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Authors: Papadakis GE, Hans D, Rodriguez EG, Vollenweider P, Waeber G, Marques-Vidal P, Lamy O

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Menopausal hormone therapy is associated with reduced total and visceral adiposity, the OsteoLaus cohort

AUTHORS: Georgios E. Papadakis[1], Didier Hans[2], Elena Gonzalez Rodriguez [1, 2], Peter Vollenweider[3], Gerard Waeber[3], Pedro Marques-Vidal[3], Olivier Lamy[2, 3]

[1] *Service of Endocrinology, Diabetes and Metabolism, Lausanne University Hospital, Switzerland.*

[2] *Center of Bone Diseases, CHUV, Lausanne University Hospital, Switzerland.*

[3] *Service of Internal Medicine, CHUV, Lausanne University Hospital, Switzerland.*

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Corresponding author's contact information and address for requests of reprint:

Georgios Papadakis

Service of Endocrinology, Diabetes and Metabolism, CHUV, Lausanne University Hospital

Avenue de la Sallaz 8-10, CH-1011 Lausanne, Switzerland

Phone: +41 79 556 03 08 /+41 79 695 63 48, Fax: +41 21 314 94 51. Email: Georgios.Papadakis@chuv.ch

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ABSTRACT

Context: Following menopause fat mass (FM) and visceral adipose tissue (VAT) increase, while non-bone lean 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 (50-80 years old) who underwent dual-energy X-ray absorptiometry (DXA) with body composition assessment. After excluding women with estrogen-modifying medications, the 1053 participants were categorized into current (CU), past (PU) and never (NU) MHT users.

Intervention(s): none.

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

Conclusions: MHT is associated with significantly decreased VAT, BMI and android FM. No benefit is detected for LBM. The benefits are not preserved in PU, suggesting caution when MHT is discontinued.

PRECIS:

Menopausal hormone therapy is associated with decreased visceral adipose tissue and prevention of the age-associated gain of fat mass. These benefits are not preserved in past users.

INTRODUCTION

Menopause is accompanied by significant changes of 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 due to declining ovarian hormone secretion or to ageing remains an open question (2). Data are more robust regarding the effect of menopause on regional fat. Several prospective studies have shown a stronger increase of abdominal fat after menopause, leading to a shift from a gynoid to an android pattern of fat distribution (5, 6, 7). The causal association with estrogen deficiency is supported by preclinical data demonstrating that disruption of estradiol signaling by deletion of estrogen receptor (ER) or ovariectomy (OVX) accelerates fat accumulation (8). It is important to underline 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 non-bone lean body mass (LBM), also referred as fat-free and skeletal muscle mass, has been described across menopause (3, 4). It remains unclear whether this finding is a consequence of estrogen deficiency or indirect factors such 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 be due to differences on population studied, studies' design (natural versus induced menopause), type of MHT and method for assessing body composition. Conversely, most studies evaluating the effect of GnRH agonists ($GnRH_{AG}$), 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 direct effect on adipocytes or indirect mechanisms such altered energy intake and/or energy expenditure (8) and/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 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 above.

MATERIAL AND METHODS:

Setting

We analyzed data from the OsteoLaus study (14). 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 (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 participants of OsteoLaus 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

1500 postmenopausal women, aged 50-80 years, were questioned on current or past MHT use, its type and duration if applicable. All participants underwent a spine and hip DXA scan by Discovery A System (Hologic, Inc., Marlborough, MA, USA). 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 BMI values (BMI > 37 kg/m^2) and uninterpretable or incomplete DXA scans (low quality images). The remaining participants were divided into 3 groups: current (CU), past (PU) and never users (NU) of MHT.

CU were taking MHT at trial entry or discontinued treatment since less than 6 months. PU discontinued MHT at least 6 months before trial entry (otherwise considered as CU). MHT use for less than 6 months, reported in 25 participants (< 3 months in 23/25), was considered unlikely to cause considerable changes of body composition and these subjects were classified as NU.

DXA measurements

All body composition measurements were in accordance with published guidelines by 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 (ROI) were defined by the analytical program including: total body, trunk, head, pelvic, upper limbs, lower limbs, android and gynoid region. The lower boundary of the android region was defined at the pelvis cut and the upper boundary above the pelvis cut 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 (SAT). Android lean and fat mass, gynoid lean and fat mass, and VAT were analyzed in a second step from the initial images of body composition. For technical reasons, 87 exams could not be reanalyzed rendering the analysis of the above parameters impossible in these participants.

Outcomes:

<u>Body composition:</u> 1. VAT; 2. Subtotal FM (by extracting head FM from the total FM); 3. Android and gynoid FM; 4. Fat mass index (FMI) computed as the ratio of total body FM over height squared; 5. LBM: subtotal, android and gynoid by analogy to FM; 6. Lean mass index (LMI) defined as the ratio of total LBM over height squared; 7. Sarcopenia indices (18): appendicular lean mass index (ALMI) computed as the ratio of appendicular lean mass (ALM) over height squared; and ALM divided by body mass index (ALM/BMI).

Grip strength: Assessment of muscle strength *via* handgrip was available for 990 participants. Participants of the CoLaus aged over 50 were invited to participate in a sub-study on frailty, which included grip strength, assessed using the Baseline® Hydraulic Hand Dynamometer. 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 and wrist between 0 and 30° of dorsiflexion. Three measurements were performed consecutively at the dominant hand and the highest value (expressed in kg) was used for the analysis.

Potential confounders

Energy intake: Dietary intake was available for 988 participants. Dietary intake was assessed using a self-administered, semi quantitative Food Frequency Questionnaire (FFQ), which has been validated against 24 hour recalls among 626 volunteers from the Geneva population (20). Briefly, this FFQ assesses the dietary intake of the previous 4 weeks and consists of 97 different food items that account for more than 90% of the intake of calories, proteins, fat, carbohydrates, alcohol, cholesterol, vitamin D and retinol, and 85% of fibre, carotene and iron. Conversion of the 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 (PAFQ). The questionnaire lists 70 activities or groups of activities and was validated against measurement 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 when the participant spent less than 10% of her total daily energy expenditure in activities with an intensity over 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 (CHUV) Clinical Laboratory on fresh blood samples within 2 hours of blood collection. Glucose was assessed by glucose dehydrogenase with a maximum inter-assay 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, USA) with a maximum intra-assay CV of 13.7%; HOMA-IR was calculated according to the formula (glucose × insulin)/22.5. Adiponectin and leptin levels were measured using a multiplexed particle-based flow cytometric cytokine assay with maximum intra-assay CV of 8.4 and 9.5% respectively (22). The analysis was conducted using a conventional flow cytometer (Guava EasyCyte Plus, Millipore, Zug, Switzerland). HOMA-IR and serum adipokines levels were available for 1046 and 977 participants, respectively

Psychiatric assessment: Screening for current or past depression was performed using the Diagnostic Interview for Genetic Studies (DIGS) 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 ATC (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 using Stata v14.1 (StataCorp, College Station, Texas, USA) for Windows. Due to their skewed distributions, leptin and adiponectin concentrations were log transformed prior to analysis. 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. Multivariable 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 Scheffe's 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 **Figure 1**. After application of exclusion criteria (n=26), the remaining 1053 women were classified in the 3 groups: 549 NU (52.14%), 216 CU (20.51%) and 288 PU (27.35%). Android composition, gynoid composition and VAT were available for 966/1053 participants (91.7%: 510 NU, 255 PU and 201 CU).

Characteristics of participants

Almost all participants were Caucasian (> 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 PU, CU and NU respectively (CU vs. NU: *p=0.04*; PU vs. NU: *p<0.001*). Accordingly, all results were adjusted for age. In the unadjusted analysis, there was a trend for BMI differences with CU<NU<PU: 24.9 ± 4.1, 25.7 ± 4.3 and 25.8.0 ± 4.3 kg/m2 (CU vs. NU: *p=0.052*; CU vs. PU*: p=0.049*). Average MHT duration was 12.2 ± 8.8 in CU and 7.9 ± 6.3 years in PU. The latter had an average of 8.5 ± 5.8 years since MHT withdrawal at study entry.

Association between menopausal hormone therapy and measures of body fat, muscle mass and strength

The age-adjusted values of body composition parameters according to MHT are presented in **Table 1**. CU exhibited significantly lower VAT values than NU. Similarly, a consistently significant advantage of CU over NU was found for BMI, android FM, percentage of subtotal FM and FMI (***p<0.05***). PU showed no advantage in comparison to NU 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 CU (CU vs. NU, *p=0.05*). The ratio ALM/BMI was the only parameter that CU clearly exceeded both PU and NU without reaching statistical significance.

We further 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 NU for BMI, subtotal FM, android FM, VAT and FMI, while being relatively flat for both CU and PU. Between groups comparison confirmed a significant benefit for both MHT groups (*p for interaction < 0.05*) for all the above 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 **Figure 2**. There was no difference between groups for the slopes of LBM outcomes with tendency for loss of muscle mass in all three groups. When selectively analyzing 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 adipokines levels. Insulin resistance tended to decrease in treatment groups: CU<PU<NU. Adiponectin was higher in MHT groups and leptin levels were lower in CU (ns for both parameters). Caloric intake differed between groups but in favor of NU (NU<CU<PU, NU vs. PU, *p=0.039*). There was no difference between groups for 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 CU according to MHT duration and of PU according to MHT duration and time since MHT withdrawal. Three subgroups were compared: 0-2, 2-5 and > 5 years. There was no difference between subgroups for any of the outcomes studied. Similar results were noted when repeating the analysis of PU between two groups of time since MHT discontinuation: < 5 years versus > 5 years. The effect of time since MHT withdrawal was further explored by a hinge analysis, which did not identify a reliable inflexion point (data not shown).

DISCUSSION

Menopausal hormone therapy 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, Supplementary Figure 1**). The

significant increase of VAT with age in NU was completely prevented in CU, 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.

Menopause is accompanied by significant changes in body composition (1, 2). Although the menopause-associated bone loss is effectively reversed by MHT (16), the evidence for its effect on FM is less consistent. Randomized controlled trials have yielded mixed results: some showing a slight decrease of BMI and total FM by MHT (24, 25), while a subgroup analysis of the WHI trial (26) did not detect any significant advantage. Despite conflicting results about total FM, most studies detected a reduction of central fat by 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 CT (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 non-obese, early postmenopausal women (31) which showed no benefit of MHT for intra-abdominal fat (assessed by CT at L4-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 sensitivity, even though another prospective non-randomized study implementing a continuous MHT regimen detected a benefit regarding android shift of fat distribution (27).

Current users of menopausal hormone therapy have lower BMI, FMI and android fat

Our data also pointed out a slight but significant superiority of CU regarding lower BMI, android fat and FMI. Interestingly, all the studies showing significant decrease of total and/or central adiposity recruited early post-menopausal 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 notice, even in the studies with significant benefits the effect size was small.

The only published metanalysis (32) showed a significant reduction of waist circumference and abdominal fat (measured by dual energy photon or DXA) by 0.8% (5 trials) and 6.8% (4 trials), respectively.

Menopausal hormonal therapy prevents the age-associated gain of body fat

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

Potential confounders do not seem to explain the MHT effect on fat mass

It remains controversial whether the beneficial effect of MHT on FM is due to a direct effect on adipocytes, mediated by other hormones or by modifying intermediary factors such as nutrition and/or physical activity. In the current study, CU tended to be less sedentary (61.4% versus 65.4 and 67.6 for NU and PU respectively) without reaching statistical significance. Caloric intake was significantly higher in PU than in NU; CU 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), adipokines levels did not differ significantly in our cohort after adjustment for age and subtotal FM (data not shown). Finally, no difference was found regarding 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 (REE), while OVX rats developed an additional increase in energy intake (8). The latter was not seen in OVX mice, in line with our data in NU. In menstruating women, REE is higher in mid-luteal phase when E2 is elevated, low in early follicular phase when E2 is lower and further reduced by $GnRH_{AG}$ (35). An indirect effect via increase of sedentarity was postulated by Lovejoy *et al*.

who prospectively followed physical activity annually by accelerometry in women going through menopause and detected a decrease of 50% over 4 years (4).

The benefit of menopausal hormone therapy on fat mass does not seem to persist after its withdrawal

Another interesting point of our study is the clear absence of residual effect of MHT in PU. PU 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 BMD (16) and suggesting a very rapid rebound effect following MHT withdrawal. However, the regression analysis detected significantly less steep slopes in PU than in NU 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 PU. Studies with $GnRH_{AG}$ (11, 36) have shown significant increases of 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 of FM (+ 21.3%) only 8 weeks after training cessation in elite Taekwondo athletes (37). The observed increase in caloric intake of PU 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.

Menopausal hormone therapy 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 BMD, previously demonstrated (29, 38), support a potential link. Surprisingly we did not detect any benefit among MHT users for LBM nor muscle strength. These results were confirmed even after excluding women with 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 already existing conflicting evidence of available studies with the only available metanalysis (33) showing a slight but significant increase (+3.3%) of LBM in MHT users. One possible

explanation might be the type of MHT. Certain progestagens, such as the norethisterone acetate used by Arabi *et al* (29) have androgenic properties that could have anabolic effect on LBM. More importantly, the effect of MHT on LBM can be selective for early post-menopausal 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 light decrease of 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 analyzing only younger post-menopausal women (< 60 years old). It is possible that this time-dependent effect is limited to a much shorter period after menopause (for example, up to 5 years) as suggested by the studies discussed above (28, 39).

Strengths and limitations

This study has several limitations. The crossectional 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. Further, we were unable to verify the adherence of participants to MHT. The vast majority of participants were Caucasians, limiting the generalization 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 for adequate statistical power. Body composition assessment was performed by DXA using last generation software which allowed for reliable measurement of VAT, differentiating it from SAT (40). To the best of our knowledge, this is the first large prospective study of postmenopausal women that has explored the MHT effect on VAT by reliably distinguishing it from other components of fat tissue.

In conclusion, current MHT use prevents the increase of visceral adiposity. This finding may have important cardiovascular, metabolic and bone implications which should be taken into account when assessing the benefit-risk ratio for MHT prescription. Nevertheless, the effect size on BMI and total FM is relatively 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 as well as 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 as well as in early postmenopausal women.

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LEGEND OF FIGURE 1

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

Abbreviations: BMI, Body mass index; DXA, Dual energy X-ray absorptiometry; E2, estrogen.

LEGEND OF FIGURE 2

Linear association between age at study inclusion and body mass index (panel A), subtotal fat mass (panel B), android fat mass (panel C) and visceral adipose tissue (panel D), according to menopausal hormone therapy group. Results are expressed as slope and 95% confidence interval for current users (*light grey*), past users (*medium grey*) and never users (*dark grey*).

TABLES:

Table 1: Age-adjusted values of body composition parameters according to menopausal hormone therapy status.

	Never users	**Past users**	**Current users**	**Global p-value**	**CU vs. NU**	**CU vs. PU**	**PU vs. NU**
Sample size	*549*	*288*	*216*				
Body mass index (kg/m^2)	25.8 ± 0.2	25.6 ± 0.3	24.9 ± 0.3	0.03	**0.03**	0.21	0.78
Fat mass (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
Fat mass (% 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
Fat mass index (kg/m^2)	10.1 ± 0.1	10.0 ± 0.2	9.4 ± 0.2	0.01	**0.02**	0.08	0.95
Lean mass index (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 ± standard error. PU, past users; NU, never users; CU, current users; ALMI, appendicular lean mass index; ALM, appendicular lean mass. Between-group comparisons performed using analysis of variance; post-hoc pairwise comparisons performed using Scheffe's method.

Table 2: Regression between the body composition variables and age at study inclusion (10-year increments), stratified by menopausal hormone therapy status

	Never	Past	Current	P-value §
Sample size	*549*	*288*	*216*	
Body mass index (kg/m^2)	**0.97 (0.52 ; 1.41)**	-0.15 (-0.94 ; 0.63)	0.15 (-0.68 ; 0.97)	0.025
Fat mass (kg)				
Subtotal	**1.78 (1.00 ; 2.57)**	-0.21 (-1.55 ; 1.13)	0.19 (-1.28 ; 1.66)	0.018
Android	**0.18 (0.08 ; 0.27)**	0.02 (-0.15 ; 0.18)	-0.08 (-0.25 ; 0.09)	0.023
Gynoid	0.04 (-0.10 ; 0.18)	-0.15 (-0.37 ; 0.08)	-0.05 (-0.29 ; 0.19)	0.375
Visceral	**0.10 (0.07 ; 0.12)**	0.05 (-0.01 ; 0.09)	0.02 (-0.03 ; 0.07)	0.014
Fat mass (% total body weight)				
Subtotal	**2.13 (1.48 ; 2.79)**	0.75 (-0.36 ; 1.85)	0.54 (-0.73 ; 1.80)	0.022
Lean mass (kg)				
Subtotal	**-0.66 (-1.23 ; -0.09)**	**-1.55 (-2.44 ; -0.65)**	-0.62 (-1.67 ; 0.44)	0.258
Android	0.01 (-0.06 ; 0.07)	-0.06 (-0.16 ; 0.04)	-0.08 (-0.19 ; 0.03)	0.322
Gynoid	**-0.17 (-0.27 ; -0.06)**	**-0.24 (-0.40 ; -0.08)**	**-0.20 (-0.38 ; -0.02)**	0.771
Fat mass index (kg/m^2)	**0.80 (0.47 ; 1.12)**	0.15 (-0.42 ; 0.71)	0.09 (-0.50 ; 0.69)	0.041
Lean mass index (kg/m^2)	0.13 (-0.07 ; 0.34)	-0.24 (-0.55 ; 0.08)	-0.12 (-0.52 ; 0.28)	0.143
ALMI (kg/m^2)	-0.15 (-0.11 ; 0.08)	**-0.17 (-0.31 ; -0.02)**	-0.14 (-0.32 ; 0.03)	0.180

ALMI, appendicular lean mass index; § p-value for interaction. Results are expressed in slope (95% confidence interval) for a ten-year increment. Significant ($p<0.05$) slopes are indicated in Bold. Statistical analysis by linear regression and interaction analysis by ANCOVA.

Table 3: Age-adjusted values for possible confounders of body composition parameters, stratified by menopausal hormone therapy status

	Never	**Past**	**Current**	Global, p-value
*Sample size**	*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[§]
Adiponectin (ng/ml)	6406 ± 234	6709 ± 327	6697 ± 369	0.24[§]
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

*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).
Results are expressed as age-adjusted mean ± standard error or as percentages for sedentarity and depression prevalence. MHT, menopausal hormone treatment. Between-group comparisons performed using analysis of variance. [§]Statistical analysis performed on log-transformed data.

Table 4: Body composition parameters in menopausal hormone therapy past users according to duration of and time since discontinuation.

	BMI (kg/m2)	Subtotal FM (kg)	Subtotal FM (%)	Android fat (kg)	VAT (kg)	FMI (kg/m2)
CURRENT USERS *Sample size*	*215*	*215*	*215*	*200*	*200*	*200*
Duration of MHT (years)						
[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-value	0.856	0.389	0.614	0.924	0.827	0.910
PAST USERS *Sample size*	*274*	*274*	*274*	*242*	*242*	*242*
Duration of MHT (years)						
[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-value	0.334	0.850	0.946	0.878	0.588	0.816
Time since discontinuation (years)						
[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-value	0.985	0.927	0.960	0.807	0.813	0.988

MHT, menopausal hormone therapy; BMI, body mass index; FM, fat mass; VAT, visceral adipose tissue; FMI, FM index. Results are expressed as adjusted mean ± standard error. Statistical analysis was performed using an ANOVA model including age, body mass index, duration of menopausal hormonal therapy and time since discontinuation.

507 **FIGURE 1:** Flow chart of the study

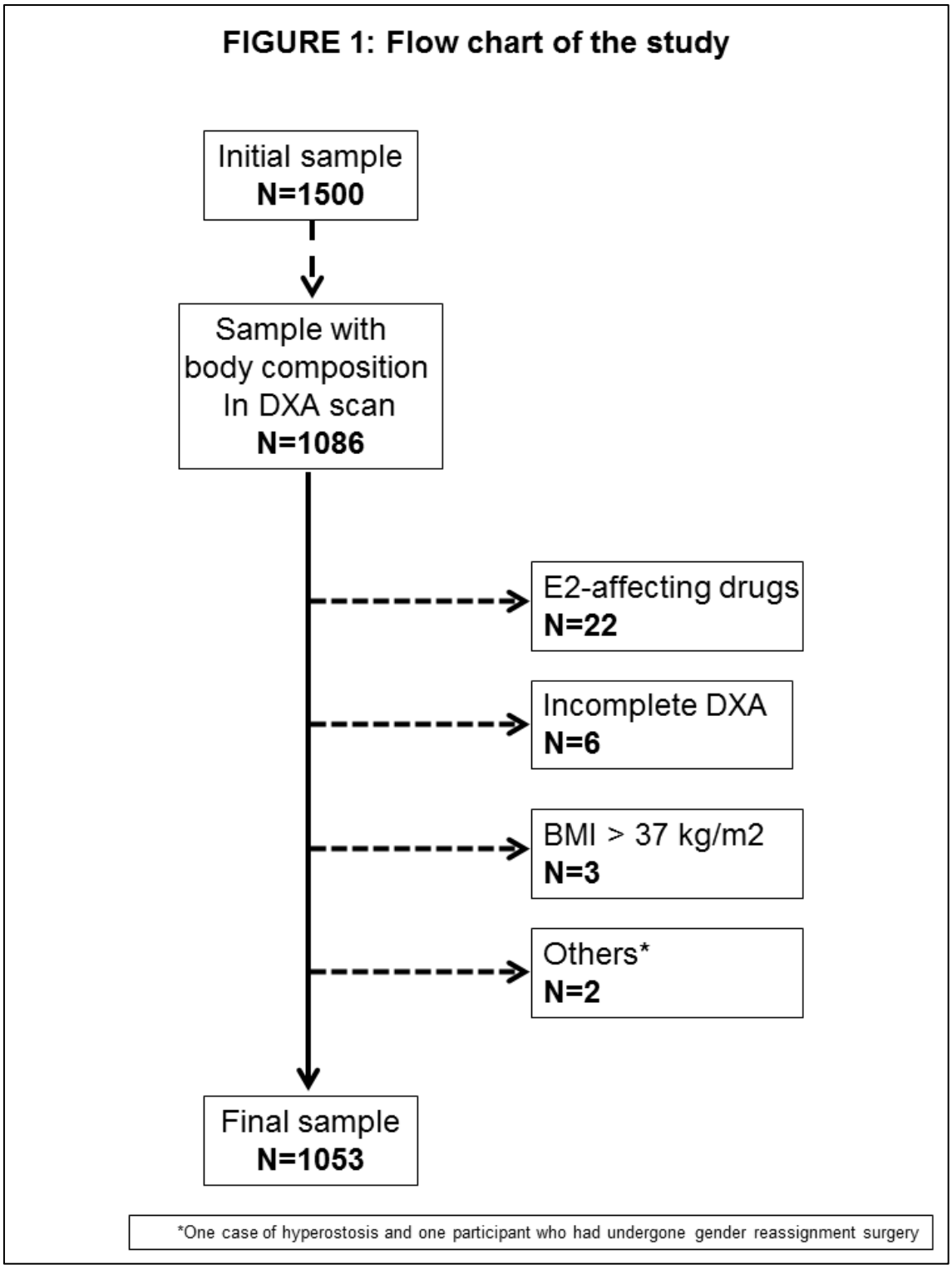

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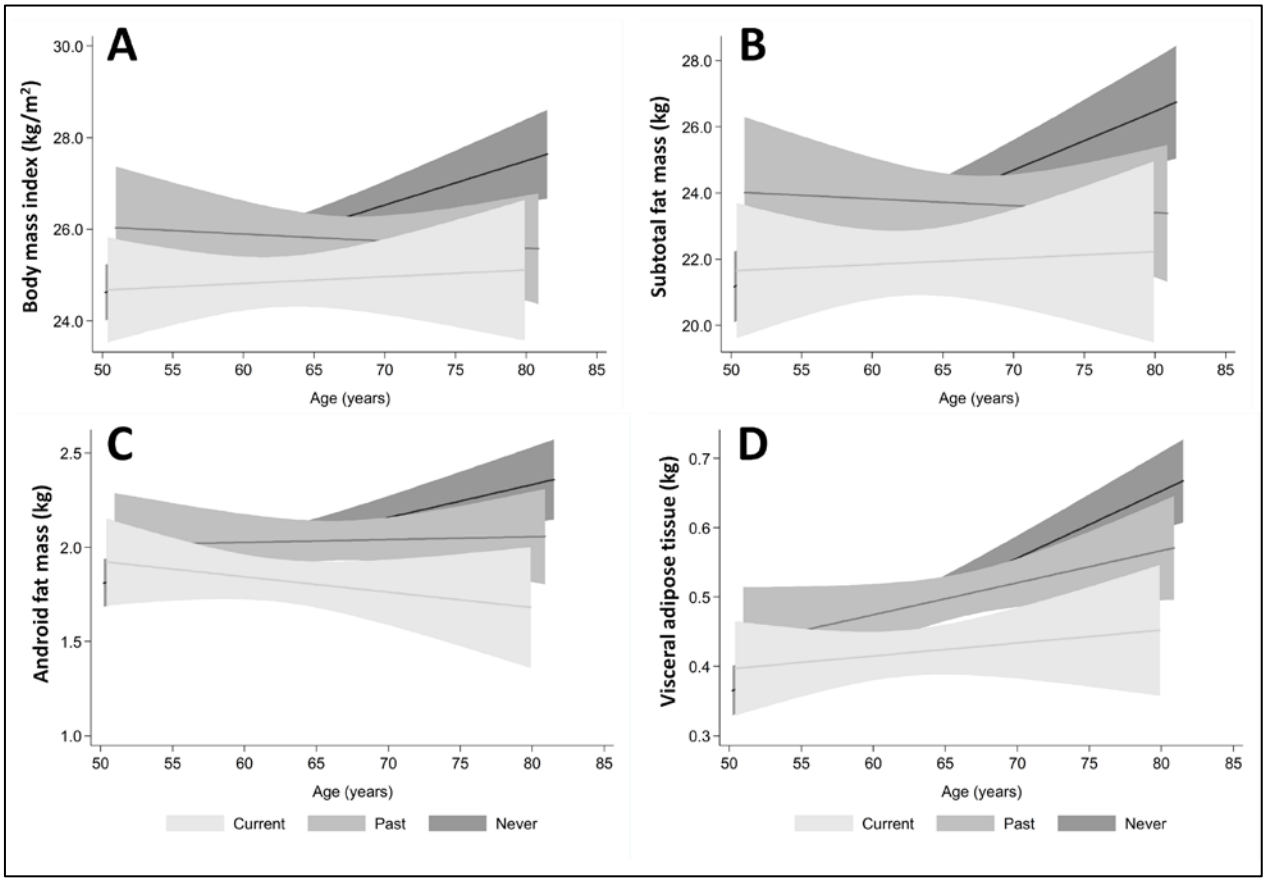
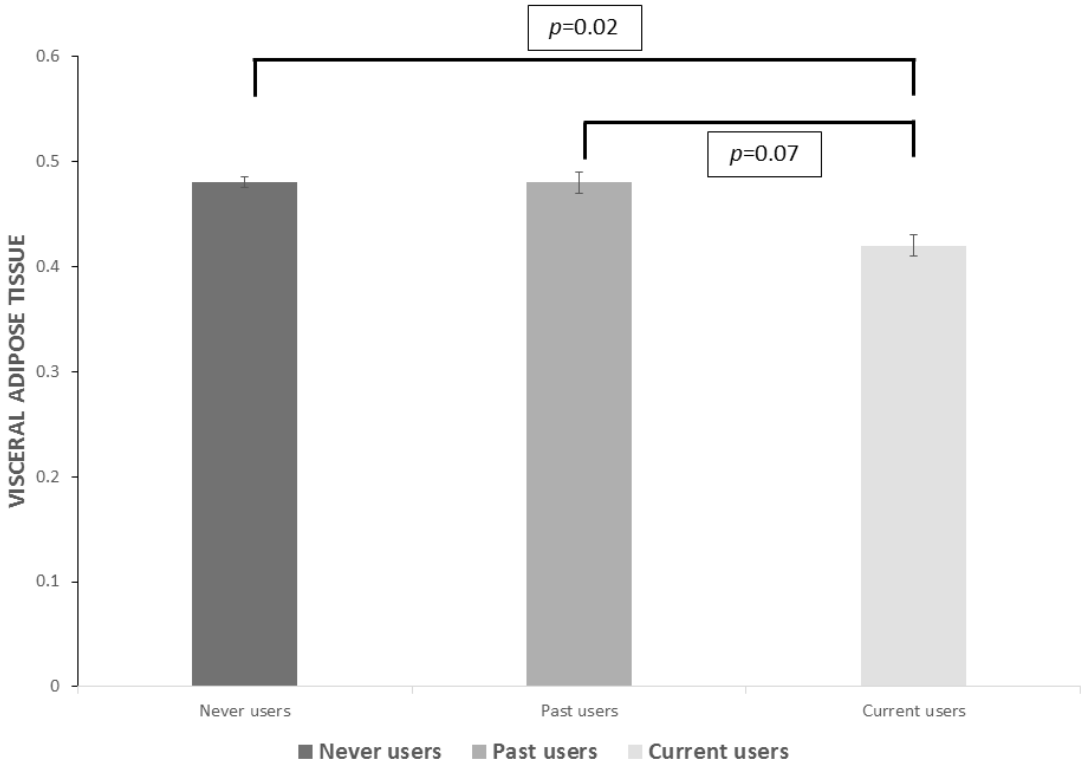
FIGURE 2: Graphic representation of regression analysis of different outcomes with age at study inclusion according to menopausal hormone therapy status



SUPPLEMENTARY FIGURE 1: Age-adjusted values of visceral adipose tissue stratified by menopausal hormone therapy status.



LEGEND OF SUPPLEMENTARY FIGURE 1

Graphic representation of age-adjusted values of visceral adipose tissue (VAT) according to menopausal hormone therapy (MHT) status. The results are shown as histograms corresponding to mean VAT values, accompanied by vertical bars corresponding to standard errors. Each MHT

groups is portrayed by a different color: current users (*light grey*), past users (*medium grey*) and never users (*dark grey*). When comparing current to never users, a statistically significant difference was seen ($p<0.05$).