Peak Perimenopause Bone Mineral Density Predicts Incident Fracture—population-based, Canada-wide data from the Canadian Multicentre Osteoporosis Study (CaM*os*)

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Short Title: ***Peak Perimenopause BMD and* CaM*os Menopausal Women's Incident Fracture Risk***

Population: A random national Canadian 9-centre sample of community dwelling women 35-58 in CaM*os*

Primary Objective:

To prospectively assess the incidence of fractures in relationship to areal bone mineral density (BMD) values at the L1-4 spine, Total Hip and Femoral Neck sites when women have attained Peak Perimenopausal BMD (PP-BMD) defined by new onset of night sweats in regular cycles or irregular cycles.

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Background Information and Scientific Rationale

Background Information

Fragility fractures occurring in older women are a recognized public health issue in many developed countries (1) and increasingly also in less developed ones. Improving our knowledge of the pathophysiology of osteoporosis is a key step in designing measures for fracture prevention, early osteoporosis diagnosis, and, if needed, appropriate treatment.

Two variables are considered crucial in osteoporosis and fracture risk in older women: the peak areal bone mineral density (BMD) achieved in puberty or young adulthood, and the rapid bone loss early in menopause (the life phase beginning one year after the final menstrual flow). Peak youth/premenopausal BMD is acquired during growth in the late teens for hip sites (2) and in the 30s for the lumbar spine BMD (2) based on prospective data from the Canadian Multicentre Osteoporosis Study (CaM*os)* Adult and Youth Cohorts. This ideal peak BMD is believed to be crucial for subsequent fracture risk in both men and women (3).

"Postmenopausal osteoporosis" is the way osteoporosis in older women is currently understood (4). This concept rests on the idea that rapid bone loss occurs during the first few menopausal years (although sometimes that inappropriately includes the perimenopausal first year after the final menstrual flow) (4, 5). Since it is known that dropping estradiol levels cause rapid bone loss (5), and that non-menstruation indicates "estrogen deficiency" for the majority of physicians, the assumption has been that it is the rapid bone loss occurring when cycles stop that accounts for (the majority of) women's risk for fractures during their menopausal years (sometimes called 'postmenopausal') (6-8). Likewise, during the menstruating years it is generally believed that estrogen is the most important factor in preserving premenopausal BMD (9, 10). Thus, menopausal "estrogen deficiency" is commonly understood to be the main cause of rapid bone loss and thus osteoporotic fractures in older women (4, 11-13).

However, significant evidence suggests that considerable bone loss may occur during the premenopausal years (14-17). Also, although poorly recognized, rapid bone loss occurs from the onset of skipped menstrual cycles in the Late Menopausal Transition and during the year after the final menstrual flow (Late Perimenopause) which are all still part of perimenopause (18-21). Strong evidence from prospective studies in premenopausal women with regular menstrual cycles in whom ovulatory characteristics have been documented by progesterone levels and or hormonally validated methods such as Quantitative Basal Temperature© (22, 23), show significant bone loss with subclinical ovulatory disturbances (14-16, 23). The duration of menstruating life (from 25-40+ years) and other reproductive characteristics such as infertility, androgen excess (24), starting during adolescence and likely duration of combined hormonal contraceptive (CHC) use (25) along with important lifestyle factors such as vitamin D intakes (26), dietary patterns (27), physical activity experiences (28), genetic background (29), family history of fracture (30) and medical risk factors such as Type 1 or 2 diabetes mellitus (T2DM) and other comorbidities (31) can all affect the risk of incident fragility fracture later in life (32, 33).

Peak bone mineral density is attained during early adulthood. Results from Ca*Mos,* a national sample of community dwelling women (from both the Adult and Youth Cohorts), showed that lumbar spine

peak BMD occurred at ages 33 to 40 years and total hip and femoral neck peak BMD occurred at ages 16 to 19 years (2). The universal assumption has been that women would maintain a stable BMD with probably insignificant bone loss before menopause (8). Therefore, if premenopausal bone mineral density is not maintained, BMD at the Perimenopausal Peak, as defined by the recent onset of night sweats in regular cycles and/or irregular cycles that typically begin 3-10 years before the onset of menopause (34), should influence subsequent (incident) rates of fracture. **Figure 1** shows our current concepts about the lifecycle of bone in women.

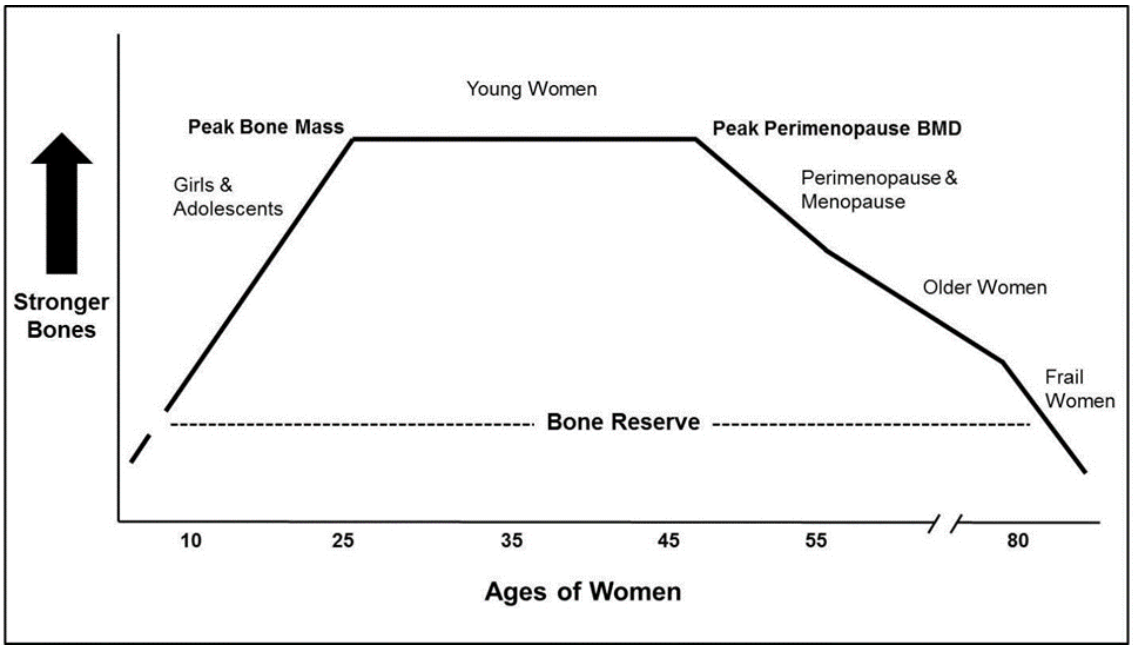


Figure 1: A theoretical model of BMD changes over the course of women's reproductive lifespan and its different phases with Peak Perimenopausal BMD being indicated by new night sweats in women with regular cycles or the onset of irregular flow. Fractures are at high risk below "bone reserve."

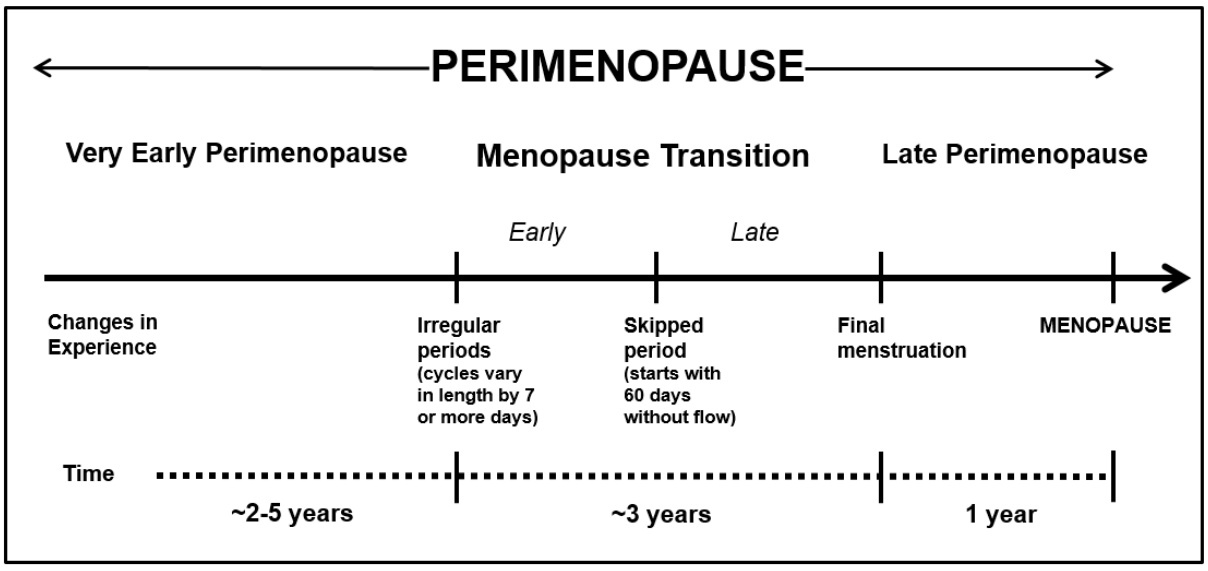
However, several reproductive events and factors affect BMD in premenopausal women (as previously discussed). For example, changes in menstrual cycle length (even within the normal range of 21-35 days) and subclinical ovulatory disturbances (within clinically normal cycles) resulting from underlying hormonal imbalances have an impact on rate of bone change and thus on bone health (32, 35-37). Amenorrhea (primary or secondary) can affect the adolescent peak bone mass and/or the rate of early post pubertal bone loss (38). An imbalance of estrogen to progesterone is present in subclinical ovulatory disturbances (occurring in regular cycles with sufficient estradiol but including short luteal phase cycles and anovulation providing insufficient progesterone). These can and do commonly occur within regular, normal-length cycles based on a population-based point prevalence study in Norway that documented serum estradiol and progesterone in over 3000 women with cycle-timed values (39). These silent ovulatory disturbances in 37% of the population (39) therefore could accelerate the rate of bone loss in apparently healthy, regularly cycling premenopausal women (14, 15, 40-43).

Not only does overt lowering of the reproductive hormones, estradiol and progesterone, occur with amenorrhea, but silent bone loss in ***regularly cycling*** women can and does occur in premenopausal women (18). Previous prospective data (from the population-based CaMos cohort) and a meta-

analysis have both shown that the most rapid BMD loss (prior to a very old/frail state) happens in the few years *before* a year has passed since the last flow (menopause) (18, 21). Ovulatory disturbances during premenopause, and the rapidly swinging (and thus downward estradiol excursions) as well as gradually decreasing progesterone levels and decreased ovulation frequency with eventually lower estrogen levels in later perimenopause will all increase the rate of BMD loss (14). Thus, assessing the relationship between BMD early in perimenopause (before rapid loss of BMD in later perimenopause (18, 21)) and the rate of incident fractures in the early menopause years will provide evidence, that is currently lacking, about the importance of other factors, especially premenopausal subclinical ovulatory disturbances, for risk of osteoporosis in older women and subsequent fracture.

We have been granted access to previously collected data from (Ca*Mos*) (44) by approval of an application to the Ca*Mos* Data, Analysis and Publication (DAP) committee and a proposal that resulted in an Osteoporosis Canada-Canadian Multicentre Osteoporosis Study fellowship (to AG, June 12, 2018).

Ca*Mos* was a longitudinal 9-centre Canada-wide cohort study of a population-based sample of 9,423 noninstitutionalized adult Canadian women (2/3) and men (1/3) ages 25-80+ who were followed for 20 years (*https://www.maelstrom-research.org/mica/individual-study/camos*, Accessed September 21, 2020*)* to ascertain the number of prevalent and incident fractures, obtain clinical measures of BMD and describe the distribution of proposed risk factors for osteoporosis" (25).



We will assess BMD in Very Early Perimenopause (45), in other words when cycles are still regular but hormone levels and experiences (such as night sweats, sleep problems) have changed (46); these women are selected based on still regular cycling but the onset of night sweats. Participants in this protocol may also be in the Early Menopause Transition with the start of irregular cycles (47). In neither case are women yet rapidly losing BMD (18, 48, 49). Access to this dataset will allow us to evaluate whether a lower Peak Perimenopausal BMD will be associated with a greater risk for incident fractures over the subsequent nine years (48).

Should we be able to confirm that there is a greater rate of incident (future) fracture in women with lower BMD levels at their BMD peak in early perimenopause (Peak Perimenopausal BMD, PP-BMD), it will aid in better understanding the impact of premenopausal women's risks for osteoporosis. We can then examine differences between women who, at Peak Perimenopause, have lower versus higher BMD to learn the *premenopausal variables* that are risk factors for sustaining menopausal osteoporotic fractures. If subclinical ovulatory disturbances are identified as a risk factor, we already know from randomized controlled trial data, that cyclic medroxyprogesterone (acting through the progesterone osteoblast receptor) and thus similar to thus ***Cyclic*** oral micronized ***Progesterone***, will effectively and safely prevent that bone loss (50). Thus, these data may stimulate development of tools to detect subclinical ovulatory disturbances in regular cycles and will, therefore, potentially aid in preventing premenopausal bone loss.

Understanding and preventing osteoporosis in pre-/perimenopausal women would be an important change from the current policy of most major osteoporosis scientific organizations, such as Osteoporosis Canada (OC). OC's focus is on secondary prevention of osteoporotic fracture (i.e. after adults have already sustained a fragility fracture) (https://osteoporosis.ca/health-care-professionals/clinical-practice-guidelines/osteoporosis-guidelines. Accessed May 25, 2020). However, identifying women at risk *before fracture occurrence* will allow primary prevention and thus will reduce the burden of disease and likely therefore improve disease-specific quality of life for those affected.

Premenopausal reproductive characteristics have not yet been well documented in relation to elevated rates of incident fracture in longitudinal population-based data. Most published studies that have reported the association of reproductive factors and BMD are cross-sectional (24, 25, 31) and relied on self-reported recall of these variables (51, 52). However, in CaM*os*, reproductive features for the adult cohort of women, in addition to BMD measurements and other important lifestyle variables, were collected prospectively and reliably at baseline, and intermittently (3-4 times) over 16+ years. These valuable data make the assessment of innovative reproductive/bone /fracture relationships possible. In addition, the large number of women (initially over 6,500) who have been followed and have had documentation/measurements on 3-4 occasions over years means that those who were originally premenopausal (the youngest age was 25) have been documented into perimenopause and/or menopause. This allows us to determine whether there are associations among reproductive factors while in premenopause or early in perimenopause, and in Peak Perimenopausal BMD values before menopause onset and subsequent fracture incidence. Since other risk factors for osteoporosis (diet, exercise, medications, variables related to anovulation and habits) are available in CaMos, we can adjust our results for these potential confounders.

Potential benefits and risks

This is a new analysis of available data. There is no risk to participants. All have signed informed consent to protocols approved by the McGill University ethics and site-specific ethics in each of the nine centres.

OBJECTIVES:

Objective 1:

Assess fracture incidence, degree of trauma and sites of non-vertebral and morphological vertebral fractures (assessed by standardized radiographs) over nine years following Peak Perimenopausal BMD (PP-BMD) values at each of the bone measurement sites (L1-4 lumbar spine [LS], femoral neck [FN] and total hip [TH]).

Objective 2:

In women with an available BMD value prior to the identified PP-BMD:

a) Determine the rate of BMD change between the first available CaMos BMD value (surrogate for Youth Peak Bone Mass-PBM) and the Peak Perimenopausal BMD for each eligible woman.
b) Determine whether this rate of premenopausal and early perimenopausal BMD change (mean/year) is associated with the 9-year following PP-BMD incident fracture rate.
c) Describe the variables associated with BMD change from PBM to PP-BMD.

Study Design

Research question

Do incident fractures early in menopause relate to the BMD value at the time of Peak Perimenopausal BMD?

Hypotheses

1. Women with lower BMD values at the time of "Peak Perimenopause" (PP-BMD) are at a greater risk of incident fractures after accounting for other important fracture-related variables.

2. Change in BMD between the first available CaMos BMD and the PP-BMD in perimenopausal women will relate to variables that are associated with disturbances in ovulation

a) Differences in the change in premenopausal BMD from youth peak BMD to PP-BMD will predict the risk for future incident fractures.

b) Reproductive factors such as adolescent CHC use, infertility and ovulatory disturbances will be associated with the rate of BMD change between the youth peak BMD and Peak Perimenopausal BMD

This is an *ad hoc* analysis of prospective population-based Canada-wide 9-centre Ca*Mos* data. Our **primary outcome** is post PP-BMD incident non-vertebral and morphological vertebral fractures. We will include fragility as well as higher trauma fractures anticipating small numbers; pathological fractures, i.e. related to focal Paget's disease or metastases, will be excluded. We will consider related to osteoporosis, fractures at all anatomical sites except those of the hands, feet, and

skull/face. We will include incident radiographic vertebral fractures assessed using the morphological criteria of modified ABQ as recently described (53).

We will be able to infer subclinical ovulatory disturbances if these premenopausal women report regular cycles and key other characteristics yet there is BMD loss from the first measured BMD to the Peak Perimenopausal BMD. Why? Because there is increasing animal (54) and human (55) clinical and experimental evidence that progesterone is women's bone formation-stimulating hormone. In a cohort study of healthy premenopausal women, 20% of cancellous volumetric spinal bone change (by quantitative computed tomography, QCT) over one year was accounted for by the mean luteal phase length (14). In DXA-assessed BMD change, we could assess ovulation and luteal phase lengths in several of our studies. In the prospective 2-year study of younger women (mean age 22) over 8% of BMD change in the hip region was related to subclinical ovulatory disturbances (23). In our meta-analysis of prospective premenopausal studies in women with documented menstrual cycles, ovulatory disturbances as well as spinal BMD change, the mean greater spinal BMD loss of -0.86% per year was documented in those with more than the median proportion of cycles having ovulatory disturbances versus fewer (15).

This study is an epidemiological prospective evaluation and there are only a certain number of women that meet our inclusion criteria and are in the Very Early Perimenopause (n=45) or the Early Menopause Transition (n=47), their peak perimenopause age, at CaM*os* years 5 or 10 examinations. It is thus inappropriate and as well as simply not possible to calculate a sample size for this exploratory study.

The **secondary outcomes** will be to examine pre-PP BMD change/year over the numbers of years for which we have premenopausal BMD change data for each woman at Peak Perimenopause. We will determine whether that rate of pre-PP-BMD change is related to risk of menopausal incident fractures and document the characteristics associated with the greatest premenopausal rate of bone loss at each of the three BMD measurement sites.

STUDY POPULATION

Selection of the study population:

We are granted access to previously collected data from **Ca*Mos*** by acceptance of our submitted and revised CaM*os* Data Analysis and Publication proposal (June 18, 2018).

CaM*os* (44) is a prospective cohort study of 9423 community-dwelling and randomly selected participants. The initial Adult CaM*os* cohort included women (n = 6539) and men (n = 2884), ≥25 year of age at baseline (mean approximate 63 years), living within 50 km of nine Canadian cities (St John's, Halifax, Quebec City, Toronto, Hamilton, Kingston, Saskatoon, Calgary, and Vancouver) that began cohort recruitment in 1995–1997 and continued for 16-21 years. Each of the nine centres enrolled an age-stratified (toward older ages) and sex-stratified (2 women:1 man) sample of about 1000 people. (Note-although most centres have now closed given our lack of success in obtaining further CIHR funding, the Vancouver Centre is still following over 400 participants with an annual questionnaire and fracture documentation).

Baseline CaM*os* data collection included an extensive interviewer-administered questionnaire and clinical assessments. The questionnaire included socio-demographic information, medical and

fracture history, family history, reproductive history, dietary intake, physical activity, cigarette and alcohol use, and quality of life determinations. Clinical assessments included height, weight, and BMD by DXA in all, plus lateral spine radiographs in those aged ≥50 years. This radiograph spanned the spinal anatomy from T4 to L4; from these data we made assessments of subclinical morphological vertebral fractures (that involved disruption of the superior, inferior or medial cortex) (56).

Each of the examinations at Year-5 and Year-10 included an interviewer-administered questionnaire and clinical assessment of height, weight, BMD, and lateral vertebral radiographs.

Information regarding fracture was gathered annually in a mailed questionnaire and was then confirmed through a structured telephone interview, which included in-depth questions regarding the fracture site and the circumstances leading to fracture. The majority of fractures were ultimately verified by radiographic or medical report data (78% of all self-reported fractures) (57).

Inclusion/Exclusion Criteria

Women were first evaluated for inclusion at their Year 5 follow-up. If they did not meet the inclusion criteria, we then evaluated them for inclusion at their Year 10 follow-up.

At the time of the interview (Y5 or Y10), **included** women satisfied the following criteria:

- Aged 35-57

AND

- non-menopausal

AND

- ≥2 years before menopause

AND

- Had new onset of new experience of night sweats in regular cycles or irregular menses.

We **excluded** women satisfying the following criteria:

- Bilateral oophorectomy
  OR
- Ever used of antiresorptive bone medications (such as bisphosphonates) or parathyroid hormone
  OR
- Ever used of pharmacological dose oral or parenteral steroids (glucocorticoid medications), (but not excluding those using inhaled steroids as for asthma)
  OR
- Ever use of ovarian hormone therapy (estrogen alone or estrogen/progesterone-progestin),
  OR
- Being pregnant in the year before PP-BMD

Type of information that will be gathered:

We will extract demographic, clinical, reproductive data along with BMD and all incident fracture information.

Overall duration of the project: N/A

Privacy of participants:

Data are coded and anonymized. We do not have access to any personal identifiers in the data files created for this analysis.

LABORATORY EVALUATIONS:

Not applicable.

STATISTICAL CONSIDERATIONS

Study outcome measures:

- Incident fracture: incident fractures for the nine years after PP-BMD measurement will be assessed for a relationship with PP-BMD with adjustment for confounding variables including comorbidities.

- Premenopausal bone change per year: BMD change will be defined as the difference between PP-BMD and the first available CaMos BMD, divided by the number of days elapsed between both DXA assessments to obtain an annual change for which negative values would indicate a decrease over time. We will consider BMD at the LS, TH and FN.

- **PP-BMD** will be chosen as the BMD closest to:
  Ages ≥35-57 years at the Year 5 or Year 10 CaM*os* examination plus the following:
  1. New experience of night sweats in regular cycles, and/or
  2. New onset irregular cycles

The incidence of fractures will be presented as a crude value, plus as adjusted for all other important influencing factors such as comorbidities, medications, reproductive variables, lifestyle, nutrition, and family history of fracture.

Co-Variables for consideration in regression related to ovulatory disturbances

The clinical experiences associated in the literature with anovulatory cycles and included in the CaM*os* questionnaire will be used. The co-variable considered will include:

- BMI at age 18 (≥24 is related to anovulation) (58)
- Age at menarche (Late age at menarche (≥16 years) occurred in 5.1% in CaM*os* premenopausal women's data (24))
- History of hormone-related infertility (that occurred in 2.7% of CaM*os* premenopausal women (20) and includes chronic or recurrent short luteal phases or anovulation. We are excluding tubal obstruction or male-factor related infertility (59))
- Weight cycling of ≥10 pounds (yes/no) (60)
- Cycle lengths of <24 days (61) and oligomenorrhea (62) [>35] days (since both are associated with anovulation) (61).

- Cognitive dietary restraint—we have 3 selected questions in the CaM*os* database that are most reflective of the entire Three Factor Restraint Score (63). We will use the top tertile of the CaM*os* eating attitudes questions to indicate cognitive dietary restraint which is strongly associated with ovulatory disturbances (64).

Participant enrolment and follow up:

We are not recruiting new participants. Available data from previously enrolled participants will be used.

Enrollment for the main study was through random selection of households (based on postal codes within 50 km of the middle of each of the nine CaM*os* research centres obtained using residential phone numbers); participants were then randomly selected within households by a sex- and age-stratified protocol(44). Ethics approval was obtained from participating centres' ethics review boards—the local BC Clinical Research Ethics Board approval is #H96-70123.

Written informed consent was obtained from all participants who were enrolled in the study. We chose a sub-sample of CaM*os* women with follow-up clinical, BMD and fracture data who were aged 35-57 at Years 5 and 10.

ANALYSIS PLAN:

Objective 1:

a) Descriptive statistics at the time of Peak Perimenopausal BMD measurement. The time of PP_BMD is either in Year 5 or 10.

b) Calculation of all fragility and higher trauma vertebral (by radiographs with morphological assessment) and non-vertebral fracture incidence rate during the available up to 9 year follow up after PP-BMD.
I have deleted the Cox regression model because it was not included in objective 1 written above.

Objective 2:

a) Descriptive statistics at baseline (at their enrolment interview and clinical examination): including BMD, demographic, reproductive (including variables related to anovulation) and clinical characteristics

b) Calculation of BMD change per year from baseline to Peak Perimenopausal BMD at each of the three measurement sites: LS, TH, and FN

c) Linear regression analysis to assess the association of the potential risk factors with change in BMD from baseline to the time of Peak Perimenopause.

Subject Confidentiality

Data files generated for the purpose of this analysis do not contain any personal information such as name, date of birth, personal health number, address or other contact information.

The files are kept on UBC encrypted drives and only authorized research team members will have access to them. The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any

unauthorized third party. We do not seek to contact CaM*os* participants or access to CaM*os* paper records. Use of data by the PI and her team is restricted to the goals of this protocol.

Summary

Using unique Canadian Multicentre Osteoporosis Study (CaM*os*) nation-wide population-based prospective cohort data we are proposing, for the first time, to document a relationship between incident fractures and the areal bone mineral density when women are regularly menstruating with night sweats (Very Early Perimenopause) or have just begun to develop irregular cycles (Early Menopause Transition). We will thus assess BMD before the rapid perimenopausal bone loss that begins with skipped periods (Late Menopause Transition) or in the year after the last flow (Late Perimenopause) (21, 48). If we can show that PP-BMD predicts incident fractures, it will suggest that something other than rapid *(post)**menopausal bone loss*** is important for women's osteoporosis risk. If we are able to show BMD loss between the baseline and PP-BMD, and the women with the most BMD loss have regular cycles but more subclinical ovulatory disturbances (short luteal phases and anovulation-related variables), it will suggest that it is not premenopausal estrogen insufficiency but lack of adequate progesterone that is related to later life osteoporosis and to fractures that occur when women are early in menopause.

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