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Journal: The Journal of clinical endocrinology and metabolism

Year: 2016 Dec

Issue: 101

Volume: 12

Pages: 5004-5011

DOI: 10.1210/jc.2016-2695

The benefit of menopausal hormone therapy on bone density and microarchitecture persists after its withdrawal

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Abbreviated title: The benefit of MHT on bone, the OsteoLaus cohort

Key words: menopausal hormone treatment; bone mineral density; trabecular bone score; OsteoLaus

Word count (excluding abstract, figure captions, and references): 3113

Number of tables: 5 **Figures**: 1 **References**: 37

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DISCLOSURE STATEMENT: Didier Hans is co-owner of the TBS patent and has corresponding ownership shares. The corresponding author ensures that none of the other authors has any conflicts of interest.

FUNDING: This work did not require any external funding. No grant or fellowship supported the writing of this paper.

ABSTRACT

Context: Menopausal hormone therapy (MHT) favorably affects bone mineral density (BMD). Whether MHT also affects bone microarchitecture, as assessed by trabecular bone score (TBS), has never been evaluated.

Objective: To assess the effect of MHT on TBS and BMD before and after its withdrawal.

Design: Cross-sectional study.

Setting: General community.

Patients or other participants: Data from the OsteoLaus cohort (1500 women aged 50-80 years). After exclusion of women with bone-modulating treatments, 1279 women were categorized according to MHT status into current (CU), past (PU) and never (NU) users.

Intervention(s): none.

Main outcome measure(s): Spine TBS and BMD at lumbar spine, femoral neck and total hip were assessed by dual X-ray absorptiometry.

Results: Age- and BMI-adjusted analysis showed higher TBS values in CU vs. PU or NU (1.31±0.01, 1.29±0.01 and 1.27±0.01 respectively, $p<0.001$). All BMD values were significantly higher in CU vs. PU or NU. Compared to NU, PU exhibited higher lumbar spine (0.94±0.01 vs. 0.91±0.01 g/cm^2, $p=0.017$) and total hip (0.86±0.01 vs. 0.84±0.01 g/cm^2, $p=0.026$) BMD and a trend for higher TBS ($p=0.066$). The 10-year loss of TBS and BMD at lumbar spine and total hip was significantly lower for both CU and PU vs. NU. MHT duration had no effect on bone parameters. In PU, the residual effect on TBS and BMD was significantly more prominent in early discontinuers (< 2 years).

Conclusion: MHT is associated with bone microarchitecture preservation, as assessed by TBS. The effect of MHT on TBS and BMD persists at least 2 years after withdrawal.

Abstract word count: 250 words

PRECIS: A crossectional analysis of the OsteoLaus cohort showed that MHT is associated with enhanced bone density and microarchitecture and the benefits persist for at least 2 years after its withdrawal.

INTRODUCTION:

Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, resulting in increased bone fragility and susceptibility to fracture (1). Bone mineral density (BMD), measured by dual X-ray absorptiometry (DXA), is a major determinant of bone strength and fracture risk (2). Nevertheless, half of fragility fractures occur in individuals with BMD values in the osteopenic or even normal range (3), highlighting the role of other factors on bone strength, like bone microarchitecture. A noninvasive assessment of the latter has been proposed by the use of trabecular bone score (TBS). TBS is a textural index that evaluates pixel grey-level variations in the lumbar spine DXA image, providing an indirect index of trabecular microarchitecture (4). Multiple case-control studies, prospective trials (5, 6) as well as a meta-analysis (7) have shown that TBS predicts fracture risk in postmenopausal women, independently of clinical risk factors, BMD and FRAX® tool. In 2015, TBS was added in the FRAX® tool to evaluate the 10-year fracture risk.

Menopausal hormone therapy (MHT) was for many years a first-line therapy in the prevention of postmenopausal osteoporosis. This practice was supported by observational data. The Women's Health Initiative (WHI) randomized trial was the first study to prove that MHT reduces the incidence of all osteoporosis-related fractures in postmenopausal women, even those at low risk of fracture (8, 9). The trial concluded, however, that the bone benefits are outweighed by other adverse events, particularly an increase in breast cancer, coronary heart disease and strokes in the estrogen/progestin arm and an excess of strokes in the estrogen-only arm. These conclusions led to a diminishing clinical use of estrogen (10), regulatory bodies downgrading MHT to second-line therapeutic choice for postmenopausal osteoporosis.

However, controversy persists about the validity of these conclusions (11). Subgroup analysis of women aged 50-59 years at entry in the WHI trial showed favorable long-term outcomes for cardiovascular disease and global index of health (12, 13). Promising evidence for protection against bone loss has been reported with lower doses of oral estrogen or transdermal administration (14). The latter causes probably less adverse vascular events (15). As a result, the latest guidelines re-establish MHT as a first-line treatment for the prevention of fracture in at-risk women before age of 60 years or within 10 years after menopause without any mandatory time limit for the duration of treatment (16). The initiation of MHT after the age of 60 years remains not recommended due to the risk of long-term complications and the existence of alternatives medications with a better safety profile.

Though the positive effect of MHT on BMD is well established, there are no available data on the direct influence of MHT on TBS. A small retrospective cohort study assessed the longitudinal changes of BMD and TBS in two different states of estrogen deprivation: natural menopause and aromatase inhibitor treatment (17). After a follow up of 2-3 years, TBS declined in both group but to a lesser extent than BMD.

Given the renewed interest for MHT, we aimed to explore the effect of MHT on TBS and BMD before and after its withdrawal using data from a large cross-sectional, population-based study on osteoporosis in women conducted in Lausanne, Switzerland.

MATERIAL AND METHODS:

Setting: Data from the OsteoLaus study were used (18). OsteoLaus is a sub-study of the CoLaus study, an ongoing prospective study aiming to assess the determinants of cardiovascular disease using a population-based sample drawn from the city of Lausanne, Switzerland (19). Between September 2009 and September 2012, all women aged between 50 and 80 years from the CoLaus study were invited to participate in the OsteoLaus study and 85% accepted. The OsteoLaus study was approved by the Institutional Ethics Committee of the University of Lausanne. All participants signed an informed consent.

At baseline, each patient had: 1. A questionnaire on potential clinical risk factors for fracture/osteoporosis (including Swiss FRAX® assessment) and on conditions affecting bone metabolism; 2. Determination of the type, dose and duration of MHT; 3. A spine (L1 to L4) and femur DXA scan using the Discovery A System (Hologic, USA); 4. A blind central processing of TBS (TBS iNsight v2.1, medimaps, France) based on a previously acquired antero-posterior spine DXA scan; 5. A vertebral fracture assessment (VFA) by two experimented clinicians using the semiquantitative approach of HK. Genant (20).

Participants: 1500 women were included in the OsteoLaus cohort. 58 women were excluded from our analysis due to non-interpretable lumbar spine images (extreme BMI defined as <15 Kg/m^2 or >37 Kg/m^2, severe spine deformations, osteosynthesis material, less than two evaluable vertebrae). 137 women were excluded for current or past osteoporotic treatment other than MHT and 23 for current or past exposure to aromatase inhibitors or tamoxifen. Three others cases were excluded because of congenital hyperostosis, androgen treatment and transsexuality.

According to MHT status, the remaining participants (n=1279) were divided into 3 groups: current (CU), past (PU) and never users (NU). CU were on MHT at trial entry or discontinued MHT since less than 6 months (otherwise considered as PU). CU had followed MHT for at least 6 months (otherwise considered as NU). PU followed MHT for at least 6 months (otherwise considered as NU) and discontinued MHT at least 6 months before trial entry (otherwise considered as CU).

Variables: BMD at lumbar spine, femoral neck and total hip, as well as spine TBS were defined as the primary outcomes of the analysis. Age and BMI were considered as major confounders and effect modifiers. Other variables of interest were prevalence of vertebral fractures in VFA (defined as ≥ 1 fracture of grade 2/3 and/or ≥ 2 fractures of grade 1), history of fragility fractures (defined as low-trauma fractures, symptomatic or asymptomatically discovered on VFA), history of major osteoporotic fractures (defined as fragility fractures in vertebrae, hip, proximal humerus, distal forearm and pelvis), use of supplements (defined as current or past use of calcium and/or vitamin D). The 10-year fracture risk was calculated with the Swiss FRAX® tool.

Dietary calcium intake was assessed using a validated, semi-quantitative food frequency questionnaire (FFQ), which also includes portion size (21). This FFQ has been validated in the Geneva population (22).

Plasma 25-hydroxyvitamin D levels were available for the majority of the participants (n=1204, NU=582, PU=359, CU=263) from the second CoLaus visit that took place within 6 months before the OsteoLaus visit. Plasma 25-hydroxyvitamin D level was measured using an ultra-high pressure liquid chromatography-tandem mass spectrometry system (23). The inter-day CV% was 4.6% at 40 nmol/L.

Statistical analysis: Statistical analyses were conducted using Stata v14.1 (StataCorp, College Station, Texas, USA) for windows. Descriptive results were expressed as number of participants (percentage) or as average ± standard deviation. Bivariate analyses were conducted using chi-square for categorical variables and analysis of variance for continuous variables. Multivariate analyses for continuous variables were conducted using analysis of variance or multiple regression; results were expressed either as adjusted average ± standard error or as slope and (95% confidence interval). Post-hoc pairwise comparisons were performed using the method of Scheffe. The association between bone outcomes and time since MHT discontinuation was modeled by nonlinear regression in order to identify possible hinges in the relationship. For each bone parameter, the hinge and the slopes before and after the hinge (when present) were estimated and their 95% confidence levels were assessed by bootstrap with 100 replications. Statistical significance was considered for a bivariate test with a p-value <0.05.

RESULTS:

Of the 1279 women included in the analysis, 282 (22%) were CU, 380 (30%) were PU and 617 (48%) were NU. The vast majority of participants were of Caucasian ethnicity (1256/1279, 98.2%). Their baseline characteristics according to MHT group are shown in **Table 1**. PU were significantly older, had a higher prevalence of fractures, took more frequently calcium and vitamin D supplements and had a higher 10-year fracture risk. The latter was no longer present after adjustment for age. No differences were found for body mass index and dietary intake of calcium. There was a significant

difference regarding plasma 25-hydroxyvitamin D levels (CU>PU>NU), which however remained slight with an average difference of 5.5 nmol/l between CU and NU.

The results for the primary bone outcomes according to MHT group, both unadjusted and after adjustment for age and BMI are shown in **Table 2**. CU had consistently higher values of TBS and BMD at all sites compared with NU and PU in both unadjusted and adjusted models. After adjustment for age and BMI, PU showed higher lumbar spine and total hip BMD than NU (*p=0.017* and *p=0.026* respectively). A trend in favor of PU versus NU was noted for TBS values (*p=0.066*).

The effect of MHT was also assessed by a multivariate analysis with age as an independent variable (**Table 3**). The adjusted slopes for 10-year increments showed a decrease of all sites BMD and TBS loss according to MHT status: CU<PU<NU ($p<0.05$ for trend). **Figure 1** provides a graphic representation of BMI-adjusted slopes for association of BMD and TBS with age, according to MHT group. CU exhibited significantly less steep slopes for loss of TBS as well as BMD at all sites. BMI-adjusted slopes for PU were significantly less steep than the ones for NU, with the exception of the BMD at femoral neck.

Table 4 shows the bone parameters according to MHT duration and time since MHT withdrawal. No association was found between MHT duration and bone outcomes in the combined CU+ PU group. In PU, all BMD and TBS values were significantly higher when the time since discontinuation was less than 2 years in comparison to more than 5 years. A multivariate regression analysis using time since MHT discontinuation as a continuous variable led to similar conclusions, whereas a hinge analysis (**Table 5**) allowed the identification of an inflexion point between 2 and 4 years since MHT discontinuation, beyond which the benefit of MHT on bone outcomes has disappeared. No statistically significant difference was noted between the different bone outcomes (TBS, BMD at different sites) in terms of inflexion points.

DISCUSSION:

To maximize anti-fracture efficacy, agents against osteoporosis should ideally have an effect on both bone mass and bone microarchitecture. TBS has emerged as a non-invasive, easily-acquired and

reliable indirect indicator of bone microarchitecture, providing an additional surrogate marker for fracture risk assessment (4). The effect of anti-osteoporotic drugs on TBS has been shown to be smaller in magnitude than the one on BMD (4, 24). In a substudy of the randomized TEAM trial, Kalder *et al* explored the effect of exemestane and tamoxifen on BMD and TBS in postmenopausal women with hormone-sensitive breast cancer (25). After 2 years, TBS increased by 3.3% in the tamoxifen group compared to a decrease of 2.3% in the exemestane group. The positive impact of tamoxifen on TBS could suggest a potential advantage related with its estrogenic agonist properties.

This crossectional analysis of the OsteoLaus cohort demonstrates for the first time that MHT is associated with higher levels of TBS. CU presented significantly higher TBS values than NU and PU. After adjustment for age and BMI, a trend for higher TBS values in PU versus NU was observed. In the multivariate analysis, slopes for 10-year increments were significantly less steep in both CU and PU, indicating that MHT slows down the age-associated loss of TBS. Interestingly, the slopes for age-associated decline in TBS were significantly more pronounced than those in spine BMD, a finding which was attributed to TBS being less influenced by age-induced osteoarthritic changes (4), which falsely elevate spine BMD.

The only clinical study that has analyzed the effect of MHT on bone microarchitecture is the Kronos Early Estrogen Prevention Study (26). In a subset of this trial (MHT n=45 vs. placebo n=30), bone microarchitecture was assessed by high-resolution peripheral quantitative computed tomography at the distal radius. After 4 years of follow-up, MHT prevented the decrease of cortical volumetric BMD, as well as the increase of cortical porosity at distal radius. Nevertheless, the degradation of trabecular microarchitecture at the distal radius was not halted by MHT. No trabecular assessment of the lumbar spine was performed in the Kronos study, thus not allowing for a direct comparison with our data. It is possible that the beneficial effect of MHT on trabecular bone is not identical at all skeletal sites.

Our results confirm that current MHT use is associated with higher BMD values at all relevant sites, a finding consistent with previous randomized trials as well as a meta-analysis (27). The

postmenopausal estrogen/progestin intervention (PEPI) trial investigated the effect of MHT in women in the early menopausal phase. After 3 years, those assigned to MHT regimens had an average gain of 5.1% and 2.4% at spine and total hip BMD respectively, compared to a loss of 2.8 and 2.2% in the placebo group (28). In the relatively older population of the WHI trial (29), after 3 years of follow-up, the percentage difference in favor of the MHT group was 4.5% and 3.6% for BMD at lumbar spine and total hip respectively. MHT was effective despite the absence of osteoporosis at baseline (mean T-scores: -1.3 at lumbar spine, -0.94 at total hip).

If the beneficial effect of MHT on BMD is well established, this is not the case for the BMD preservation after its withdrawal. Our data argue in favor of a partially persistent effect in PU, who showed higher BMD values in the age-adjusted analysis, as well as a less rapid decrease of age-associated BMD loss at lumbar spine and total hip compared to NU. Further analysis confirmed that time since MHT withdrawal is a crucial factor, with early discontinuers (< 2 years) presenting with significantly higher BMD levels than the late ones (> 5 years). The inflexion point, beyond which the BMD benefit disappears, is estimated between 2-4 years.

Conflicting data exist about an eventual rebound effect after MHT withdrawal resulting in rapid loss of the previously acquired benefit. Several non-randomized studies (30, 31, 32) did not find any accelerated bone loss after MHT discontinuation. In the PERF study (30), the dosage of bone remodeling markers did not show increased bone resorption following MHT withdrawal. However, in a randomized placebo-controlled trial, Greenspal *et al* (33) revealed significant loss of BMD (4.5%, 2.4% and 1.8% at lumbar spine, femoral neck and total hip respectively) 1 year after estrogen withdrawal in comparison to alendronate discontinuation in 425 hysterectomized women, previously treated with these agents versus placebo for 2 years. The loss of BMD observed in the estrogen group was associated with an increase of bone resorption markers. Similar results were described in other trials of recently menopaused (34, 35) or older women (36). In the latter study, there were no significant BMD differences between PU and placebo group already 2 years after estrogen withdrawal.

Possible explanations for these divergent results can be hypothesized. In the PERF trial, the authors reported heterogeneous rates of bone loss with faster decrease of BMD in women with lower BMI. Trémollieres et al (35) also detected large variations among MHT withdrawers. The rate of bone loss correlated with age and vertebral BMD at the time of MHT cessation but not with BMI. Differences regarding MHT type and dose between different studies, as well as the non-assessment of 25-hydroxyvitamin D status in some trials constitute additional factors possibly contributing to the contradictory results. Due to our large sample covering the whole age spectrum of post-menopausal women, we consider our results to be less dependent on individual variables, thus being reliable for generalization to clinical practice. In our cohort, the mean BMI was in the slightly overweight range without differences between groups. Plasma 25-hydroxyvitamin D levels differed slightly but remained higher than 50 nmol/l (= 20 µg/l) in all groups. Relatively high BMI and adequate plasma 25-hydroxyvitamin D levels may have lessened excessive bone resorption post MHT withdrawal, in contrast to other studies.

Another interesting point is the between-sites discrepancy with absence of residual effect on femoral neck, whose content is rich in cortical bone. This finding has not been reproduced in previous studies and should be considered cautiously until further evaluation, given that estrogen-deprivation states have been preferentially linked to loss of trabecular bone.

BMD and TBS are surrogate markers of osteoporotic fracture risk. Consequently, given our results in favor of a residual effect in PU, we would expect decreased fracture incidence in this group in comparison with NU. The recently published NICE guidelines (37) on the effect of MHT recency and duration on fracture incidence support this hypothesis. Indeed, after an exhaustive meta-analysis and based mostly on observational trials, the authors concluded that bone benefits of MHT seem to persist after its withdrawal; however they have vanished by 5 years since MHT discontinuation. Data derived from randomized controlled trials showed no effect of MHT duration on fracture risk, whereas some observational studies suggested additional benefits only for MHT duration longer than 10 years.

In agreement with these conclusions, we did not detect any BMD differences according to MHT

duration in the combined CU+PU group. The most plausible explanation is the accelerated gain of bone density at all sites during the first 2 years of treatment, seen with MHT (29) and also described for other antiresorptives. The increase of bone density slows down during the next years of treatment with a subsequent flattening of the changes, thus constituting the overall duration of MHT a less decisive factor on bone outcomes.

Our study has several limitations. First, the beginning and the end of MHT were self-reported. Second, for the same reason we could not reliably distinguish the different routes of administration of MHT (oral, transdermal, vaginal) or the type of MHT (estrogen-alone or estrogen/progestin). Third, we could not determine the dose of estrogen per participant, which can be important given the potentially dose-dependent effects on bone outcomes. On the other hand, our study has many strong points to be taken into account. The large sample of the OsteoLaus cohort allows for adequate statistical power to detect differences between groups. Another strength is the quality of the data collected in the cohorts CoLaus and OsteoLaus. Radiological tests were performed in high quality and standardized devices allowing for accurate measurement of BMD and TBS.

In conclusion, we report herein for the first time that MHT is associated with better preservation of bone microarchitecture, as assessed by TBS. In addition to the well-established positive effect of MHT on BMD, the bone microarchitecture preservation probably contributes to the anti-fracture efficacy. The protective effect of MHT on BMD and TBS seems to persist after its withdrawal for at least 2 years. Given the renewed interest in MHT, our results provide encouraging informations for the impact of this treatment and its withdrawal on bone health.

REFERENCES:

1. Consensus development conference. Prophylaxis and treatment of osteoporosis. *Am J Med* 1991; 90: 107-10.
2. Johnell O, Kanis J a, Oden A, et al. Predictive Value of BMD for Hip and Other Fractures. *J Bone Miner Res*. 2005;20(7):1185-1194. doi:10.1359/JBMR.050304.
3. Siris ES, Brenneman SK, Barrett-Connor E, et al. The effect of age and bone mineral density on the absolute, excess, and relative risk of fracture in postmenopausal women aged 50-99: Results from the National Osteoporosis Risk Assessment (NORA). *Osteoporos Int*. 2006. doi:10.1007/s00198-005-0027-4.
4. Silva BC, Leslie WD, Resch H, et al. Trabecular bone score: A noninvasive analytical method based upon the DXA image. *J Bone Miner Res*. 2014;29(3):518-530. doi:10.1002/jbmr.2176.
5. Hans D, Goertzen AL, Krieg M-A, Leslie WD. Bone microarchitecture assessed by TBS predicts osteoporotic fractures independent of bone density: The manitoba study. *J Bone Miner Res*. 2011;26(11):2762-2769. doi:10.1002/jbmr.499.
6. Leslie WD, Johansson H, Kanis JA, et al. Lumbar spine texture enhances 10-year fracture probability assessment. *Osteoporos Int*. 2014;25(9):2271-2277. doi:10.1007/s00198-014-2761-y.
7. Mccloskey E V., Odén A, Harvey NC, et al. A Meta-Analysis of Trabecular Bone Score in Fracture Risk Prediction and Its Relationship to FRAX. *J Bone Miner Res*. 2015;xx(xx):1-9. doi:10.1002/jbmr.2734.
8. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA*. 2002;288(3):321-333. doi:10.1001/jama.288.3.321.
9. Banks E, Beral V, Reeves GK, Balkwill A, Barnes I, Collaborators MWS. to the Pattern of Use of Hormone Therapy in Postmenopausal Women. *J Am Med Assoc*. 2004;291(18):2212-2220. doi:10.1001/jama.291.18.2212.
10. Parente L, Uyehara C, Larsen W, Whitcomb B, Farley J. Long-term impact of the women's health initiative on HRT. *Arch Gynecol Obstet*. 2008;277:219-224. doi:10.1007/s00404-007-0442-1.

11. de Villiers TJ, Stevenson JC. The WHI: the effect of hormone replacement therapy on fracture prevention. *Climacteric*. 2012;15(3):263-266. doi:10.3109/13697137.2012.659975.
12. Roehm E. A reappraisal of women's health initiative estrogen-alone trial: long-term outcomes in women 50–59 years of age. *Obstet Gynecol Int.* 2015; 2015:7132955.
13. Santen RJ, Allred DC, Ardoin SP, Archer DF, Boyd N, Braunstein GD, et al. Postmenopausal hormone therapy: an Endocrine Society scientific statement. *J Clin Endocrinol Metab*. 2010; 95:s1–s66. doi: 10.1210/jc.2009-2509
14. Ettinger B, Ensrud KE, Wallace R, et al. Effects of ultralow-dose transdermal estradiol on bone mineral density: a randomized clinical trial. *Obstet Gynecol*. 2004;104(3):443-451. doi:10.1097/01.AOG.0000137833.43248.79.
15. Mohammed K, Abu Dabrh AM, Benkhadra K, et al. Oral vs Transdermal Estrogen Therapy and Vascular Events: A Systematic Review and Meta-Analysis. *J Clin Endocrinol Metab*. 2015;100(11):4012-4020. doi:10.1210/jc.2015-2237.
16. Baber RJ, Panay N, Fenton A. 2016 IMS Recommendations on women's midlife health and menopause hormone therapy. *Climacteric*. 2016;19(2):109-150. doi:10.3109/13697137.2015.1129166.
17. Pedrazzoni M, Casola A, Verzicco I, Abbate B, Vescovini R, Sansoni P. Longitudinal changes of trabecular bone score after estrogen deprivation: Effect of menopause and aromatase inhibition. *J Endocrinol Invest*. 2014;37(9):871-874. doi:10.1007/s40618-014-0125-2.
18. Lamy O, Krieg M, Metzger M, Aubry-Rozier B, Stoll D, Hans D.. The OsteoLaus Cohort Study. *Osteologie*. 2012;21(2):77-82.
19. Firmann M, Mayor V, Marques Vidal P, Bochud M, Pécoud A, Hayoz D, Paccaud F, Preisig M, Song KS, Yuan X, et al. The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. *BMC Cardiovasc Disord.* 2008;8(1):6.
20. Genant HK, Li J, Wu CY, Shepherd JA. Vertebral fractures in osteoporosis: a new method for clinical assessment. *J Clin Densitom*. 2000;3(3):281-290. doi:10.1385/JCD:3:3:281.

21. Bernstein L, Huot I, Morabia A. Amélioration des performances d'un questionnaire alimentaire semi-quantitatif comparé à un rappel des 24 heures. *Santé Publique*. 1995;7:403-13.
22. Beer-Borst S, Costanza MC, Pechère-Bertschi A, Morabia A. Twelve-year trends and correlates of dietary salt intakes for the general adult population of Geneva, Switzerland. *Eur J Clin Nutr.* 2009;63:155-64.
23. Bruce SJ, Rochat B, Béguin A, et al. Analysis and quantification of vitamin D metabolites in serum by ultra-performance liquid chromatography coupled to tandem mass spectrometry and high-resolution mass spectrometry--a method comparison and validation. *Rapid Commun Mass Spectrom*. 2013;27(1):200-206. doi:10.1002/rcm.6439.
24. Popp AW, Guler S, Lamy O, et al. Effects of zoledronate versus placebo on spine bone mineral density and microarchitecture assessed by the trabecular bone score in postmenopausal women with osteoporosis: a three-year study. *J Bone Miner Res*. 2013;28(3):449-454. doi:10.1002/jbmr.1775.
25. Kalder M, Hans D, Kyvernitakis I, Lamy O, Bauer M, Hadji P. Effects of exemestane and tamoxifen treatment on bone texture analysis assessed by TBS in comparison with bone mineral density assessed by DXA in women with breast cancer. *J Clin Densitom*. 2014;17(1):66-71. doi:10.1016/j.jocd.2013.03.003
26. Farr JN, Khosla S, Miyabara Y, Miller VM, Kearns AE. Effects of estrogen with micronized progesterone on cortical and trabecular bone mass and microstructure in recently postmenopausal women. *J Clin Endocrinol Metab*. 2013;98(2):249-257. doi:10.1210/jc.2012-3406.
27. Wells G, Tugwell P, Shea B, et al. V. Meta-analysis of the efficacy of hormone replacement therapy in treating and preventing osteoporosis in postmenopausal women. *Endocr Rev*. 2002;23(4):529-539. doi:10.1210/er.2001-5002.
28. Writing group for the PEPI trial. Effects of hormone therapy on bone mineral density: results from the postmenopausal estrogen/progestin interventions (PEPI) trial. *JAMA.* 1996;276(17):1389-1396. doi:10.1001/jama.276.17.1389.
29. Cauley JA, Robbins J, Chen Z, et al. Effects of Estrogen Plus Progestin on Risk of Fracture and Bone Mineral Density. *JAMA.* 2003; 290(13):1729-1738. doi:10.1001/jama.290.13.1729.

30. Bagger YZ, Tankó LB, Alexandersen P, et al. Two to three years of hormone replacement treatment in healthy women have long-term preventive effects on bone mass and osteoporotic fractures: the PERF study. *Bone*. 2004;34(4):728-735. doi:10.1016/j.bone.2003.12.021.
31. Greendale GA, Espeland M, Slone S, et al. Bone Mass Response to Discontinuation of Long-term Hormone Replacement Therapy: Results From the Postmenopausal Estrogen/Progestin Interventions Safety Follow-up Study. *Arch Intern Med.* 2002; 162(6):665-672. doi:10.1001/archinte.162.6.665.
32. Middleton ET, Steel S a. The effects of short-term hormone replacement therapy on long-term bone mineral density. *Climacteric*. 2007;10(3):257-263. doi:10.1080/13697130701370435.
33. Greenspan SL, Emkey RD, Bone HG, et al. Significant differential effects of alendronate, estrogen, or combination therapy on the rate of bone loss after discontinuation of treatment of postmenopausal osteoporosis: A randomized, double-blind, placebo-controlled trial. *Ann Intern Med*. 2002;137(11):875-883. doi:200212030-00008.
34. Wasnich RD, Bagger YZ, Hosking DJ, et al. Changes in bone density and turnover after alendronate or estrogen withdrawal. *Menopause*. 2004;11(6):622-630. doi:10.1097/01.
35. Tremollieres FA, Pouilles JM, Ribot C. Withdrawal of hormone replacement therapy is associated with significant vertebral bone loss in postmenopausal women. *Osteoporos Int*. 2001;12(5):385-390. doi:10.1007/s001980170107
36. Christopher Gallagher J, Rapuri PB, Haynatzki G, Detter JR. Effect of discontinuation of estrogen, calcitriol, and the combination of both on bone density and bone markers. *J Clin Endocrinol Metab*. 2002;87(11):4914-4923. doi:10.1210/jc.2002-020727.
37. National Institute for Health and Care Excellence. Menopause: diagnosis and management of menopause. (NICE guideline 23) 2015. *www.nice.org.uk/guidance/ng23*

TABLES:

Table 1: Baseline characteristics of participants according to menopausal hormone therapy status

	Never (n=617)	**Past (n=380)**	**Current (n=282)**	**p-value**
Age (years)	62.1 ± 8.0	67.4 ± 6.2	64.0 ± 6.8	<0.001
BMI (kg/m^2)	25.9 ± 4.5	26.0 ± 4.2	25.3 ± 4.0	0.084
Fractures (%)				
VFA	32 (5.2)	30 (7.9)	6 (2.1)	0.005
All fragility	94 (15.2)	89 (23.4)	39 (13.8)	0.001
Major osteoporotic	69 (11.2)	58 (15.3)	17 (6.0)	0.001
Calcium intake				
Diet (mg)	954 ± 524	982 ± 506	1038 ± 571	0.102
Supplements (%)	215 (34.9)	212 (55.8)	105 (37.2)	<0.001
Plasma 25-hydroxyvitamin D (nmol/L)	51.1 ± 22.5	55.7 ± 23.1	56.6 ± 24.7	0.002
FRAX ®, 10 years risk	11.2 ± 7.1	13.4 ± 7.5	10.5 ± 5.6	<0.001

BMI, body mass index; BMD, body mineral density; TBS, trabecular bone score; VFA, vertebral fracture assessment. Results are expressed as number of participants (percentage) or as average ± standard deviation. Between groups analysis was performed by chi-square or analysis of variance.

385 **Table 2**: Bone mineral densities and trabecular bone score of the sample according to menopausal
386 hormone therapy status

	Never (n=617)	Past (n=380)	Current (n=282)
Unadjusted			
BMD lumbar spine	0.91 ± 0.15 [a]	0.94 ± 0.17 [b]	0.98 ± 0.15 [c]
TBS lumbar spine	1.28 ± 0.10 [a]	1.27 ± 0.10 [a]	1.31 ± 0.10 [b]
BMD femoral neck	0.73 ± 0.11 [a]	0.73 ± 0.10 [a]	0.76 ± 0.11 [b]
BMD total hip	0.85 ± 0.12 [a]	0.85 ± 0.11 [a]	0.89 ± 0.11 [b]
Age and BMI-adjusted			
BMD lumbar spine	0.91 ± 0.01 [a]	0.94 ± 0.01 [b]	0.98 ± 0.01 [c]
TBS lumbar spine	1.27 ± 0.01 [a]	1.29 ± 0.01 [a]	1.31 ± 0.01 [b]
BMD femoral neck	0.72 ± 0.01 [a]	0.73 ± 0.01 [a]	0.76 ± 0.01 [b]
BMD total hip	0.84 ± 0.01 [a]	0.86 ± 0.01 [b]	0.89 ± 0.01 [c]

387 BMD, bone mineral density; TBS, trabecular bone score. Results are expressed as adjusted mean ±
388 standard error. Between groups analysis were performed using analysis of variance. Post-hoc pairwise
389 comparisons using the method of Scheffe; values with different superscripts are significantly different
390 at $p<0.05$ for pairwise comparisons (a versus b, b versus c).

Table 3: Multivariate analysis of bone parameters according to menopausal hormone therapy status with age as an independent variable

				p-value for parallelism		
	Never (n=617)	**Past (n=380)**	**Current (n=282)**	**All**	**Current vs. never**	**Past vs. Never**
BMD LS						
Bivariate	-0.016 (-0.031; -0.001)	0.015 (-0.013; 0.043)	0.026 (-0.001; 0.052)	0.013	0.009	0.042
BMI-adjusted	-0.026 (-0.040; -0.011)	0.014 (-0.013; 0.041)	0.025 (-0.001; 0.050)	<0.001	0.001	0.008
TBS LS						
Bivariate	-0.054 (-0.063; -0.045)	-0.033 (-0.048; -0.017)	-0.022 (-0.039; -0.005)	0.002	0.001	0.024
BMI-adjusted	-0.051 (-0.060; -0.041)	-0.032 (-0.048; -0.017)	-0.022 (-0.038; -0.005)	0.005	0.003	0.048
BMD FN						
Bivariate	-0.031 (-0.042; -0.020)	-0.018 (-0.035; -0.001)	-0.009 (-0.028; 0.010)	0.099	0.044	0.212
BMI-adjusted	-0.038 (-0.048; -0.028)	-0.019 (-0.035; -0.002)	-0.010 (-0.027; 0.008)	0.011	0.006	0.055
BMD TH						
Bivariate	-0.039 (-0.051; -0.028)	-0.022 (-0.041; -0.004)	-0.011 (-0.029; 0.008)	0.025	0.011	0.115
BMI-adjusted	-0.048 (-0.059; -0.038)	-0.023 (-0.040; -0.006)	-0.011 (-0.028; 0.007)	<0.001	<0.001	0.012

BMI, body mass index; BMD, bone mineral density; TBS, trabecular bone score. LS, lumbar spine; FN, femoral neck; TH, total hip. Results are expressed as adjusted slope (95% confidence interval) for a ten-year increment. Statistical analysis by analysis of covariance.

Table 4: Bone parameters according to duration of menopausal hormone therapy and time since withdrawal

	BMD lumbar spine	TBS lumbar spine	BMD femoral neck	BMD total hip
Duration of MHT (years), n=644				
[0-2]	0.92 ± 0.02	1.27 ± 0.01	0.73 ± 0.01	0.85 ± 0.01
[2-5]	0.95 ± 0.02	1.28 ± 0.01	0.73 ± 0.01	0.87 ± 0.01
[5+]	0.94 ± 0.01	1.28 ± 0.01	0.72 ± 0.01	0.85 ± 0.01
P-value	0.485	0.640	0.672	0.326
P-value for trend	0.379	0.679	0.506	0.823
Time since withdrawal (years), n=357				
[0-2]	1.02 ± 0.03 [a]	1.33 ± 0.02 [a]	0.77 ± 0.02 [a]	0.90 ± 0.02 [a]
[2-5]	0.93 ± 0.02 [b]	1.28 ± 0.01 [a, b]	0.71 ± 0.01 [b]	0.85 ± 0.01 [a, b]
[5+]	0.93 ± 0.01 [b]	1.27 ± 0.01 [b]	0.72 ± 0.01 [b]	0.85 ± 0.01 [b]
P-value	0.007	0.002	0.007	0.009
P-value for trend	0.003	<0.001	0.005	0.002

MHT, menopausal hormone therapy; BMD, bone mineral density; TBS, trabecular bone score; Results are expressed as adjusted mean ± standard error. Information on duration of MHT was available for the majority of current (CU, n=278) and past (PU, n=366) MHT users, consisting a total of 644 participants. Information on time since MHT discontinuation was available for the majority (n=357) of PU. Statistical analysis was performed using an ANOVA model including age, body mass index, duration of menopausal hormonal therapy and time since discontinuation. Post-hoc pairwise comparisons using the method of Scheffe; values with different superscripts are significantly different at $p<0.05$ for pairwise comparisons (a versus b).

Table 5: Piecewise regression between bone parameters and time since discontinuation of menopausal hormone therapy in past users of menopausal hormone therapy

	BMD lumbar spine	**TBS lumbar spine**	**BMD femoral neck**	**BMD total hip**
Unadjusted				
Hinge (years)	3.486 (-1.540 ; 8.512)	2.750 (0.612 ; 4.887)*	2.250 (1.001 ; 3.499)***	2.405 (-0.171 ; 4.981)
Slope before hinge	-0.033 (-0.094 ; 0.028)	-0.032 (-0.060 ; -0.003)*	-0.061 (-0.135 ; 0.014)	-0.051 (-0.126 ; 0.024)
Slope after hinge	0.001 (-0.003 ; 0.005)	-0.002 (-0.004 ; 0.000)*	0.001 (-0.0004 ; 0.003)	0.000 (-0.002 ; 0.002)
Multivariate-adjusted §				
Hinge (years)	3.468 (0.324 ; 6.613)*	2.750 (0.071 ; 5.429)*	2.282 (1.007 ; 3.557)***	3.804 (1.628 ; 5.980)***
Slope before hinge	-0.032 (-0.089 ; 0.025)	-0.028 (-0.057 ; 0.001)	-0.054 (-0.113 ; 0.005)	-0.022 (-0.082 ; 0.037)
Slope after hinge	0.002 (-0.002 ; 0.006)	0.001 (-0.001 ; 0.003)	0.001 (-0.0004; 0.003)	0.002 (0.000 ; 0.003)

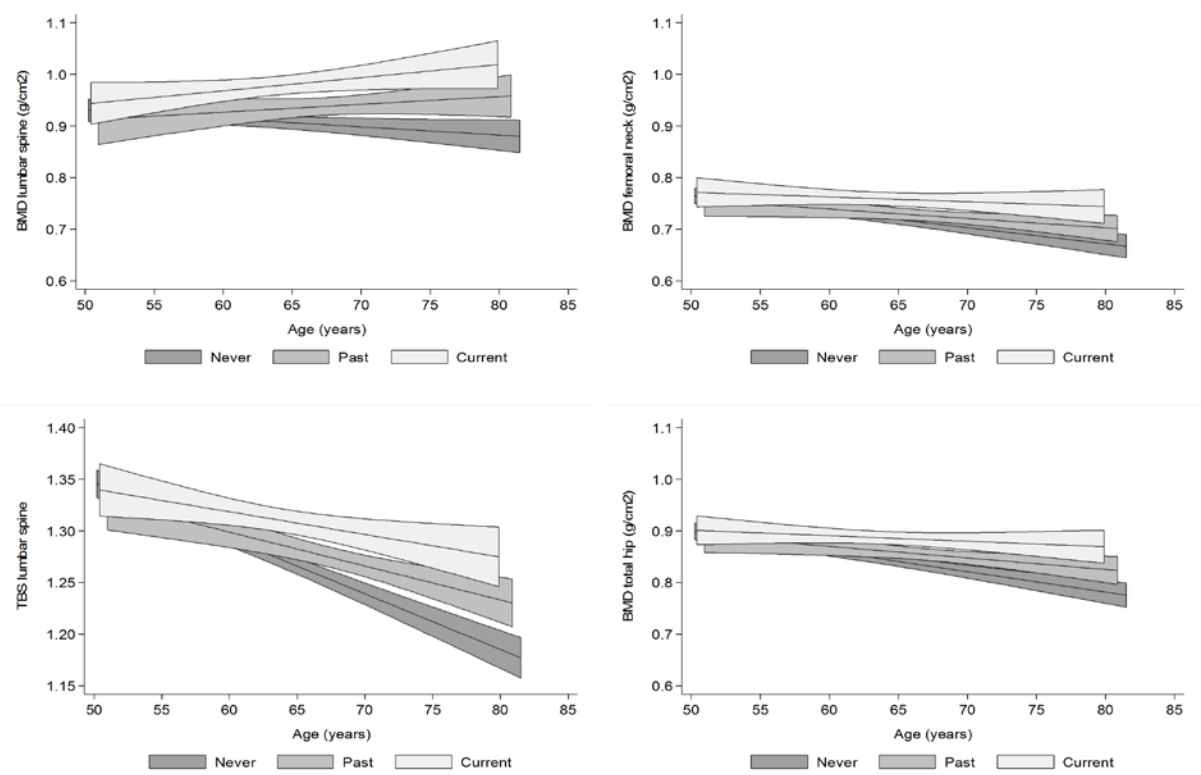
The analysis was conducted on past users of menopausal hormone therapy (MHT), for which information on time since MHT discontinuation was available **(n=357)**. BMD, bone mineral density; TBS, trabecular bone score; § adjusted on age, body mass index, and duration of menopausal hormone therapy. Non-linear regression performed on the residuals following multivariate linear regression of the different bone parameters on age, body mass index, and duration of menopausal hormone therapy.*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

411 ***LEGEND OF FIGURE 1*:**

412 Graphic representation of the body mass index-adjusted association and corresponding 95%
413 confidence interval of bone mineral density (BMD) and trabecular bone score (TBS) with age,
414 according to menopausal hormone therapy group: current users (CU) (*light grey*), past users (PU)
415 (*medium grey*) and never users (NU) (*dark grey*).

416

Figure 1. BMI-adjusted slopes for association of BMD and TBS with age, according to MHT group



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