinin MT, Van Aerde JE, Parrott A, Field CJ, Euler AR, Lien EL. Assessment of the efficacious dose of arachidonic and docosahexaenoic acids in preterm infant formulas: Fatty acid composition of erythrocyte membrane lipids. Pediatr Res 1997; 42: 819-825.

31. Sprecher H, Luthria DL, Mohammed BS, Baykousheva SP. Reevaluation of the pathways for the biosynthesis of polyunsaturated fatty acids. J Lipid Res 1995; 36: 2471-2477.

32. Innis SM. Essential fatty acids in growth and development. Prog Lipid Res 1991; 30: 39-103.

33. EPSGAN Committee of Nutrition. Comment on the content and composition of lipids in infant formulas. Acta Paediatr Scand 1991; 80: 887-896.

34. Makrides M, Gibson RA. Long-chain polyunsaturated fatty acid requirements during pregnancy and lactation. Am J Clin Nutr 2000; 71(suppl): 307S-311S.

35. Birch EE, Garfield S, Hoffman DR, Uauy R, Birch DG. A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. Dev Med Child Neurol 2000; 42: 174-181.

36. Wiegand RD, Koutz CA, Chen H, Anderson RE. Effect of dietary fat and environmental lightning on the phospholipid molecular species of rat photoreceptor membranes. Exp Eye Res 1995; 60: 291-306.

37. Delton-Vandenbroucke I, Grammas P, Anderson RE. Polyunsaturated fatty acid metabolism in retinal and cerebral microvascular endothelial cells. J Lipid Res 1997; 38: 147-159.

38. Uuay R, Pierano P, Hoffman D, Mena P, Birch D, Birch E. Role of essential fatty acids in the function of the developing nervous system. Lipids 1996; 31(Suppl): S 167-76.

39. Birch EE, Hoffman DR, Castaneda YS, Fawcett SL, Birch DG, Uauy RD. A randomized controlled trial of long-chain polyunsaturated fatty acid supplementation of formula in term infants after weaning at 6 wk of age. Am J Clin Nutr 2002; 75: 570-580.

40. Auestad N, Halter R, Hall RT, Blatter M, Bogle ML, Burks W, Erickson JR, Fitzgerald KM, Dobson V, Innis SM, Singer LT, Montalto MB, Jacobs JR, Qiu W, Bornstein MH. Growth and development in term infants fed long-chain polyunsaturated fatty acids: A double-masked, randomized, parallel, prospective, multivariate study. Pediatrics 2001; 108(2): 372-381.

41. Lovelady CA, Nommsen-Rivers LA, McCrory M A, Dewey KG. Effects of exercise on plasma lipids and metabolism of lactating women. Med Sci Sports Exerc 1995; 27: 22-28.

42. Borsheim E, Knardahl S, Hostmark AT. Short-term effects of exercise on plasma very low density lipoproteins (VLDL) and fatty acids. Med Sci Sports Exerc 1999; 31: 522-530.

43. Oscai LB, Essig DA, Palmer WK. Lipase regulation of muscle triglyceride hydrolysis. J Appl Physiol 1990; 69: 1571-1577.

44. Krogh A, Lindhard J. The relative value of fat and carbohydrate as sources of muscular energy. Biochem J 1920; 14: 290-363.

45. Hodgetts V, Coppack SW, Frayn KN, Hockaday TDR. Factors controlling fat mobilization from human subcutaneous adipose tissue during exercise. J Appl Physiol 1991; 71: 445-451.

46. Romijn JA, Coyle EF, Sidossis L, Gastaldelli A, Horowitz JF, Endert E, Wolfe RR. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity. Am J Physiol Endocrinol Metab 1993; 265: 380-391.

47. Romijn JA, Coyle EF, Sidossis LS, Zhang XJ, Wolfe RR. Relationship between fatty acid delivery and fatty acid oxidation during strenuous exercise. J Appl Physiol 1995; 79: 1939-1945.

48. Mulla NA, Simonsen L, Bulow J**.** Post-exercise adipose tissue and skeletal muscle lipid metabolism in humans: the effects of exercise intensity. J Pysiol 2000; 524 Pt 3: 919-28.

ORIGINAL RESEARCH ARTICLE

Maternal Diet and Exercise: Effects on Long-Chain Polyunsaturated Fatty Acid Concentrations in Breast Milk.

Bopp MJ, Lovelady CA, Hunter CP, Kinsella TC.

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ABSTRACT

Background: Long-chain polyunsaturated fatty acids (LCPUFA) are essential for infant growth and development. The amount of long-chain PUFA in breast milk depends on maternal diet and body stores. Because exercise increases mobilization and utilization of fatty acids, maternal activity may also influence the amount of LCPUFA in breast milk.

Objective: To investigate the effects of exercise on α-linolenic acid (LNA), linoleic acid (LA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA) concentrations in maternal plasma and breast milk. In addition, to determine if lactating women consume adequate amounts of LCPUFA to compensate for those used for energy during exercise.

Design: LCPUFA in plasma and breast milk were measured at 12 weeks postpartum in exercising and sedentary women. Dietary intake was recorded for three days. A subsample of women participated in exercise and rest sessions to examine the acute effects of exercise on breast milk LCPUFA.

Results: There were no differences in dietary intake between the two groups. Intake of LA was 11.05 ± 1.39 and 9.34 ± 0.97 and LNA was 0.96 ± 0.12 and 0.82 ± 0.09 grams/day by the sedentary and exercise groups, respectively. These amounts are close to the Adequate Intakes of LA and LNA for lactation (13 and 1.3 grams/day, respectively). No differences were found in LCPUFA in plasma and breast milk between groups. After 30 minutes of exercise, there was a trend for an increase in LA and LNA, with no change in DHA, EPA and AA concentrations in breast milk.

Applications/Conclusions: These results suggest that women consuming adequate amounts of LCPUFA can exercise moderately without decreasing the LCPUFA in their breast milk.

INTRODUCTION

It is well established that breast milk is the optimal food for infants, providing all the nourishment they need to grow physically and mentally for the first six months of life (1, 2). Long-chain polyunsaturated fatty acids (LCPUFA), specifically α-linolenic acid (LNA; 18:3n-3), linoleic acid (LA; 18:2n-6), docosahexaenoic acid (DHA; 22:6n-3), eicosapentaenoic acid (EPA; 20:5n-3), and arachidonic acid (AA; 20:4n-6) are some of the many components in human milk that promote infant growth and development.

LNA and LA are essential fatty acids that can be desaturated and elongated into the longer chain fatty acids EPA, DHA, and AA, respectively. DHA is an important nutrient in breast milk that is crucial for neural and visual development of the infant (3). During lactation, 70-80 mg of DHA is utilized per day for breast milk production (4). AA has also been shown to affect infant growth and development (5).

Intake of LCPUFA by the breast fed infant is dependent on maternal diet, body fat stores, and possibly activity level. Dietary fatty acids of lactating women can be utilized in three ways: 1) stored in the adipose tissue, 2) transferred to the mammary gland for incorporation into milk, and/or 3) used for energy, especially during exercise. Exercise mobilizes fatty acids from body stores for energy. Increased metabolism during exercise may lead to a decrease in concentrations of LCPUFA available for incorporation into breast milk. However, the increased loss due to oxidation of the fatty acids may be countered by an increase in fatty acid mobilization from adipose stores. This phenomenon has not been investigated in lactating women.

Current recommendations of the Centers for Disease Control and Prevention and the American College of Sports Medicine (6) are to exercise moderately 30 minutes per day, every day of the week, whereas the Institute of Medicine (7) recommends incorporating 60 minutes of moderate exercise per day to achieve optimal health benefits. In addition, the American Academy of Pediatrics and the American Dietetic Association recommend that all women breastfeed their infants during the first year of life (1, 2). However, there is a paucity of research on the effects of exercise by lactating women on the composition of their breast milk. Therefore, the purpose of this study was to investigate the effects of chronic and acute exercise on plasma and breast milk LCPUFA. Another objective was to determine if breastfeeding women consume adequate amounts of LCPUFA to compensate for those used for energy during exercise.

METHODS

Participants

Healthy (absence of chronic disease), nonsmoking, exclusively breastfeeding women were recruited from prenatal classes and obstetricians' offices. Participants were eligible if 1) their body mass index (BMI) was between 20 and 30 kg/m^2, 2) their infants' birth weight was greater than 2500 grams, and 3) there were no birth complications. Mothers were assigned to one of two groups based on their self-reported exercise history. The exercise group (EG) consisted of mothers who exercised at least 30 minutes a day, three days per week, for the past six weeks (n=30). Women were assigned to the sedentary group (SG) if they exercised once a week or less (n=23). A subsample (n=14) of women from the EG completed a second study examining effects of acute exercise, in which subjects participated in an exercise session and a rest session. This research study was approved by the Institutional Review Board of the University of North Carolina at Greensboro, and written, informed consent was obtained from all participants. This research was conducted prior to establishment of the HIPPA guidelines.

Experimental Design

This was a cross-sectional study that measured the LCPUFA concentrations in plasma and breast milk of sedentary and exercising lactating women at 12 (± 2) weeks postpartum. Breast milk was collected by each woman on the morning of her laboratory measurements. Milk (30 ml) from the first feed after 5 AM was expressed and stored in the subject's home freezer until transported to the lab, where it was frozen at –80 ˚C until

analysis. Blood samples were drawn and then body composition and cardiorespiratory fitness were measured.

Fourteen of the exercising women returned for two more tests to determine the effects of acute exercise on concentrations of fatty acids in breast milk. The women participated in both a rest and an exercise session, with the order of sessions randomized. Sessions were separated by approximately two days. Exercise consisted of brisk walking or jogging at an intensity of approximately 75% of predicted maximum heart rate for 30 minutes. During the exercise session, breast milk was expressed before the onset of exercise and 10 and 60 minutes after the exercise bout. During the rest session, milk was collected at the same time points, with the women resting in a seated position for the 30-minute test period. All milk was completely expressed with a Medela electric breast pump (McHenry, IL) from both breasts at each time point. Samples were frozen at –80 ˚C until analysis.

Dietary Analysis

Prior to laboratory measurements, women weighed their food and beverage intake on a portable digital gram scale (OHAUS, Florham Park, NJ) and recorded their consumption for three days. The Food Processor Analysis Program (version 2.2, Salem, Oregon), along with manufacturer information, food label nutrient content information, and other food composition tables (8), were used for dietary analysis.

Anthropometrics

Weight to the nearest 0.1 kg was measured on a stationary beam balance with women wearing only a bathing suit. Height was measured, using a stadiometer, to the

nearest 0.1 cm. Body composition was measured by hydrostatic weighing. Body density and percent body fat were calculated using the equations of Brozek et al. (9).

Cardiorespiratory Fitness Level

To assess the cardiorespiratory fitness level of the participants, a submaximal graded treadmill test was used (10). Women wore a heart rate monitor (Polar, Inc., Woodbury, NY). Heart rate and perceived exertion were measured and recorded every minute for the duration of the test. Participants exercised until their heart rate reached 85% of their predicted maximal heart rate, and predicted oxygen consumption (VO_2) was determined using the formulas of the American College of Sports Medicine (10). Linear regression was used to determine the subject's predicted VO_{2max} using heart rate as the independent variable and predicted VO_2 as the dependent variable.

Plasma and Breast Milk Fatty Acid Analysis

Blood was drawn by venipuncture from an antecubital vein by a trained phlebotomist, after a 12-hour fast. The plasma layer was separated and stored at -80˚C until analyzed for fatty acid content. Plasma fatty acids were extracted by an adapted method of Bligh and Dyer (11). Breast milk was extracted using a modified Folch procedure as detailed by Bitman et al. (12). The fatty acid profiles of plasma and breast milk were determined by a gas chromatograph (GC) (5890 Gas Chromatograph, Hewlett Packard, Avondale, PA). Plasma was collected from a subsample of 38 (SG, n=14; EG, n=24) women, because measurement of plasma FA was started after 15 women had already completed the study of breast milk fatty acids. Milk was not collected from one participant in the SG due to laboratory error.

Statistical Analysis

SPSS-PC (Version 10.0, SPSS, Chicago, IL) statistical software was used for all data analysis. Student's t test was used to assess differences between experimental groups. Repeated measures ANOVA was used to determine if breast milk LCPUFA concentrations changed during exercise or rest sessions. Pearson's correlations were identified for relationships between breast milk fatty acids and diet, plasma, parity, and percent body fat. Multiple linear regression analysis was used to predict concentrations of breast milk fatty acids from the above variables. Statistical significance was set at $P<0.05$.

RESULTS

There were no significant differences between groups in characteristics of participants, except that exercising women exhibited a higher level of cardiorespiratory fitness (Table 1). They reported exercising an average of 47 minutes per day, four days per week. The majority of the women walked briskly and/or participated in aerobic exercise classes. Their average fitness level was in the 80th percentile, according to values given by the American College of Sports Medicine (10) for cardiorespiratory fitness. The sedentary women had an average fitness level in the 40th percentile.

There were no differences in percent body fat (Table 1) or dietary intake (Table 2) between the two groups; except that arachidonic acid intake was significantly higher in the SG. Both groups consumed a diet of approximately 55% carbohydrates, 15% protein, and 30% fat; and the ratio of LA to LNA was approximately 11.5:1.

There were no significant differences in the concentrations of the LCPUFA in the breast milk (Table 3) of exercising and sedentary lactating women. Breast milk and plasma of the subgroup (n=38) were also not significantly different between groups (data not shown). Due to the lack of difference between groups, the data from all subjects were pooled together for further analysis. Breast milk AA and dietary AA were correlated ($r=0.29$, $p=0.04$); however, there were no correlations between breast milk and dietary intake of the other LCPUFA's. Plasma LA and AA were significantly correlated with milk LA ($r=0.33$, $p=0.04$) and AA ($r=0.45$, $p=0.005$), respectively. There was no correlation between parity or percent body fat and breast milk fatty acid concentrations in this group. When these were controlled for, there were still no significant differences in

dietary fatty acid concentrations between groups. In multiple regression analysis, dietary intake, parity, and percent body fat did not predict fatty acid concentrations in breast milk.

Characteristics of the subsample who participated in the second study were similar to the characteristics of all the women in the EG, shown in Table 1. Between session changes over time were noted (Figure 1) for LA and LNA; however, these changes were not significant (LA, $p=0.18$; LNA, $p=0.23$). During the exercise session, LA increased from time 0 to 60, but in the rest session, LA decreased from time 0 to 60. Similarly, LNA increased in the exercise session from time 0 to 60. However, in the rest session, LNA increased from time 0 to 10, but decreased from time 10 to 60, with time 60 falling to a concentration lower than the initial measurement (Figure 1). There were no significant differences in changes over time for AA, EPA, or DHA (data not shown). Interestingly, percent body fat was significantly correlated with concentrations of LA in milk at time 60 after the exercise session ($r= 0.62$, $p= 0.02$) and with concentrations of LNA in milk at time 10 ($r=0.53$, $p= 0.04$) and time 60 ($r= 0.77$, $p= 0.001$) after the exercise session. There were no significant correlations between percent body fat and milk LCPUFA concentrations during the rest sessions.

DISCUSSION

In this study, dietary intake and exercise did not significantly affect the plasma and breast milk of lactating women. However, there was a trend towards an increase in LA and LNA after an acute exercise bout. With the exception of AA intake, there were no differences in the diets of the women in this study. On average, these women ate diets sufficient in calories, protein, and fat, and were similar to dietary intakes of lactating women reported by other researchers (13, 14). It is possible that the observation of no effect of diet or exercise on the LCPUFA concentrations (except AA) in breast milk was because there was insufficient variation in intakes among the women.

The optimal ratio of n-6 to n-3 fatty acids has not been established; however, researchers suggest a ratio of less than 10:1 (15). This ratio is estimated to be 25:1 in American diets due to increased consumption of processed foods in place of natural foods (16). Women in the current study consumed diets with an n-6/n-3 ratio at about 11.5:1, which is better than the reported national average. The recommended Adequate Intakes of LA and LNA for lactation are 13 grams/day and 1.3 grams/day, respectively (7). Diets of breastfeeding mothers in this study were close to these recommendations (Table 2). Conversion of LNA into DHA and EPA is quite inefficient (15); therefore, in addition to consuming adequate amounts of LA and LNA, breastfeeding women should make efforts to consume adequate amounts of DHA and EPA. These results suggest that active, breastfeeding women are able to meet the new dietary recommendations for LA and LNA; however, specific recommendations are not yet available for DHA, EPA, and AA.

Current recommendations for LA and LNA for infants aged 0-6 months are 4.4 and 0.5 g/day, respectively (7). Based on the recommended total fat intake of 31 g/day for this age group (7), the percent of total fat coming from the individual fatty acids (LA and LNA) can be calculated as 14.2% and 1.6%, respectively. Average concentrations of LA and LNA in our breast milk samples were similar to these recommendations (Table 3), indicating that the women in this study were able to provide sufficient LA and LNA for their infants.

Concentrations of fatty acids were similar to those reported by others in breast milk (17) and plasma (18). Only dietary and breast milk AA concentrations were significantly related in our study. However, isotope tracer studies indicate that maternal body stores, rather than diet, are the major source of breast milk fatty acids, suggesting that dietary fatty acids are first stored in the body and later mobilized for incorporation into breast milk (19). In addition, concentrations of LCPUFA in breast milk were lower than concentrations of LCPUFA in plasma, with the exception of LNA, which was slightly higher in breast milk than in plasma. This suggests that the availability of LCPUFA in the plasma is not a limiting factor for concentrations in breast milk.

Fatty acid concentrations decrease as parity increases (20). The observation of no effect of parity on concentrations of fatty acids in breast milk may be, in part, due to the limited range of parity in this group. Most women had a parity of 1 (n=18) or 2 (n=23), with only 13 women having a parity of 3 or greater. While breast milk fatty acid concentrations did decrease as parity increased, this relationship was not significant.

Exercise leads to the mobilization of fatty acids from the adipose tissue triacylglycerol; plasma fatty acid concentrations increase three fold compared to rest (21). We observed that 30 minutes of moderate exercise increased concentrations of LA and LNA in breast milk; however, the increase was not significant. Borsheim et al. (22) observed a similar increase in LA in the plasma of exercising as compared to resting men, and Mougios et al. (23) reported that male handball players had an increase in plasma LA after two 30-minute playing sessions. However, we observed no effect of exercise on concentrations of AA in breast milk, in contrast to the decrease in plasma AA reported by Borsheim (22).

The observation of no exercise effect on breast milk DHA may be related to DHA being one of the fatty acids stored in small quantities in the human body. And during lactation, storage concentrations are much lower than normal (4). Therefore, there may be much lower amounts of DHA mobilized during exercise, as compared to LA and LNA. Plasma lipid fractions also contain very little EPA and AA. Turnover of these free fatty acids is quite rapid in the time between meals in order to provide tissues with nourishment (24).

Since exercise stimulated lipid mobilization can continue for an extended period of time after cessation of exercise (22), fatty acids may have been transferred to breast milk from plasma after the 60-minute measurement point. In our sample, there was a trend towards an increase in the concentrations of LA and LNA in the breast milk from 10 to 60 minutes after exercise. This increase may have been higher if we had continued our measurements beyond 60 minutes. These results suggest that instead of experiencing

a decrease in breast milk LA and LNA concentrations after exercise, women may, in fact, have increased concentrations of LA and LNA in their breast milk.

The significant relationship between percent body fat and concentrations of LA and LNA 60 minutes after the exercise session suggests a greater mobilization of LA and LNA during exercise in women who have a higher percent body fat. This is likely due to a greater availability of fat for mobilization. The trend towards an increase in concentrations of LA and LNA after exercise indicates that LA and LNA may actually increase in breast milk after exercise, especially among women with adequate body fat stores. This may not be true for women who are underweight.

Moderate exercise is not only safe during lactation, but also improves the cardiorespiratory fitness levels of postpartum women, and provides many other health benefits (25-27). We have previously reported no effect of exercise on breast milk concentrations of macronutrients (25, 27), vitamin B6 (28), or immunological compounds (29). This study found no effect of exercise on the LCPUFA concentrations in breast milk. Therefore, a lactating woman with adequate body fat stores, and consuming a diet sufficient in calories and essential fatty acids (including total fatty acid intake and LA to LNA ratio), can perform moderate exercise without decreasing, and potentially increasing, the concentrations of LCPUFA in her breast milk.

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REFERENCES

1. American Academy of Pediatrics, Work Group on Breastfeeding. Breastfeeding and the use of human milk. *Pediatrics* 1997; 100: 1035-9.

2. Position of the American Dietetic Association: Breaking the barriers to breastfeeding. *J Am Diet Assoc* 2001; 101: 1213.

3. Innis SM. Essential fatty acids in growth and development. *Prog Lipid Res* 1991; 30: 39-103.

4. Makrides M and Gibson RA. Long-chain polyunsaturated fatty acid requirements during pregnancy and lactation. *Am J Clin Nutr* 2000; 71(suppl): 307S-11S.

5. Innis SM, Adamkin DH, Hall RT, Kalhan SC, Lair C, Lim M, Stevens DC, Twist PF, Diersen-Schade DA, Harris CL, Merkel KL, Hansen JW. Docosahexaenoic acid and arachidonic acid enhance growth with no adverse effects in preterm infants fed formula. *J Pediatr* 2002; 140: 547-54.

6. Pate RR, Pratt M, Blair SN et al. Physical activity and public health: a recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. *JAMA* 1995; 273: 402-407.

7. Institute of Medicine. *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids*. Food and Nutrition Board. Washington, DC: National Academy Press 2002.

8. Pennington JAT. Bowes and Church's food values of portions commonly used, 17th ed. Philadelphia, PA: J.B. Lippincott Co., 1998.

9. Brozek J, Grande F, Anderson JT, Keys A. Densitometric analysis of body composition; revision of some quantitative assumptions. *Ann N Y Acad Sci* 1963; 110: 113-140.

10. American College of Sports Medicine. *ACSM's Guidelines for Exercise Testing and Prescription, 6th ed*. Philadelphia, PA: Lippincott, Williams & Williams; 2000.

11. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959, 37; 8: 911-7.

12. Bitman J, Wood DL, Mehta NR, Hamosh P, Hamosh M. Lipolysis of triacylglycerols in human milk at low temperatures: a note of caution. *J Pediatr Gastroenterol Nutr* 1983; 2: 521-4.

13. Otto SJ, van Houwelingen AC, Badart-Smook A, Hornstra G. Comparison of peripartum and postpartum phospholipid polyunsaturated fatty acid profiles of lactating and nonlactating women. *Am J Clin Nutr* 2001; 73: 1074-9.

14. Fidler N, Sauerwald T, Pohl A, Demmelmair, Koletzko B. Docosahexaenoic acid transfer into human milk after dietary supplementation: a randomized clinical trial. *J Lipid Res* 2000; 41: 1376-83.

15. Davis BC, Kris-Etherton PM. Achieving optimal essential fatty acid status in vegetarians: current knowledge and practical implications. *Am J Clin Nutr* 2003 Sep; 78(3 Suppl): 640S-646S.

16. Simopoulos AP. Omega-3 Fatty Acids, Part I: Metabolic effects of omega-3 fatty acids and essentiality. *Handbook of Lipids in Human Nutrition*, CRC Press 1996: 51-73.

17. Makrides M, Neumann MA, Gibson RA. Effect of maternal docosahexaenoic acid (DHA) supplementation on breast milk composition. *Eur J Clin Nutr* 1996; 50: 352-7.

18. Francois CA, Connor SL, Bolewicz LC, Connor WE. Supplementing lactating women with flaxseed oil does not increase docosahexaenoic acid in their milk. *Am J Clin Nutr* 2003; 77: 226-33.

19. Del Prado M, Villalpando S, Elizondo A, Rodriguez M, Demmelmair H, Koletzko B. Contribution of dietary and newly formed arachidonic acid to human milk lipids in women eating a low-fat diet. *Am J Clin Nutr* 2001; 74: 242-7.

20. Jensen RG. Lipids in Human Milk. *Lipids* 1999; 34: 1243-1271.

21. Hodgetts V, Coppack SW, Frayn KN, Hockaday TDR. Factors controlling fat mobilization from human subcutaneous adipose tissue during exercise. *J Appl Physiol* 1991; 71: 445-51.

22. Borsheim E, Knardahl S, Hostmark AT. Short-term effects of exercise on plasma very low density lipoproteins (VLDL) and fatty acids. *Med Sci Sports Exerc* 1999; 31: 522-30.

23. Mougios V, Kotzamanidis C, Koutsari C, Atsopardis S. Exercise-induced changes in the concentration of individual fatty acids and triacylglycerols of human plasma. *Metabolism* 1995; 44: 681-8.

24. Spector, AA. Lipid Metabolism: Essential fatty acids. Biochemical and Physiological Aspects of Human Nutrition. Martha H. Stipanuk. W.B. Saunders Co. 2000: 365-83.

25. Lovelady CA, Lonnerdal B, Dewey KG. Lactation performance on exercising women. *Am J Clin Nutr* 1990; 52: 103-9.

26. Lovelady CA, Nommsen-Rivers LA, McCrory M A, Dewey KG. Effects of exercise on plasma lipids and metabolism of lactating women. *Med Sci Sports Exerc* 1995; 27: 22-8.

27. Dewey KG, Lovelady CA, Nommsen-Rivers, McCrory MA, Lonnerdal B. A randomized study of the effects of aerobic exercise by lactating women on breast-milk volume and composition. *N Engl J Med* 1994; 330: 449-53.

28. Lovelady CA, Williams JP, Garner KE, Moreno KL, Taylor ML, Leklem JE. Effect of energy restriction and exercise on vitamin B-6 status of women during lactation. *Med Sci Sports Exerc* 2001; 33: 512-8.

29. Lovelady CA, Hunter CP, Geigerman C. Effect of exercise on immunologic factors in breast milk. *Pediatrics* 2003; 111: 148-52.

TABLE 1. Characteristics of Participants*

Characteristic	Sedentary Group (n=23)	Exercising Group (n=30)
Age (yrs)	31.5 ± 1.0	31.5 ± 0.6
Height (cm)	163.1 ± 1.1	164.6 ± 1.2
Weight (kg)	66.0 ± 1.6	64.0 ± 1.5
Body Mass Index (kg/m^2)	24.9 ± 0.7	23.6 ± 0.5
% Body Fat	28.0 ± 1.4	25.5 ± 1.1
Predicted maximal oxygen consumption (O_2/kg/min)	32.2 ± 1.0	39.6 ± 1.0**

* Values are means ± standard errors of the means.

** Significantly different from sedentary group, $p < 0.01$

TABLE 2. Dietary Intake*

	Sedentary Group (n=23)	Exercising Group (n=30)
Total energy intake (kcal)	2276 ± 93	2142 ± 106
Kcal/kg body weight	35 ± 2	34 ± 2
Total protein intake (grams)	85 ± 5	83 ± 3
% kcal from protein	15 ± 1	16 ± 1
Total carbohydrate intake (grams)	313 ± 13	306 ± 18
% kcal from carbohydrate	55 ± 1	56 ± 2
Total fat intake (grams)	80 ± 5	69 ± 5
% kcal from fat	32 ± 1	29 ± 1
Fatty acid intake (grams)		
18:2 n-6 (LA)	11.05 ± 1.39	9.34 ± 0.97
18:3 n-3 (LNA)	0.96 ± 0.12	0.82 ± 0.09
20:4 n-6 (AA)	0.10 ± 0.01	0.06 ± 0.01**
20:5 n-3 (EPA)	0.03 ± 0.01	0.03 ± 0.01
22:6 n-3 (DHA)	0.06 ± 0.02	0.05 ± 0.02

* Values are means ± standard errors of the means.

** Significantly different from sedentary group, $p < 0.05$

LA=linoleic acid, LNA=linolenic acid, AA=arachidonic acid, EPA=eicosapentaenoic acid, DHA=docosahexaenoic acid

TABLE 3. Skinfold Measurements*

	Sedentary Group (n=23)	Exercising Group (n=30)
Triceps	23.45 ± 1.45	19.89 ± 1.02**
Subscapular	20.42 ± 1.55	15.96 ± 1.12**
Midaxillary	13.80 ± 0.95	11.03 ± 0.82**
Abdominal	21.46 ± 1.39	20.33 ± 1.52
Suprailiac	13.87 ± 1.18	12.06 ± 0.89
Thigh	42.53 ± 2.72	36.10 ± 2.21

* Values are means ± standard errors of the means.

** Significantly different from sedentary group, $p < 0.05$

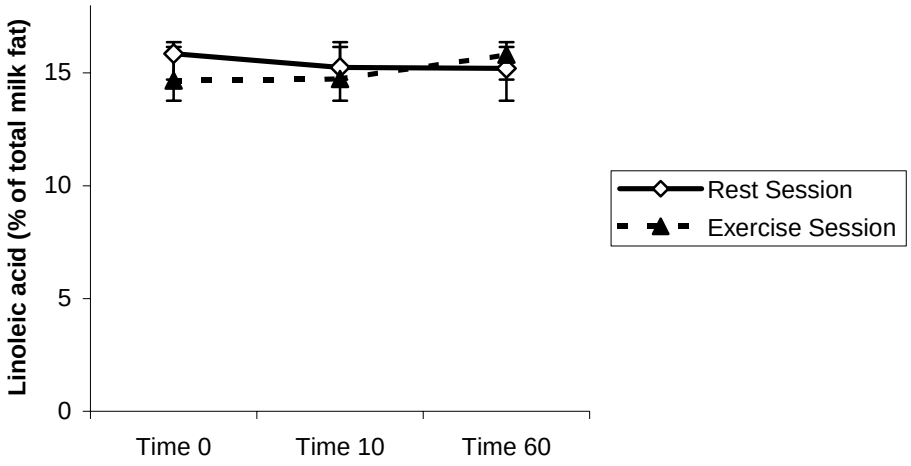
TABLE 4. Fatty acid Composition of Breast Milk & Plasma*

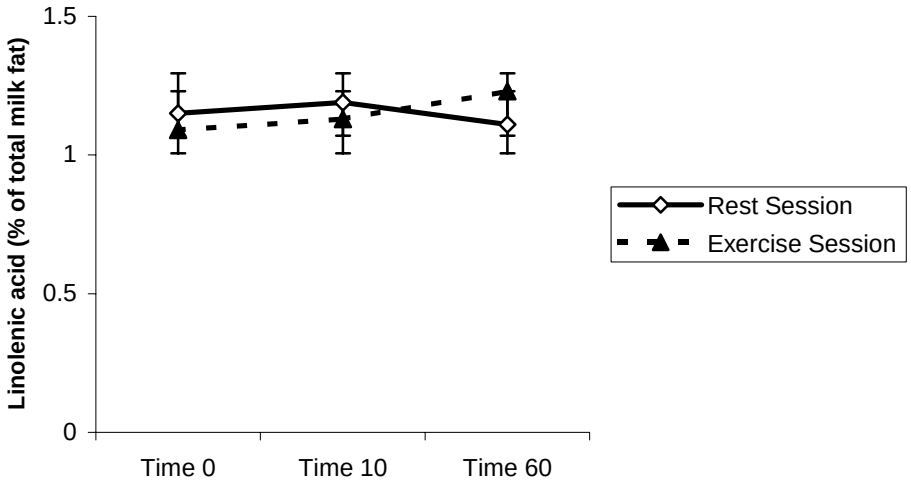
	Sedentary Group (milk n=22) (plasma n=14)	Exercising Group (milk n=30) (plasma n=24)
% of total fat		
18:2 n-6 (LA)		
Breast Milk	16.94 ± 0.97	14.71 ± 0.72
Plasma	32.43 ± 1.46	31.38 ± 0.80
18:3 n-3 (LNA)		
Breast Milk	1.14 ± 0.11	1.03 ± 0.09
Plasma	0.97 ± 0.14	0.98 ± 0.09
20:4 n-6 (AA)		
Breast Milk	0.41 ± 0.03	0.38 ± 0.02
Plasma	6.72 ± 0.36	6.14 ± 0.21
20:5 n-3 (EPA)		
Breast Milk	0.30 ± 0.08	0.39 ± 0.09
Plasma	0.42 ± 0.05	0.54 ± 0.06
22:6 n-3 (DHA)		
Breast Milk	0.21 ± 0.08	0.43 ± 0.09
Plasma	0.92 ± 0.09	1.01 ± 0.10

* Values are means ± standard errors of the means.

LA=linoleic acid, LNA=linolenic acid, AA=arachidonic acid, EPA=eicosapentaenoic acid, DHA=docosahexaenoic acid

FIGURE 1. Percent linoleic acid (LA) and linolenic acid (LNA) in breast milk of women before (Time 0) and after (Time 10 and Time 60) the 30-minute rest session or exercise session. Values are means ± SEM. LA concentration decreased during rest and increased during exercise. Changes were similar with LNA concentrations. However, these changes were not significant (RMANOVA).





CHAPTER II

THE EFFECTS OF DIET AND EXERCISE ON BODY COMPOSITION, BONE MINERAL DENSITY AND BONE TURNOVER MARKERS DURING LACTATION

REVIEW OF LITERATURE

Bone Density Changes During Lactation

During lactation, maternal bone density decreases due to hormonal changes and increased calcium losses to breast milk. Bone losses during lactation average from 3 to 5%, ranging up to 10% in some women (1). Reductions in bone mineral density (BMD) occur mostly in the spine and hip regions (2), due to a greater percentage of trabecular bone. Bone density losses are the greatest in the first 3-5 months of lactation (1-3% per month), and evidence indicates that in later lactation and early weaning mineral stores may be somewhat replenished (3, 4). At 6 months postpartum, lumbar spine BMD was decreased in lactating women (-6.5 %) as compared to BMD measurements at 2 days postpartum (5). Lactating women had more significant lumbar spine and total bone losses than non-lactating controls at 6 months postpartum. After weaning, lactating women showed greater bone gains than the non-lactating controls (6).

The majority of BMD losses appear to occur in the first 3-5 months of lactation, but recovery to normal BMD levels does not take place until lactation ceases. It is important to note that BMD does not always completely recover upon cessation of lactation. Studies examining bone loss during lactation found that BMD levels at 5 months post-weaning were lower than BMD levels at pre-pregnancy and delivery (4, 7, 8). Therefore, minimizing losses of bone density during the lactation period may promote quicker and more complete recovery of bone mineral density upon weaning.

Whether or not bone density losses in pregnancy and lactation lead to poor bone mineral status later in life, and possibly osteoporosis, is still somewhat controversial (9). A recent retrospective twin study by Paton *et al*., found no deleterious effect of pregnancy or lactation on bone mineral density later in life. In fact, women who had been pregnant had higher bone density than never pregnant women, and women who breastfed their infants had bone densities higher than their non-breastfeeding twin counterparts (10). Therefore, even though research studies are still not conclusive concerning the long term effects of BMD loss during lactation, improving bone density during lactation will benefit mothers later in life.

Markers of Bone Turnover

Bone turnover is comprised of a resorption phase and a formation phase. Some of the markers of bone resorption include urinary pyridinium cross links, deoxypyridinoline (DPD) and pyridinoline (PYD), hydroxyproline, and helicopeptide (HP). Commonly used markers of bone formation include serum osteocalcin (OC) and bone-specific alkaline phosphatase (BAP). In studies investigating the effects of lactation on bone turnover, markers are usually measured within the first few days or weeks of partuition (11, 12, 13) and at 6 months postpartum (11, 12). Sowers et al. also measured bone markers at 2, 4, 12, and 18 months postpartum (11), while others set their endpoint measures at 3 months postpartum (13). Markers of bone turnover will fluctuate over a 6 month study period; however, based on the above studies (12, 13) it is sufficient to measure these markers at baseline and endpoint to assess change over time in this population. This is the model for the current research study to follow.

DPD is highly specific to bone and its crosslinks are found in the blood and urine following the breakdown of crosslinked collagen. The crosslinks serve to strengthen the collagen molecules. DPD levels in the urine indicate the degradation of mature collagen, as opposed to newly formed collagen. Other measures of bone resorption (i.e. hydroxyproline) can be contaminated by the breakdown of this newly formed collagen, making DPD a more reliable and sensitive indicator of bone resorption. Urinary DPD and PYD are also widely used bone resorption markers because they are not influenced by dietary intake (14). PYD is also one of the major crosslinks of bone collagen, however, it is also found in cartilage, bone, ligaments and blood vessels. Turnover is much higher in bone than in cartilage, ligaments and vessels; therefore, amounts of PYD found in urine come primarily from bone resorption. Currently, PYD and DPD are considered the best measures of bone resorption (15-20). HP is a urinary measure of the helical part of the type I collagen. HP is a relatively new assay, and has been well correlated to C-terminal cross-linking type I collagen, which is a well-established assay (21). Type I collagen is the most prevalent protein found in the bone matrix. When bone resorption occurs, type I collagen is cleaved from both the helicoidal and telopeptide regions. DPD, as described above, is a measure of cross-links from the telopeptide region. HP is a measure of the helicoidal region of the type I collagen, which is the major portion of the protein. Assessing both the telopeptide and helicodal regions allows for increased understanding of bone resorption.

Alkaline phosphatase is thought to play a role in bone mineralization, or bone formation, however, the specific function of the enzyme is not yet known. There are

multiple isoforms of alkaline phosphatase, including bone, liver, and kidney. In healthy adults, absent of liver problems, total alkaline phosphatase is comprised of 50% liver, and the other 50% comes from the bone. However, there is a specific assay that detects the bone-specific alkaline phosphatase (BAP). BAP is a direct product of osteoblasts. In studies looking specifically at bone turnover, i.e. osteoporosis, where small changes in bone turnover are potentially detectable, BAP is the preferred assay. BAP is a specific assay; however, as with many assays there is the potential for contamination, up to 20%, with liver alkaline phosphatase (22).

Osteocalcin (OC), another marker of bone formation, is also a specific marker of the function of osteoblasts. OC is a more delicate assay, in that it is rapidly degraded in serum. It has also been suggested that because OC is a part of the bone matrix, some OC may be released during the bone resorption phase. This may be especially true for individuals with a high rate of bone turnover, in which the smaller fragments of OC are released (22). Keeping the disadvantages in mind, OC is still a commonly used, well-accepted measure of bone formation. Used along with BAP, these two indicators provide a good understanding of the rate of bone formation. With the exception of HP, all of the above markers have been used to study the lactating population (11-13).

Hormones and Bone Mineral Density

Prolactin, a hormone necessary to stimulate and maintain lactation, is 10-20 times higher in pregnancy as compared to the non-pregnant state. Postpartum, prolactin levels gradually decline, independent of lactation. Lactating women have prolactin levels that continue to be elevated (above that of non-lactating and pregnant women) if they are

exclusively breastfeeding until 6 months postpartum (5); however, prolactin levels also decrease significantly in breastfeeding women during the first 6 months postpartum. Basal prolactin levels are lower in multiparous mothers, as compared to primiparous mothers, yet, milk intake is greater in infants of multiparous mothers. This may be due to an increased uptake by prolactin receptors in the mammary gland of multiparous mothers, leading to lower prolactin levels in serum (23). During lactation, bone loss takes place due to a hyperprolactinemic and hypoestrogenic state. Multiple hormones are involved in calcium homeostasis during pregnancy and lactation. These hormones include calcitonin, parathyroid hormone (PTH), and 1,25- dihydroxyvitamin D (1,25D). Calcitonin levels are variable during lactation, and may in fact be unaltered in some women. PTH and 1,25D may be necessary for serum calcium regulation, and may also be necessary for BMD recovery during late lactation and weaning. Serum PTH may be slightly depressed in lactation and 1,25D is not elevated during lactation, but may be higher in lactating women as compared to non-lactating controls (3). Parathyroid hormone-related protein (PTHrP) is now considered to be one of the prime regulators of calcium and bone metabolism during lactation (9, 24, 25), however this concept is still under investigation. PTHrP is produced by the lactating mammary gland, possibly due to the effects of prolactin. PTHrP is secreted into both breast milk and into maternal circulation, and has characteristics similar to PTH. PTHrP promotes the synthesis (via stimulation of renal 1-α -hydroxylase activity and nephrogenous cyclic adenosine monophosphate) of 1,25D and the renal tubular reabsorption of calcium. Therefore, during lactation, PTHrP may be the cause of increased 1,25D when PTH levels are low.

Laskey *et al.* (2) examined the role of an individual's vitamin D-receptor genotype because of the interaction of genes with nutrients and the effects on calcium and bone metabolism during lactation. This study, however, did not find any relationship of vitamin D-receptor genotype on bone area adjusted bone mineral content at any body site, or with maternal weight or height.

Growth hormone (GH) is an anabolic hormone that is produced by the pituitary gland and is involved in many metabolic systems. Insulin-like growth factor-1 (IGF-1) is a growth-regulating, insulin-like peptide. Together, GH and IGF-1 are key players in the bone remodeling system (26). In addition, GH is known to increase milk production during lactation (27, 28); this effect is mediated by IGF-1 (29). Further, GH and IGF-1 are increased during exercise, and this increase may stimulate an increase in bone turnover. The role of GH and IGF-1 on bone remodeling during lactation in women who are exercising proves to be an interesting investigation; however, studies are limited in this area of research.

During lactation, estrogen levels rapidly drop back to pre-pregnancy levels, or may even drop lower, in order to promote lactation. Low estrogen levels can lead to decreased BMD, and coupled with excessive calcium losses to breast milk, can lead to rapid bone remodeling. Normal bone remodeling occurs over a period of 4-8 months; however, during lactation the remodeling may occur within 3-4 months (25).

According to Frost's (30) hypothesis of mechanical usage set points, estrogen may be responsible for decreasing bone modeling and remodeling set points. The set point is the level at which a given mechanical stress promotes bone remodeling at a level

to maintain current BMD. Stressing the bones above this level will increase bone density, while not stressing the bones enough to reach the set point will result in bone loss. During pregnancy when estrogen levels are high, the set point is lowered, and therefore increased mechanical stress (i.e. increased weight) above the low set point, should theoretically increase bone density. When pregnancy ends, and estrogen levels decrease rapidly, the set point increases. This means that bone stress needs to increase to levels above the new higher set point to increase BMD. The extra weight of pregnancy also decreases, leading to decreased mechanical stress. This theory would suggest that pregnant women experience increases in bone mineral density, but this is not commonly observed. The other factors of pregnancy, including increased calcium needs by mother and fetus and many other hormonal changes, lead to BMD losses. Frost's theory possibly explains some of the BMD losses seen in lactation. Decreased estrogen leads to an increased set point of mechanical usage. Therefore, the lactating woman has to stress the bones at a level much higher than during pregnancy in order to prevent BMD losses and to promote BMD gains.

Exercise and Bone Mineral Density

Exercise, especially weight bearing exercise, has been shown to improve bone density. Specific sites on the human body are exposed to greater mechanical stress on a regular basis; the legs have to support the body when walking and the spine has to constantly hold the body in an upright position. These stresses help to maintain the BMD of the bones in these areas. As suggested in Frost's (30) theory, as body weight increases (i.e. during pregnancy) the mechanical stress on these specific bone regions will be

greater, and bone mineral density will increase. In order to increase bone, it is necessary for the bone to be stressed at a level greater than that under normal conditions. Trabecular bone, the predominant bone in the vertebral column, responds to stress more rapidly than cortical bone. In response to regular stimuli, or stress, new collagen fibers can be deposited, forming the new bone matrix, after approximately 8 to 12 weeks. Mineralization of this new bone will occur in the subsequent weeks or months (31-33), with the whole process totaling approximately 4-8 months. This process may be upregulated to a total of 3-4 months during lactation (25).

Weight bearing exercises are beneficial for increasing BMD in the region under stress. Muscle contraction and gravity are mechanical forces that can increase bone mineralization when the body is exercising. It is important to provide resistance to specific bone areas to produce the desired bone strengthening. The axial skeleton needs proper loading through an appropriate training program. Squats, deadlifts, high pulls, standing shoulder press, isometric pushups, ball bench presses and abdominal exercises work the core of the body, as well as some areas of the appendicular skeleton.

Exercise and estrogen hormone replacement therapy (HRT) increased lumbar spine and femoral neck BMD individually and when used together in postmenopausal women, possibly due to decreased bone turnover (34). Decreases in osteocalcin in response to HRT and HRT + exercise, but not in an exercise only group, suggests that the observed increases in BMD were due to a decrease in bone resorption and not an increase in bone formation (34). In a recent study, women who exercised (aerobic, weight-bearing, and weight-lifting) 3 days/week had improved femoral neck and lumbar spine

BMD when HRT was also used. Improved trochanteric BMD was observed in exercising women using HRT as well as those not using HRT (35). Postmenopausal women undergoing a one-year strength training program, half of which were currently using HRT, experienced improvements in BMD that were linear to amount of weight lifted over the year (36). These studies suggest that exercise and HRT may have both individual and synergistic effects on bone mineral density in postmenopausal women.

Effects of Exercise and Lactation on Bone Turnover Markers

Markers of both bone formation and bone resorption have been investigated in terms of their response to exercise. In adult male soccer players with levels of activity ranging from 12 hours/week (premier players) to 6 hours/week (sixth league players), who were compared to older former players and matched controls, it was determined that the active players had higher OC, BAP, and carboxyterminal crosslinked telopeptide of type I collagen (ICTP). Activity was positively correlated with BAP in those participants who exercised 6 hours per week or less; however, there was no difference in the higher activity levels (above 6 hours/week). The authors suggested that bone adapts to current levels of exercise, with duration above the needed level not providing additional benefits (37). Interestingly, a substudy of the premier athletes from the previous study was performed, in which these athletes underwent a 4-week rest period between two seasons followed by 10 active days into the second season. During the rest period, bone formation markers carboxy-terminal propeptide of type I collagen (PICP) and total alkaline phosphatase (TAP) were decreased and resorption marker ICTP was increased. Upon resumption of exercise in the new season PICP increased and ICTP decreased.

Osteocalcin (OC) was higher in exercising athletes as compared to controls at baseline, and decreased after 7 days of rest, however this change was not significant (38). Taken together, these two studies suggest that weight-bearing exercise (soccer playing, running, and jumping) provide benefits to bone. In addition, the 6-hour threshold effect should be considered in the design of studies examining changes in bone turnover markers, and studies should not exceed this upper limit. Further studies can also attempt to determine how this 6-hour activity level should be met (i.e. 3 vs. 6 sessions/wk). Bone formation markers are higher and resorption markers are lower in adults who engage in aerobic activity on a regular basis, and when this activity ceases, bone formation decreases and resorption increases. Shibata et al. (39) also found that one year of walking training promotes bone formation, indicated by an increase in BAP, with this effect greater in the group who added a jumping session each day in addition to walking.

Resistance exercise training also provides an increase in bone formation markers and a decrease in bone resorption markers, with changes occurring early in the training program (40). High intensity resistance exercise training increased bone formation in older adults (41), and aerobic training decreased bone resorption in young males (42). It has been suggested that the effects of physical activity (aerobic and anaerobic) on bone turnover is dependent on the type of activity performed, and that anaerobic activity may increase overall bone turnover (42). Changes in bone turnover are often apparent before changes in BMD via dual energy x-ray absorptiometry (DXA) can be noted (11).

Postpartum, the mother's body is readjusting bone metabolism from pregnancy. In late pregnancy, in order to provide minerals for the growing fetus, bone resorption is

very high, and bone formation is low. In the first few months postpartum, a rapid decline in resorption, with a slower increase in formation, has been shown. The resorption and formation will eventually balance at levels closer to pre-pregnancy (43, 44). During lactation, bone formation remains low, and amenorrhea (with or without lactation) was associated with increased bone resorption; however, in lactation (with amenorrhea) resorption is even higher. Overall bone turnover appears to be elevated with lactation (13).

The effects of exercise on bone turnover markers during lactation have not been identified. The overall elevation in bone turnover during lactation, or the rapid decline in resorption in the postpartum period, may override any effects that may be due to an exercise program; however, this is not yet known.

Effect of Exercise on Bone Mineral Density during Lactation

Little and Clapp (45) detected no effect of exercise on inhibition of bone mineral loss in eleven lactating women who participated in self-selected recreational exercise (walking, running, aerobics, step aerobics, stair machine). Subjects in the exercise group had to exercise at least 3 days per week, for at least 20 minutes per session, at a minimum of 50% of maximal oxygen consumption. These women were compared to non-exercising controls. All subjects (both exercisers and non-exercisers) presented with femoral neck and lumbar spine losses at 3 months postpartum. The non-exercisers, however, had greater losses at the lumbar spine than the exercisers, but the difference was not significant. It is suggested, by these authors, that a planned intervention, utilizing both resistance and aerobic exercise may provide the necessary stimulus to improve

BMD during lactation (45). A successful intervention to improve bone mineral density during lactation could be modeled after strength training programs for postmenopausal women (35).

Drinkwater and Chesnut (46) observed decreases in femoral neck and radial shaft BMD in six female athletes during pregnancy, but found an increase in tibial BMD. After 6 months of lactation in these same six subjects, radial shaft BMD returned to normal, while femoral neck BMD continued to decrease. A non-significant decrease in lumbar spine BMD was also detected after 9 months of pregnancy and during lactation. This study compared these active pregnant and subsequently lactating women to a group of non-pregnant, non-lactating women. Decreases in bone mineral density observed in the lactating women were not seen in the non-pregnant, non-lactating group. There was no comparison of exercising lactating women to non-exercising lactating women in this study. It is well established that BMD decreases during lactation, especially during the first few months. Losses range from 3-5%, with some losses seen as high as 10%. Women in the above study were very active athletes prior to and during the first two trimesters of pregnancy, however many tapered down their exercise during the third trimester. Intensity and duration of exercise during the lactation period was not reported (46). The non-significant decrease in lumbar spine observed in these athletes suggests that exercise may slow bone loss during lactation in this area. Although this study did not have a control group, other studies report a significant decline in the lumbar spine among sedentary, lactating women (4, 8). Taken together, these two studies (45, 46)

provided preliminary data that were used to design a clinical trial utilizing a specific aerobic and strength training program in breastfeeding women.

Effect of Dietary Intake on Bone Changes During Lactation

During the last trimester of pregnancy, calcium is deposited in the fetal skeleton, at approximately 200-250 mg/day. Absorption efficiency is increased during pregnancy, suggesting no need for increased calcium intake above normal female needs (7). While up to 300 mg of calcium is lost daily to breast milk, maternal calcium intake does not appear to affect BMD during lactation (2). Calcium absorption efficiency returns to that at pre-pregnancy; however, renal calcium retention is increased (47), and urinary calcium losses are decreased during lactation (48). At 2 months postpartum, breast milk calcium appears to come mostly from spinal trabecular bone, in a study of women consuming recommended levels of calcium (approximately 1200 mg Ca/day) (7). Laskey *et al.* (2) also examined bone density loss during lactation, finding that the greatest losses occurred in the spine and femoral neck. Women in the second study also had an adequate calcium intake at approximately 1400 mg/day. Neither of these two studies found any relationship between calcium intake, breast milk calcium, or any bone loss factors. Both research groups suggest that maternal metabolic changes (increased renal calcium retention and decrease urinary calcium losses) contribute to meeting breast milk calcium needs. However, these metabolic changes are often not sufficient to meet breast milk calcium needs, therefore calcium is resorbed from the bone. Long-term observational calcium supplementation studies in postmenopausal women, a group at high risk for poor bone density, have shown little effect of calcium intake alone on reducing hip fractures

(49, 50). Similarly, in lactating women, calcium supplementation did not improve BMD (48). In addition, dietary calcium intake is not related to bone turnover markers in lactating women (11). Recently, in women with habitually low calcium intakes (<500 mg/d), increasing calcium intake improved calcium balance, which, in turn may lessen bone losses during lactation (51). The improvement in calcium balance in women with low calcium intakes (51), and the lack of effect of calcium supplementation during lactation (48), provides further evidence that there may be a threshold above which increased calcium intake will have no effect on bone loss (52). This threshold is likely 800-1000 mg, which is the recommended intake in adults.

Adequate vitamin D intake is necessary for effective calcium absorption. With sufficient vitamin D intake, postmenopausal women had a lower risk for osteoporotic hip fractures (53). While vitamin D supplementation is unnecessary for lactating women consuming dietary vitamin D and receiving adequate sun exposure (54), low vitamin D intakes may affect proper calcium absorption, thereby affecting bone mineral density. In women who do not receive adequate sun exposure, which is more common due to damaging effects of the sun, supplementing with high doses (4000 IU/d) of vitamin D increased circulating 25-hydroxyvitamin D in the mother and her breast milk (55). The effect of vitamin D intake on bone mineral density during lactation has not been thoroughly investigated.

While calcium and vitamin D are both necessary for bone health, an adequate total energy intake is also important for the health of bones, especially in women. Until recently, reproductive function was linked to body mass, especially fat mass. It was

suspected that amenorrhea was partially the result of a low percent body fat. However, recent research indicates that, in fact, energy deficit (whether from caloric restriction or energy expenditure) is the actual culprit inducing hormonal changes. Maintaining reproduction function and bone health is the result of adequate energy availability and is not based on body fatness (56). Interestingly, a decrease in energy availability (through caloric restriction and increased exercise) suppressed bone formation and increased bone resorption in young exercising women, with lower energy availability exhibiting greater negative effects on bone turnover (57). Increased aerobic capacity and decreased body weight and BMI, but not decreased body fatness, in women, was associated with increased femoral neck BMD (58).

Recently, high protein, especially dairy protein, diets have been investigated for their effects during weight loss and on bone health (59). Bowen *et al.* (59) reported that overweight women on a weight loss diet containing either high mixed protein or high dairy protein had increased losses of bone resorption markers in the urine, with higher losses in the mixed protein group. The mixed protein group, however, also had an increase in bone formation. Researchers concluded that the dairy protein group had less overall bone turnover, which is considered beneficial for bone health.

Numerous other nutrients may play a role in bone health. Macdonald *et al*. (60) investigated the effects of a variety of nutrients on bone loss in pre- and postmenopausal women. After adjustment for energy intake and other confounding factors, calcium was associated with less femoral neck (FN) BMD loss; conversely, increased polyunsaturated and monounsaturated fatty acids, retinol, and vitamin E were associated with increased

FN BMD loss. There was a high correlation between retinol and vitamin E, and the poly- and monounsaturated fatty acids, which may indicate covariance, but cannot be overlooked in their effects of BMD loss (60). A higher ratio of n-6 to n-3 fatty acids has also been shown to be associated with lower BMD in both men and women (61). In premenopausal women there were also positive associations with vitamin C, magnesium and potassium, nutrients found in fruits and vegetables, on FN BMD. Whether these associations are related to the nutrients themselves, or with some other factor in fruits and vegetables, is not known (60). It was also reported that increased potassium intake (via increased fruit and vegetable intake), or decreased renal acid load may have positive effects on bone health (62).

The role of dietary intake on bone formation, loss, and total turnover is complex. Identifying the role of individual dietary factors, and how multiple dietary factors work together (e.g. the ratio of protein to calcium) to impact bone health has not been determined.

Summary

Breast milk is the best food for infants, and lactation is a normal process of the female body. Breast milk formation induces the resorption of calcium and other minerals from the maternal skeleton, resulting in decreased bone mineral density during this period. While most women regain these bone losses upon cessation of lactation, some women do not fully recover bone losses incurred during lactation. Calcium intake does not appear to have an effect on bone mineral losses during lactation. On the other hand, exercise, especially resistance-type, has been shown to improve bone mineral density in

postmenopausal women. The effect of a resistance and aerobic exercise program has not been studied in lactating women, and was, therefore, the purpose of the following research study.

REFERENCES

1. Kalkwarf HJ and Specker BL. Bone mineral changes during pregnancy and lactation. Endocrine 2002; 17: 49-53.

2. Laskey MA, Prentice A, Hanratty LA, Jarjou LMA, Dibba B, Beavan SR, Cole TJ. Bone changes after 3 mo of lactation: influence of calcium intake, breast-milk output, and vitamin D-receptor genotype. Am J Clin Nutr 1998; 67: 685-92.

3. Prentice A. Micronutrients and the bone mineral content of the mother, fetus and newborn. J Nutr 2003; 133: 1693S-99S.

4. Karlsson C, Obrant KJ, Karlsson M. Pregnancy and lactation confer reversible bone loss in humans. Osteoporos Int 2001; 12: 828-34.

5. Hayslip CC, Klein TA, Wray HL, Duncan WE. The effects of lactation on bone mineral content in healthy postpartum women. Obstet Gynecol 1989; 73: 588-592.

6. Kalkwarf HJ, Specker BL. Bone mineral loss during lactation and recovery after weaning. Obstet Gynecol 1995; 86: 26-32.

7. Ritchie LD, Fung EB, Halloran Bp. A longitudinal study of calcium homeostasis during human pregnancy and lactation and after resumption of menses. Am J Clin Nutr 1998; 67: 693-701.

8. More C, Bettembuk P, Bhattoa HP, Balogh A. The effects of pregnancy and lactation on bone mineral density. Osteoporos Int 2001; 12: 732-7.

9. Prentice A. Calcium in pregnancy and lactation. Annu Rev Nutr 2000; 20: 249-272.

10. Paton LM, Alexander JL, Nowson CA, Margerison C, Frame MG, Kaymakei B, Wark JD. Pregnancy and lactation have no long-term deleterious effect on measures on bone mineral in healthy women: a twin study. Am J Clin Nutr 2003; 77: 707-14.

11. Sowers M, Eyre D, Hollis BW, Randolph JF, Shapiro B, Jannausch ML, Crutchfield M. Biochemical markers of bone turnover in lactating and nonlactating postpartum women. J Clin Endocrinol Metab 1995; 80: 2210-2216.

12. More C, Bhattoa HP, Bettembuk P, Balogh A. The effects of pregnancy and lactation on hormonal status and biochemical markers of bone turnover. Eur J Obstet Gynecol Reprod Biol 2003; 106: 209-13.

13. Holmberg-Marttila D, Leino A, Sievanen H. Bone turnover markers during lactation, postpartum amenorrhea and resumption of menses. Osteoporos Int 2003; 14: 103-9.

14. Colwell A, Russell RGG, Eastell R. Factors affecting the assay of urinary 3-hydroxypyridinium crosslinks of collagen as markers of bone resorption. Eur J Clin Invest 1993; 23: 341-9.

15. Eyre DR, Dickson IR, Van Ness KP. Collagen crosslinking in human bone and cartilage: age-related changes in the content of mature hydroxypyridinium residues. Biochem J 1988; 252: 495-500.

16. Eyre DR, Paz MA, Gallop PM. Cross-linking in collagen and elastin. Annu Rev Biochem 1984; 53: 717-48.

17. Robins SP, Duncan A. Pyridinium crosslinks of bone collagen and their location in peptides isolated from rat femur. Biochem Biophys Acta 1987; 914: 233-9.

18. Siebel MJ, Robins SP, Bilezikian JP. Urinary pyridinium crosslinks of collagen: specific markers of bone resorption in metabolic bone disease. Trends Endocrinol Metab 1992; 3: 263-70.

19. Kraenzlin EM, Siebel MJ. Measurement of biochemical markers of bone resorption. In Siebel MJ, Robins SP, Bilezikian JP, editors. Dynamics of bone and cartilage metabolism. San Diego: Academic Press, 1999: 411-26.

20. Brixen K, Eriksen EF. Validation of local and systemic markers of bone turnover. In Siebel MJ, Robins SP, Bilezikian JP, editors. Dynamics of bone and cartilage metabolism. San Diego: Academic Press, 1999: 427-36.

21. Garnero P, Delmas PD. An immunoassay for type I collagen α1 helicoidal peptide 620-633, a new marker of bone resorption in osteoporosis. Bone 2003; 32: 20-6.

22. Seibel MJ. Molecular markers of bone turnover: biochemical, technical and analytical aspects. Osteoporos Int 2000; Suppl 6: S18-29.

23. Zuppa AA, Tornesello A, Papacci P, Tortorolo G, Segni G, Lafuenti G, Moneta E, Diodato A, Sorcini M, Carta S. Relationship between maternal parity, basal prolactin levels and neonatal breast milk intake. Biol Neonate 1988; 53(3): 144-7.

24. Kovacs CS. Calcium and bone metabolism in pregnancy and lactation. J Clin Endocrinol Metab 2001; 86: 2344-8.

25. Kalkwarf HJ, Specker BL, Ho M. Effects of calcium supplementation on calcium homeostasis and bone turnover in lactating women. J Clin Endocrinol Metab 1999; 84: 464-70.

26. Canalis E. Role of growth hormone and insulin-like growth factors in the function of the osteoblast. In: Targets for Growth Hormone and IGF-1 Action. Ed. R. Bouillon. BioScientifica Ltd, Bristol UK. 2001; 117-30.

27. Breier BH, Milsom SR, Blum WF, Schwander J, Gallaher BW, Gluckman PD. Insulin-like growth factors and their binding proteins in plasma and milk after growth hormone stimulated galactopoiesis in normally lactating women. Acta Endocrinol (Copenh) 1993; 129: 427-35.

28. Gunn AJ, Gunn TR, Rabone DL, Breier BH, Blum WF, Gluckman PD. Growth hormone increases breast milk volumes in mothers of preterm infants. Pediatrics 1996; 98: 279-82.

29. Flint DJ, Knight CH. Interactions of prolactin and growth hormone (GH) in the regulation of mammary gland function and epithelial cell survival. J Mammary Gland Biol Neoplasia 1997; 2: 41-8.

30. Frost HM. The role of changes in mechanical usage set points in the pathogenesis of osteoporosis. J Bone Miner Res 1992; 7: 253-261.

31. Eriksen EF, Axelrod DW, Melsen F. The cellular basis for remodeling. In: *Bone Histomorphometry*. New York: Raven Press. 1994. pp. 13-28.

32. Kaplan FS, Hayes WC, Keaveny TM, Boskey A, Einhorn TA, Iannotti JP. Form and function of bone. In: *Orthopaedic Basic Science*, SR Simon, ed. Rosemont, IL: American Academy of Orthopaedic Surgeons. 1994. pp. 1-184.

33. Marcus R. Skeletal aging: Understanding the functional and structural basis of osteoporosis. Trends Endocrinol Metab 1991; 2: 53-8.

34. Kohrt WM, Snead DB, Slatopolsky E, Birge SJ. Additive effects of weight-bearing exercise and estrogen on bone mineral density in older women. J Bone Miner Res 1995; 10: 1303-1311.

35. Going S, Lohman T, Houtkooper L, Metcalfe L, Flint-Wagner H, Blew R, Stanford V, Cussler E, Martin J, Teixeira P, Harris M, Milliken L, Figueroa-Galvez A, Weber J. Effects of exercise on bone mineral density in calcium-replete postmenopausal women with and without hormone replacement therapy. Osteoporos Int. 2003; 14: 637-43.

36. Cussler EC, Lohman TG, Going SB Houtkooper LB, Metcalfe LL, Flint-Wagner HG, Harris RB, Teixeira PJ. Weight lifted in strength training predicts bone change in postmenopausal women. Med Sci Sports Exerc 2003; 35: 10-17.

37. Karlsson KM, Karlsson C, Ahlborg HG, Valdimarsson O, Ljunghall S. The duration of exercise as a regulator of bone turnover. Calcif Tissue Int 2003; 73: 350-5.

38. Karlsson KM, Karlsson C, Ahlborg HG, Valdimarsson O, Ljunghall S, Obrant KJ. Bone turnover responses to changed physical activity. Calcif Tissue Int 2003; 72: 675-80.

39. Shibata Y, Ohsawa I, Watanabe T, Miura T, Sato Y. Effects of physical training on bone mineral density and bone metabolism. J Physiol Anthropol Appl Human Sci 2003; 22: 203-8.

40. Fujimura R, Ashizawa N, Watanabe M, Mukai N, Amagai H, Fukubayashi T, Hayashi K, Tokuyama K, Suzuki M. Effect of resistance exercise training on bone formation and resorption in young male subjects assessed by biomarkers of bone metabolism. J Bone Miner Res 1997; 12: 656-62.

41. Vincent KR and Braith RW. Resistance exercise and bone turnover in elderly men and women. Med Sci Sports Exerc 2002; 34: 17-23.

42. Woitge HW, Friedmann B, Suttner S, Farahmand I, Muller M, Schmidt-Gayk H, Baertsch P, Ziegler R, Seibel MJ. Changes in bone turnover induced by aerobic and anaerobic exercise in young males. J Bone Miner Res 1998; 13: 1797-1804.

43. Naylor KE, Iqbal P, Fledelius C, Fraser RB, Eastell R. The effect of pregnancy on bone density and bone turnover. J Bone Miner Res 2000; 15: 129-37.

44. Black AJ, Topping J, Durhan B, Farquharson RG, Fraser WD. A detailed assessment of alterations in bone turnover, calcium homeostasis, and bone density in normal pregnancy. J Bone Miner Res 2000; 15: 557-63.

45. Little KD, Clapp JF. Self-selected recreational exercise has no impact on early postpartum lactation-induced bone loss. Med Sci Sports Exerc 1998; 30: 831-836.

46. Drinkwater BL, Chesnut CH. Bone density changes during pregnancy and lactation in active women: a longitudinal study. Bone and Mineral 1991; 14: 153-160.

47. Chan SM, Nelson EAS, Leung SSF, Cheng JCY. Bone mineral density and calcium metabolism of Hong Kong Chinese postpartum women-a 1-y longitudinal study. Eur J Clin Nutr 2005; 59: 868-76.

48. Prentice A, Jarjou LMA, Cole TJ, Stirling DM, Dibba B, Fairweather-Tait S. Calcium requirements of lactating Gambian mothers: Effects of a calcium supplement on breast-milk calcium concentration, maternal bone mineral content, and urinary calcium excretion. Am J Clin Nutr 1995; 62: 58-67.

49. Cumming RG, Cummings SR, Nevitt MC. Calcium intake and fracture risk: results from the Study of Osteoporotic Fractures. Am J Epidemiol 1997; 145: 926-34.

50. Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, Cauley J, Black D, Vogt TM. Risk factors for hip fracture in white women. Study of osteoporotic fractures research group. N Engl J Med 1995; 332: 767-73.

51. O'Brien KO, Donangelo CM, Vargas Zapata CL, Abrams SA, Spencer EM, King JC. Bone calcium turnover during pregnancy and lactation in women with low calcium diets is associated with calcium intake and circulating insulin-like growth factor 1 concentrations. Am J Clin Nutr 2006; 83: 317-23.

52. Barger-Lux MJ, Davies KM, Heaney RP. Calcium supplementation does not augment bone gain in young women consuming diets moderately low in calcium. J Nutr 2005; 135: 2362-6.

53. Feskanich D, Willett WC, Colditz GA. Calcium, vitamin D, milk consumption, and hip fractures: a prospective study among postmenopausal women. Am J Clin Nutr 2003; 77: 504-11.

54. Specker BL. Do North American women need supplemental vitamin D during pregnancy and lactation? Am J Clin Nutr 1994; 59 (Suppl): 484S-91S.

55. Hollis BW and Wagner CL. Vitamin D requirements during lactation: high-dose maternal supplementation as therapy to prevent hypovitaminosis D for both the mother and the nursing infant. Am J Clin Nutr 2004; 80(suppl): 1752S-8S.

56. Loucks A. Energy availability, not body fatness, regulates reproductive function in women. Med Sci Sports Exerc 2003; 31: 144-8.

57. Ihle R and Loucks AB. Dose-response relationships between energy availability and bone turnover in young exercising women. J Bone Miner Res 2004; 19: 1231-40.

58. Stewart KJ, Bacher AC, Hees PS, Tayback M, Ouyang P, Jan de Beur S. Exercise effects on bone mineral density. Am J Prev Med 2005; 28: 453-60.

59. Bowen J, Noakes, Clifton PM. A high dairy protein, high-calcium diet minimizes bone turnover in overweight adults during weight loss. J Nutr 2004; 134: 568-73.

60. Macdonald HM, New SA, Golden MHN, Campbell MK, Reid DM. Nutritional associations with bone loss during the menopausal transition: evidence of a beneficial effect of calcium, alcohol, and fruit and vegetable nutrients and of a detrimental effect of fatty acids. Am J Clin Nutr 2004; 79: 155-65.

61. Weiss LA, Barrett-Connor E, von Muhlen D. Ratio of n-6 to n-3 fatty acids and bone mineral density in older adults: the Rancho Bernardo Study. Am J Clin Nutr 2005; 81:934-8.

62. Macdonald HM, New SA, Golden MHN, Campbell MK, Reid DM. Low dietary potassium intakes and high dietary estimates of net endogenous acid production are associated with low bone mineral density in premenopausal women and increased markers of bone resorption in postmenopausal women. Am J Clin Nutr 2005; 81: 923-33

ORIGINAL RESEARCH ARTICLE

Effects of diet and exercise on body composition, bone mineral density and bone turnover markers during lactation.

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INTRODUCTION

An estimated 44 million Americans either have or are at risk for osteoporosis, eighty percent of whom are women. In 2002, $18 billion was spent nationally on direct costs to treat osteoporosis, and costs continue to rise (1). Not only is this a considerable financial drain, but the quality of life for these individuals will continue to decline. Improving bone status earlier in life may reduce the likelihood of developing poor bone mass in later adulthood.

Bone loss during lactation is normal, is necessary to provide calcium for breast milk production (2, 3), and can range from 3-9% (4). These losses usually reverse upon weaning (5, 6); however, some lactating women do not experience full reversal of these losses after cessation of breastfeeding (7). Susceptible women include those nursing multiple babies, adolescent mothers, and women in the later childbearing years who may not be able to regain lost bone mass before transitioning into menopause (4).

Minimizing bone losses during lactation may improve bone density post-weaning and decrease the risk of osteoporosis later in life. Resistance type exercise has been shown to improve bone mineral density (BMD) in young women (8). The effects of exercise on BMD during lactation have only recently been investigated (9, 10); however, no randomized intervention studies investigating the effects of specific resistance and aerobic exercise on BMD during lactation have been reported. Drinkwater and Chesnut observed a decrease in femoral neck BMD in six active women during lactation (9), whereas Little and Clapp saw decreases at the femoral neck and lumbar spine in lactating women who were either sedentary or were participating in self-selected recreational

exercise (10). The researchers of the second study (10) suggested that the type of exercise and short duration of the study were both factors that may have contributed to the lack of difference in BMD changes between groups.

Bone mineral density has been studied extensively in postmenopausal women, who experience similar hormonal changes (low estrogen levels) and bone loss as those that occur during lactation. Exercise interventions to improve BMD in postmenopausal women have been shown to be effective (11); therefore, it can be rationalized that a similar intervention would be beneficial for the lactation period. It is clearly established that exercise is safe during lactation and does not negatively impact breast milk volume and composition (12, 13).

Lactation has been suggested as a possible contributing factor for postmenopausal osteoporosis in women whose bone density does not completely recover to pre-pregnancy levels upon weaning; however, results of studies are inconclusive (2). In fact, recent epidemiological research suggests higher bone density in women who breastfed their infants (14), compared to women who did not breastfeed their infants.

Measurement of bone turnover markers, in addition to BMD measured by dual energy x-ray absorptiometry (DXA), will greatly improve the understanding of bone changes during lactation. While correlations between BMD and bone turnover markers are not strong, each measurement provides important insight. Bone turnover markers are representative of current physiological status of bone, whereas BMD via DXA represents the cumulative effects of many factors (15), (i.e. genetics, diet, and exercise).

While evidence is growing to support resistance exercise for increased bone during the postmenopausal period, there is a paucity of data available examining the role of moderate exercise on bone changes during lactation. The aim of the present study was to determine the effects of a specific resistance and aerobic training program on BMD and bone turnover markers during lactation. We also examined the role of dietary intake, specifically calcium, protein, and energy intake, on BMD and bone turnover during lactation.

SUBJECTS AND METHODS

Subjects

Healthy (free from chronic disease and hormonal disturbances), non-smoking, sedentary (no exercise during the prior three months), exclusively breastfeeding (no more than 4 ounces of formula fed only occasionally) women, aged 23-37 yrs, with a body mass index of 20 to 30 (kg/m^2) at 2 to 3 weeks postpartum were recruited through prenatal education classes offered at the Women's Hospital of Greensboro and flyers (Appendix B) posted at local obstetricians' offices. Women were screened over the telephone and were excluded if birth was by a cesarean section. Based on studies of bone loss during lactation (10, 16), power calculations estimate that a final sample size of 20 (10 women per group) will provide significant power to detect a 10% difference in change in BMD between groups. Written informed consent was obtained from all participants prior to admission into the study, and all women obtained medical clearance from their personal physician (Appendix B). This research project was approved by the Institutional Review Board of the University of North Carolina at Greensboro.

Measurements

All measurements were taken at baseline (3 ± 2 wks; range 2.5 – 4.75) and endpoint (21 ± 2 wks; range 19 - 23), before a 16 week intervention. Participants came to the Human Performance Laboratory at the University of North Carolina at Greensboro for anthropometric, fitness and strength assessment, as well as for blood collection. Women traveled to Wake Forest University Baptist Medical Center, J. Paul Sticht Center on Aging (Winston-Salem, NC) for dual energy x-ray absorptiometry (DXA)

measurements. Participants were met by a research assistant at the DXA facility to assist with childcare and to obtain DXA data from the participant.

Upon completion of baseline measurements, participants were subsequently randomly assigned to an intervention or control group, stratified for parity. Prior to the endpoint measurements, a urine sample was collected and tested to verify that the woman was not pregnant before she had the final bone density measurement. After birth, menstruation will not resume for at least 8 weeks in non-lactating women. During lactation, menstrual cycles may not resume for 18 months (17); therefore, a pregnancy test was unnecessary at the baseline test. Postpartum return of menses was documented in all women as return of menses has been shown in numerous studies to decrease bone losses during lactation (15, 18). Menstrual cycles return at various times during the lactation period, and are highly individual. Return of menses is not subject to alteration for research purposes, and therefore was documented and considered in the analysis.

Anthropometrics and Body Composition

Weight to the nearest 0.1 kg was measured on a stationary beam balance with subjects wearing only light clothing. Height was measured without shoes by a stadiometer to the nearest 0.1 cm.

BMD was measured using DXA (Hologic Delphi A, Delphi XP software version 12.3; Hologic, Bedford, MA). DXA measurement sites included whole body, lumbar spine (L1-L4), and hip (left, including femoral neck, trochanter and Wards triangle). Subjects were placed in a supine position while an x-ray beam scanned the entire body at 1-cm intervals. Changes in BMD, as well as bone mineral content (BMC) and bone area

(BA), are reported as percent change from baseline to endpoint, to account for individual variability. Lean and fat mass were also measured by DXA.

Bone turnover markers

Serum and urine samples were collected at baseline and at endpoint for analysis of bone turnover markers. Blood was collected by a trained phlebotomist in the morning after an overnight fast. Urine (from first or second morning void) was collected, by the participants, in the morning on the day of their visit to the laboratory. Serum (5 ml) was collected into an EDTA free vacutainer for analysis of bone-specific alkaline phosphatase (U/L; BAP), a bone formation marker. Serum was frozen at -70˚C until analyzed by enzyme immunoassay (Metra BAP EIA kit, Quidel Corporation, San Diego, CA). Urine (5ml) was collected for analysis of pyridinium crosslinks [deoxypyridinoline (DPD) and pyridinoline (PYD)], a bone resorption marker. Urine was frozen at -70˚C until analyzed by enzyme immunoassay (Metra PYD EIA kit, Quidel Corporation, San Diego, CA). Pyridinium crosslinks were corrected for urine concentration by measurement of creatinine (mmol/L), which was measured by a standard colorimetric (alkaline picric acid) assay (Oxford Biomedical Research, Oxford, MI).

Exercise Testing

To assess the cardiovascular fitness level of the participants, a modified Balke protocol (19) submaximal graded treadmill test was used. Subjects wore a heart rate monitor (Polar, Inc., Woodbury, NY) throughout the exercise bout, and resting heart rate (RHR) was measured immediately prior to exercise. Submaximal heart rate was determined for each subject using the heart rate reserve formula [(220-age-RHR) x 85%

+ RHR] (19). To avoid injury, subjects were asked to stretch before beginning the test, and the subjects warmed up on the treadmill for two minutes. Treadmill speed was chosen by the participants, at what they felt was a challenging level. This speed remained constant throughout the test. Heart rate was measured and recorded every minute, as well as level of perceived exertion. Treadmill grade was increased by 2.5% every two minutes. Participants walked on the treadmill until their heart rate reached 85% of their predicted maximal heart rate, unless discomfort or fatigue was expressed. Predicted oxygen consumption (VO_2) was determined using the formulas of the American College of Sports Medicine (ACSM) (19) as follows:

VO_2 (walking) = (3.5 ml/kg x min) + (speed in m/min x 0.1) + (grade x m/min x 1.8)

VO_2 (running) = (3.5 ml/kg x min) + (speed in m/min x 0.2) + (grade x m/min x 0.9)

Linear regression was used to determine the subject's predicted VO_{2max} using heart rate as the independent variable and predicted VO_2 as the dependant variable.

Muscular strength was measured by the 1 repetition maximum (RM) method as described by the ACSM (19), in the Human Performance Laboratory. Subjects warmed-up the muscles to be tested by lifting 40-60% of perceived maximum, 5-10 times. After a 1-minute rest, 5 more repetitions were performed at 60-80% of perceived maximum. After 3-5 minutes of rest, a small amount of weight was added until the weight could not be lifted. The 1 RM was determined as the last weight successfully lifted.

Dietary Analysis

Dietary intake was determined by 24-hour recall over the telephone using the Minnesota Nutrition Data System for Research (NDS-R, Nutrition Coordinating Center,

University of Minnesota, MN) software, on two randomly selected days in the week prior to and during the last week of the intervention period. Whenever possible, this included one weekday and one weekend day. The software is designed to allow for immediate dietary input during the interview, and dietary analysis is subsequently generated. A trained interviewer (MJB) used a five-pass method to collect detailed information about food consumed in the previous 24 hours. Interviews were completed in approximately 20-30 minutes. Women were informed that they would be called in the week prior to and during the last week of the study period, but were not given the randomly selected day. Before the women were telephoned for their interview, they were given handouts of two-dimensional visual food portions. These tools aided the subjects in determining portion sizes of food they consumed. This method has been used in the National Health and Nutrition Examination Surveys, and has been validated against both in-person administrated recalls (20) and observation (21). All women were given a multivitamin (provided by the researchers, containing vitamin D) supplement; however, the supplement did not have any minerals (Appendix B), and was to be taken daily during the 16-wk intervention period. Many postpartum women are encouraged to take a multivitamin by health care professionals. The provided vitamin contained folic acid, which is vitally important if the participants were to become pregnant during the study period. Women were asked about multivitamin compliance every two weeks during the intervention. All women in the study were instructed not to change their dietary intake or restrict their calorie intake during the 16-week intervention period.

Exercise Program

In the intervention group, women participated in a 16-week home-based exercise program. Women were provided with a heart rate monitor, an exercise ball and handheld weights to facilitate the exercise in the home. All women (exercise and non-exercise) were given the exercise ball and weights as compensation for participation in the study. Research assistants traveled to participants' homes 3 days per week to train mothers in the exercise program and to ensure exercise compliance (i.e. observation of exercise and/or checking the data saved on participant's heart rate monitors) during the study. The intervention consisted of aerobic (3 days/week) and resistive exercise (3 days/week). Participants were sedentary at the onset of the exercise program and the progression of the aerobic and resistance exercise program was individualized. The aerobic program consisted of 45-minute sessions at an intensity of 65 to 80 percent of the woman's predicted maximum heart rate. Women wore heart-rate monitors (Polar, Port Washington, NY) to confirm that they were exercising at the prescribed intensity. Duration of aerobic exercise began at 15 minutes per day, progressing to 45 minutes per day by increasing the time spent in their target heart-rate range by 5 minutes each day for the first week and by 3 minutes per day thereafter. At the beginning of the intervention, when aerobic exercise duration was short, aerobic and resistance training was completed on the same day. Once aerobic exercise duration increased to at least 30 minutes, subjects trained aerobically 3 days per week and did resistance training on alternating days, for a total of 6 days/week of training. Each exercise session was preceded by a 5-minute warm-up, and ended with a 5-minute cool-down period, to avoid injury.

The resistance program focused on structural exercises that involved direct force through the axial skeleton. Exercises included squats, deadlifts, high pulls, standing shoulder press, bent-over dumbbell row, isometric pushups, ball bench press, abdominal plank, crunches, and a wall sit. Subjects completed 3-4 sets of each exercise using a weight that was equal to 60%, and progressed to 85-90%, of their initial 1 RM. Subjects had 1-3 minutes of rest between sets. All of these exercises were completed in the home with handheld weights and exercise balls provided by the researchers.

Women in the control group were instructed not to perform vigorous aerobic or resistance exercise more than once per week. They were offered the exercise program and equipment after they had completed the baseline and endpoint measurements. This incentive aided in subject recruitment and encouraged women to agree to random assignment to the research groups.

Statistical analysis

Data were analyzed with JMP (v.5.1.2, SAS Institute, Cary, NC) statistical software. Student's t tests were used to determine differences in baseline subject characteristics. Pearson's correlations were conducted to determine any existing relationships between dietary intake, especially energy, calcium, and protein, and bone mineral density and bone turnover markers to determine factors for analysis of covariance (ANCOVA). ANCOVA was used to determine actual differences between groups, using baseline measurements as the covariate, in BMD, BA, BMC, bone turnover markers, strength and predicted maximal oxygen consumption. Differences between groups with respect to changes in BMD, BMC and BA were evaluated with ANCOVA, with the

differences between the endpoint and baseline measurements of BMD, BMC and BA as the outcome variables and parity, diet (energy, calcium and protein), and measurements of BMD, BMC and BA at the beginning of the study as the covariates. ANCOVA was also performed to determine the effects of diet and exercise on the changes in bone turnover markers, controlling for baseline measurements. In the ANCOVA models, covariates with $p<0.10$ were removed from the model.

RESULTS

One hundred twenty four women were screened for this study, 26 were not eligible to participate. Of the remaining 98 eligible women, 74 were no longer eligible after delivery due to the one of the following reasons: change in health status (n=3), personal reasons (time constraints, work status, scheduling conflicts, n=30), delivery was cesarean section (n=21), or unwillingness to randomize to group (exercise vs. non-exercise; n=20). Twenty-four women were recruited and completed baseline measurements. A final sample of 20 women (control n=10, exercise n=10) completed baseline and endpoint measurements. Four women did not complete the study because they were no longer exclusively breastfeeding (there were no significant differences in their baseline characteristics as compared to the women who completed the study). At baseline, characteristics of the participants were not significantly different between groups (**Table 1**). Participants in this study were non-obese, aged 25-35, and were either Caucasian (n=19) or Asian (n=1). Eight women [control group (CG) n=3, exercise group (EG) n=5] started hormonal birth control (progesterone-only pill or IUD), and two women (CG n=1, EG n=1) resumed menses during the study period. All of the multiparous participants breastfed their previous children for at least 3 months.

At baseline, no significant differences in cardiovascular fitness (**Table 2**) or muscular strength were observed between groups. Both groups experienced a non-significant increase in VO_2 over the study period; however, there was no significant difference between groups. The EG had increased muscular strength (actual and % change) after the intervention period in all areas tested ($p<0.05$), except for push-ups

(p=0.07) (**Table 3**). Compliance for exercise was calculated as a percentage (number of sessions completed out of the total required over the 16 weeks) for both the aerobic training and the resistance training portions of the program. Average aerobic compliance was 85%, and resistance compliance was 94% of the total requirement.

Total body mass lost (kg) between groups was not significantly different. There was a trend for the EG to have lost significantly less LBM than the CG (p=0.05). Percent body fat decreased in both groups over the intervention period, with the EG losing a greater percentage body fat than the CG (p=0.09) (**Table 4, Figure 1**).

Losses of lumbar spine (LS) BMC and BMD were significantly less in the EG as compared to the CG ($p<0.05$); however, no significant differences were detected between groups in whole body or hip BMC or BMD changes, and there were no differences in BA between groups in any body region (**Table 5**). Multiparous women (n=10), who had all breastfed their previous children, lost significantly less whole body BMC and BA than the primiparous women ($p<0.05$), and there was a trend towards less loss of LS BMC (p=0.08) and LS BMD (p=0.08) (data not shown).

There were no significant differences in dietary intake between groups at baseline or after the 16 wk intervention period (**Table 6**). Because baseline and endpoint Ca intakes were highly correlated, calcium intake was averaged over the 4 days (including baseline and endpoint). Because dietary intake did not differ between groups, all participants were grouped together (n=20) for analysis of the effects of dietary intake (4 day average) on bone changes. Calcium intake was correlated with vitamin D intake ($R^2=0.83$, $p<0.05$) and the calcium to protein ratio (Ca:Pro) ($R^2=0.74$, $p<0.05$). In

addition, there was a weak association with Ca:Pro and protein intake (R^2=0.11, $p<0.05$). Whole body BMD losses were minimized as calcium intake increased (R^2=0.28, $p<0.05$), as protein intake increased (R^2=0.15, p=0.09) and with an increase in the calcium to protein ratio (Ca:Pro) (R^2=0.18, p=0.059). Calcium and protein intake were not correlated with whole body BA, or LS and hip BMD, BMC, or BA (data not shown). After determining that there was a difference in BMC and BA between multiparous and primiparous women, a model (ANCOVA) was constructed that included calcium intake, group (exercise vs. non-exercise), and parity. This model determined that calcium intake was a significant predictor of changes in whole body BMD, when parity was included in the model (R^2=0.73, $p<0.0001$), but not group. In the same manner, a model including Ca:Pro ratio and parity was a significant predictor of whole body BMD changes (R^2=0.70, $p<0.001$). There was no relationship between change in energy intake and BMD, BMC or BA in whole body, LS or hip.

Markers of bone turnover were analyzed in all participants; however, one participant in the exercise group was diagnosed with hypothyroidism at the end of the study period and her bone turnover markers were greater than two standard deviations higher than the rest of the participants, therefore her data were excluded. Markers of bone formation (BAP) and bone resorption (pyridinium crosslinks) were not significantly different between groups at baseline or after the intervention period; however, the CG had an increase, while the EG experienced a decrease, in bone formation (from baseline and endpoint) ($p<0.05$) (**Table 7**). A model (ANCOVA) that included calcium intake,

group and parity did not significantly account for the variability of markers of bone turnover.

In addition, an ANCOVA model was constructed to determine the effects of parity, group (exercise vs. non-exercise), calcium intake, BAP, and pyridinium crosslinks on whole body, LS, and hip BMD, BMC, and BA. Parity, change (decrease) in pyridinium crosslinks, and group accounted for 65% of the variability of LS BMC (R^2=0.65, $p<0.01$), i.e. a higher parity, a decrease in bone resorption, and exercise predicted that there would be less loss of LS BMC. Parity, change (increase) in BAP, and group accounted for 71% of the variability of LS BMD (R^2=0.71, $p<0.001$), i.e. a higher parity, an increase in bone formation, and exercise predicted that there would be less loss of LS BMD. Bone turnover markers were not significant predictors of whole body BMD, BMC or BA, or LS BA.

DISCUSSION

The results of this study demonstrated that exercise minimized losses of LS BMD and LS BMC in breastfeeding women. It has been observed that breastfeeding causes a loss of BMD in the spine and hip regions, but losses have been less pronounced in the whole body (17, 22). Breastfeeding women undergoing a 16-week resistance and aerobic training program lost significantly less BMD and BMC ($p<0.05$) in the lumbar spine region, but not in the hip region or whole body.

This is the first study, to our knowledge, to investigate the effects of a very specific exercise program on bone changes during lactation. Few studies have examined the effects of exercise on BMD during lactation (9, 10), and to our knowledge there are no published studies investigating the effects of exercise on bone turnover markers during lactation. Little and Clapp (10) detected no effect of exercise on inhibition of bone mineral loss in lactating women who participated in self-selected recreational exercise (walking, running, aerobics, step aerobics, stair machine). These women were compared to non-exercising controls. All subjects (both exercisers and non-exercisers) presented with femoral neck and lumbar spine BMC or BMD losses at 3 months postpartum. The non-exercisers, however, had greater losses at the lumbar spine than the exercisers, but the difference was not significant. It was suggested, by these authors, that a planned intervention, utilizing both resistance and aerobic exercise may provide the necessary stimulus to improve BMD during lactation (10). Similarly, Drinkwater and Chesnut (9) observed decreases in femoral neck and radial shaft BMD in six female athletes during pregnancy, but found an increase in tibial BMD. After 6 months of lactation in these

same six subjects, radial shaft BMD returned to normal, while femoral neck BMD continued to decrease. A non-significant decrease in lumbar spine BMD was also detected after 9 months of pregnancy and during lactation. Their study compared these active pregnant and subsequently lactating women to a group of non-pregnant, non-lactating women. Decreases in bone mineral density observed in the lactating women were not seen in the non-pregnant, non-lactating group. There was no comparison of exercising lactating women to non-exercising lactating women in that study. In contrast to these two studies (9, 10), our study utilized a specific resistance and aerobic training program to determine the effects of exercise on BMD and bone turnover during lactation.

The exercise program developed for this study was 16 weeks in duration. Strength training programs, at a minimum, should be at least 8 weeks in duration to allow for neural adaptations (23), after which muscular hypertrophy will occur. The first few weeks of our program were designed to familiarize the participants to the program, to decrease likelihood of injury. By week 5, the aerobic portion of the program was at full intensity. While a program lasting longer than 16 weeks may be more effective, it was not feasible for this study. The bone turnover cycle is upregulated to 3-4 months during lactation (4); therefore, the 16 weeks of this intervention would allow for a full cycle of bone turnover to occur. Introduction of solid foods to the infant normally begins around 4-6 months, therefore, the first 5-6 months of exclusive breastfeeding was our focus, in that the greatest turnover of the mother's bones will occur in this time frame. Our program consisting of 3 days of aerobic exercise and 3 days of resistance training per week was effective in increasing strength in all areas assessed; however, there was no

difference between groups in the improvement in cardiovascular fitness. This may be, in part, due to some lack of compliance (too great of a time commitment for mothers of small children), or that in order to see greater improvements in cardiovascular fitness, more than 3 days a week are necessary. A research assistant was present in the home 3 days per week, usually during the strength training sessions, which may also explain the lower compliance with the aerobic training. Another possible explanation for the lack of difference between groups is that the non-exercising group also experienced improvements in cardiovascular fitness. The study began at around 3 weeks postpartum, and all women participating in this study were still recovering from the birth process and would likely have fitness improvements between baseline and endpoint measurements.

As hypothesized, the exercising women had substantial improvements in body composition. Mean weight loss was not significantly different between groups; however, the decrease in % body fat was greater in the exercising women ($p=0.09$). Exercising women were able to maintain most of their lean body mass, while the non-exercising group lost lean mass ($p=0.05$). While it may have been expected that the exercising women would have gained lean body mass (i.e. muscle mass), this gain in muscle mass may have been masked by early postpartum fluid losses (i.e. at baseline, women may have appeared to have more lean body mass because they had not yet lost excess fluid retained from pregnancy). Therefore, the net loss of lean body mass (-0.7 kg) experienced by the exercising group is likely an effect of fluid changes and a decrease in bone mineral density, and not a lack of increase in muscle mass. Body water was not

measured in this study, and likely would have provided insight into lean body mass changes.

Women in this study were stratified into groups by parity, and all but one of the multiparous women had two children. Whole body BMC and BA losses were significantly less in our multiparous women ($p<0.05$), and a trend was noticed for multiparous women to have less loss of LS BMC and BMD. The beneficial role of parity on bone during lactation may not necessarily be from simply having other children; rather, having breastfed previous children may be providing the beneficial effects (24). Caird *et al.* reported that femoral neck BMD was lower in multiparous women, as compared to nulliparous women (1.1 % BMD loss per live birth); however, they found a 1.5% increase in LS BMD per breast-fed child (24).

Postpartum amenorrhea, postpartum return of menses, and use of hormonal birth control have all been associated with changes in bone in breastfeeding women, yet results are inconclusive (5, 22, 25). In the current study one women in each group experienced return of menses during the study period. Eight women started hormonal birth control during the study; however, all were either taking oral progesterone-only mini pills, or were using an intrauterine device that did not involve systemic hormonal changes (i.e. hormones were retained in the uterus). Bone changes in these women were not significantly different from the other participants.

In breastfeeding women, approximately 300 mg of calcium is lost to breast milk each day; however, BMD during lactation has not been thought to be affected by maternal calcium intake (21). Metabolic changes during pregnancy (increased calcium

absorption efficiency) and lactation (calcium absorption efficiency returns to pre-pregnancy levels, increased renal calcium retention, and decreased urinary calcium losses) (26) are necessary to provide calcium for breast milk. Calcium supplementation studies have shown that there is no positive effect of calcium supplementation on BMD or bone turnover markers during lactation in women with sufficient intakes (27), therefore it has been proposed that metabolic changes have a stronger effect on calcium regulation than dietary intake. In addition, the role of protein on maintenance of bone mass has come under investigation. It is proposed that protein (28), rather than energy intake may help to maintain bone mass. Therefore, the ratio of calcium to protein may also be an indicator of a person's ability to maintain bone mass. In the current study, calcium intake and the calcium to protein ratio were both weak, yet significant, predictors of whole body BMD. The strength of this prediction was improved when parity was a covariate. Women with higher calcium intakes, and who were multiparous, lost significantly less whole body BMD. The same effect was seen with the calcium to protein ratio.

Caloric intake was not significantly different from baseline to endpoint within or between groups. Interestingly, change in caloric intake from baseline to endpoint was greater in the non-exercising group [CG = -422(438), EG = -192(388) kcal], yet there was no difference in body mass lost [CG = -3.5(1.6), EG = -3.6(2.5)]. Exercising women were able to consume more calories, offset by increased exercise, while losing the same amount of total body mass, yet maintaining lean body mass. In a recent study, a decrease in energy availability (through caloric restriction and increased exercise) suppressed bone

formation and increased bone resorption, with lower energy availability exhibiting greater negative effects on bone turnover (29). While differences in caloric intake were not significant between groups in our study, there was a decrease in bone formation in the exercising group, possibly indicating a slowing of overall bone turnover.

Bone resorption (pyridinium crosslinks) decreased in both groups over the study period. Bone resorption is very high at the end of pregnancy and decreases postpartum in both breastfeeding and non-breastfeeding mothers (30, 31). Measuring bone turnover markers at baseline and endpoint is sufficient in 6 month studies (32, 33); however, due to fluctuations in bone response, an effect of the training program may have occurred before our endpoint measurement. Bone formation was not significantly different between groups at baseline or endpoint. When parity (individual R^2=0.11, NS), change in pyridinium crosslinks (individual R^2=0.09, NS), and exercise grouping (individual R^2=0.36, $p<0.001$) were modeled to predict changes in LS BMC, 65% of the variability of LS BMC was explained, corrected for baseline LS BMC (individual R^2=0.13, NS). Having breastfed previously, participation in our exercise program, and experiencing a decrease in bone resorption are all factors that contribute to preserving LS BMC. A similar model was created for LS BMD, and 71% of the variability of LS BMD, corrected for baseline LS BMD (individual R^2=0.24, $p<0.05$), was explained by parity (individual R^2=0.07, NS), group status (individual R^2=0.3, $p<0.05$), and change in bone formation markers (individual R^2=0.06, NS). We did not observe a relationship between bone turnover markers and whole body or hip BMD or BMC. Others have seen only moderate correlations (ranging from –0.29 to –0.48) between BMD and bone turnover

markers in postpartum women (15). It is not surprising that our exercise program had no effect on whole body BMD, while having positive effects on LS BMD. The lumbar spine (axial skeleton) is composed of the highly metabolic trabecular bone, which has a much higher turnover rate, as compared to the whole body, and is more susceptible to rapid gains and losses (10, 27).

In conclusion, women who participated in our 16-week resistance and aerobic training program lost significantly less LS BMC and LS BMD than non-exercising controls. The exercise program was also successful in improving body composition (decreased % body fat and maintenance of lean body mass). In breastfeeding women who had adequate caloric intakes, calcium intake and the calcium to protein ratio were positively correlated with whole body BMD, especially in women who have breastfed other children. Additional studies are needed to determine the effects of this exercise program upon weaning, in both the exercising and non-exercising women, and also in women who continue to exercise after participating in the program and those who do not.

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REFERENCES

1. National Osteoporosis Foundation website: http://www.nof.org/osteoporosis/diseasefacts.htm. Accessed February 27, 2006.

2. Prentice A. Calcium in pregnancy and lactation. Annu Rev Nutr 2000; 20: 249-272.

3. Kovacs CS. Calcium and bone metabolism in pregnancy and lactation. J Clin Endocrinol Metab 2001; 86: 2344-8.

4. Kalkwarf HJ, Specker BL, Ho M. Effects of calcium supplementation on calcium homeostasis and bone turnover in lactating women. J Clin Endocrinol Metab 1999; 84: 464-70.

5. Kalkwarf HJ, Specker BL, Bianchi DC, Ranz J, Ho M. The effect of calcium supplementation on bone density during lactation and after weaning. N Engl J Med 1997; 337: 523-8.

6. Krebs NF, Reidinger CJ, Robertson AD, Brenner M. Bone mineral density changes during lactation: maternal, dietary, and biochemical correlates. Am J Clin Nutr 1997; 65: 1738-46.

7. Ritchie LD, Fung EB, Halloran Bp. A longitudinal study of calcium homeostasis during human pregnancy and lactation and after resumption of menses. Am J Clin Nutr 1998; 67: 693-701.

8. Snow-Harter C, Bouxsein M, Lewis BT, Carter DR, Marcus R. Effects of resistance and endurance exercise on bone mineral status of young women: a randomized exercise intervention trial. J Bone Miner Res 1992; 13: 133-42.

9. Drinkwater BL, Chesnut CH. Bone density changes during pregnancy and lactation in active women: a longitudinal study. Bone and Mineral 1991; 14: 153-160.

10. Little KD, Clapp JF. Self-selected recreational exercise has no impact on early postpartum lactation-induced bone loss. Med Sci Sports Exerc 1998; 30: 831-836.

11. Snow CM, Shaw JM, Winters KM, Witzke KA. Long-term exercise using weighted vests prevents hip bone loss in postmenopausal women. J Gerontol A Biol Sci Med Sci 2000; 55: 489-91.

12. Dewey KG, Lovelady CA, Nommsen-Rivers, McCrory MA, Lonnerdal B. A randomized study of the effects of aerobic exercise by lactating women on breast milk volume and composition. N Engl J Med 1994; 330: 449-53.

13. Lovelady CA, Garner KE, Moreno KL, Williams JP. The effect of weight loss in overweight, lactating women on the growth of their infants. N Engl J Med 2000; 342: 449-53.

14. Paton LM, Alexander JL, Nowson CA, Margerison C, Frame MG, Kaymakei B, Wark JD. Pregnancy and lactation have no long-term deleterious effect on measures on bone mineral in healthy women: a twin study. Am J Clin Nutr 2003; 77: 707-14.

15. Sowers M, Eyre D, Hollis BW, Randolph JF, Shapiro B, Jannausch ML, Crutchfield M. Biochemical markers of bone turnover in lactating and nonlactating postpartum women. J Clin Endocrinol Metab 1995; 80: 2210-2216.

16. Hayslip CC, Klein TA, Wray HL, Duncan WE. The effects of lactation on bone mineral content in healthy postpartum women. Obstet Gynecol 1989; 73: 588-592.

17. Olds SB, London ML, Ladewig PW. Postpartal adaptation and nursing assessment. In V.S. Jutson & M Earl (Eds.), Maternal Newborn Nursing (6th ed.). New Jersey, Prentice-Hall. 2000.

18. Kalkwarf HJ, Specker BL. Bone mineral loss during lactation and recovery after weaning. Obstet Gynecol 1995; 86: 26-32.

19. American College of Sports Medicine. Guidelines for exercise testing and prescription, 6th ed. Baltimore, MD: 2000.

20. Tran KM, Johnson RK, Soultanakis RP, Matthews DE. In-person vs telephone-administered multiple-pass 24-hour recalls in women: validation with doubly labeled water. J Am Diet Assoc 2000; 100: 777-83.

21. Conway JM, Ingwersen LA, Moshfegh AJ. Accuracy of dietary recall using the USDA five-step multiple-pass method in men: an observational validation study. J Am Diet Assoc 2004; 14:595-603.

22. Laskey MA, Prentice A, Hanratty LA, Jarjou LMA, Dibba B, Beavan SR, Cole TJ. Bone changes after 3 mo of lactation: influence of calcium intake, breast-milk output, and vitamin D-receptor genotype. Am J Clin Nutr 1998; 67: 685-92.

23. Izquierdo M, Hakkinen K, Ibanez J, Anton A, Garrues M, Ruesta M, Gorostiaga EM. Effects of strength training on submaximal and maximal endurance performance capacity in middle-aged and older men. J Strength Cond Res 2003; 17: 129-39.

24. Hreshchyshyn MM, Hopkins A, Zylstra S, Anbar M. Associations of parity, breast-feeding, and birth control pills with lumbar spine and femoral neck bone densities. Am J Obstet Gynecol 1988; 159: 318-22.

25. Caird LE, Reid-Thomas V, Hannan WJ, Gow S, Glasier AF. Oral progesterone-only contraception may protect against loss of bone mass in breast-feeding women. Clin Endocrinol 1994; 41: 739-45.

26. Prentice A, Jarjou LMA, Cole TJ, Stirling DM, Dibba B, Fairweather-Tait S. Calcium requirements of lactating Gambian mothers: Effects of a calcium supplement on breast-milk calcium concentration, maternal bone mineral content, and urinary calcium excretion. Am J Clin Nutr 1995; 62: 58-67.

27. Cross NA, Hillman LS, Allen SH, Krause GF. Changes in bone mineral density and markers of bone remodeling during lactation and postweaning in women consuming high amounts of calcium. J Bone Miner Res 1995; 10: 1312-20.

28. Bonjour JP. Dietary protein: an essential nutrient for bone health. J Am Coll Nutr 2005; 24: 526S-36S.

29. Ihle R and Loucks AB. Dose-response relationships between energy availability and bone turnover in young exercising women. J Bone Miner Res 2004; 19: 1231-40.

30. Naylor KE, Iqbal P, Fledelius C, Fraser RB, Eastell R. The effect of pregnancy on bone density and bone turnover. J Bone Miner Res 2000; 15: 129-37.

31. Black AJ, Topping J, Durhan B, Farquharson RG, Fraser WD. A detailed assessment of alterations in bone turnover, calcium homeostasis, and bone density in normal pregnancy. J Bone Miner Res 2000; 15: 557-63.

32. More C, Bhattoa HP, Bettembuk P, Balogh A. The effects of pregnancy and lactation on hormonal status and biochemical markers of bone turnover. Eur J Obstet Gynecol Reprod Biol 2003; 106: 209-13.

33. Holmberg-Marttila D, Leino A, Sievanen H. Bone turnover markers during lactation, postpartum amenorrhea and resumption of menses. Osteoporos Int 2003; 14: 103-9.

TABLE 1
Subject characteristics at baseline[1, 2]

	Control Group (n=10)	Exercise Group (n=10)
Age (y)	31.6 (2.8)	31.1 (3.0)
Parity[3]	Primiparous=4 Multiparous=6	Primiparous=5 Multiparous=5
Prepregnancy wt (kg)	60.6 (9.7)	62.7 (11.8)
Baseline wt (kg)	68.7 (12.2)	68.7 (10.1)
Height (in)	65.3 (3.1)	63.9 (2.2)
BMI (kg/m^2)	24.8 (2.9)	26.1 (3.3)

[1] Mean (SD)
[2] $p > 0.05$ for all variables
[3] All multiparous mothers breastfed their previous infants for at least 3 months

TABLE 2
Cardiovascular fitness at baseline and endpoint[1, 2]

	Control Group (n=10)			Exercise Group (n=10)		
	Baseline	Endpoint	% change	Baseline	Endpoint	% change
Predicted VO_2 (ml/kg/min)	32.3 (4.8)	34.4 (4.2)	6.9 (5.5)	31.1 (3.7)	34.7 (4.7)	11.4 (6.5)
Predicted VO_2 (L/min)	2.21 (0.46)	2.24 (0.48)	1.17 (6.17)	2.13 (0.31)	2.24 (0.37)	5.15 (5.44)
Test duration (min)	12 (3)	13 (3)	15 (11)	12 (3)	13 (2)	8 (19)
Resting Heart Rate	68 (3)	72 (9)	6 (13)	71 (10)	73 (13)	2 (11)
85% predicted max HR	172 (3)	172 (3)	--	171 (4)	171 (4)	--
HR at end of test	172 (5)	176 (5)	3 (4)	175 (5)	177 (6)	1 (3)
Test speed (mph)	2.9 (0.4)	2.9 (0.4)	0.4 (6.3)	2.9 (0.4)	3.1 (0.4)	9.5 (17.5)
Highest grade (%)	12.3 (3.4)	14 (3.4)	16.4 (13.1)	12.5 (4.1)	13.3 (2.9)	11.1 (22.6)

[1] Mean (SD)
[2] $p>0.05$ for differences between groups for baseline, endpoint, and % change

TABLE 3 Muscular strength at baseline and endpoint[1]

	Control Group (n=10)			Exercise Group (n=10)		
	Baseline	Endpoint	% change	Baseline	Endpoint	% change
Squats (lbs)	66 (15)	74 (21)	12 (11)	59 (10)	85 (14)	46 (25)[2]
Bench Press (lbs)	43 (14)	44 (16)	1 (5)	37 (5)	59 (7)[2]	63 (32)[2]
Ab Plank (sec)	69.2 (40.2)	60.4 (29.5)	-2.7 (37.4)	61.2 (50.8)	115.7 (64.0)[2]	203.3 (249.2)[2]
Military Press (lbs)	35 (9)	36 (8)	5 (16)	33 (5)	44 (7)[2]	34 (20)[2]
Deadlift (lbs)	66 (15)	71 (22)	7 (15)	57 (10)	92 (11)[2]	63 (13)[2]
Push-ups (#)	11 (7)	14 (7)	66 (161)	10 (6)	26 (9)[2]	221 (198)[3]
High Pulls (lbs)	42 (9)	44 (13)	4 (13)	40 (8)	62 (9)[2]	58 (22)[2]
Dumbell Row (lbs)	44 (14)	45 (9)	8 (28)	38 (15)	66 (13)[2]	89 (46)[2]
Wall sit (sec)	36.1 (14.7)	37.9 (28.5)	-5.7 (40.9)	26.7 (17.9)	56.6 (32.2)	161.0 (118.2)[2]
Crunches (#)	61 (72)	63 (85)	-2 (25)	51 (36)	106 (91)	128 (83)[2]

[1] Mean (SD)
[2] Significantly different from control group, $p<0.05$.
[3] Trend towards significant difference from control group, $p=0.07$.

TABLE 4 Body composition at baseline and endpoint[1]

	Control Group (n=10)			Exercise Group (n=10)		
	Baseline	Endpoint	% change	Baseline	Endpoint	% change
Weight (kg)	68.7 (12.2)	65.2 (13.0)	-5.4 (2.9)	68.7 (10.0)	65.1 (11.1)	-5.5 (3.6)
Fat Mass (FM)						
kg	23.1 (6.9)	21.2 (7.4)	-9.4 (7.5)	23.5 (7.2)	20.5 (8.4)	-14.3 (9.6)
% FM	33.1 (5.0)	31.8 (5.8)	-4.3 (5.8)	33.56 (6.2)	30.6 (7.6)	-9.5 (7.1)[2]
Lean Body Mass (LBM)						
kg	45.6 (6.2)	44.0 (6.4)	-3.6 (2.3)	45.23 (4.9)	44.5 (5.0)	-1.6 (2.3)[2]
% LBM	66.9 (5.0)	68.2 (5.8)	1.9 (2.3)	66.4 (6.2)	69.4 (7.6)	4.3 (3.5)[2]

[1] Mean (SD)
[2] Trend towards significant difference from control group, $P<0.10$.

TABLE 5
Percent change in bone mineral density (BMD), bone mineral content (BMC), and bone area[1]

	Control Group (n=10)			Exercise Group (n=10)		
	Baseline	Endpoint	% change	Baseline	Endpoint	% change
Whole Body						
BMD (g/cm^2)	1.07 (0.09)	1.06 (0.09)	-0.8 (0.8)	1.09 (0.08)	1.09 (0.07)	-0.6 (1.2)
BMC (g)	2093 (269)	2064 (267)	-1.4 (1.0)	2105 (285)	2076 (290)	-1.4 (1.7)
Area (cm^2)	1953 (161)	1943 (167)	-0.6 (1.0)	1919 (146)	1904 (159)	-0.8 (1.2)
Lumbar Spine						
BMD (g/cm^2)	1.07 (0.1)	1.0 (0.1)	-7.02 (1.9)	1.05 (0.1)	1.0 (0.1)	-4.8 (1.0)[2]
BMC (g)	62.4 (10.9)	58 (10.3)	-7.1 (2.2)	59 (11.1)	56.7 (10.8)	-4.0 (1.9)[2]
Area (cm^2)	58 (6.4)	58 (6.4)	-0.04 (2.2)	55.9 (6.2)	56 (6.4)	0.9 (1.5)
Total Hip						
BMD (g/cm^2)	0.95 (0.1)	0.93 (0.1)	-2.2 (2.9)	0.96 (0.1)	0.93 (0.1)	-2.8 (2.4)
BMC (g)	30.3 (5.6)	29.8 (4.5)	-1.1 (6.1)	29.2 (4.1)	29 (3.7)	-0.4 (6.0)
Area (cm^2)	31.6 (3.0)	31.9 (2.4)	1.2 (4.2)	30.5 (3.0)	31.2 (2.1)	2.4 (5.1)
Femoral Neck						
BMD (g/cm^2)	0.9 (0.1)	0.8 (0.1)	-3.82 (6.0)	0.9 (0.1)	0.8 (0.1)	-4.2 (3.5)
BMC (g)	4.0 (0.6)	3.8 (0.5)	-5.3 (5.7)	4.1 (0.6)	3.9 (0.6)	-4.8 (6.5)
Area (cm^2)	4.7 (0.4)	4.6 (0.5)	-1.5 (5.7)	4.8 (0.4)	4.7 (0.5)	-0.6 (5.2)
Trochanter						
BMD (g/cm^2)	0.7 (0.1)	0.7 (0.1)	-2.1 (2.8)	0.7 (0.1)	0.7 (0.1)	-1.0 (3.3)
BMC (g)	6.4 (1.4)	6.4 (1.3)	-0.3 (5.3)	6.42 (1.6)	6.5 (1.4)	1.8 (9.4)
Area (cm^2)	9.41 (1.13)	9.6 (1.2)	2.0 (5.4)	9.26 (1.3)	9.5 (1.2)	2.7 (6.3)
Ward's Triangle						
BMD (g/cm^2)	0.8 (0.1)	0.8 (0.1)	-1.7 (8.1)	0.9 (0.1)	0.8 (0.1)	-7.1 (5.6)
BMC (g)	0.9 (0.2)	0.9 (0.1)	4.4 (19.3)	1.0 (0.1)	1.0 (0.1)	-1.6 (6.2)
Area (cm^2)	1.2 (0.1)	1.2 (0.1)	5.6 (13.7)	1.2 (0.1)	1.3 (0.1)	6.4 (10.7)

[1] Mean (SD)
[2] Significantly different from control group, $p<0.05$.

TABLE 6
Dietary intake at baseline and endpoint[1, 2]

	Control Group (n=10)	Exercise Group (n=10)
Kcal		
Baseline	2112 (510)	2109 (556)
Endpoint	1690 (375)	1918 (388)
Kcal change	-422 (438)	-192 (388)
Fat (g)		
Baseline	82 (21)	79 (26)
Endpoint	69 (17)	72 (21)
Protein (g)		
Baseline	74 (16)	87 (20)
Endpoint	79 (13)	75 (23)
Vitamin D (mcg)[3]		
Baseline	4.3 (2.1)	6.3 (3.1)
Endpoint	4.5 (1.4)	6.4 (3.9)
Calcium (mg)		
Baseline	1125 (380)	1360 (555)
Endpoint	949 (352)	1207 (563)
Average[4]	1037 (279)	1284 (504)
Ca:Protein		
Baseline	15.2 (3.5)	15.8 (6.1)
Endpoint	12.2 (4.5)	15.5 (5.3)
Average[4]	13.4 (2.5)	15.6 (5.1)

[1] Mean (SD)
[2] $p > 0.05$
[3] Supplemental vitamin D not included
[4] Average of 4 days (2 baseline, 2 endpoint)

TABLE 7
Markers of bone turnover[1]

	Control Group (n=10)	Exercise Group (n=9)
BAP[2]		
baseline	25.1 (8.6)	31.0 (6.3)
endpoint	28.3 (9.7)	30.0 (6.1)
change	3.2 (4.7)	-1.1 (3.4)[4]
Pyridinium crosslinks[3]		
baseline	88.8 (39.7)	80.4 (36.2)
endpoint	42.0 (17.2)	31.1 (11.1)
change	-46.7 (28.2)	-49.3 (38.4)

[1] Mean (SD)
[2] Bone-specific Alkaline Phosphatase, U/L
[3] nmol/mmol creatinine
[4] Significantly different from control group, $p<0.05$.

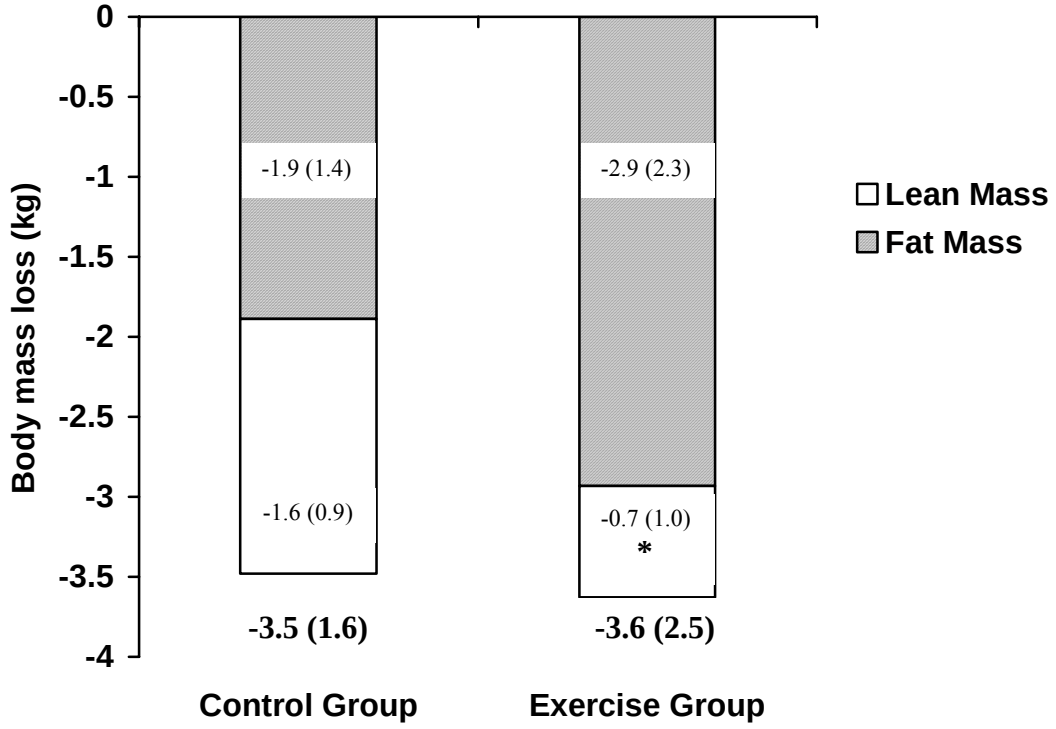

FIGURE 1. Mean (SD). Difference in lean and fat mass loss from baseline to endpoint. There was no difference in total body mass lost between groups; however, the EG lost more fat mass, while preserving lean mass during the intervention period (* p=0.0549), compared to the CG.

EPILOGUE

The research presented in this dissertation suggests that maternal exercise provides many benefits to mothers during lactation. There is no negative effect of exercise on breast milk fatty acid concentrations, and there may actually be a positive effect. A strength and aerobic training program minimized lumbar spine bone density losses in breastfeeding women.

The two research studies presented in this dissertation were a collaborative effort of many individuals, including both the research teams and the women who volunteered to participate in the studies. Recruitment for the bone study took much longer than expected, due to many reasons. Rate of cesarean section deliveries was extremely high, and these women had to be excluded from the study, therefore prolonging recruitment. In addition, we had many women who were unwilling to randomize to group, which is understandable for this population.

The exercise program designed for this study was effective not only in minimizing bone losses, but also for improving body composition in these women. On a more personal level, seeing first hand the changes in these women as they progressed through the program week by week was wonderful. Body image improved tremendously in the women who were in our exercise group, and all were so thankful of the personal attention that they received throughout the study period.

Compliance was acceptable, considering the population studied. These were free-living mothers of newborns, some first time mothers, and most of the rest were mothers of young toddlers. Exercising six times a week is a challenge; one has to be understanding of sleep deprivation, child and parent illness, and time management (especially in those participants who returned to work outside the home).

If this study were to be repeated, I would definitely consider changing the dietary intake protocol. The NDS-R program is excellent; however, asking postpartum women to complete 24-hour recalls was more of a challenge than was expected. Pregnancy and lactation are a time marked by forgetfulness and fatigue. Asking a mother what she ate 24 hours before was harder than most would understand. For many of the recalls, the participants asked if they could just write it down and call back, because that was easier and less stressful. In the future I would use the 3-day weighed food records (as used in the fatty acid study), and then enter that information into the NDS-R program. With the 3-day record, the mothers can tape record their intake and/or write it down at their convenience, thereby reducing subject burden.

A study following these women after weaning their infants is currently in progress. This study should provide interesting data on bone recovery in the post-weaning period. Some of the women in the exercise group have continued to exercise while others have not, and some of the women in the non-exercising group have started to exercise. Comparing the bone densities of all of these women will be very interesting. One could also consider studies that would vary the exercise program to determine exactly what components are effective (i.e. aerobic only, resistance only, other

combinations of the two types of exercise, higher intensity vs. lower intensity, any threshold levels above or below which exercise is more or less effective in minimizing bone loss). If time and funding were available, conducting midpoint measurements of bone density and bone turnover markers would also give insight into the changes over the study period.

Overall, this study was designed to find positive effects of exercise on bone density in breastfeeding women. These results can be built on further research. Our study was very specific with its methods and inclusion criteria for participants. This may have lengthened recruitment time, but made the study much more relevant, and the findings more significant. I was involved in every component of this study; including conception, design, recruitment, implementation, applied and basic laboratory assessment, data analysis, and even participation. I look forward to seeing this study potentially replicated on a larger scale and the follow up weaning study results. My time working on these studies, while lengthy, was enjoyable.

APPENDIX A: CHAPTER 1 PROTOCOLS

PLASMA FATTY ACID PROFILE ANALYSIS
(Du, January 1994)

Principle

This method used is an adaptation of Bligh and Dyer's (1959)

Materials

Test tubes: 125 x 16mm
120 x 10mm
100 x 10mm
Pipetman pipettes: 100µl
1000µl
Pasteur pipettes
N_2 evaporator
Water aspirator
Repipettors
Test tube shaker
Vortex mixer
Centrifuge
Heat block
5ml volumetric flask

Reagents

Internal standard: 17:0 methyl ester at approximately 10 mg/ml in iso-octane
HPLC grade: hexane, methanol, chloroform, and iso-octane
BHT (Butylated hydroxytoluene)
Sodium sulphate
Boron trifluoride in methanol
Benzene

Procedures

1. Preparation of reagents

Mix one part chloroform with two parts methanol, add 50 mg BHT in each liter of the mixture. Place the mixture in a repipettor calibrated for 3.75 ml delivery. Chloroform and distilled water are placed in another 2 repipettors calibrated for 2.5 ml delivery.

2. Extraction

Put 15µl (5µl for LDL) 17:0 internal standard in a 100 X 10mm test tube with teflon cap. Blow dry on N_2 evaporator.
Add 0.2ml plasma in the test tube, add 0.8 0.9% saline for making a volume of 1ml.
Add 3.75ml of 2:1 methanol and chloroform mixture in test tube and shake for 1 hour on the shaker.
After shaking, centrifuge at 2000 rpm for 10 minutes. Transfer the supernatant into a 120 x 10mm test tube (with teflon cap) by pasteur pipette.

Extract the residue with 1ml distilled water and 3.75ml methanol-chloroform mixture. Vortex. Centrifuge again for 10 minutes. Transfer the supernatant to the same 120 x 10mm test tube as in the first extraction.
Add 2.5ml distilled water and 2.5ml chloroform in the 120 x 10mm test tube. Mix. Centrifuge again for 10 minutes.
Suck the upper portion out by using the water aspirator. Withdraw the lower chloroform layer by pasteur pipette to another 100 x 10mm test tube. Dry under nitrogen.
3. Methylation
Add 0.2ml benzene and 1ml boron trifluoride-methanol in the nitrogen dried test tube. Cap tightly. Place tube in 95ºC heat block for 90 minutes.
Remove test tube from the heat block and cool to room temperature. Add 5ml distilled water and 5ml hexane to the tube and mix for 2 minutes on vortex mixer.
Centifuge tube at 1500 rpm for 10 minutes. Transfer the top hexane layer with pasteur pipette to 125 x 16mm test tube.
Add 2ml hexane to the first tube and mix again for 2 minutes on vortex mixer.
Centrifuge at 1500 rpm for 10 minutes. Remove hexane layer and combine it with first hexane portion.
Add about 0.3g Na_2SO_4 to the combined extracts and mix for 45 seconds on vortex mixer.
Transfer the extract to a 100 x 10mm test tube and evaporate hexane under nitrogen until dry.
Reconstitute sample in 0.3ml iso-octane for plasma.
Inject 1µl in GC.

Modified Folch Extraction of Total Lipids in Human Milk (Bitman et al.)

Materials

Test tubes: 50 ml
120 x 10mm
100 x 10mm
Pipetman pipettes: 100µl
1000µl
Pasteur pipettes
N_2 evaporator
Vortex mixer
Centrifuge
Heat block
Homogenizer

Reagents

Internal standard: 17:0 methyl ester at approximately 10 mg/ml in iso-octane
HPLC grade: hexane, methanol, chloroform, and iso-octane
BHT (Butylated hydroxytoluene)
Sodium sulfate
Boron trifluoride in methanol
Benzene
Sodium Chloride

Extraction

1. Add 150 µl of 10 mg/ml 17:0. Dry under nitrogen.
2. After heating to 80C for 1 minute to inactivate lipases, 1.0 ml of milk is placed in a 50 ml glass test tube with Teflon-lined screw cap containing 18 ml chloroform:methanol (2:1 containing 0.01% butylated hydroxytoluene). Dichloromethane can be substituted for chloroform. Heating can be omitted if a fresh, or frozen and thawed, sample is extracted immediately. If the sample has been lipolyzed, it should be acidified to pH 2.0 before extraction in order to free fatty acid soaps.
3. Homogenize for 30 seconds using a Polytron homogenizer (KINEMATICA GmbH, KRIENS-LUZERN, Switzerland).
4. Add 6 ml of 0.7% NaCl and invert tube a few times.

5. Centrifuge at 1000rpm (260 x g) for 10 minutes.
6. Remove upper (methanol/water) phase by suction pipette and discard.

7. Weigh large tube before aliquoting.
8. Transfer and 8.0 ml aliquot of the lower chloroform phase to a tared (0.0000 place) 15 ml culture tube.
9. Transfer a 3.0 ml aliquot of the chloroform phase to a 4.0 ml amber vial. The lipid in this vial will be used for thin-layer chromatographic separation and gas liquid chromatographic analysis of fatty acid methyl esters.
10. Evaporate solvent under nitrogen (tube from step 6) and re-weigh.
11. Determine % fat: ((tube + fat) – tube)) x 150 = % fat

Methylation

Dry 3.0 ml aliquot under nitrogen. (tube from Step 9)

Add 0.2ml benzene and 1ml boron trifluoride-methanol in the nitrogen dried test tube.

Cap tightly. Place tube in 95ºC heat block for 90 minutes.

Remove test tube from the heat block and cool to room temperature. Add 5ml distilled water and 5ml hexane to the tube and mix for 2 minutes on vortex mixer.

Centifuge tube at 1500 rpm for 10 minutes. Transfer the top hexane layer with pasteur pipette to 125 x 16mm test tube.

Add 2ml hexane to the first tube and mix again for 2 minutes on vortex mixer.

Centrifuge at 1500 rpm for 10 minutes. Remove hexane layer and combine it with first hexane portion.

Add about 0.3g Na_2SO_4 to the combined extracts and mix for 45 seconds on vortex mixer.

Transfer the extract to a 100 x 10mm test tube and evaporate hexane under nitrogen until dry.

Reconstitute sample in 0.3ml iso-octane for plasma. Vortex. Transfer 50 µl to small GC vial. Add 450 µl iso-octane to vial.

Inject 1µl in GC.

APPENDIX B: CHAPTER 2 FORMS

Want to GET IN SHAPE after you have your baby??

*** ARE YOU EXPECTING OR DO YOU HAVE A NEW BABY??**

*** ARE YOU INTERESTED IN PARTICIPATING IN AN *EXERCISE PROGRAM* AFTER YOU HAVE YOUR BABY??**

*** DO YOU WANT TO *GET IN SHAPE* BY IMPROVING YOUR FITNESS LEVEL AND MUSCULAR STRENGTH??**

*** DO YOU PLAN TO EXCLUSIVELY BREASTFEED FOR THE FIRST 5 MONTHS POSTPARTUM??**

YES?

Then GIVE US A CALL!

(You must be pregnant or your baby must be less than 2 weeks old)

Research Study

Lactation and Bone Mineral Density

UNCG Departments of Nutrition and Exercise Science

CALL: Dr. Cheryl Lovelady or Melanie Bopp at **336-256-1090**

UNIVERSITY OF NORTH CAROLINA AT GREENSBORO

Consent to Act as a Human Subject

PARTICIPANT'S NAME _____

DATE OF CONSENT _____

PROJECT TITLE: Effect of Exercise Training During Lactation on Mother's Bone Status

INVESTIGATORS: Cheryl Lovelady Ph.D., R.D., Laurie Wideman Ph.D., Melanie Bopp, and Heather Kennedy

DESCRIPTION AND EXPLANATION OF PROCEDURES: The purpose of this study is to determine the bone status of the lactating mother as a result of resistance and aerobic exercise training. The study will begin two to three weeks after you deliver and will continue until you are at your twentieth week postpartum. If you consent to participate, you will be assigned by chance to one of two groups. The first group will participate in the measurements and the exercise program. If you are assigned to the other, or control group, you will participate in all the measurements described below but will not participate in the exercise program. After completion of the project, the control group will be offered the opportunity to learn the procedures used in the experimental group to promote increased bone status through a personalized exercise prescription.

Participants in both groups will be asked to do the following:

1. Receive medical clearance from your physician, through the form provided, to participate in the exercise program.
2. Participate in three short dietary recall sessions. You will be called three times in one week at your convenience at the beginning and end of the study. This diet record will be used to determine your nutritional intake.
3. At 2 to 3 weeks and at 20 weeks postpartum you will be given a body scan by dual energy x-ray absorptiometry (DXA). This whole-body scan is necessary to determine your bone density. The scan will be completed at the J. Paul Sticht Center on Aging at the Wake Forest University Medical Center in Winston Salem. You will lay still and flat on an x-ray table, and the scanner will move back and forth several feet above you. The entire procedure takes approximately 30-45 minutes, depending on your height. Your breast milk will not be affected by the DXA scan.
4. At 20 weeks postpartum, you will be given a pregnancy test to ensure that you are not pregnant when the DXA scan is administered.
5. Visit the Human Performance laboratory at UNCG for several measurements at the beginning (4 weeks) and end (20 weeks) of the study. This visit should take less than 2 hours.

a) Your height and weight will be recorded first.
b) Then your cardiovascular fitness will be determined through an exercise test on a treadmill. You will walk or run on the treadmill, beginning at a low level, and will increase until you reach 85% of your calculated maximum heart rate. A researcher certified in cardiopulmonary resuscitation (CPR) will be present at the exercise session. Heart rate and rating of perceived exertion will be measured throughout this test.
c) You will be asked to provide approximately 4 tbls of venous blood after an overnight fast (no alcohol for 24 hours prior to blood draw). The blood will be drawn in the morning at the lab. Venipuncture will be performed by a trained phlebotomist. The blood is needed to assess your bone status.

6. Muscular strength will be assessed at the beginning and end of the study. We will be testing the strength of your muscles using hand weights.
7. You will be asked to collect a small urine sample in the morning on the day of your visit to the lab. The first void of the morning is preferable. You will collect the urine sample into a sterile urine collection cup, and will need to store the sample in your home refrigerator until you come into the lab. You will bring the sample with you to the lab.
8. During the morning feeding on the day you visit the lab, you will collect approximately 4 tablespoons of breast milk. While the infant is nursing on one breast, the milk sample will be obtained from your other breast (a breast pump will be provided if needed). All samples must be chilled immediately in household refrigerator. You will bring the sample with you to the lab.
9. After 20 weeks of the study, you will be asked to repeat all of the measurements above.

Those assigned by chance to the exercise group will also be asked to do the following:
1. Participate in resistance exercise sessions (30-45 minutes) three times each week at your home. All necessary equipment will be provided. A video will be provided for instruction on proper weight training technique. You will also participate in aerobic exercise sessions (45 minutes) three times a week. A qualified research assistant certified in CPR and educated on proper resistance training technique will be present at exercise sessions at least 3 times a week to monitor your training technique (to prevent injury), exercise intensity level, and heart rate.
2. Participate in strength tests every six weeks to determine any necessary changes to your personalized resistance-training program.

RISKS AND DISCOMFORTS: The risk of injury during exercise exists for you; temporary muscle fatigue and/or respiratory discomfort may result from the graded exercise test. Exercise sessions may result in temporary muscle soreness. Insertion of the needle during venipuncture may be slightly painful. Every precaution will be taken to minimize the risks involved with venipuncture (air emboli, infection, bruising, and fainting). You will be exposed to very mild radiation from the DXA scan, equivalent to 1/10 the exposure from a routine chest x-ray, and less than the exposure of a dental x-ray. There is no risk to your breast milk.

POTENTIAL BENEFITS: Results of all the tests conducted will be provided to you at no cost. Mothers participating in the study will undergo two free bone density scans, which provide valuable bone density and body composition information. All participants will receive, at no cost, a stability ball, hand weights, and video for home exercise; however, women in the control group will not receive these materials until completion of the study. Benefits to the exercising mothers also include the potential for increased cardiovascular fitness, increased muscular strength, and increased lean muscle tissue.

COMPENSATION/TREATMENT FOR INJURY: In the case of injury, you will be referred to your personal physician for treatment. You are responsible for paying for your treatment for injury. Upon completion of the study, you will receive a $50 stipend.

CONSENT: Your signature on this consent form indicates that you have read the procedures, risks and benefits involved in this research. You are free to refuse to participate or to withdraw your consent to participate in this research at any time without penalty or prejudice; your participation is entirely voluntary. Your privacy will be protected because you will not be identified by name as a participant in this project. All collected data will be stored in a locked file cabinet and will be shredded when it is no longer needed.

The research and this consent form have been approved by the University of North Carolina at Greensboro Institutional Review Board that ensures that research involving humans follows federal regulations. Questions regarding your rights as a participant in this project can be answered by calling Eric Allen at (336) 334-5878. Questions regarding the research itself can be answered by Dr. Cheryl Lovelady by calling (336) 256-0310. Any new information that develops during the project will be provided to you if the information might affect your willingness to continue participation in the project.

By signing this form, you are agreeing to participate in the project described to you by _____

_____	_____
Subject's Signature	Witness to Signature

MEDICAL CLEARANCE FORM APPLICANT'S SIDE

This form is required for acceptance in the lactation study and must be completed by you and your attending physician. This information along with your physician's statement will be used for your participation in a graded exercise test and for prescription of an exercise program. Please check any of the conditions that apply to you.

Name (Print) _____

Age _____ **Weight** _____ **Height** _____

1. Do you have any of the following risk factors for heart disease?

_____Inactive lifestyle
_____Stressful lifestyle
_____Stroke
_____Diabetes Mellitus
_____Smoke Cigarettes (if yes, # per day _____)
_____Heart disease in family (if yes, please specify _____
_____High Blood Triglycerides
_____High Blood Cholesterol
_____High Blood Pressure
_____Obesity

2. Have you ever experience any of the following?

_____Chest pain
_____Chest Pressure
_____Palpitations/skipped heart beats

Discomfort/pain in the: __throat _____wrist _____elbow _____teeth _____jaw

3. Do you have or has a physician diagnosed you as having any of the following?

_____Heart murmur _____Chronic Bronchitis _____Muscle/Bone/Joint problems
_____Emphysema _____Arthritis _____Neurological problems
_____Asthma _____Allergies _____Other (explain) _____

4. Have you ever undergone surgery for any of the following?

_____Varicose veins _____Leg surgery _____Abdominal Surgery
_____Hernia repair _____Musculoskeletal _____Other (explain) _____

5. Have you ever had any of the following tests?

_____Exercise Stress test _____Coronary Angiography_Echocardiogram
_____Holter Monitor _____Exercise Stress test with Isotope

6. Please list all medications/supplements that you are taking.

Name of medication	Dosage	Doses/Day

7. Are you taking any diet pills or ephedra for weight loss? YES NO
8. Did you exercise vigorously before pregnancy?(circle one) YES NO
9. Did you exercise regularly during your third trimester?(circle one) YES NO

_____ _____
Applicant's Signature Date

MEDICAL CLEARANCE FORM PHYSICIAN'S SIDE

Please check and/or comment on additional history and all pertinent physical findings. This information will be used for the applicant's participation in a graded exercise test and exercise program.

Name of Applicant (print) _____
Name of Physician (print) _____
Physician's Telephone (_____) _____
Physician's Address _____

1. Additional history not mentioned on APPLICANT'S SIDE:

_____
_____
_____
_____

2. Significant abnormal findings:

HEENT _____ Pulses _____
Chest _____ Extremities _____
Heart _____ Neurologic _____
Abdomen _____ Orthopedic _____
Other _____
Comments: _____
_____

3. Please provide the following, if available:

Total cholesterol _____ Blood pressure /_____
Triglycerides _____ Resting pulse _____
LDL _____ HDL _____

4. I have examined the above-named individual and find no reason why she should not participate in a graded exercise test or other physical activities.

_____ _____
Physician's Signature Date

PARTICIPANTS: PLEASE BRING THIS FORM DIRECTLY TO CHERYL LOVELADY OR MELANIE BOPP. PLEASE DO NOT HAVE YOUR PHYSICIAN'S OFFICE MAIL IT TO US. THANKS.

For further information, please contact:
Cheryl Lovelady, Ph.D., R.D.
UNCG Department of Food and Nutrition
Phone: (336) 256-0310

Multivitamin given to all participants:

Vitamin A	5000 IU
Vitamin C	60 mg
Vitamin D	400 IU
Vitamin E	30 IU
Thiamin	1.5 mg
Riboflavin	1.7 mg
Niacin	20 mg
Vitamin B6	2 mg
Folic Acid	400 mcg
Vitamin B12	6 mcg
Pantothenic Acid	10mg

Resistance training Week 1 - Familiarization

3 days per week – 60% - 1 set per exercise **Please record date and weight lifted each day

3 second lift – hold for 0 seconds – lower for 3 seconds 45-60 second rest between exercises

Day 1		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets	1	1	1	1	
	Number of Reps					
	Pounds lifted:					
		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
	Number of Sets	1	1	1	1	1
	Number of Reps					
	Pounds lifted:					
Day 2		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets	1	1	1	1	
	Number of Reps					
	Pounds lifted:					
		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
	Number of Sets	1	1	1	1	1
	Number of Reps					
	Pounds lifted:					
Day 3		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets	1	1	1	1	
	Number of Reps					
	Pounds lifted:					
		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
	Number of Sets	1	1	1	1	1
	Number of Reps					
	Pounds lifted:					

Resistance training Weeks 2-6

Alternate days – 70% - 10-15 reps per set 3 sets per exercise

3 second lift – hold for 0 seconds – lower for 3 seconds **Please record date, weight lifted and number of reps each day

45-60 second rest between exercises, straight or supersets

WEEK 2

Day 1		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 2		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 3		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					

WEEK 3

Day 1		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 2		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					

Day 3		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					

WEEK 4

Day 1		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 2		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 3		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					

WEEK 5

Day 1		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 2		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 3		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank

Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					

WEEK 6

Day 1		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 2		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 3		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					

Resistance training Weeks 7-11

Alternate days – 85% - 5-8 reps per set 4-5 sets per exercise

2 second lift – hold for 0 seconds – lower for 2 seconds **Please record date, weight lifted and number of reps each day

2 minute rest between exercises, supersets

WEEK 7

Day 1		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					

Day 2		Squats	Bench Press	Military Press	Sit ups
Date:	Number of Sets				
	Number of Reps				
	Pounds lifted:				

Day 3		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					

WEEK 8

Day 1		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 2		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 3		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					

WEEK 9

Day 1		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 2		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 3		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					

WEEK 10

Day 1		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 2		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 3		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					

WEEK 11

Day 1		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 2		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 3		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					

Resistance training Weeks 12-16

Alternate days – 90% - 3-5 reps per set 5 sets per exercise

3 second lift – hold for 1 second – lower as quickly as possible **Please record date, weight lifted and number of reps each day

2-3 minute rest between exercises, supersets

WEEK 12

Day 1		Squats	Bench Press	Military Press	Sit ups
Date:	Number of Sets				
	Number of Reps				
	Pounds lifted:				

Day 2		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					

Day 3		Squats	Bench Press	Military Press	Sit ups
Date:	Number of Sets				
	Number of Reps				
	Pounds lifted:				

WEEK 13

Day 1		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 2		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 3		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					

WEEK 14

Day 1		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 2		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 3		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					

WEEK 15

Day 1		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 2		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 3		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					

WEEK 16

Day 1		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 2		Stiff leg deadlift	Push up	High Pulls	Dumbbell row	Ab plank
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					
Day 3		Squats	Bench Press	Military Press	Sit ups	
Date:	Number of Sets					
	Number of Reps					
	Pounds lifted:					

Stages of Exercise:

Week 1: Familiarization

3 days per week, 1 set of all exercises, 60% of One Repetition Max (RM). 3-0-3 tempo: 3 seconds down, hold for 0 seconds, and 3 seconds up. 45-60 seconds between each exercise. 10-15 reps of each exercise.

Weeks 2-6: Hypertrophy

Alternating days of the split routine. 70% 1RM, 10-15 reps per set, 3 sets, 3-0-3 tempo, 45-60 seconds rest. Straight set method (doing all 3 sets of one exercise and then moving onto next exercise) or superset method (doing one set of one exercise and then one set of the next until you have done all exercises for that day and then cycle back through all exercises until you finished all 3 sets for each).

Weeks 7-16: Strength

Alternating days, 85% 1RM, 5-8 reps per set, 4-5 sets, 2-0-2 tempo, 2 minutes rest between each. Superset method.

Day 1 Exercises:

Squats: Start standing up with dumbbells at side with feet shoulder width apart and slowly sit down as if sitting in a chair. Make sure knees do not go in front of ankles. Go down until butt is at a 90 degree angle with knees. Arms can be at side or above shoulders with elbows bent.

Bench Press: Can be done lying on ball or floor. On floor, lay down with back flat on floor and legs straight. Hold dumbbells in each hand and raise arms extending from shoulders, straight up. Arms should be directly over chest with elbows extending as you raise arms and elbows bending as you come back down. On ball, rest upper back on the ball in a backbend position with feet flat on the floor, shoulder width apart and extend arms from shoulders, straight up. Make sure to keep abs parallel with the floor and push hips up toward ceiling.

Standing Military Press: Stand straight up with feet shoulder width apart. Hold dumbbells in each hand and raise arms straight up toward ceiling directly from shoulders (i.e. start with elbows bent and raise arms up).

OR Push Press: Same thing as military press, but for variation add squatting down like as in the squats and as the legs become straight then push arms up in the air overhead.

Abdominal Sit-up: Lay down on floor with feet flat on the floor and knees raised. Raise upper back until elbows (or hands, depending on position) touch knees.

Easiest: Start sitting up and slowly with control lower back to the floor.

Next: Start lying down and go up to knees and back down with arms straight out towards knees.

Next: Same as above, but cross arms over chest.

Next: Same as above, but arms are behind neck with elbows bent. Make sure your arms are not pulling on neck and your stomach muscles are doing the work.

Hardest: Same as above, but do all variations on the ball. Position similar to bench press on the ball (i.e. back bend position). The farther back your back and hips are on the ball, the harder the work-out on the stomach muscles.

OR Crunches: Lay down on floor with knees bent and feet either up in the air or flat on floor. Raise upper back to a slight curl; do not come up all the way. Variations for arms are the same as abdominal sit-ups.

Wall Sits: Position back against a wall as if sitting in a chair. Make sure tops of upper leg is parallel to the floor and calf-side of lower leg is parallel to the wall. Let arms rest against back of the wall (i.e. – do not rest hands on hips or legs). Hold for as long as possible while keeping the correct technique.

Day 2 Exercises:

Stiff Leg Deadlift: Start standing up with feet about six inches apart. Keep knees slightly bent and hold dumbbells in each hand. Slowly bend over as if touching toes.

Pushups: Lay down with stomach on the floor. Feet should be about six inches apart. With hands shoulder width apart at chest/shoulder level, slowly rise up and then back down and repeat. Shoulders and elbows should be at a 90 degree angle and back should remain parallel to the floor, not arched or sagging.

Easiest: Bend knees and let knees touch the floor and back should be at an angle to the floor as you descend, but make sure back never arches. Just do the down motion and then come up to knees to get back up and then repeat the down motion again.

Next: Same position as above but do both ups and downs.

Hardest: First example, on feet (no knees), doing both ups and downs.

High Pulls: With feet shoulder width apart and knees slightly bent start with arms straight by side with dumbbells in hand and lift fists straight up so that elbows are bent and straight out and then go back down to straighten elbows. Like rowing, but pulling up towards ceiling.

Bent Over Dumbbell Row: With back straight, but slightly bent over, hold dumbbells in each hand and keep arms in 90 degree angle, while raising arms straight up, while keeping elbows bent. Feet should be shoulder width apart with knees slightly bent.

Abdominal Plank (or straight leg sit-up): Start with stomach on the floor and place elbows on the floor with arms bent. Elbows should be anywhere from under chest to further out towards the head, but inside the body. Hold body up and keep back straight, not arched or sagging. Hold position for as long as you can. Start with 5-10 seconds and work up to 30 seconds. When you can do that, move up to the next level trying to achieve one minute or more.

Easiest: Elbows under chest and on knees with legs bent and back at an angle.

Next: Extend elbows further out toward head or further out.

Next: Move up to feet only and keep legs straight.

Hardest: On feet and elbows bent on floor under head or further out.