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**Determinants of Bone Mineral Density Changes in Women Transitioning to
Menopause: A MONET Group Study**

By

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THESIS

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ABSTRACT

Menopause is an important period for bone health in women. **Objective:** To assess the determinants of bone mineral density (BMD) changes in women transitioning to menopause.

Method: A secondary data analysis of the MONET (Montreal-Ottawa New Emerging Team) study. Outcome measures included yearly assessment of menopause status, body composition, BMD, physical activity energy expenditure (PAEE) and dietary calcium and vitamin D intakes.

Results: 84 of the original 102 women had complete data for the purpose of the present study. Repeated measures analysis revealed significant decreases in lumbar spine and femoral neck BMD ($P < 0.01$). Regression analysis revealed that baseline femoral neck BMD, changes in PAEE and trunk fat explained 31% of the variation of BMD changes at the femoral neck, while changes in both PAEE and trunk fat account for 27% of BMD change at lumbar spine.

Conclusion: Baseline femoral neck and changes in physical activity energy expenditure and trunk fat are determinants of the reduction of bone mineral density in women transitioning to menopause.

Keywords: Menopausal, bone mineral density, body composition, physical activity, dietary calcium and vitamin D.

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LIST OF ABBREVIATIONS

BW	Body Weight
% BF	% Body Fat
BM	Bone Mass
BMC	Bone mineral content
BMD	Bone mineral density
Ca	Calcium
CNS	Central nervous system
DEXA	Dual Energy X-ray Absorptiometry
FFM	Fat Free Mass
FM	Fat Mass
GH-IGF-1	Growth hormone insulin-like growth factor 1
HRT	Hormone replacement therapy
NOF	National Osteoporosis Foundation
PAEE	Physical Activity Energy Expenditure
PTH	Parathyroid hormone
Vit. D	Vitamin D
WC	Waist Circumference
YSM	Years since menopause
25(OH) D	25-hydroxyvitamin D

CHAPTER 1

A. INTRODUCTION

Women reproductive system strongly affects the growth and development of the skeleton throughout adult life (1). It is known that hormonal status of an individual is one of the major determinants of bone mineral density (BMD) which is expressed as grams of bone minerals per unit area (2, 3). Studies have shown that estrogens directly stimulate bone formation, while estrogen deficiency can result in bone loss (2, 4). Reproductive aging is a natural process that started at menarche then followed by 3 overlapping phases for women: reproduction, menopausal transition and postmenopause (5, 6) (figure 1). The menopausal transition is divided into two stages: early defined as when menstrual cycles vary by more than 7 days and late when menstrual cycles may be skipped two or more times. During the later stage weight gain and vasomotor symptoms, such as hot flashes and night sweats are most likely to occur. The first year after the final menstrual period occurring in the postmenopausal phase, is also considered part of the perimenopausal period where women may continue to experience vasomotor symptoms which can last for up to five years after the last menstrual period. Perimenopause, start from the initiation of the menopause transition to one year after the final menstrual period which typically occurs in women aged between 40 to 65 years old (7).

								Final Menstrual Period (FMP)		
								0		
Stages:	-5	-4	-3	-2	-1		+1	+2		
Terminology:	Reproductive			Menopausal Transition			Postmenopause			
	Early	Peak	Late	Early	Late*		Early*	Late		
				Perimenopause						
Duration of Stage:	variable			variable			a 1 yr	b 4 yrs	until demise	
Menstrual Cycles:	variable to regular	regular		variable cycle length (>7 days different from normal)	≥2 skipped cycles and an interval of amenorrhea (≥60 days)		Amen x 12 mos	none		
Endocrine:	normal FSH		↑ FSH	↑ FSH				↑ FSH		

Figure 4: Stages of Normal Reproductive Aging in Women.
Adapted from Soules et al. (5).

Since menopause represents a critical endocrine and metabolic period in women's life, it has been suggested to have a strong impact on bone mass (BM) (8). Menopause is defined as “the permanent cessation of menstruation, resulting from the progressive loss of ovarian follicular activity”(9). When a female reaches puberty there is a rapid increase in BMD until the late teens, which is then followed by a slower increase in BMD during the second decade and consolidation of skeletal mineral content during the third decade until peak BMD is achieved between 25 to 35 years old (1, 10). Numerous studies have reported that body weight, body composition, dietary calcium intake, physical activity and normal pubertal development are considered major determinants of peak bone mass (2, 11-13).

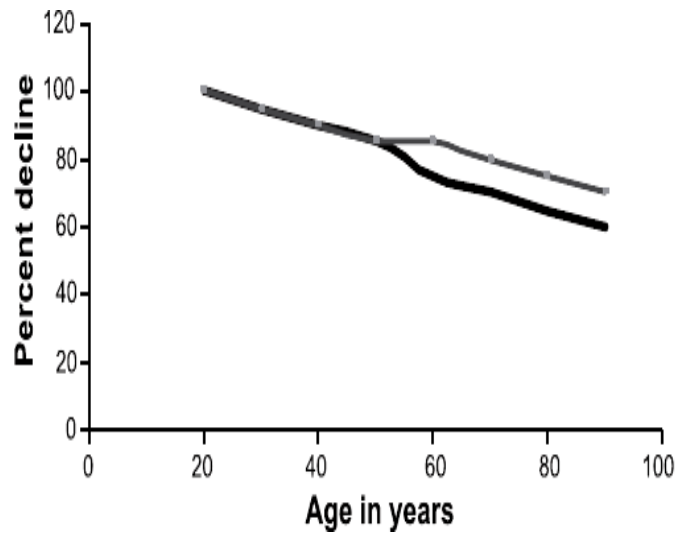


Figure 5: The peak bone mass and progressive bone loss throughout a woman's life
 The black line represents women, who have not received HRT,
 While the light line represents women who have received HRT.
 Source: Adapted from Brown, M. et al. (4).

Bone mass is defined as the amount of minerals (mostly calcium and phosphorus) contained in a certain volume of bone (14). While calcium (Ca) and vitamin D (Vit.D) intake and mechanical loading are the most important determinants of peak BMD, they also contribute to maximize the bone strength and display the genetic influence in the level of bone mineral density (15). There are two primary cell types which control the amount of bone tissue: the osteoblasts which produce bone tissue and the osteoclasts which cause the resorption of bone. In a healthy individual, there is a balance between osteoblast and osteoclast activity such that the amount of bone being lost is compensated by the amount of bone produced (4). Both osteoclasts and osteoblasts activity are influenced by the estrogen plasma levels.

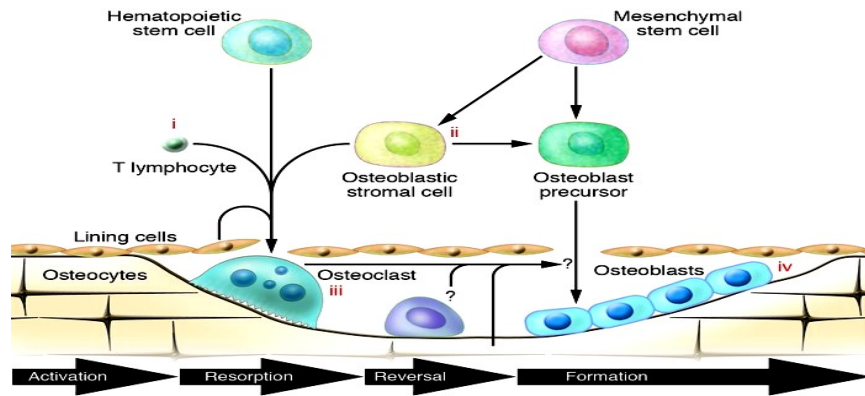


Figure 6: Bone cell formation and resorption with possible sites of estrogens action
 Source: Adapted from Brown, M. et al. (4).

At menopause, the ovarian estrogen plasma levels significantly decreased by 85-90% in comparison to the premenopausal estrogens plasma levels (1). This hormonal change could explain the shift in BM balance that occurs at menopause. In fact, bone resorption increases by 90% while bone formation increases only by 45% as assessed by markers of bone resorption and formation respectively (5). Therefore, women naturally undergo a phase of rapid bone loss that begins approximately 2-3 years before the cessation of menstruation and continues for up to 5 years postmenopausal (16-18). The perimenopausal (transition to menopause) and early postmenopausal period are periods of high bone turnover as a result of progressive ovarian failure (19).

Bone mass and BMD are considered the major determinants of risk of fractures accounting for > 60% of the individual variation in breaking bone strength threshold. Also, as aging progresses, bone loss gradually increases (20-22). When age-related bone loss is higher than the physiological norms, it contributes to the development of osteoporosis, which is defined as BMD that is at least 2.5 standard deviations below the mean BMD for young healthy individuals (20). The potential bone fractures secondary to osteoporosis is a serious health problem (23).

Despite the fact that up to 80% of the bone strength (including BMD and quality) might be genetically determined (24), many other factors such as age, smoking, weight, height, fat free mass (FFM), fat mass (FM), nutritional habits and physical activity have an effect on BMD (4, 13, 25-27). It has been demonstrated that menopausal women undergo several metabolic, physiologic and biochemical changes that may affect their body composition and body fat distribution, nutritional habits and physical activity as well (1) . All of these factors may therefore alter the pattern of bone loss during premenopausal, perimenopausal and postmenopausal years (28).

The association between dietary Ca and Vit.D intake, bone mass and BMD is documented in many studies (12, 14, 23, 29). The influence of weight-bearing physical activities and the role of both FFM and FM on BMD were also investigated (12, 28). To our knowledge, those scientific evidences are mainly based on cross-sectional studies and many had focused on postmenopausal BMD, while studies had compared pre- and postmenopausal women and few used longitudinal studies that follow women throughout the menopause transition. Therefore, there still are discrepancies between studies observations and reports. Also, the influence of perimenopausal period is obviously neglected with scanty investigations available at the present time on the effect of this specific hormonal transition period on BMD.

Thus, the first objective of this secondary analysis study was to document the change in BMD and the yearly rate of bone loss at the lumbar spine and femoral neck in women transitioning to menopause. The second objective was to identify the anthropometric and lifestyle determinants of changes in BMD at lumbar spine and femoral neck in women transitioning to menopause.

B. LITERATURE REVIEW

1. Bone mineral density in premenopausal women

1.1 Body weight

Body weight (BW) is considered as one of the BMD determinants (19). Significant negative associations between FM and bone mass were reported in premenopausal women after controlling for BW (11, 30-32). Fogelholm et al. (33) reported that BW, and especially the FFM are major determinants of BM. Consequently and in line with the weight-bearing effect theory, obese women have, in general, higher BMD and bone mineral content (BMC) (expressed as grams of bone minerals) than normal weight women. Because the increased risk of health problems associated with an excessive amount of body fat, obese people often try to lose weight. However, few studies explored the influence of weight changes on BMD in premenopausal women (33, 34). Although reduction of excess weight reduces the risk of several chronic cardiometabolic diseases, it may also lead to bone loss. In fact, Fogelholm et al. (33) noticed a reduction in total body BMD following a voluntary weight reduction without a corresponding change in total body BMC. Similar results were reported by Van Loan et al. (34) in premenopausal obese women. On the other hand, studies have found that changes in total body BMD were reflected by corresponding changes in BMC (35-37). The reasons for these discrepancies among studies are not clearly identified. Although a majority of studies suggest that total BMD and/or BMC decrease following a significant weight reduction, less is known about these changes in specific bone sites such as at the lumbar spine and femoral neck. It has been reported that BMD was maintained at the lumbar spine, but reduced at the femoral neck in premenopausal women after losing weight following a calorie restricted diet (35, 38). In contrast, some research groups found a BMD reduction at the lumbar spinal and not at the

femoral neck (39, 40) after controlling for weight loss. The reported change in BMD at the greater trochanter after weight loss are also inconsistent (35, 38).

Several factors may explain the change in BMD following weight loss. Simply, it may be secondary to the decrease in the mechanical loading on bone as a result of reduced BW as well as FFM (40). This mechanism, however, could not explain the positive associations found between weight and BMD of non-weight-bearing bone such as the radius (33). Some researchers explained these positive associations through the endocrinal alterations accompanying the changes in body FM which affect the production of cytokine-like hormone such as leptin secreted by fat cells. In fact, leptin is considered an important candidate molecule to explain the link between changes in body composition and bone formation and resorption. It has been found that an increase in BW secondary to an increase in FM, is associated with an increased leptin plasma levels. Furthermore, it has been found that leptin has a direct anabolic effect on osteoblasts, and also an indirect effect through the central nervous system (CNS) by stimulating the growth hormone insulin-like growth factor 1 (IGF-1) axis which in turn stimulate periosteal bone formation and suppression of neuropeptide Y, a powerful inhibitor of bone formation (41-43). A second potential explanation for the discrepancies related to the associations between changes in BW and BMD is the limitation associated with the use of the dual-energy X-ray absorptiometry (DXA). It has been reported that there is a mean precision error varying between 2.7% to 3.4% for BMD measure by DEXA which is mostly due to the variation of the positioning of the subject's skeleton. Therefore, correct and careful positioning of patients is essential to obtain reliable BMD values (44). Given that the BMD and BMC changes following weight loss are clinically small and partly reversible, more studies are needed to clarify whether

these observations are physiological or secondary to the inaccuracies and/or technical measurement limitations (33).

1.2 Body composition and body fat distribution

Most of the studies conducted to assess the contribution of body composition to BMD in premenopausal women suggested that FFM is the strongest predictor of BMD, as positive correlations were observed only between FFM and both total and segmental BMD (45-50). The authors explained the absence of positive correlation between FM and BMD by the amount of estrogens secreted by the ovaries which is, in premenopausal women, many times higher than the adipose tissue estrogens production through aromatization process. Another study suggested that the risks of osteoporosis, and non spine fractures in premenopausal women were significantly higher for subjects with a higher percentage body fat (%BF) independent of BW, physical activity, and age. Therefore, FM could have a negative effect on bone mass which is in contrast with the positive effect of weight-bearing itself on BMD in premenopausal women (51). Also, Hsu, Y. et al. (51) conducted a community-based, cross-sectional study in 4585 premenopausal women, and 2248 postmenopausal women aged 25–64 years, they measured total-body and hip BMC, BMD and body composition by DXA. The results showed a higher risk of osteoporosis and lower BMD in women with a high %BF.

It is well documented that FM acts as a peripheral site for the conversion of androgens to estrogens as a result of activation of aromatase enzyme in the adipose tissue and this production seems to increase with aging (52). Also, it is suggested that the most active adipose tissue sites for the production of estrogens in women are located at the hip, followed by the thigh, then the abdomen (52). Therefore, the lower extra-glandular estrogens production and its effect on BMD may be masked by the higher amount of ovarian estrogens secretion in premenopausal women.

Other factors that could explain the positive correlation between FFM and BMD are daily physical activity levels, nutritional status and age. All of these factors could affect the amount of bone loss in premenopausal women (50).

Douchi et al. (46) reported that premenopausal women with upper (android) body fat distribution have greater BMD than women with lower (gynoid) body fat distribution. The researchers suggested firstly, that higher central accumulation of body fat is associated with lower sex-hormone binding globulin levels, resulting in higher free plasma estrogens and testosterone levels, the biologically active forms of these hormones, consequently promoting bone formation. Secondly, another possible explanation for a higher BMD in women with an android body fat distribution is that elevated androgen plasma levels are associated with the development of structural and functional male muscle physical characteristics. Thus, the higher BMD observed in women with an android body fat distribution may be secondary to the development, in part, of a greater muscle mass (46, 53). Therefore, taking these observations into consideration, we could speculate that the effect of FM on BMD is mediated not only by its weight-bearing effect, but also by other related hormonal factors as well. Moreover body fat distribution, especially central obesity, rather than total adiposity is an important predictor of BMD in premenopausal women.

1.3 Dietary calcium and vitamin D intake

Although many aspects of the diet and lifestyle affect bone status, the main key environmental factors which determine BMD are daily dietary Ca and Vit. D intake (13). Calcium is the most plentiful mineral in the human body and represents 1.5% of body mass. Approximately 99% of the total Ca is stored in the skeleton. The other storage sites are in the cells of soft tissue (0.9%), blood stream and extracellular fluid (0.1%) (14) , where they act on

the cardiovascular, nervous, and muscular systems (14). Women daily Ca requirement is 1000 mg. to attain the nutritional bone benefits of Ca. Adequate Vit. D (25-hydroxyvitamin D or 25(OH)D) status is needed and could be defined as serum 25(OH)D of 30 ng/mL (usually achieved with a daily oral intake of at least 400 to 600 IU of Vit.D) (33). The North American Menopause Society (NAMS) 2006 (14) stated that dietary sources are the ideal forms to meet the adequate daily Ca intake because there are many other essential nutrients in high-calcium foods. Dairy products offer the most value of high Ca content and at a relatively low cost. Non dairy food sources of Ca include leafy green vegetables, a few types of nuts like almonds, and some beans, but the Ca content is less concentrated than in dairy products. Moreover, the Ca in some foods (eg, spinachs) is not well absorbed in presence of a Vit.D insufficiency. Other foods containing high levels of Ca include canned salmon and sardines, but only if eaten with bones (14). On the other hand, Vit.D is present mainly in fish oils, tuna meat, milk products and eggs (33). Determining the relationship between dietary Ca and Vit.D intake and BMD is the key for identifying nutritional strategies to decrease age-related bone loss during menopausal years (54). Overall, evidence from previous studies, randomized, placebo-controlled clinical trials suggests a positive relationship between BMD and Ca intake of 1377.8 ± 631.9 in premenopausal women and to smaller extent in women around the onset of menopause (13, 18). While some other studies reported no significant association between daily dietary Ca intake of 1088 ± 489 and BMD values in premenopausal women (31, 55). Bacon et al. (55) had noticed, in obese premenopausal women, negative relationships between the number of times women dieted to lose weight, the cognitive dietary restraint score and the current BMC. This may be due to insufficient amount of Ca and Vit. D intake, in absence of minerals supplementation, during the diet restrain periods as it was reflected by the high prevalence of osteoporosis (31%) in their

sample. This observation emphasizes the fact that frequent dieting episodes could negatively influence bone health, even in obese women who are not considered as bone losers. In contrast to these results, another study reported that overweight premenopausal women could maintain their BMD status, if they meet the recommended amount of daily Ca intake during the weight loss periods. This could partly be explained because of a sufficient amount of Ca was absorbed (31). Since the amount of total Ca absorbed is an important factor in establishing Ca balance and prevention of bone loss, it is possible that a daily adequate Ca intake (1000 mg) combined with an adequate Vit. D plasma levels could contribute to maintain bone mass during a weight loss intervention in overweight or obese women. There are two potential mechanisms that may elucidate the above finding. Firstly, higher Vit. D plasma levels is associated with a lower rate of bone resorption and tended to decrease urinary Ca excretion. Secondly, a high Ca intake may help the skeleton to respond to hormonal cues which may contribute to reduce bone resorption in premenopausal women (56) Thirdly, high Ca intake significantly amplified weight and fat loss secondary to energy restriction and increased the percentage of fat loss from the trunk region which in turn decrease the circulating leptin levels which could explain the link between the reduction of body weight and associated bone loss as described earlier (41, 57). Von Hurst et al. (27) observed a decrease in BMD at the lumbar spine (-40%) and hip (-32%) in premenopausal women although they reported consuming the recommended daily Ca intake. The authors attributed the low BMD and bone loss to the low serum 25-hydroxyvitamin D plasma levels. Consequently, it is possible that bone health in those women was influenced more by their poor Vit.D status than their daily dietary Ca intake. The main cause of Vit.D deficiency is the lack of sun exposure the major source of Vit.D for most humans (58). Also, it well documented that unprotected sun exposure increases the risk of skin cancer, increasing the use of sunscreen with

a sun protection which reduce Vit.D production by 98% (59). Based on these observations, the national osteoporosis foundation (60) recommends a daily intake of 800 to 1000 IU of Vit.D for women during menopausal transition in order to achieve an optimal Ca absorption and maintain bone health (60). This daily recommended amount of Vit.D, particularly in the sufficient sun exposure, could not be meet from dietary source alone therefore, raising the importance of Vit.D supplementation to achieve the required serum threshold levels of 80–90 nmol/L of Vit.D to maximize Ca absorption (60). Lips, et al (103) reported in a large overview paper of the available data from epidemiological and intervention studies that Vitamin D status is related to BMD either combined with Ca intake or not. In most of clinical trials they reported increase BMD and reduce risk of fractures in participants who received Ca supplement of 800 IU/d and 1200 mg/d Vit.D meanwhile, participants who received less than 1000 mg/d Ca and less than 400 IU/d Vit.D showed none significant improvements in both BMD and risk of fractures (103). It is important to emphasizing that dietary Ca absorption seems to be proportionally related to the plasma levels of Vit.D. This strong association between Ca and Vit.D intake and bone health has been shown in several studies and support the importance of sufficient daily Vit. D intake to maintain healthy BM in premenopausal women and decrease the risk of osteoporosis and fractures (61-63).

1.4 Physical activity

Physical activity is a well recognized determinant of BMD (64), particularly the effect of regular physical activity on BMD during the stage of bone growth and development. In fact, physical activity helps to develop strong skeleton and achieving higher peak BMD and muscle strength (64-66). Physical activity performed during the teenage years is found to be the main predictor of BMD later in life (64). Participating in sports and/or loading physical activity, such

as walking on a regular basis before the age of 18, is associated with higher BMD (64). The mechanical weight loading of physical activity is thought to be an important factor to positively affect BM and its strength (67). The greater the straining force of physical activity on bone surfaces, greater the osteogenic (bone formation) effects (67, 68).

Previous studies have shown that premenopausal women with regular menstruation who reported practicing physical activity during teenage years had significant greater BMD than women who did not engage in any regular physical activity (69). On the other hand, previous studies have shown inconsistent effects of resistance training on BMD in premenopausal women (70-72). For instance, Warren et al. (67) reported that two years of strength training had no effect on BMD at different bone sites in premenopausal women. However, they suggested that, even in the absence of a significant increase in BMD, strength training may affect bone structure (size and dimensions). These results were supported by another study which reported that strength training for more than nine months did not significantly affect either the total body or regional BMD in premenopausal women (71). Some researchers investigated the effect of different types of physical activities on BMD such as work and/or active living lifestyle and they did not observed any effect on total and regional BMD (73, 74). Others study that examined the effects of climbing stairs and daily movements on calcaneal BMD, did not find a significant difference between the low and high physical activity levels group in premenopausal women. Finally, studies suggested that physically active lifestyle may help to minimize bone loss and maintain healthy bone status up to premenopausal years (75-77).

Overall, the results of the studies on the effect of the type, intensity, duration or frequency of physical activity or strength training on BMD in premenopausal women are conflicting.

Further research is required to identify the optimal volume and/or type of strength training needed in order to positively affect BMD in premenopausal women.

2. Bone mineral density in perimenopausal women

2.1 Body weight

Little is known about whether BW or weight change influence bone loss during the perimenopausal period. Furthermore, the effects of diet and/or physical activity levels remain largely not documented. Macdonald et al. (19) conducted a large sample based study of 1,064 premenopausal women (mean age \pm SD, 48.0 \pm 1.5 years) randomly chosen. They measured their BMD, BW and height and asked them to complete a food frequency and physical activity questionnaire two times between (1990- 1993) and (1997- 1999). The authors observed that in perimenopausal women BW change, either increase or decrease through the course of the study was associated with femoral neck BMD while BW at follow-up was associated with lumbar spine BMD change in women not taking hormone replacement therapy (HRT) meanwhile both energy intake and expenditure not correlated nor predicting to lumbar spine BMD. On the other hand, in HRT users, neither BW nor weight changes were associated with changes in BMD perhaps because of the dominant effect of supplement estrogens on bone. These results are in line with the observation of Sirola et al (79) who reported that, weight change is a significant determinant of bone loss at both the lumbar spine and femoral neck in 940 perimenopausal women follow-up for 5 years meanwhile.

Longitudinal studies are necessary to further examine the relationship between BW and weight changes and rate of bone loss and BMD during the perimenopausal period.

2.2 Body Composition and body fat distribution

The perimenopausal period has been associated with an increase in BW and FM and a decrease in FFM (78). Li et al. (78) conducted a study in order to determine the independent

effect and relative contribution of FFM and FM to BMD in a sample of 43 sedentary perimenopausal women. Total body BMD, regional BMD, and soft tissue body composition were measured by DEXA. They reported that FFM rather than FM was a significant predictor of femoral neck BMD and that the endocrine effect of FM on BMD remains minimal or masked by the main effect of ovarian estrogens secretion during perimenopause.

Although FFM is more related to BM than FM in premenopausal women, whether an increase in FFM in perimenopausal women would lead to healthier bone mass or reduce bone loss, is still unknown. Therefore, longitudinal studies are necessary to examine the relationship between changes in body composition and rate of bone loss and BMD in perimenopausal women.

2.3 Dietary calcium and vitamin D intake

Few studies examined the effects of dietary Ca intake on BMD in perimenopausal women. The results of the majority of the studies supported the concept that adequate daily Ca intake in the presence of sufficient Vit.D status contribute to reduced bone loss during the perimenopausal period (14, 80, 81). Moreover, it has been reported that building a healthy BM before menopause is associated with a reduced bone loss during the first years of menopause (15, 28, 82). Picard et al. (28) studied the BM across menopause transition in 141 women already assessed 10 years before while in premenopause status. They reported the following observations: first, they highlight the importance of the influence of both past and current daily Ca intakes on BMD status, secondly, that the level of premenopausal BMD was a more important determinant of current BM health than the rate of bone loss during the menopause transition, emphasizing the importance of building a healthy BM before menopause; thirdly, that Ca derived from dairy products has shown to have the most beneficial effect on BMD; and fourthly, that Vit.D intake is essential to minimize BM loss during menopausal years.

There is a lack of scientific evidences on the effect of dietary Ca intake and Vit. D status on BMD in perimenopausal women. Therefore, further investigation is needed in this sub-population to identify if this period of women's life is critical to attenuate bone loss and/or maximize BMD, and if so, develop strategies to increase awareness among perimenopausal women to the benefits of a healthy lifestyle during the perimenopausal period.

2.4 Physical activity

It is a well known that menopause is accompanied by an increased rate of bone loss, mainly within the 5 years around the onset of menopause, even though bone resorption may take place prior to menopause (76, 83). Regular physical activity has been known to play an important role in maintaining or increasing total and/or regional BMD (55, 69, 84). Few studies have been done to investigate the influence of different types of physical activities on BMD in perimenopausal women. Puntilla et al. (84) reported, following a 12 months longitudinal study comparing habitual leisure-time physical activity levels to moderate intensity exercises in a population based random sample of 1873 peri- and postmenopausal women, that regular weight bearing exercising was ineffective to reduce the BMD or BMC loss at the femoral neck. Other authors suggested that being physically active, particularly practicing weight bearing physical activities, and reporting healthy dietary habits throughout life is associated with maintaining BMD during perimenopausal period (65, 85, 86).

The type and/or volume of physical activity necessary to reduce BM loss during the perimenopausal period are still not well documented. Further studies are needed to document the effects of physical activity in perimenopausal women.

3. Bone mineral density in postmenopausal women

3.1 Body weight

Body weight and weight changes in postmenopausal women are thought to be strong predictors of BMD (87). It has been demonstrated in postmenopausal women who had not taken HRT, that weight changes was a significant predictor of the individual variation in BMD loss at the lumbar spine and femoral neck (accounting for 8.4 % and 2.6 % respectively) (19). There is a study documented the effect of energy restriction for weight loss on the rate of bone turnover in postmenopausal women. Ricci et al. (87) reported that after serial BMD measurements, the rates of bone resorption increased during an energy restriction diet when compared to weight maintenance in postmenopausal women. In addition, the reduction in FM with weight loss was directly associated with a decrease in plasma estrones levels. Ricci et al. (87) attributed the elevated rates of bone resorption during energy restriction diet to the increase in plasma levels of parathyroid hormone (PTH) observed after 6 months of weight loss intervention. Although the absolute PTH plasma levels was within the clinical normal reference range (87), its increased levels could have contributed to increased bone resorption. A rise in PTH may occur as a result of reduced Ca intake, 25-hydroxyvitamin D or hypercalciuria. In the same study, estrogen (estradiols and estrones) plasma levels were positively related to BMD and negatively related to bone loss. On the other hand, the bone loss could also result from the reduction in mechanical loading associated with weight loss and concomitant decrease in FM and FFM. In contrast to the above results, Milliken et al. and Choi et al. (2, 21) reported that although there was significant increase in BW, but BW was not a significant predictor of BMD changes in postmenopausal women not receiving HRT. Meanwhile, both BW and change in BW were significant predictors for BMD in women receiving HRT.

3.2 Body composition and body fat distribution

Body composition is one of the major factors modulating the BM in postmenopausal women (22). Most studies observed a significant positive correlation between FM and total BMD in postmenopausal women (11, 19, 45, 50, 88, 89) . The authors of these studies explained this association through the role of the extra-glandular adipose tissue estrogen production as a mediator between FM and BMD. The association between FM and BMD could also be explained by the fact that lower FM is associated with low plasma levels of estrogen which are often found to be associated with low BMD and higher risk of fractures among elderly women (88). Other studies reported associations between FM and regional BMD. Cui et al. (45) observed a significant positive correlation between FM and BMD at all regional sites, such as at the lumbar spine and femoral neck. FM was the only predictor of BMD at the lumbar spine, distal forearm, and calcaneus sites, while both FM and FFM contributed to individual variation in BMD at the hip, with the effect of FFM being a little greater than the FM. These findings were consistent with Milliken et al. (2) results which stated that despite the exposure of individual skeleton to similar systemic conditions, bone sites respond differently, even after accounting for the effects of physical activity and Ca intake. For example, two areas at the hip, the femoral neck and greater trochanter sites, responded in a different way, particularly for women not taking HRT. In contrast, Gnudi et al. (22) reported in postmenopausal women that both FM and FFM influence bone density, with different physiological and/or pathological conditions modulating this relationship, body FM did not affect any of the bone density parameters considered either separately or when tested with FFM in postmenopausal women. Moreover, women with a higher FM were older and even if they present a significantly higher BW and BMI, they present a lower BMD, BMC than women with a higher FFM. However, among women with a higher FFM, both

FM and FFM were significantly associated with all BMD measurements. The author explained these results through the action of muscles, which apply mechanical stress on bone and therefore stimulated bone production (90, 91). In addition, some studies minimized the role of FM on BM. They claimed that BW alone is not effective as a bone production mechanical stimulus because of the lack of evidence regarding the bone response to static loads (92-94). Therefore, they suggested that the FM load act on BM by increasing the muscle-mediated skeletal dynamic load. However, other researchers have reported an independent action of FM on BMD mediated by the effect of estrogens, leptin as we mentioned previously or the effect of insulin and amylin, as peptide hormones secreted from pancreatic beta cells, to stimulate bone formation and decrease bone resorption (95, 96). In conclusion, the previous data suggests that both body FM and FFM can affect BM in postmenopausal women. Also, changes in FM and FFM explained between 6–32% of the individual variation in BMD (2). However, their relative effect on bone mass may be modulated by their absolute amount and/or by their ratio to total body mass weight in postmenopausal women (22).

On the basis of the results of the previous studies, the relationship between soft tissue body composition and BMD has been mainly examined in pre- and postmenopausal women. Although it is generally established that BW is an important determinant of BMD, there is considerable argument regarding the relative contributions of its two major components: FM and FFM. Some studies have shown that FFM is the major predictor of BMD and FM has little or no significant effect on bone physiological in either pre- or postmenopausal women. In contrast, others suggested that FM is the main determinant of BMD while others claimed that FM and FFM have an effect on BMD. Some studies had reported different effect of BW and body composition based on the site studied (e.g., total BMD, lumbar spine, or femoral neck), the bone parameters

(e.g., BMD vs. BMC) used in analysis, as well as the participants' menopausal status. Finally, in most of these studies the researchers did not separate pre- and postmenopausal women in their analyses and did not consider perimenopausal women as a distinct menopausal status.

3.3 Dietary calcium and vitamin D intake

Many studies investigated the association between dietary Ca and Vit.D intake and BMD in postmenopausal women in order to reduce bone loss and lower the risk of fractures and osteoporosis. Most studies conducted to Canadian and Chinese women who recorded a minimum Ca intake of 1000 mg/d, reported that daily Ca intake is significantly associated with changes in BMD and BMC in postmenopausal women (12-14, 54, 97), whatever the method of dietary Ca assessment used (54). Another study reported associations between Ca intake and BMD at specific bone sites such as the greater trochanter and Ward's triangle, whereas, no significant association was found at the lumbar spine. Therefore, they recommended that increasing the dietary Ca intake above 1000 mg /day was helpful to prevent cortical bone loss among early postmenopausal Chinese women (12). Others recommended increase daily Ca intake up to 1200 mg at menopause due to the acceleration of bone resorption rate in response to the decline in ovarian estrogen production and secondary to the decreased efficiency of dietary Ca utilization resulting from estrogen related shifts, the reduction of intestinal absorption and renal resorption as a result of aging process (54). Some studies reported that weight loss in overweight women leads to greater bone loss compared to obese women reporting the same daily amount of dietary Ca intake. They attributed these difference in bone loss to the greater weight bearing effect on skeleton of obese women (23, 31). In contrast to the above results, Hassa et al. (16) reported no relationship between lumbar spine and femur neck BMD and daily dietary Ca intake in postmenopausal women. They suggested that achieving adequate peak bone mass is

important to maintain bone mass and minimize the risk of fractures during the post menopausal period. In another study, women were categorized according to the quartiles of dietary Ca weekly servings. in order to investigate the effect of dietary Ca intake on BM, and found that low dietary Ca intake increase the BM and the risk of osteoporosis in early postmenopausal women (99). Also, Macdonald et al. (100) divided women into groups according to tertiles of Ca intake and found that higher daily dietary Ca intake ($\geq 1200\text{mg/d}$) in postmenopausal women reduced BM at the femoral neck.

The role of Vit. D in maintaining BMD in postmenopausal women had been investigated in many studies. Moore et al. 2004 (101) reported that less than 30% of postmenopausal women meet the current recommendations for daily Vit. D intake. The recommended plasma levels of Vit.D is 50 ng/ml (14, 31) could not be obtained only from dietary sources as mentioned before (58, 59) . Thus, most studies were conducted in postmenopausal women receiving recommended amount of Vit. D (600-800 IU/day) from dietary combined with supplementary sources. The results confirmed the positive relationship between adequate daily Vit. D intake and the reduction of bone loss and risk of osteoporosis (62, 102, 103). They also indicated that diet alone as a source of Vit. D could not maintain the BM in postmenopausal women. However, further investigations are needed.

Taken together, the above results revealed inconsistency between study findings which may be attributed to the weak association reported between daily dietary Ca, Vit. D intake and bone loss, small sample size, differences in population study (e.g. age, years since menopause (YSM)), bone sites measure, dietary Ca assessment tools, and range of daily Ca intake. Identifying significant associations between dietary Ca intake and BMD require accurate dietary

Ca assessment tools, controlling for co-factors affecting BMD and taking into account that these associations may vary between menopausal status and YSM as well.

3.4 Physical activity

Early postmenopause is a period during which important reduction in both muscular strength and BM are observed in women (104-106). Most studies that investigated the effect of physical activity in postmenopausal women reported that exercise training increase or maintain BMD in postmenopausal women (55, 74, 75, 84, 107-110). A strong association had been found between FFM and lumbar spine BMD in physically active postmenopausal women (75, 110). In contrast, in sedentary postmenopausal women, increased FM and % BF were positively associated with BMD (108). Another study reported that BW was strongly associated with BMD in active nonathletic postmenopausal women (111). The author emphasized that long term practicing of mild to moderate physical activity, even if they were not in competitive events, could play a role in maintaining BMD in postmenopausal women (111). These results were confirmed by Chien et al. (65) intervention study which showed that, the combination of mild and moderate intensity aerobic exercises reduced bone loss and contributed to maintaining BMD. Cussler, E. et al.(107) and Douchi, T. et al.(108) also showed an influence of strength exercises training on BMD in postmenopausal women aged 50 – 60 years old. Many researchers had found that the volume of physical activity required to stimulate BM and to generate health benefits is less than the volume of physical activity needed to enhance physical fitness (74, 112). Therefore, an active lifestyle have a positively impact on BMD, even if it is in the form of habitual daily activities such as climbing stairs, walking at work, etc.; it helps to reduce bone loss or maintain BMD in comparison to sedentary postmenopausal women (69, 74, 113, 114). Meanwhile, Puntila et al. (84) reported that habitual leisure time physical activity levels did not

have a positive effect on BMD and BMC in postmenopausal women. In addition, other researchers claimed that there was no relationship between physical activity levels and BMD in postmenopausal women (16). Moreover, Bemben, D.A., et al.,(104) reported even a decrease in BMD after a 6-months resistance strength training program- in early postmenopausal estrogen-deficient women. Finally, it has been reported that high physical activity levels in teenagers and increased BW during premenopausal period minimize bone loss or help maintaining healthy bones prior to the onset of menopause, consequently decreasing the risk of osteoporosis and fractures after menopause (64).

From the above findings, most of studies showed that regular physical activity, muscle strength and BW (FM and/or FFM) apply mechanical load on the skeleton which stimulate bone production.

C. SUMMARY

Throughout this section we have presented factors that have been documented to affect BMD during different menopausal status such as the hormonal status, BW, body composition and body fat distribution, dietary Ca and Vit.D intake and physical activity. Some of these factors play a role by increasing bone formation while others by reducing bone resorption through the entire women life, especially physical activity and dietary Ca and Vit. D intake. Other factors like hormonal status play a major role in the process of bone development at critical period like at the puberty and menopause transition, while other factors like body composition (FM and FFM) and body fat distribution seem to play a major role at the premenopausal and/or the postmenopausal periods consequently. However, most of the studies done to document the effects of these determinants on BMD used a cross sectional design and mainly focused on premenopausal or postmenopausal women giving less attention to the intermediate stage of perimenopausal. In addition, other factors may have affected the results like, the sample characteristics and size.

Thus, the present study was performed in order to identify the determinants of BMD changes in women from premenopause to postmenopause. To investigate this goal we performed a secondary data analysis of the MONET (Montreal, Ottawa, and New Emerging Team) observational, 5 year longitudinal study.

CHAPTER 2

2.1 Specific problem

Women are losing BMD more rapidly around the age of menopause which increase their risk of osteoporosis and bone fractures. Many population-based cross-sectional studies have been performed but relatively few prospective studies have followed women through the menopausal transition to document the anthropometrics and lifestyle determinants of change in BMD. Furthermore, there is a lack of knowledge, especially in regard to the factors affecting BMD changes during the perimenopausal period of women's life.

2.2 Objectives

The objectives of the present study were firstly to document the change in BMD and the yearly rate of bone loss at the lumbar spine and femoral neck in women transitioning to menopause. Secondly, to identify the determinants of changes in BMD at lumbar spine and femoral neck in women transitioning to menopause.

2.3 Hypothesis

The first hypothesis is that the rate of bone loss is increased following menopausal years. Our second hypothesis is that changes in body composition, physical activity energy expenditure, dietary calcium and vitamin D intake are strong determinants of BMD changes in women transitioning to menopause.

2.4 Limitations

Our study presents some limitations. First, the study is observational and the population studied was composed of healthy non-smoking, women with a BMI of 23.4 ± 2.2 kg/m², thus our findings are limited to this subgroup of the population. Secondly, besides the measurement of the plasma follicular-stimulating hormone (FSH) level at baseline to verify the menopausal status, we did not measure other plasma sex hormones, leptin, adiponectine or markers of bone metabolism. Thirdly, serum 25-hydroxyvitamin D was not measured in our participants and Ca and Vit. D supplements were not included in our analysis because of lack of information.

2.5 Strengths

Despite these limitations, the present study enriches the scientific evidence because of its longitudinal nature by following up a well-characterized cohort of premenopausal women throughout menopausal transition. We used gold standard measures methods (DXA and CT scan) for the measurement of bone mineral density and body composition (55). For dietary Ca and vit. D intake we used a 7 day food diary which is considered as an accurate measurement of long-term habitual dietary intake (115). Also, our assessment of physical activity energy expenditure (PAEE) was performed by accelerometers, which have been shown to be a reliable tool (116, 117).

CHAPTER 3

A. Methods

Methods used in the present study are detailed in the article format within the methodology section of the article in chapter 4 entitled: **Determinants of Bone Mineral Density Changes in Women Transitioning to Menopause: A MONET Group Study**

CHAPTER 4

Article

This chapter presents the major findings of our secondary analysis and the discussion of the results of the present study. They are presented in article format entitled:

Determinants of Bone Mineral Density Changes in Women Transitioning to Menopause: A MONET Group Study

**A. Determinants of Bone Mineral Density Changes in Women Transitioning to Menopause: A
MONET Group Study.**

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1. ABSTRACT

Transitioning to menopause is an important period for bone health in women. **Objective:** To assess the anthropometric and lifestyle determinants of bone mineral density (BMD) changes in women transitioning to menopause. **Method:** A secondary data analysis of the MONET (Montreal-Ottawa New Emerging Team) prospective 5-year longitudinal study of 102 premenopausal women (age: 49.9 ± 1.9 yrs; body mass index: 23.3 ± 2.2 kg/m²). Outcome measures included yearly assessment of menopause status, body composition, body fat distribution and BMD (dual-energy x-ray absorptiometry), physical activity energy expenditure (PAEE) (accelerometer) and dietary calcium and vitamin D intakes (7-day food diary). **Results:** 84 women had complete data for the purpose of the present study. In this group, 2 women were still premenopausal, 26 peri-menopausal and 56 postmenopausal at the end of the study. Repeated measures analysis revealed significant decreases in lumbar spine and femoral neck BMD ($P < 0.01$) at the end of the 5 years. Regression analysis revealed that in our cohort of women transitioning to menopause baseline femoral neck BMD, changes in PAEE and trunk fat explained 31% of the individual variation of absolute BMD changes at the femoral neck, while changes in both PAEE and trunk fat accounted for 27% of the variance of BMD change at lumbar spine. Daily dietary calcium and vitamin D intake did not show any significant correlation with BMD changes through the 5 years. **Conclusion:** Our results suggest that baseline femoral neck and changes in physical activity energy expenditure and trunk fat are determinants of the reduction of bone mineral density observed in our cohort of women transitioning to menopause.

Keywords: Menopausal transition, bone mineral density, body composition, physical activity, dietary calcium and vitamin D.

2. INTRODUCTION

Transitioning to menopause is a progressive process that starts with reproductive-aged women with regular, cyclic, predictable menses that are characteristic of ovulatory cycles, to a final menstrual period associated with ovarian senescence and menopause (1). Numerous studies have reported that normal pubertal development, body weight, body composition, body fat distribution, dietary calcium and vitamin D intake, physical activity levels and smoking influence bone mass throughout a woman's life (2-6). It is also known that a woman's hormonal profile is one of the major determinants of bone mineral density (BMD) (5, 7, 8). Studies have shown that estrogen directly stimulates bone formation, while estrogen deficiency can result in bone loss (5, 9). Since menopause represents an important perturbation of endocrine and metabolic processes, it has been suggested to have a strong impact on bone mass (10, 11).

In a healthy individual, there is a balance between osteoblast and osteoclast activity such that the amount of bone being lost is compensated by the amount of bone being produced (9). Both osteoclasts and osteoblasts activity are influenced by estrogen plasma levels. At menopause, ovarian estrogen levels decrease by 85-90% in comparison to premenopausal state (12). This hormonal change partly explains the shift in bone mass balance that occurs at menopause (8). It has been suggested that perimenopause and early postmenopause are periods of high bone turnover as a result of progressive ovarian failure (13). Therefore, women naturally undergo a phase of rapid bone loss (4.3 mg/cm^2 per year (-3.2%)) (14) that begins approximately 2-3 years before the cessation of menstruation up to 5 years after menopause (15-17). The influence of body composition (fat mass (FM) and fat free mass (FFM)), body fat distribution, especially abdominal fat (18), weight-bearing physical activities and dietary calcium and vitamin D intakes on BMD have been investigated in pre, peri and postmenopausal women (3, 19). Some

studies suggested that FFM is the strongest determinant of both total and segmental BMD in premenopausal women (13, 17, 20-23). FM and body fat distribution (visceral and peripheral) are the strongest determinants of BMD in both perimenopausal and postmenopausal women (24). Also, positive associations between dietary calcium and vitamin D intakes have been reported with bone mass (4, 20, 25) and BMD (19, 26). However, much of the scientific evidence is mainly based on cross-sectional studies (21, 27, 28) with some focusing on postmenopausal women (5, 15). To our knowledge, only 2 longitudinal study designs following women throughout the menopause transition are currently available (13, 19). Therefore, this could partly explain the discrepancies observed between BMD determinants among these studies. This observation also highlights the need to perform additional observational longitudinal studies to identify the determinants of BMD change in women transitioning to menopause.

Thus, the objectives of the present study were first, to document the changes in BMD and the yearly rate of bone loss at the lumbar spine and femoral neck in women transitioning to menopause. Second, to identify the anthropometric and lifestyle determinants of changes in BMD at lumbar spine and femoral neck in women transitioning to menopause. We hypothesized that: 1) the rate of bone loss is increase following menopausal years, and that 2) the changes in body composition, physical activity energy expenditure (PAEE), dietary calcium and vitamin D intake are the strongest determinants of BMD changes in women transitioning to menopause.

3. METHODS

3.1 Participants

The study included 102 healthy premenopausal women participating in one of the Montreal-Ottawa New Emerging Team (MONET) group studies, which comprised a 5-year observational longitudinal study (2004 to 2009) on the effects of menopausal transition on body composition, energy balance and cardiometabolic risk factors. Participants were recruited using community advertising and referrals from the Ob/Gyn clinics. Premenopausal women were included if they met the following criteria: 1) premenopausal status (two menstruations in the last 3 months, no increase in cycle irregularity in the 12 months before testing, and a plasma follicular-stimulating hormone level < 30 IU/L as a mean of verification), 2) aged between 47 and 55 years; this age range was selected to maximize the likelihood of women becoming postmenopausal by the end of the study, 3) no surgically induced menopause, 4) non-smoker, 5) BMI between 20 and 29.9 kg/m², and 6) reported weight stability (± 2 kg) for 6 months or more before enrollment in the study. Exclusion criteria were 1) pregnancy or having plans to become pregnant, 2) medical problems that could have interfered with outcome variables including cardiovascular and/or metabolic diseases, 3) taking oral contraceptives or hormone therapy, 4) high risk for hysterectomy, and 5) history of drug and/or alcohol abuse.

As described by Abdunour *et al.* (29), of the 314 called received, 102 women were found eligible. Among them, 11 dropped out of the study for personal reasons. Consequently, a total of 91 women completed the 5-year study. Since not all women had completed BMD measurements, 84 participants with completed data set were included in this secondary analysis. This study

received approval from the University of Ottawa and the Montfort Hospital ethics committees, and written consent was obtained from each participant.

3.2 Anthropometric assessment

Body weight and height were measured with a BWB-800AS digital scale and a Tanita HR-100 height rod (Tanita Corporation of America, Inc, Arlington Heights, IL), respectively, while participants were wearing a hospital gown. Waist circumference was measured with a flexible measuring tape at the midpoint between the last floating rib and the upper part of the iliac crest. An average of 2 measurements was taken. Body composition indices, lumbar spine and femur neck BMD were measured using dual-energy X-ray absorptiometry (DXA; GE-LUNAR Prodigy module; GE Medical Systems, Madison, WI.). Coefficient of variation and correlation for percentage of body fat (% BF) measured in 12 healthy subjects tested in our laboratory were 1.8% and $r = 0.99$, respectively. Meanwhile in our laboratory the coefficient of variation for measuring BMD by DXA was <1% using PHANTOM.

3.3 Physical activity energy expenditure assessment

Assessment of physical activity was performed using multidirectional accelerometry units (Actical; Mini Mitter Co, Inc, Bend, OR), which have been shown to be reliable (30). The accelerometer measurements were used to estimate mean daily PAEE. Participants put on the accelerometer upon waking up and took it off just before going to bed. Accelerometry and dietary data were collected simultaneously for 7 consecutive days. Such duration was chosen because it resulted in 90% reliability for PAEE measurement in both males and females (31). The accelerometer was worn on the right hip (anterior to the iliac crest), secured with an elastic

belt with the arrow pointing up, because that placement was the best predictor of energy expenditure ($r = 0.92 - 0.97$) (32).

3.4 Daily dietary calcium, vitamin D intake assessment

Daily energy and macronutrient intakes were assessed with a food diary. Subjects were asked to record the type and amount of food and beverages consumed for 7 consecutive days. Participants received oral and written instructions on recording their food intake. They were asked to be as specific as possible in their description by indicating all main ingredients and the quantity, the brand of products, and the cooking method. Participants were also asked to bring food labels, when possible, to facilitate the analysis of the food diary.. Data were carefully verified on the return of the food diary to obtain forgotten data or to correct misreported data. The food diaries were analyzed with FOOD PROCESSOR SQL software version 10.8 including Canadian nutrient file 2007; ESHA Research, Salem, OR). Participants were also asked to report if they were taking calcium and/or vitamin D supplements but we could not include them in our analysis because the information about the exact amount and type of supplements were not enough precise to be analyzed.

3.5 Statistical analysis

Results are presented as the mean \pm standard deviation. Repeated Measure ANOVAs were used to determine the main effects of variables of interest, with time (year 1 to 5) as a within-subject factor and menopause status (premenopause, perimenopause and postmenopause) as a between-subject factor. Because only 2 women still premenopausal at the end of the study, we combined them with perimenopausal women for repeated measures analysis. ANCOVA were performed to further explore the effect of menopausal transition on BMD. Therefore, the

database was transformed into cases. For premenopausal status, year 1 values were selected for all participants (n= 84); for perimenopausal status, the last year values during which the participant was in perimenopause were selected (n= 81); and for the postmenopausal status, the 5th year values were selected (n = 56) and we adjusted variables for age. Change of a variable was obtained by subtracting year 1BMD value from year 5 ($\Delta = \text{year 5} - \text{year 1}$). In order to calculate the yearly rate of bone loss for menopausal status, we determined the difference in BMD between the first and last year spent in the specific menopausal status for every participant, then divided it by the number of years spent in that menopausal period and then performed an ANOVA. For example, if a participant spent 3 years in premenopause, the equation was $((\text{BMD in year 3} - \text{BMD in year 1}) / 3) = \text{gm/cm}^2/\text{year}$ for premenopause and 2 years in perimenopause $((\text{BMD in year 5} - \text{BMD in year 3}) / 2) = \text{gm/cm}^2/\text{year}$ for perimenopause. Pearson correlations were used to determine the associations between the absolute and/or changes in BMD and dependent variables. Stepwise multiple regression analyses were used to identify independent determinants of change in BMD at lumbar spine and femoral neck. A P value ≤ 0.05 was considered as significant. Statistical analyses were performed using SPSS 17.0 for windows (SPSS Inc. Chicago, Illinois, USA).

4. RESULTS

4.1 Characteristics of the participants

Characteristics of the 84 participants included in the current secondary analysis are presented in Table 1. At baseline, women were all premenopausal, non-obese (based on BMI) and had normal BMD at lumbar spine and femoral neck. There were only 6 % of our participants who met the Canadian Guideline (33) recommendations for daily calcium intake (1000mg/day), while none of our participants met the recommendations for daily vitamin D intake (600 IU/day) throughout the 5-year study. The average PAEE varied between 472 and 1683 Kcal/day during the study. By the end of year-5, 2 (2.0%) women were still premenopausal, 26 (31.0%) were perimenopausal and 56 (67.0%) had become postmenopausal.

4.2 Menopausal status and bone mineral density over time

A repeated-measure analysis of variance was performed to determine absolute changes of BMD at lumbar spine and femoral neck throughout the 5 years (Table 2). The results showed a significant effect of time on the lumbar spine and femoral neck BMD ($P \leq 0.01$), showing an overall decrease throughout the 5 years, while menopausal status did not show any significant effect at both sites. However, a significant interaction was observed between time and menopausal status for BMD at both lumbar spine and femur neck ($P < 0.01$).

To further analyze the effect of menopausal status on BMD, we generated menopausal status cases (see methods). We used the BMD value of the first year for premenopausal status ($n = 84$ cases), BMD value of the last year in which the participant was in perimenopause status ($n = 81$ cases); and the BMD value of the 5th year for postmenopausal status ($n = 56$ cases) and we

performed an ANCOVA adjusted for age. The results showed no significant effect of menopausal status on both lumbar spine and femur neck BMD (results not shown).

One-way ANOVA was performed to determine the effect of the menopausal status on the yearly rate of BMD loss. The results showed no significant difference in yearly BMD loss at both site between women in perimenopausal (N= 63 cases) and postmenopausal status (N=36 cases). However, the lumbar spine yearly rate of BMD loss was significantly higher in both perimenopausal and postmenopausal compared to premenopausal women (N=33 cases), whereas at the femoral neck, only the postmenopausal women presented a significant higher yearly rate of BMD loss than the premenopausal women (Figure 1).

4.3 Associations between independent variables and bone mineral density

Person's correlations performed between the independent variables of interest and BMD revealed that BW ($0.22 \geq r \leq 0.32$; $0.05 > P < 0.01$) and FFM ($0.33 \geq r \leq 0.42$; $P < 0.01$) were the only two independent variables that were positively associated with lumbar spine and femoral neck BMD each year, from year 1 to year 5, whereas PAEE ($r = 0.32$; $P < 0.01$) was positively associated with femoral neck BMD only at year 2. Also, the 5 years mean PAEE did not show significant correlation with BMD at either bone sites (data not shown).

Persons' correlations were conducted to explore the associations between absolute changes (Δ) of the independent variables of interest and BMD changes at lumbar spine and femoral neck throughout the 5 years. Because we observed significant negative correlations between baseline age and Δ lumbar spine BMD ($r = -0.30$; $P < 0.01$), and between baseline BMD and Δ femoral neck BMD ($r = -0.27$; $P < 0.05$), we adjusted lumbar spine Δ BMD for age and femoral neck Δ BMD for baseline BMD. Following these adjustments, Δ total PAEE was positively correlated

with Δ BMD at lumbar spine ($r = 0.33$; $P < 0.05$) and femoral neck ($r = 0.41$; $P < 0.01$). Change in moderate PAEE positively correlated with Δ Lumbar spine BMD ($r = 0.29$; $p < 0.05$) mean while change in mild PAEE is positively correlated with Δ Femoral BMD ($r = 0.30 < 0.05$). Change in trunk fat showed positive correlations with Δ BMD at lumbar spine ($r = 0.34$; $P < 0.05$) and femoral neck ($r = 0.25$; $P < 0.01$). Changes in BW, BMI, %FM also showed significant positive correlations with Δ BMD at lumbar spine ($0.29 \geq r \leq 0.35$; $P < 0.05$) whereas changes in FFM, peripheral fat, daily dietary calcium and vitamin D intake were not significantly correlated with Δ BMD at lumbar spine or femoral neck (Table 3).

4.5 Determinants of changes in lumbar spine and femoral neck bone mineral density

Multiple regression analyses were conducted to identify the anthropometric and lifestyle determinants of BMD absolute changes in women from premenopause to postmenopause (Table 4). A stepwise multiple regression analyses were performed with age, baseline and changes of independent variables that were significantly correlated with lumbar spine and/or femoral neck BMD changes. As a result, Δ PAEE and Δ trunk fat together explained 27% of the individual variance of BMD changes at lumbar spine ($P < 0.001$), while Δ PAEE, baseline femoral neck BMD, and Δ trunk fat explained 31% ($p < 0.001$) of the individual variance of BMD changes at femoral neck.

5. DISCUSSION

Our results show a significantly higher yearly rate of BMD loss at the lumbar spine during perimenopause and following menopausal years and an increased femoral neck BMD loss after menopause. These findings are in line with a number of studies which suggested that the process of bone loss starts to accelerate during perimenopause and reaches its maximum 8-10 months after menopause (12, 13, 16, 28). Clarke et al. (37) reported that the obvious decrease in BMD in postmenopausal women is not only secondary to the increase in the rate of bone loss, but also due to the marked reduction of bone formation. On the other hand, our results are in contradiction with other studies suggesting that perimenopausal women have the highest rate of bone loss due the correspondent hormonal and body composition changes observed (increase FM and/or decrease FFM) during that period (13, 35, 38). Moreover, in the present study, menopausal status per se, as opposed to time, was not an independent factor to explain the bone loss during the 5-year follow-up. This observation could be partly explained by the characteristics of our participants; we included only non-obese (based on BMI) healthy premenopausal women and among whom only 40 % and 50% of participants progressed to an early phase of perimenopause (10-19 months) and postmenopause (≤ 10 months), respectively by the end of the study.

The main objective of the present observational longitudinal study was to identify the determinants of BMD changes during the transition to menopause. As expected, the change in PAEE was the strongest determinant of BMD changes at both lumbar spine and femoral neck sites. First, the influence of the increased PAEE to decrease BMD loss could be partly explained through the mechanical stress generated by muscle contraction (muscle power) on bone surfaces leading to stimulation of bone formation (5, 21, 36, 39). Second, the weight bearing effect of

daily habitual activities such as stair climbing and walking are effective in maintain bone mass and attenuate bone loss (36). Meanwhile, women who increased their mild and moderate physical activity living, show less reduction in BMD at femoral neck and lumbar spine respectively. On the other hand, women who increased their sedentary living increased their BMD loss at both lumbar spine and femoral neck. These findings re-emphasize the importance of increasing physical activity to minimize BMD loss in women transitioning to menopause. Unfortunately, we did not document the type of the physical activity performed in our cohort.

Baseline BMD is shown to be a determinant of femoral neck BMD change in our study. We observed a negative correlation between baseline femoral BMD and the change in BMD at femoral neck. This means that, a greater BMD at baseline is associated with a greater loss in BMD at femoral neck, which contradicts previous studies that have reported that building a healthy BM before menopause is associated with a reduced bone loss during the menopause years (19, 35, 38). However, the reason for this observation to our knowledge is unknown and further studies are needed to confirm these results and investigate the underlying mechanisms of this finding.

Surprisingly, we observed that change in trunk fat, independently of the change in FM, was one of the determinants of the individual variation of BMD change at lumbar spine and femoral neck. This finding suggests that an increase in trunk fat may attenuate the reduction in BMD during the menopause transition. This observation could be due to the weight bearing effect of a higher trunk fat mass on bone mass by increasing muscle-mediated skeletal dynamic load on the lumbar spine as well as on the femoral neck. Also, subcutaneous fat mass has an independent hormonal action on BMD metabolism by increasing the plasma levels of extra glandular estrogens through the conversion of androgens to estrogens as a result of activation of aromatase

enzyme in the adipose cell, a process that seems to be higher in postmenopausal women and increasing with aging (24). Furthermore, in the original MONET study, Abdunour J, et al. (29) observed an increase in visceral fat after the third year of the study using computed tomography measurement. This increase in visceral fat could explain the biochemical non-weight bearing influence of trunk fat on BMD change. In fact, some studies reported significant inverse correlations between plasma levels of adipokines such as adiponectin, which is produced mainly by visceral fat, and lumbar spine and femoral neck BMD (41, 42). Regulation of bone formation by adiponectin has been studied in animal models (43). They reported that adiponectin could regulate bone cells through 3 mechanisms; 1) the positive action of the autocrine/paracrine pathway by locally produced adiponectin; 2) the direct endocrine negative effect of circulating adiponectin by inhibiting osteoblasts activity; 3) the indirect positive action of adiponectin on enhancing the insulin signaling effect on bone formation. In regard to the latest mechanism, we did not observe significant changes in fasting insulin levels and insulin sensitivity (HOMA score) in our sample (data not shown). Considering the fact that adiponectin is negatively correlated with obesity in general and central adiposity in particular (44, 45), we speculate that the increase in trunk fat could be associated with a reduction of the adiponectin plasma levels which could result in an attenuation of the reduction of BMD at the lumbar spine and femoral neck. However, other researchers did not report association between adiponectin and changes in BMD (46). Also, studies have reported an association between plasma levels of leptin, which is produced mainly by subcutaneous fat, and BMD suggesting that leptin may play a role by enhancing bone formation and/or decreasing bone loss through stimulating osteoblasts and inhibiting osteoclasts activity through central and peripheral mechanisms (47, 48). However,

some other studies still have found no evidence to support the relationship between plasma leptin levels and BMD changes (41, 49).

Although studies (4, 17, 52, 53) reported that daily calcium and vitamin D intake influence BMD, our results do not show any associations between the absolute values and/or changes in daily dietary calcium and vitamin D intake and absolute or changes in BMD in line with some studies (24, 52). This conflicting finding may be due to the fact that only 6% of our participants met the Canadian Guideline recommendations (33) for dietary calcium intake (1000 mg/day) and none of them met for dietary vitamin D (15 mcg/day) during the 5 years of the current study. In addition, we may have underestimated the calcium and/or vitamin D intake in a percentage of our participants (19 women = 22 %) who had reported taking calcium and vitamin D supplements during the 5-years of the study, but failed to specify dosage and frequency. Also, differences in population characteristics, bone sites measured and methods used for assessment of dietary calcium and vitamin D intake may contribute to this discrepancy.

Our study presents some limitations. First, the study is observational and the population studied was composed of healthy non-smoking, women with a BMI of 23.4 ± 2.2 kg/m², thus our findings are limited to this subgroup of the population. Second, beside the measurement of the plasma follicular-stimulating hormone (FSH) level at baseline to verify the menopausal status, we did not measure other plasma sex hormones, leptin, adiponectine or makers of bone metabolism. Third, serum 25-hydroxyvitamin D was not measured in our participants and calcium and vitamin D supplements were not included in our analysis. Despite these limitations, the present study enriches the scientific evidence because of its 5-years longitudinal nature by the follow-up of a well-characterized cohort of premenopausal women throughout menopausal

transition. We used gold standard measures methods (DXA and CT scan) for the measurement of bone mineral density and body composition (54). For dietary calcium and vitamin D intake we used (a 7 day food diary) which is considered accurate measurement of long-term habitual dietary intake (55). Also, our assessment of physical activity energy expenditure was performed by accelerometers, which have been shown to be reliable (30, 56).

In summary, our results support the previous observations that bone loss accelerates during perimenopause and following the menopausal years. In our cohort, absolute changes in physical activity energy expenditure and trunk fat and baseline femoral neck BMD explained 31% of the individual variation of change in femoral neck BMD while changes in physical activity energy expenditure and trunk fat explains 27% of the individual variation of change in lumbar spine BMD. Consequently, these results support the recommendation that women should be encouraged to increase their physical activity energy expenditure during the menopause transition period. Further longitudinal studies are needed to specify the type of physical activity required to maintain bone mass and also to confirm the role and the underlying biochemical mechanisms to explain the reported association between trunk fat and bone mineral density.

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Table 1. Baseline characteristics of the participants (N = 84)

Variable	Mean \pm SD	Range
Age (years)	49.9 \pm 2.0	47-55
Body weight (kg)	61.3 \pm 6.6	46.8-78.7
Height (cm)	161.8 \pm 6.4	150.0-180.5
Body mass index (kg/m ²)	23.4 \pm 2.2	19.3-28.8
Waist circumference (cm)	78.5 \pm 7.0	62-94
Fat mass (kg)	19.4 \pm 5.0	9.6-29.9
Fat free mass (kg)	38.9 \pm 4.0	31.1-50.1
Trunk fat (kg)	9.4 \pm 3.0	3.3-18.3
Peripheral fat (kg)	10.0 \pm 2.5	5.0-15.5
% Body fat	31.6 \pm 6.0	18.2-41.7
Vitamin D (mcg/day)*	4.1 \pm 2.8	0.12-13.9
Calcium (g/day)*	901.3 \pm 273.9	397.3-1623.2
PAEE (kcal/day)*	817.5 \pm 262.6	326.3-1904.7
Lumbar spine BMD (g/cm ²)	1.19 \pm 0.13	0.80-1.50
Femoral neck BMD (g/cm ²)	0.95 \pm 0.11	0.70- 1.21

*Number (N) for calcium and vitamin D = 81 and for physical activity energy expenditure (PAEE) = 74.

Table 2. Bone mineral density (BMD) at lumbar spine and femoral neck by time point and menopausal status at year 5.

BMD (g/cm ²)	N	Perimenopause*		N	Postmenopause		ANOVA P value	Repeated measures	
		Baseline	Year 5		Baseline	Year 5		Interaction	Time
Lumbar spine	27	1.18±0.11	1.15±0.11	55	1.20±0.15	1.12±0.15	<0.01	<0.01	NS
Femoral neck	28	0.95±0.10	0.92±0.10	56	0.95±0.11	0.90±0.11	<0.01	<0.01	NS

Values are mean ± standard deviation. ; N: number of participants.*(included 2 premenopausal women).

Table 3. Pearson’s correlations between absolute changes of independent variables of interest and absolute changes in bone mineral density (BMD) at lumbar spine and femoral neck after adjusting for baseline age and femoral neck BMD.

Changes in	Lumbar Spine BMD	Femoral neck BMD
Height	-0.24	-0.17
Weight	0.31*	0.17
Body mass index	0.35*	0.19
Trunk fat	0.34*	0.25*
Peripheral fat	0.20	0.03
% Fat Mass	0.29*	0.20
Fat-free mass	-0.06	-0.11
Calcium	-0.08	0.05
Vitamin D	-0.09	-0.02
Total PAEE	0.33*	0.41**
Sedentary PAEE	-0.19	0.25
Mild PAEE	0.05	0.30*
Moderate PAEE	0.29*	0.26
Vigorous PAEE	0.09	0.19

PAEE: Physical activity energy expenditure ** P < 0.01, * P < 0.05.

Table 4. Determinants of 5-years absolute changes (Δ) in bone mineral density (BMD) at the lumbar spine and femoral neck using stepwise regressions analysis (N=60).

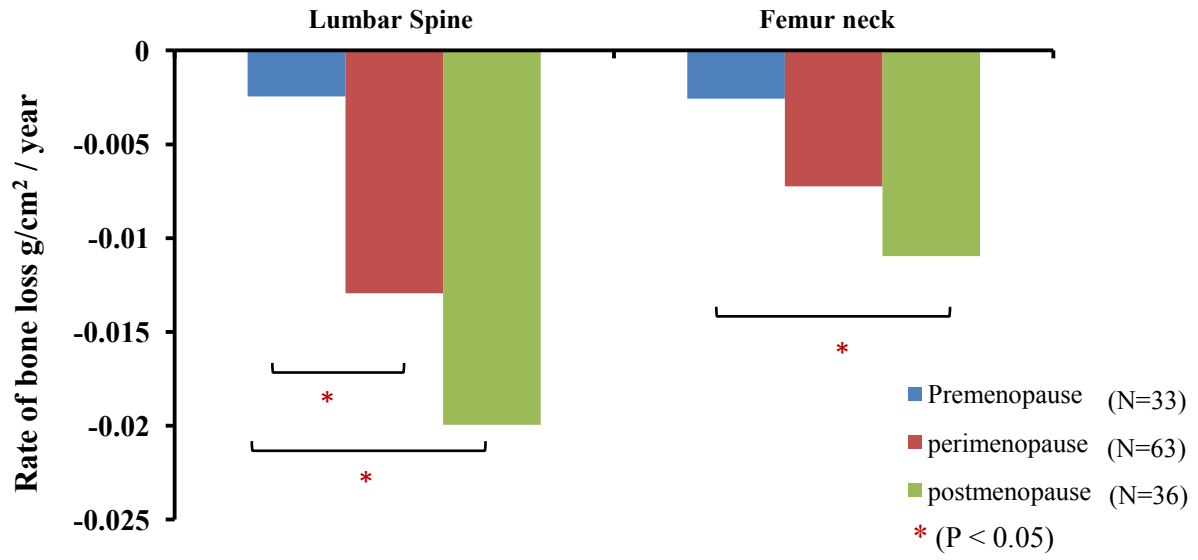
Dependent variables	Independent variables	R ² Change	P value	Total R ²
Δ Lumbar spine BMD	Δ Physical activity energy expenditure	0.157	0.002	27 %
	Δ Trunk fat	0.109	0.000	
Δ Femoral neck BMD	Δ Physical activity energy expenditure	0.116	0.001	31%
	Baseline femoral neck BMD	0.116	0.008	
	Δ Trunk fat	0.080	0.000	

Variables included in models: Lumbar spine BMD change ; Femoral neck BMD change; Baseline femur ;BMD; age change; Trunk fat change; Peripheral fat change; Physical activity energy expenditure change.

Figure legends

Figure1. Yearly rate of bone mineral density loss at the lumbar spine and femoral neck based on women menopausal status

Figure 1



CHAPTER 5

Conclusion and perspectives

Altogether, the literature reveals that women BM is influenced by hormonal fluctuations throughout her lifespan. Other factors have been reported to affect BMD changes such as body composition, body fat distribution, physical activity and dietary elements (1) . Since menopause represents a critical endocrine and metabolic period in women's life, it has been suggested to have a strong impact on bone mass (8). Studies have shown that estrogens directly stimulate bone formation, while estrogen deficiency can result in bone loss (2, 4). The influence of physical activities and the role of both FFM and FM on BMD were also investigated (12, 28). The association between dietary Ca and vit. D intake, BM and BMD is documented in many studies (55, 118). To our knowledge, most of scientific evidences are mainly based on cross-sectional studies and had focused on either premenopausal or postmenopausal BMD (18, 47). Few longitudinal studies that follow women throughout the menopause transition (19, 28). Also, the influence of important hormonal changes during the perimenopausal period is obviously neglected with scanty investigations available at the present time on the effect of this specific hormonal transition period on BMD.

Our results suggest that the process of bone loss increases at perimenopause and following menopausal years. In our cohort, absolute change in PAEE and change in trunk fat and baseline femoral BMD explain 31 % of the individual variation of change in BMD at the femoral neck while change in PAEE and change in trunk fat explains 27% of the individual variations in BMD change at the lumbar spine. Furthermore, our results show that women who increased their mild and moderate physical activity , show less reduction in BMD at the femoral neck and lumbar spine respectively, while women who increased their sedentary behavior show more bone loss at

both lumbar spine and femoral neck sites. Consequently, we can suggest that encouraging women to increase their daily physical activity energy expenditure could represent a relevant non-pharmacotherapy way to attenuate the reduction of BMD in women transitioning to menopause. Further longitudinal and long-term prospective studies involving larger numbers of premenopausal participants are needed to document the effect of premenopause, perimenopause and postmenopause on BMD as well as to document the effects of different interventions to maintain or reduce the BMD loss in women transitioning to menopause. Also further studies are needed to specify the type, intensity and levels of physical activity needed to maintain BMD during these critical menopausal periods. Also, studies are needed to confirm the role and the underlying biochemical mechanisms to explain the reported association between trunk fat and bone mineral density.

Candidate contribution

This study is a secondary data analysis of the original MONET study. Most of the data were previously collected as part of the MONET Study. For the purpose of the current study, I participated in all the post data collection process, including data entry and verification of the data base. I analysed, entered and verified participant yearly 7-day food journal for the 5 year study. I performed the statistical analysis and wrote the drafts as well as reviewing the final version of the main paper included in the thesis.

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