Menopausal Hormone Therapy Is Associated With Reduced Total and Visceral Adiposity: The OsteoLaus Cohort

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Context: After menopause, fat mass (FM) and visceral adipose tissue (VAT) increase and nonbone lean body mass (LBM) decreases. Whether menopausal hormone therapy (MHT) reverses these changes remains controversial.

Objective: To assess the effect of MHT on FM, VAT, and LBM before and after its withdrawal and evaluate potential confounders.

Design: Cross-sectional study.

Setting: General community.

Patients or Other Participants: Women of the OsteoLaus cohort (50 to 80 years old) who underwent dual-energy X-ray absorptiometry (DXA) with body composition assessment. After we excluded women with estrogen-modifying medications, the 1053 participants were categorized into current users (CUs), past users (PUs), and never users (NUs) of MHT.

Intervention: None.

Main Outcome Measures: VAT measured by DXA was the primary outcome. We assessed subtotal and android FM, LBM, muscle strength (hand grip), and confounding factors (caloric intake, physical activity, biomarkers).

Results: The groups significantly differed in age, NU $<$ CU $<$ PU. Age-adjusted VAT was lower in CUs than NUs ($P = 0.03$). CUs exhibited lower age-adjusted body mass index (BMI) (-0.9 kg/m^2) and a trend for lower FM (-1.3 kg). The 10-year gain of VAT ($P < 0.01$) and subtotal and android FM ($P < 0.05$) was prevented in CUs. No difference in LBM or hand grip was detected. No residual effect was detected for PUs, including for early MHT discontinuers. The confounding factors did not significantly differ between groups except for higher caloric intake in PUs compared with NUs.

Conclusions: MHT is associated with significantly decreased VAT, BMI, and android FM. No benefit is detected for LBM. The benefits are not preserved in PUs, suggesting caution when MHT is discontinued. **(*J Clin Endocrinol Metab* 103: 1948–1957, 2018)**

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Abbreviations: ALM, appendicular lean mass; ALMI, appendicular lean mass index; BMI, body mass index; CU, current user; CV, coefficient of variation; DXA, dual-energy X-ray absorptiometry; E2, estradiol; ER, estrogen receptor; FFQ, Food Frequency Questionnaire; FM, fat mass; FMI, fat mass index; GnRH-Ag, gonadotropin-releasing hormone agonist; HOMA-IR, homeostatic model assessment of insulin resistance; LBM, lean body mass; LMI, lean mass index; MHT, menopausal hormone therapy; NU, never user; OVX, ovariectomy; PU, past user; ROI, region of interest; SAT, subcutaneous adipose tissue; SE, standard error; VAT, visceral adipose tissue; WHI, Women's Health Initiative.

Menopause is accompanied by changes in bone, fat, and muscular compartments (1, 2). In particular, menopause transition has been linked to increased propensity for weight gain and fat mass (FM) accumulation (3, 4). Whether this association is caused by declining ovarian hormone secretion or aging remains an open question (2). Data are more robust regarding the effect of menopause on regional fat. Several prospective studies have shown a greater increase of abdominal fat after menopause, leading to a shift from a gynoid to an android pattern of fat distribution (5–7). The causal association with estrogen deficiency is supported by preclinical data demonstrating that disruption of estradiol (E2) signaling by estrogen receptor (ER) deletion or ovariectomy (OVX) accelerates fat accumulation (8). It is important to emphasize that excess of central fat, and specifically of visceral adipose tissue (VAT) in humans, is associated with insulin resistance and high prevalence of metabolic syndrome, which are risk factors for atherosclerotic cardiovascular disease (9).

A decline in nonbone lean body mass (LBM), also called fat-free or skeletal muscle mass, has been described across menopause (3, 4). It remains unclear whether this finding is a consequence of estrogen deficiency or of indirect factors such as a more sedentary lifestyle (10).

Interventional trials assessing the effect of menopausal hormone therapy (MHT) on body composition have yielded mixed results regarding total FM and LBM (8). Those inconsistent findings can reflect differences in the population studied, study design (natural vs induced menopause), type of MHT, and method for assessing body composition. Conversely, most studies evaluating the effect of gonadotropin-releasing hormone agonists (GnRH-Ags), creating an artificial menopause state, have found increased total adiposity and intra-abdominal fat (8). Interestingly, the most recent one (11) showed that this phenotype could be prevented by estrogen administration.

Another point that remains unclear is whether the eventual impact of MHT on FM is the result of a direct effect on adipocytes or indirect mechanisms such as altered energy intake or energy expenditure (8) or behavioral effects on mood and anxiety (12), which in turn might affect food intake and physical activity. In addition, insulin and adipokines (leptin, adiponectin) have been suggested as potential modifiers in the crosstalk between the reproductive axis and energy homeostasis both centrally and peripherally (7, 13).

In this cross-sectional study, we assessed the effect of MHT on FM, VAT, and LBM before and after its withdrawal and attempted to explore potential confounders as detailed earlier.

Materials and Methods

Setting

We analyzed data from the OsteoLaus study (14). OsteoLaus is a substudy of the CoLaus study, an ongoing prospective study aiming to assess the determinants of cardiovascular disease by using a population-based sample drawn from the city of Lausanne, Switzerland (15). The aims of the OsteoLaus study are to compare different models of fracture risk prediction and to assess the relationship between osteoporosis and cardiovascular diseases. Recruitment of OsteoLaus participants was detailed previously (16). CoLaus data (second visit) were collected within 6 months before the OsteoLaus visit. The study was approved by the Institutional Ethics Committee of the University of Lausanne. All participants signed an informed consent.

Participants

A total of 1500 postmenopausal women, aged 50 to 80 years, were questioned on current or past MHT use, its type, and duration, if applicable. All participants underwent a spine and hip dual-energy X-ray absorptiometry (DXA) scan on a Discovery DXA System (Hologic, Inc., Marlborough, MA). We included in this study all the women for whom body composition assessment was performed during the DXA scan (n = 1086). Exclusion criteria were intake of medication with estrogen-mediated effects (aromatase inhibitors, tamoxifen, antiandrogens), extreme body mass index (BMI) values (BMI >37 kg/m^2), and uninterpretable or incomplete DXA scans (low-quality images). The remaining participants were divided into three groups: current users (CUs), past users (PUs), and never users (NUs) of MHT. CUs were taking MHT at trial entry or discontinued treatment <6 months earlier. PUs discontinued MHT ≥ 6 months before trial entry (otherwise considered as CUs). MHT use for <6 months, reported in 25 participants (<3 months in 23/25), was considered unlikely to cause considerable changes in body composition, and these subjects were classified as NUs.

DXA measurements

All body composition measurements were in accordance with published guidelines by the International Society for Clinical Densitometry (17). The subjects were placed in a supine position with palms down and arms at sides, slightly separated from the trunk, and correctly centered on the scanning field. Regions of interest (ROIs) were defined by the analytical program and included total body, trunk, head, pelvis, upper limbs, lower limbs, and android and gynoid regions. The lower boundary of the android region was defined at the pelvis cut line and the upper boundary above the pelvis cut line by 20% of the distance between the pelvis and chin. The upper boundary of the gynoid ROI was defined below the pelvis cut line by 1.5 times the height of the android space, and gynoid ROI height was equal to 2 times the android ROI height. For each region, DXA scanned weight of total mass, FM, and LBM. VAT was measured as the fat tissue located deep in the abdomen around the internal organs, as opposed to subcutaneous adipose tissue. Android LBM and FM, gynoid LBM and FM, and VAT were analyzed in a second step from the initial body composition images. For technical reasons, 87 examinations could not be reanalyzed, rendering analysis of the aforementioned parameters impossible in these participants.

Outcomes

Body composition

Body composition outcomes were VAT; subtotal FM (calculated by extracting head FM from total FM); android and gynoid FM; fat mass index (FMI), calculated as the ratio of total body FM over height squared; subtotal, android, and gynoid LBM, by analogy to FM; lean mass index (LMI), defined as the ratio of total LBM over height squared; and sarcopenia indices (18): appendicular lean mass index (ALMI), calculated as the ratio of appendicular lean mass (ALM) over height squared, and ALM divided by BMI.

Grip strength

Assessment of muscle strength *via* handgrip was available for 990 participants. Participants of the CoLaus aged >50 were invited to participate in a substudy on frailty, which included grip strength, assessed with a Baseline® hydraulic hand dynamometer (Fabrication Enterprises, Inc., White Plains, NY). Positioning of the participants was done according to the American Society of Hand Therapists guidelines (19): subject seated, shoulders adducted and neutrally rotated, elbow flexed at 90°, forearm in neutral position, and wrist between 0° and 30° of dorsiflexion. Three measurements were performed consecutively at the dominant hand, and the highest value (expressed in kilograms) was used for the analysis.

Potential confounders

Energy intake

Dietary intake was available for 988 participants. Dietary intake was assessed with the self-administered, semiquantitative Food Frequency Questionnaire (FFQ), which has been validated against 24-hour recalls among 626 volunteers from the Geneva population (20). Briefly, the FFQ assesses dietary intake for the previous 4 weeks and consists of 97 different food items that account for >90% of the intake of calories, proteins, fats, carbohydrates, alcohol, cholesterol, vitamin D, and retinol and 85% of fiber, carotene, and iron. Conversion of FFQ responses into nutrients was based on the French CIQUAL food composition table. Total energy intake was computed, including alcohol consumption.

Sedentarity index

Physical activity was estimated in 901 participants by a self-administered physical activity frequency questionnaire. The questionnaire lists 70 activities or groups of activities and was validated against measurements of energy expenditure by heart rate monitor with satisfactory correlations (r = 0.76) between the two methods (21). For this study, only sedentary status (yes/no) was used. Sedentary status was defined as spending <10% of total daily energy expenditure in activities with an intensity >4 basal metabolic rate equivalents.

Hormonal assays

Blood sampling was performed at the second CoLaus visit. Most biological assays were performed by the Lausanne University Hospital Clinical Laboratory on fresh blood samples within 2 hours of blood collection. Glucose was assessed by glucose dehydrogenase, with a maximum interassay and intra-assay coefficient of variation (CV) of 2.1% and 1.0%, respectively. Insulin was assessed by a solid-phase, two-site chemiluminescent immunometric assay (Diagnostic Products Corporation, Los Angeles, CA), with a maximum intra-assay CV of 13.7%. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated according to the formula (glucose × insulin)/22.5. Adiponectin and leptin levels were measured with a multiplexed particle-based flow cytometric cytokine assay with maximum intra-assay CVs of 8.4% and 9.5%, respectively (22). The analysis was conducted with a conventional flow cytometer (Guava EasyCyte Plus; Millipore, Zug, Switzerland). HOMA-IR and serum adipokine levels were available for 1046 and 977 participants, respectively.

Psychiatric assessment

Screening for current or past depression was performed with the Diagnostic Interview for Genetic Studies, as described previously (23). Depression was defined as the presence of depressive personality disorder or major depressive disorder (single or recurrent episode). Antidepressant treatment was considered as present for any reported medicine with an Anatomical Therapeutic Chemical code beginning with "N06A" (antidepressants) or "N06CA" (antidepressants in combination with psycholeptics) (https://www.whocc.no/atc_ddd_index/).

Statistical analysis

Statistical analyses were conducted in Stata version 14.1 (StataCorp, College Station, TX) for Windows. Because of their skewed distributions, leptin and adiponectin concentrations were log transformed before analysis. Descriptive results were expressed as the number of participants (percentage) or as average ± standard deviation. Bivariate analyses were conducted with χ^2 for categorical variables and analysis of variance for continuous variables. Multivariable analyses for continuous variables were conducted with analysis of variance or multiple regression; results were expressed either as adjusted average ± standard error (SE) or as slope and 95% confidence interval. Post hoc pairwise comparisons were performed with the Scheffe method. Statistical significance was considered for a two-tailed test with a P value <0.05.

Results

Study population

The flowchart of the study is shown in Fig. 1. After application of exclusion criteria (n = 26), the remaining 1053 women were classified in the three groups: 549 NUs (52.14%), 216 CUs (20.51%), and 288 PUs (27.35%). Android composition, gynoid composition, and VAT were available for 966/1053 participants (91.7%: 510 NUs, 255 PUs, and 201 CUs).

Characteristics of participants

Almost all participants were white (>98% for each group). The three groups differed significantly in age: 66.8 ± 6.3, 62.6 ± 6.7, and 61.3 ± 7.9 years for PUs, CUs, and NUs, respectively (CUs vs NUs, P = 0.04; PUs vs NUs, $P < 0.001$). Accordingly, all results were adjusted for age. In the unadjusted analysis, there was a trend for BMI differences with CUs < NUs < PUs: 24.9 ± 4.1, 25.7 ± 4.3, and 25.8.0 ± 4.3 kg/m^2, respectively (CUs vs

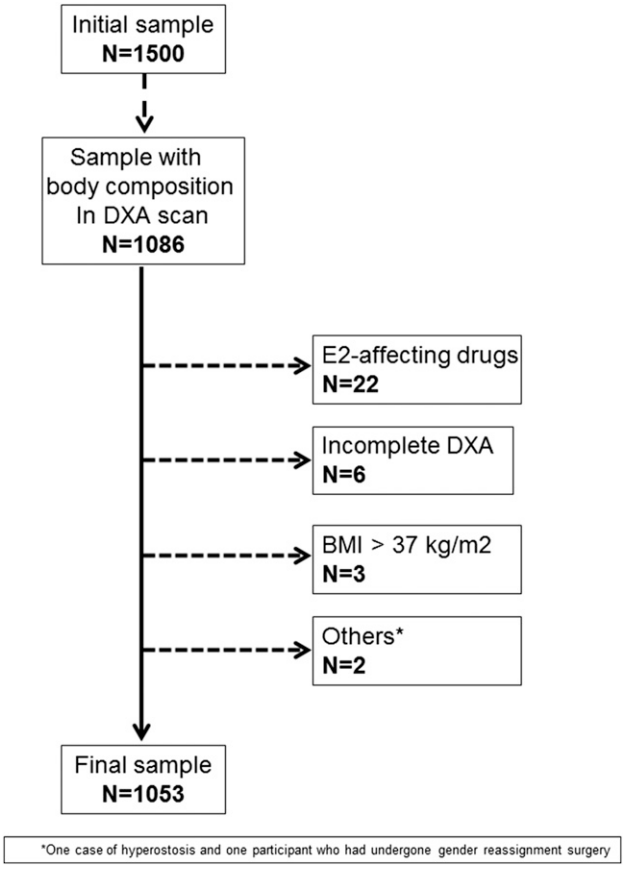

Figure 1. Flowchart of the study highlighting the inclusion and exclusion criteria. Of the 1500 postmenopausal women of OsteoLaus with DXA scan, body composition assessment was retrievable for 1086 women who were included in the current analysis.

NUs, $P = 0.052$; CUs vs PUs, $P = 0.049$). Average MHT duration was 12.2 ± 8.8 years in CUs and 7.9 ± 6.3 years in PUs. The latter had an average of 8.5 ± 5.8 years since MHT withdrawal at study entry.

Association between MHT and measures of body fat, muscle mass, and strength

The age-adjusted values of body composition parameters according to MHT status are presented in Table 1. CUs exhibited significantly lower VAT values than NUs. Similarly, a consistently significant advantage of CUs over NUs was found for BMI, android FM, percentage of subtotal FM, and FMI ($P < 0.05$). PUs showed no advantage in comparison with NUs for all FM outcomes. We did not detect any statistical benefit for the MHT groups regarding LBM, sarcopenia indices, and handgrip strength. On the contrary, there was a trend for lower LMI in the CUs (CUs vs NUs, $P = 0.05$). The ratio ALM/BMI was the only parameter for which CUs clearly exceeded both PUs and NUs without reaching statistical significance.

We also performed a regression analysis of different outcomes with age, stratified by MHT group (Table 2). The slopes for 10-year increments were significantly positive in NUs for BMI, subtotal FM, android FM, VAT, and FMI while being flat for both CUs and PUs. Between-group comparison confirmed a significant benefit for both MHT groups (P for interaction < 0.05) for all the aforementioned outcomes and percentage FM. The most prominent difference was seen for VAT ($P = 0.01$). The associations between BMI, subtotal FM, android FM, and VAT with age are represented in Fig. 2. There was no difference between groups for the slopes of LBM outcomes, with a tendency for loss of muscle mass in all three groups. When we selectively analyzed women aged <60 years, no statistical differences persisted between groups.

Comparison of potential confounders between MHT groups

In an attempt to explore potential confounders, age-adjusted results between MHT groups are shown in Table 3. No significant difference was detected for glucose, insulin, and adipokine levels. Insulin resistance tended to decrease in treatment groups: CUs < PUs < NUs. Adiponectin was higher in PUs and CUs, and leptin levels were lower in CUs (not significant for both parameters). Caloric intake differed between groups but in favor of NUs (NUs < CUs < PUs; NUs vs PUs, $P = 0.039$). There was no difference between groups in sedentary status, prevalence of depression, or use of antidepressant medications at study entry.

Subgroup analysis according to MHT duration and time since MHT withdrawal

Table 4 shows the main outcomes of CUs according to MHT duration and of PUs according to MHT duration and time since MHT withdrawal. Three subgroups were compared: 0 to 2, 2 to 5, and >5 years. There was no difference between subgroups for any of the outcomes studied. Similar results were noted when we repeated the analysis of PUs between two groups of time since MHT discontinuation: <5 years and >5 years. The effect of time since MHT withdrawal was further explored by a hinge analysis, which did not identify a reliable inflection point (data not shown).

Discussion

MHT is associated with lower visceral adiposity

This cross-sectional analysis of the OsteoLaus cohort demonstrated that active MHT use is associated with significantly lower levels of VAT measured by DXA (Table 1, Supplemental Fig. 1). The significant increase of VAT with age in NUs was completely prevented in CUs, suggesting that MHT slows down the age-associated increase of VAT. These results are in agreement with a recent randomized study in premenopausal women who experienced an increase in VAT under GnRH-Ag (11), a phenotype reversed by estrogen therapy.

Table 1. Age-Adjusted Values of Body Composition Parameters According to MHT Status

	NUs	PUs	CUs	Global *P*	CUs vs NUs	CUs vs PUs	PUs vs NUs
Sample size	549	288	216				
BMI, kg/m^2	25.8 ± 0.2	25.6 ± 0.3	24.9 ± 0.3	0.03	**0.03**	0.21	0.78
FM, kg							
Subtotal	23.3 ± 0.3	23.3 ± 0.5	22.0 ± 0.5	0.05	0.06	0.14	0.99
Android	2.01 ± 0.04	2.00 ± 0.06	1.83 ± 0.06	0.02	**0.03**	0.12	0.97
Gynoid	4.64 ± 0.05	4.71 ± 0.08	4.48 ± 0.08	0.13	0.29	0.13	0.74
Visceral	0.48 ± 0.01	0.48 ± 0.02	0.42 ± 0.02	0.01	**0.02**	0.07	0.98
FM, % total body weight							
Subtotal	35.9 ± 0.3	36.2 ± 0.4	34.6 ± 0.4	0.01	**0.03**	**0.03**	0.90
Lean mass, kg							
Subtotal	40.2 ± 0.2	39.8 ± 0.3	40.1 ± 0.4	0.62	0.95	0.86	0.62
Android	3.20 ± 0.02	3.17 ± 0.03	3.12 ± 0.04	0.24	0.24	0.60	0.86
Gynoid	6.36 ± 0.04	6.34 ± 0.06	6.29 ± 0.06	0.63	0.63	0.85	0.95
FMI, kg/m^2	10.1 ± 0.1	10.0 ± 0.2	9.4 ± 0.2	0.01	**0.02**	0.08	0.95
LMI, kg/m^2	15.9 ± 0.1	15.7 ± 0.1	15.5 ± 0.1	0.04	0.05	0.64	0.37
ALMI, kg/m^2	6.6 ± 0.04	6.5 ± 0.05	6.5 ± 0.06	0.08	0.12	0.85	0.35
ALM/BMI	6795 ± 47	6815 ± 68	6978 ± 74	0.10	0.11	0.27	0.97
Hand grip strength, kg	24.6 ± 0.2	23.9 ± 0.3	24.5 ± 0.4	0.19	0.97	0.43	0.20

Results are expressed as age-adjusted mean ± SE. Between-group comparisons performed with analysis of variance; *post hoc* pairwise comparisons performed with the Scheffe method. Boldface values correspond to statistical significant differences ($P < 0.05$) in between-group comparisons.

Menopause is accompanied by changes in body composition (1, 2). Although menopause-associated bone loss is reversed by MHT (16), the evidence for its effect on FM is less consistent. Randomized controlled trials have yielded mixed results, with some showing a slight decrease in BMI and total FM with MHT (24, 25), whereas a subgroup analysis of the Women's Health Initiative (WHI) trial (26) did not detect a significant advantage. Despite conflicting results about total FM, most studies detected a reduction in central fat with MHT, as indicated by reduced waist circumference (25), decrease in DXA-measured trunk to leg fat ratio (26), lower waist-to-hip ratio (27), reduced trunk FM measured by whole-body computed tomography (28), and reduced DXA-measured android fat (29). Several small studies have assessed the effect of MHT on VAT, as reviewed by Santen *et al.* (30). The majority showed reduced VAT, except for a randomized placebo-controlled study in nonobese, early postmenopausal women (31) that showed no benefit of MHT for intra-abdominal fat (assessed by computed tomography at L4 to L5 vertebral disk level). This result was potentially attributed to the continuous estrogen/progestin regimen used in this study and an accompanying decrease in insulin

Table 2. Regression Between the Body Composition Variables and Age at Study Inclusion (10-Year Increments), Stratified by MHT Status

	NUs	PUs	CUs	*P*[a]
Sample size	549	288	216	
BMI, kg/m^2	**0.97 (0.52 to 1.41)**	−0.15 (−0.94 to 0.63)	0.15 (−0.68 to 0.97)	0.025
FM, kg				
Subtotal	**1.78 (1.00 to 2.57)**	−0.21 (−1.55 to 1.13)	0.19 (−1.28 to 1.66)	0.018
Android	**0.18 (0.08 to 0.27)**	0.02 (−0.15 to 0.18)	−0.08 (−0.25 to 0.09)	0.023
Gynoid	0.04 (−0.10 to 0.18)	−0.15 (−0.37 to 0.08)	−0.05 (−0.29 to 0.19)	0.375
Visceral	**0.10 (0.07 to 0.12)**	0.05 (−0.01 to 0.09)	0.02 (−0.03 to 0.07)	0.014
FM, % total body weight				
Subtotal	**2.13 (1.48 to 2.79)**	0.75 (−0.36 to 1.85)	0.54 (−0.73 to 1.80)	0.022
Lean mass, kg				
Subtotal	**−0.66 (−1.23 to −0.09)**	**−1.55 (−2.44 to −0.65)**	−0.62 (−1.67 to 0.44)	0.258
Android	0.01 (−0.06 to 0.07)	−0.06 (−0.16 to 0.04)	−0.08 (−0.19 to 0.03)	0.322
Gynoid	**−0.17 (-0.27 to −0.06)**	**−0.24 (-0.40 to −0.08)**	**−0.20 (−0.38 to −0.02)**	0.771
FMI, kg/m^2	**0.80 (0.47 to 1.12)**	0.15 (−0.42 to 0.71)	0.09 (−0.50 to 0.69)	0.041
LMI, kg/m^2	0.13 (−0.07 to 0.34)	−0.24 (−0.55 to 0.08)	−0.12 (−0.52 to 0.28)	0.143
ALMI, kg/m^2	−0.15 (−0.11 to 0.08)	**−0.17 (−0.31 to −0.02)**	−0.14 (−0.32 to 0.03)	0.180

Results are expressed as slope (95% confidence interval) for each 10-year increment. Significant ($P < 0.05$) slopes are indicated in bold. Statistical analysis by linear regression and interaction analysis by analysis of covariance.

[a] *P* for interaction.

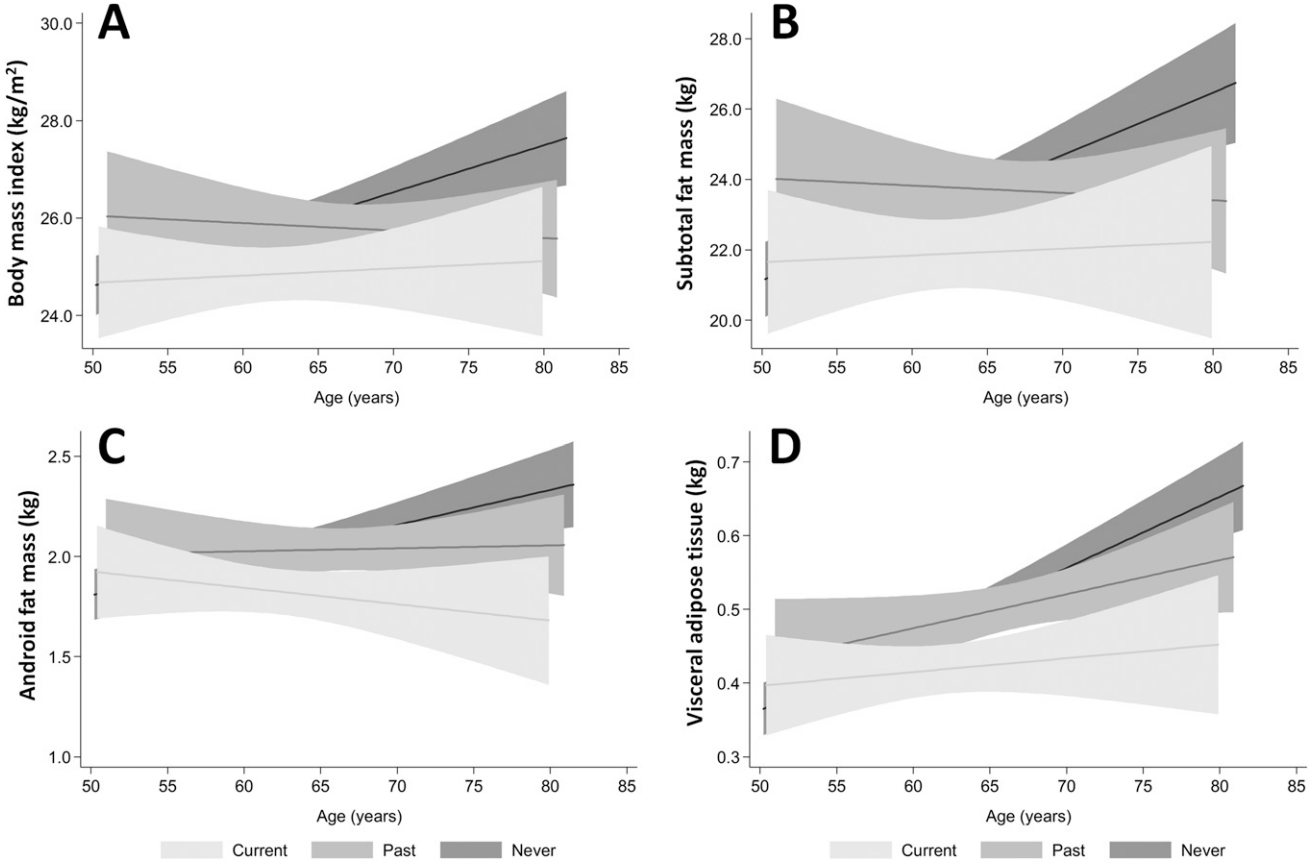

Figure 2. Linear association between age at study inclusion and (A) BMI, (B) subtotal FM, (C) android FM, and (D) VAT, according to MHT group. Results are expressed as slope and 95% confidence interval for CUs (*light gray*), PUs (*medium gray*), and NUs (*dark gray*).

sensitivity, even though another prospective non-randomized study implementing a continuous MHT regimen detected a benefit regarding android shift of fat distribution (27).

Current MHT users have lower BMI, FMI, and android fat

Our data also pointed out a slight but significant superiority of CUs regarding lower BMI, android fat, and

Table 3. Age-Adjusted Values for Possible Confounders of Body Composition Parameters, Stratified by MHT Status

	NUs	PUs	CUs	Global *P*
Sample size[a]	549	288	216	
Glucose, mmol/L	5.76 ± 0.04	5.65 ± 0.05	5.65 ± 0.06	0.18
Insulin, mU/L	7.67 ± 0.23	7.31 ± 0.32	7.06 ± 0.36	0.32
HOMA-IR	2.04 ± 0.08	1.94 ± 0.11	1.88 ± 0.13	0.53
Leptin, pg/mL	6782 ± 276	7414 ± 385	5965 ± 434	0.19[b]
Adiponectin, ng/mL	6406 ± 234	6709 ± 327	6697 ± 369	0.24[b]
Total caloric intake, kcal	1613 ± 31	1751 ± 43	1655 ± 48	0.04
Current smoking, yes, %	20.9	15.5	16.7	0.12
Sedentary	(n = 471)	(n = 241)	(n = 189)	
Yes, %	65.4	67.6	61.4	0.40
No, %	34.6	32.4	38.6	
Depression prevalence	(n = 363)	(n = 168)	(n = 147)	
Yes, %	51.5	54.2	57.8	0.43
Antidepressant medications, yes, %	11.8	14.2	15.3	0.37

Results are expressed as age-adjusted mean ± SE or as percentages for sedentarity and depression prevalence. Between-group comparisons performed with analysis of variance.

[a]The exact sample size differs according to the parameter analyzed (glucose, n = 1048; insulin, n = 1046; HOMA-IR, n = 1046; leptin, n = 977; adiponectin, n = 977; total caloric intake, n = 988; sedentarity index, n = 901; depression scale, n = 678).

[b]Statistical analysis performed on log-transformed data.

Table 4. Body Composition Parameters in MHT PUs According to Duration of and Time Since Discontinuation

	BMI (kg/m^2)	Subtotal FM (kg)	Subtotal FM (%)	Android FM (kg)	VAT (kg)	FMI (kg/m^2)
CUs						
Sample size	215	215	215	200	200	200
Duration of MHT, y						
0–2	24.51 ± 0.97	20.34 ± 1.73	33.14 ± 1.49	1.76 ± 0.19	0.39 ± 0.06	9.12 ± 0.67
2–5	24.62 ± 0.69	20.74 ± 1.23	34.52 ± 1.06	1.81 ± 0.14	0.41 ± 0.04	9.43 ± 0.48
5+	25.02 ± 0.36	22.5 ± 0.65	34.76 ± 0.56	1.84 ± 0.08	0.43 ± 0.02	9.42 ± 0.27
P	0.856	0.389	0.614	0.924	0.827	0.910
PUs						
Sample size	274	274	274	242	242	242
Duration of MHT, y						
0–2	26.71 ± 0.72	24.18 ± 1.22	36.38 ± 1.01	2.10 ± 0.14	0.54 ± 0.04	10.47 ± 0.51
2–5	25.39 ± 0.62	23.94 ± 1.05	36.70 ± 0.86	2.00 ± 0.13	0.49 ± 0.04	10.04 ± 0.47
5+	25.67 ± 0.33	23.48 ± 0.57	36.76 ± 0.47	2.03 ± 0.07	0.50 ± 0.02	10.23 ± 0.25
P	0.334	0.850	0.946	0.878	0.588	0.816
Time since discontinuation, y						
0–2	25.72 ± 0.82	24.17 ± 1.40	36.40 ± 1.15	2.14 ± 0.17	0.53 ± 0.05	10.32 ± 0.60
2–5	25.69 ± 0.63	23.54 ± 1.08	36.80 ± 0.89	2.03 ± 0.14	0.51 ± 0.04	10.21 ± 0.49
5+	25.81 ± 0.32	23.63 ± 0.55	36.71 ± 0.45	2.02 ± 0.07	0.50 ± 0.02	10.22 ± 0.24
P	0.985	0.927	0.960	0.807	0.813	0.988

Results are expressed as adjusted mean ± SE. Statistical analysis was performed with an analysis of variance model including age, BMI, duration of MHT, and time since discontinuation.

FMI. Interestingly, all studies showing a significant decrease in total or central adiposity recruited early postmenopausal women (25, 26, 28), whereas differences were less pronounced in older populations, as in the WHI trial (average age >63 years). It is possible that the beneficial effect of MHT on FM is more pronounced in the early postmenopausal period and that age-mediated changes overcome the MHT benefits later in life. Of note, even in the studies showing significant benefits, the effect size was small. The only published meta-analysis (32) showed a significant reduction in waist circumference and abdominal fat (measured by dual energy photon or DXA) by 0.8% (5 trials) and 6.8% (4 trials), respectively.

MHT prevents the age-associated gain of body fat

The benefit of MHT was confirmed in the regression analysis, which highlighted a clear divergence between CUs and NUs regarding the association between age and body fat parameters. Indeed, NUs had significantly larger slopes for increase of BMI, subtotal and android FM, and FMI. MHT prevented significantly the age-associated increase of these parameters. This type of analysis offers the benefit of a projection over time, going beyond the limits of a simple cross-sectional analysis.

Potential confounders do not seem to explain the MHT effect on FM

It remains controversial whether the beneficial effect of MHT on FM is caused by a direct effect on adipocytes, mediated by other hormones, or by modifying intermediary factors such as nutrition or physical activity. In the current study, CUs tended to be less sedentary (61.4% vs 65.4% and 67.6% for NUs and PUs, respectively) without reaching statistical significance. Caloric intake was significantly higher in PUs than in NUs; CUs did not differ from the other two groups. Despite findings of positive correlations between E2 and leptin independently of body fat in one study of premenopausal women (33), adipokine levels did not differ significantly in our cohort after adjustment for age and subtotal FM (data not shown). Finally, no difference was found regarding the prevalence of depression between groups.

Existing evidence on regulation of energy intake and expenditure by estrogens has been recently reviewed by Leeners *et al.* (34). Strong preclinical data support an important role for estrogen in bioenergetics. Both OVX mice and rats exhibited a marked reduction of spontaneous physical activity and a decrease in resting energy expenditure, whereas OVX rats developed an additional increase in energy intake (8). The latter was not seen in OVX mice, in line with our data in NUs. In menstruating women, resting energy expenditure is higher in the midluteal phase, when E2 is elevated; low in the early follicular phase, when E2 is lower; and further reduced by GnRH-Ag (35). An indirect effect via an increase in sedentarity was postulated by Lovejoy *et al.* (4), who prospectively followed physical activity annually by accelerometry in women going through menopause and detected a decrease of 50% over 4 years.

The benefit of MHT on FM does not seem to persist after its withdrawal

Another interesting point of our study is the clear absence of residual effect of MHT in PUs. PUs were classified according to MHT duration and time since MHT discontinuation; this analysis surprisingly showed no residual effect in early discontinuers, unlike our results regarding bone mineral density (16), suggesting a very rapid rebound effect after MHT withdrawal. However, the regression analysis detected significantly less steep slopes in PUs than in NUs for multiple FM outcomes, a result that deserves further exploration by a longitudinal study. To the best of our knowledge, no other study has specifically assessed body composition in PUs. Studies with GnRH-Ag (11, 36) have shown significant increases in total and central adiposity as soon as 4 months after estrogen withdrawal, consistent with our hypothesis of a rapid rebound effect. The rapid response of FM to external stimuli is also illustrated by the early increase in FM (+21.3%) only 8 weeks after training cessation in elite taekwondo athletes (37). The observed increase in caloric intake of PUs in our study provides another possible explanation for the rapid loss of FM benefits after MHT withdrawal. It would be reasonable to suggest confirmation of these results in the setting of a randomized trial to eliminate contribution of a selection bias.

MHT does not have any detectable benefit on lean mass

We hypothesized that MHT leads to increased LBM, which in turn would contribute to its favorable bone effects via increased mechanical load. Strongly positive correlations between LBM and bone mineral density, previously demonstrated (29, 38), support a potential link. Surprisingly, we did not detect any benefit among MHT users for LBM or muscle strength. These results were confirmed even after we excluded women using osteoporotic drugs other than MHT (n = 82, data not shown), thus arguing against an intermediate role of LBM in the MHT-mediated bone benefits.

Our results add to the conflicting evidence of available studies with the only available meta-analysis (33) showing a slight but significant increase (+3.3%) of LBM in MHT users. One possible explanation might be the type of MHT. Certain progestogens, such as the norethisterone acetate used by Arabi *et al.* (29), have androgenic properties that could have an anabolic effect on LBM. More importantly, the effect of MHT on LBM can be selective for early postmenopausal women, weaning off rapidly under the stronger effect of age. In favor of this hypothesis, the WHI trial revealed that MHT significantly delayed loss of LBM after 3 years (28). Nevertheless, this relation was completely reversed between year 3 and 6 of the study, with a slight decrease in LBM in all groups at the end of year 6 (39), a finding also confirmed in the subset of women with high compliance. In our analysis, no LBM benefit was revealed when we analyzed only data from younger postmenopausal women (<60 years old). It is possible that this time-dependent effect is limited to a much shorter period after menopause (*e.g.*, up to 5 years), as suggested by the studies discussed earlier (28, 39).

Strengths and limitations

This study has several limitations. The cross-sectional design is inevitably accompanied by a selection bias. Information on the beginning and the end of MHT was self-reported. This was also the case for the route of administration (oral, transdermal, vaginal), the type of MHT (estrogen-alone or estrogen/progestin), and the history of hysterectomy, preventing us from reliably assessing these factors. Furthermore, we were unable to verify participants' adherence to MHT. Most participants were white, limiting the generalizability of study's conclusions to other ethnicities. Our evaluation of confounding factors is partial. The physical activity assessment was only rough. We did not measure resting energy expenditure, which is a potential target of estrogen treatment.

On the other hand, our study has considerable strengths to be taken into account. The large sample of the OsteoLaus cohort allows adequate statistical power. Body composition assessment was performed with DXA and last-generation software, which allowed reliable measurement of VAT, differentiating it from subcutaneous adipose tissue (40). This large, prospective study of postmenopausal women has explored the effect of MHT on VAT by reliably distinguishing it from other components of fat tissue.

In conclusion, current MHT use prevents the increase in visceral adiposity. This finding may have important cardiovascular, metabolic, and bone implications that should be taken into account when assessing the benefit/risk ratio for MHT prescription. Nevertheless, the effect size on BMI and total FM is small, and MHT prescription cannot substitute for other interventions such as physical activity. Physicians should be aware that the benefit of MHT on body composition might rapidly disappear after its withdrawal and strongly encourage women to optimize nutrition and increase physical activity when stopping MHT. Future research via prospective and ideally randomized studies should assess differences depending on type of MHT and route of administration and on the evolution of body composition after MHT withdrawal. It would also be interesting to specifically investigate the effects of MHT on body composition in populations with an ethnically diverse composition and in early postmenopausal women.

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