

MENOPAUSE

Metabolic and hormonal parameters in post-menopausal women 10 years after transdermal oestradiol treatment, alone or combined to micronised oral progesterone

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Abstract

Background. In the post-Women's Health Initiative Study era few post-menopausal women complete long term hormonal treatment (HT).

Objective. To analyse metabolic/hormonal parameters and frequency of the metabolic syndrome (METS) in post-menopausal women after 10 years of HT.

Methods. Retrospective data from parallel cohorts of post-menopausal women receiving HT for 10 years was analysed. Regimens included: transdermal oestradiol (50 µg) ($n=22$), sequential cyclic HT with transdermal oestradiol (50 µg) plus 200 mg/day micronised oral progesterone (cycle days 12–25) ($n=83$), or continuous combined HT with transdermal oestradiol (50 µg) plus 100 mg/day micronised oral progesterone ($n=46$). A group of women who elected not to use HT served as a control group ($n=35$).

Results. Frequency of the METS did not significantly increase after 10 years of HT. Oestradiol and sex hormone binding globulin (SHBG) levels displayed a significant increase compared to baseline after 10 years of HT (all regimens). These values were significant higher when compared to the control group. Glucose levels were significantly higher after 10 years in women receiving the sequential cyclic regimen. Although not reaching statistical significance, there was a trend for transdermal oestradiol alone to increase HDL-C and decrease triglyceride levels.

Conclusion. Serum oestradiol and SHBG levels were significantly higher after 10 years of transdermal oestradiol, alone or combined with micronised oral progesterone, without differences observed in serum metabolic parameters. More research through randomised clinical trials is required.

Keywords: Post-menopausal women, metabolic variables, long-term hormone treatment, transdermal oestradiol, micronised progesterone

Introduction

Hormone treatment (HT) has been used for several decades in menopausal women to relieve vasomotor symptoms, treat vaginal discomfort and prevent osteoporosis. However, its use has significantly decreased basically due to the results of the Women's Health Initiative Study (WHI) that pointed out that risks were higher than benefits owing to increased gynaecological cancer and lack of cardiovascular risk protection [1–6]. Although the effect on vasomotor/

urogenital symptoms and bone metabolism is similar independent of oestrogen type and route of administration, oral users, as compared to transdermal ones, display higher cardiovascular and cancer risks. Risks are also higher when synthetic progestin compounds are used as compared to micronised progesterone treatment [7–9]. Thus, adverse effects of HT rely basically on the type of oestrogen and progestin formulation, dosage, route of administration, women's age, body weight, associated diseases and treatment duration.

The combination of transdermal oestradiol and micronised progesterone appears to be effective and relatively safe if elementary precautions are taken, and currently the best choice for HT in most post-menopausal women. Despite the fact that in general HT use has decreased and been limited to the strict period needed to neutralise vasomotor/urogenital symptoms [6,10], some women use it for long periods and feel quite confident with their results rather than those of other therapeutical recommendations. The objective of this study was to analyse metabolic/hormonal parameters and frequency of the metabolic syndrome (METS) in post-menopausal women after 10 years of HT with transdermal oestradiol, alone or combined with micronised oral progesterone.

Material and methods

Population

We previously reported a retrospective series of parallel cohorts of post-menopausal women attending the Menopause Unit of the University of Granada San Cecilio Clinic Hospital of Granada, Spain, who had completed 10 years of HT ($n=223$) and a control group ($n=50$) to whom fatal cardiovascular disease risk was measured before and after [11]. This study represents a sub-analysis of 151 subjects of the total cohort who completed 10 years of HT and had complete data on metabolic/hormonal parameters and the METS frequency. At baseline, women HT users had a mean age of 49.8 ± 3.0 years (median 50.0 years) and initiated one of the following regimens: transdermal oestradiol (50 μg) ($n=22$), sequential cyclic HT with transdermal oestradiol (50 $\mu\text{g}/\text{day}$) plus 200 mg/day natural micronised oral progesterone (cycle days 12–25) ($n=83$) and continuous combined HT using transdermal oestradiol (50 μg) plus 100 mg/day of micronised oral progesterone ($n=46$). Thirty five subjects of the original control group after 10 years presented complete metabolic/hormonal data. Total cholesterol (TC; mg/dl), high-density lipoprotein cholesterol (HDL-C; mg/dl), low-density lipoprotein cholesterol (LDL-C; mg/dl), very low-density lipoprotein cholesterol (VLDL-C; mg/dl), glucose (mg/dl), triglycerides (TG; mg/dl), oestradiol (pg/ml) and sex hormone binding globulin (SHBG; nmol/l) levels were measured at baseline and 10 years later. TC, TG, LDL-C, VLDL-C, HDL-C and glucose levels were assayed with a Hitachi DPP photometric analyser (Roche Diagnostics GmbH, Mannheim, Germany). Oestradiol and SHBG levels were determined using the electrochemiluminescence immunoassay method (oestradiol with Elecsys Oestradiol II reagents from Roche Diagnostics GmbH, Mannheim, Germany; and SHBG with Immulite 2000 reagents from Siemens Solutions Diagnostics Limited Gwynedd,

UK). Analytic sensitivity and accuracy was 5.0 pg/ml and 2.6% and 0.02 nmol/l 6.6% for oestradiol and SHBG, respectively. Body mass index (BMI, kg/m^2) and blood pressure (BP, mmHg) determinations were recorded at same period intervals.

All participants complained of mild to severe vasomotor symptoms and vaginal discomfort related to atrophy without inflammatory signs. They had ceased menstruation due to natural causes ($n=116$; 62.4%) or surgery ($n=70$; 37.6%) including bilateral oophorectomy ($n=68$) for benign conditions (fibroids, endometriosis) or previous malignant disease (one ovarian cancer and one cervical cancer who initiated HT 5 years after surgery). Post-menopausal status was confirmed by a serum oestradiol level of <20 pg/ml or by previous bilateral oophorectomy. Each participant was yearly followed up, including physical examination to determine their health status, blood tests, ultrasound and mammography. None were on any chronic medication. The METS was defined by the presence of three of the following risk factors: TG ≥ 150 mg/dl, HDL-C < 50 mg/dl, systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg, basal glucose ≥ 100 mg/l and obesity (BMI > 30 kg/m^2).

This retrospective study was approved by the San Cecilio Hospital Ethics Committee. Data was obtained from individual's medical records. Women were informed about the study purpose and signed an informed consent for this analysis.

Statistical analysis

Statistical analysis was carried out using SPSS software package (Version 13.0 for Windows, SPSS, Chicago, IL). Data are presented as mean \pm standard deviations, medians and frequencies. Kolmogorov–Smirnov's test was used to determine normal distribution. According to this, ANOVA (parametric) or Kruskal–Wallis test (non-parametric) were used to compare means between studied groups whereas the paired *t*-student's (parametric) or Wilcoxon's signed rank (non parametric) tests for differences within groups (baseline vs. after 10 years). Frequencies were compared with chi-square calculation. A *p*-value of < 0.05 was considered statistically significant.

Results

Baseline characteristics, BMI, oestradiol and SHBG levels, metabolic parameters, BP values (systolic and diastolic) and METS frequencies among studied groups at baseline and after 10 years are depicted on Table I. BMI and METS frequency did change in any of the HT regimens after 10 years. Serum oestradiol and SHBG levels were found significantly higher after 10 years of transdermal oestradiol HT, alone or combined to oral micronised progesterone.

Table I. General and metabolic variables in young post-menopausal women (HT and non-HT users) before and after 10 years.

	Non HT use (control) (n = 35)	Transdermal oestradiol (n = 22)	Transdermal oestradiol + micronised progesterone (n = 83) (sequential cyclic)	Transdermal oestradiol + micronised progesterone (n = 46) (continuous combined)
Age at baseline	49.7 ± 2.7	50.0 ± 2.7	50.0 ± 3.1	49.5 ± 3.0
Tobacco use				
Baseline (%)	0 (0%)	0 (0%)	5 (6%)	2 (4.3%)
10 years (%)	0 (0%)	0 (0%)	5 (6%)	2 (4.3%)
Type of menopause				
Natural (%)	20 (57.1%)	0 (0%)	61 (73.5%)	35 (76.1%)
Surgery (%)	15 (42.9%)	22 (100%)	22 (26.5%)	11 (23.9%)
Body mass index (BMI kg/m ²)				
Baseline (mean)	28.8 ± 4.2	29.6 ± 4.3	27.5 ± 4.3	27.3 ± 4.2
10 years (mean)	29.3 ± 6.7	29.4 ± 3.6	28.4 ± 4.4	28.5 ± 4.1
Baseline BMI 25–30 (%)	17 (48.5%)	8 (36.3%)	28 (33.8%)	16 (34.8%)
10 years BMI 25–30 (%)	14 (40%)	10 (45.5%)	31 (37.3%)	19 (41.3%)
Baseline BMI > 30 (%)	12 (34.3%)	10 (45.5%)	25 (30.1%)	12 (26.1%)
10 years BMI > 30 (%)	16 (45.7%)	8 (38.1%)	29 (34.6%)	15 (34.1%)
Oestradiol (pg/ml)				
Baseline	10.8 ± 6.3	12.2 ± 14.3	17.8 ± 21.4	21.6 ± 20.5 [†]
10 years	10.7 ± 5.0	33.0 ± 16.4*	32.4 ± 22.5*	34.8 ± 31.9* [†]
SHBG (nmol/l)				
Baseline	36.6 ± 21.2	39.9 ± 23.9	37.8 ± 26.4	32.3 ± 23.1
10 years	46.4 ± 22.6	60.6 ± 30.7*	50.9 ± 20.6*	52.7 ± 31.6*
Glucose (mg/dl)				
Baseline	90.7 ± 10.7	93.0 ± 10.1	94.6 ± 19.4	89.1 ± 18.4
10 years	94.5 ± 28.8	96.6 ± 8.0	101.2 ± 23.0*	92.4 ± 25.4
Triglycerides (mg/dl)				
Baseline	124.2 ± 73.6	120.9 ± 54.4	113.6 ± 58.7	113.4 ± 47.4
10 years	130.5 ± 91.6	102.2 ± 49.4	115.6 ± 57.4	110.4 ± 43.0
Total cholesterol (mg/dl)				
Baseline	233.7 ± 36.1	227.8 ± 30.8	227.9 ± 44.3	228.8 ± 32.3
10 years	237.0 ± 35.7	229.7 ± 28.1	226.9 ± 36.3	220.1 ± 32.8
HDL-C (mg/dl)				
Baseline	61.4 ± 15.1	60.4 ± 20.9	60.0 ± 17.0	62.9 ± 15.7
10 years	63.8 ± 21.4	70 ± 18.2	64.3 ± 15.9	60.5 ± 16.9
LDL-C (mg/dl)				
Baseline	141.1 ± 35.1	135.8 ± 43.0	141.7 ± 39.9	144.2 ± 32.3
10 years	140.9 ± 37.1	138.1 ± 33.9	131.3 ± 45.1	140.6 ± 26.0
VLDL-C (mg/dl)				
Baseline	25.3 ± 11.6	21.8 ± 10.2	21.5 ± 16.3	20.5 ± 11.6
10 years	28.6 ± 25.7	21.2 ± 10.4	22.2 ± 13.5	24.2 ± 13.6
Systolic BP (mmHg)				
Baseline	131.7 ± 16.8	128.6 ± 33.1	133.0 ± 30.5	140.5 ± 34.9
10 years	143.0 ± 20.3*	140.6 ± 15.9	139.2 ± 15.8	138.1 ± 25.6
Diastolic BP (mmHg)				
Baseline	82.4 ± 11.3	75.7 ± 23.0	81.1 ± 17.4	84.8 ± 18.0
10 years	89.4 ± 10.2*	83.9 ± 10.9	82.3 ± 15.6	81.5 ± 18.5
METS				
Baseline (%)	15 (42.9%)	9 (40.9%)	31 (37.3%)	20 (43.6%)
10 years (%)	15 (42.9%)	5 (22.7%)	35 (42.2%)	22 (47.8%)

BP: blood pressure; BMI: body mass index; METS: metabolic syndrome; HDL-C: high density lipoprotein cholesterol; VLDL-C: very low density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; SHBG: sex hormone binding globulin; METS: metabolic syndrome.

Data are presented as mean ± standard deviations or frequencies.

**p* < 0.05 as compared to baseline using paired *t*-student's or Wilcoxon's signed rank test.

[†]*p* < 0.05 among groups using ANOVA or Kruskal–Wallis test.

These values were significant higher when compared to the control group. Glucose levels after 10 years of transdermal oestradiol and cyclic micronised oral progesterone were significantly higher, with no other

changes in remaining metabolic parameters. After 10 years, both systolic and diastolic BP determinations were higher in the control group as compared to any of the HT using groups (Table I).

In the 10-year period, there were two non-fatal cardiovascular events. The first was a stroke occurring in a woman 10 years after using continuous combined oestrogen plus progesterone treatment. Patient discontinued treatment afterwards. The second was an acute myocardial infarction in a woman who previously had a hysterectomy and was treated with the transdermal oestrogen alone regimen; she decided to continue HT despite contrary clinical recommendation.

Discussion

Although non-steroid ovarian hormones present significant changes during the menopausal transition, oestradiol secretion presents a slight age dependant rise at the onset of this transition and remains unchanged until the late perimenopause, presumably in response to elevated FSH levels [12,13]. In women, SHBG levels decline with age at a slightly greater rate between 49 and 54 years reflecting chronological aging and ovarian aging factors [14]. Plasma SHBG levels regulate free and albumin-bound androgen and oestrogen levels [15]. BMI may influence serum oestradiol and SHBG levels during the natural menopausal transition. Thus, elevated BMI values correlate with decreasing oestradiol and SHBG concentrations in peri- and post-menopausal women [16–18]. These relationships between weight and hormone levels have clinically been related to hot flush presence and severity [18].

This study was drawn upon young post-menopausal women who had used HT based on transdermal oestradiol, alone (hysterectomised women), or associated to oral micronised progesterone (intact uterus) given cyclically or in a continuous combined fashion. Subjects were otherwise healthy upon HT initiation yet the majority had elevated body weight (overweight or obesity) and high METS rates. Participants were all periodically checked by routine gynaecological follow up to rule out incidental pathology or adverse events. In addition, a significant number of studied women were non-smokers throughout the 10 year observational period. These body characteristics would be representative of the general Spanish female population of similar age, with little or low physical activity. This selection bias may be related to the fact that women with elevated weight most frequently complain of vasomotor symptoms and are more prone to use HT [18,19]. Despite this, there were no differences in BMI values before and after 10 years within and between studied groups (HT users and non-users). The three different HT regimens were well accepted with a high degree of individual satisfaction and controlled for their climacteric symptoms. Transdermal oestradiol is rapidly absorbed through the skin, avoiding high initial hormonal peaking concentrations [9]. Women

using transdermal oestradiol alone in the present series displayed a mild, but significant increase in serum oestradiol levels after 10 years, value which was significantly higher than those of the control group, and yet similar to those who used transdermal oestradiol associated to continuously or cyclically oral progesterone, which also displayed a significant increase after 10 years. This is suggestive that women with elevated body weight using fixed transdermal doses (with or without progesterone) increase serum oestradiol levels to a similar extent. This effect may be positive to control urogenital and vasomotor symptoms (optimal since these were the main reasons for HT use), and also, although not determined, protect skin and bone metabolism and improve cardiovascular function. In addition, it may also favour – in some way – cancer development and haemostatic alterations [6,9,10,20]. These benefits and risks were not assessed in this report; however, we hope future proteomic research will aid in identifying individual risks before treatment is initiated. Unfortunately, we also did not have the opportunity to evaluate quality of life before and after long term HT, despite the fact that women were periodically assessed and decided to continue HT.

Controversy exists regarding the extent to which age, menopausal status, diseases and lifestyle behaviours account for the increased weight experienced by mid-aged women [6,21,22]. Our results show that both women – HT users and non-users – displayed no significant increases in BMI after 10 years. This may suggest that women – with an already elevated weight – maintained their lifestyle habits to prevent further weight gain. Nevertheless, age-related changes in body composition could not be ruled out despite BMI remaining stable moreover if abdominal circumference or other anthropometric measures were not assessed.

Observational studies support the hypothesis that post-menopausal HT may reduce the risk of atherosclerotic disease manifestations, although the cardiovascular protective effect of such therapy is still controversial. Oestradiol plus progestogen favour positive changes in cardiovascular risk markers and outcomes [9,11,23–26]. Oral oestrogen therapy improves lipid profile, insulin resistance and carotid blood flow, which is reduced when a progestagen is associated [27]. Transdermal oestradiol treatment has beneficial effects on glucose metabolism and TG levels that may contribute to maintain endothelium function [9,25,28–31]. In women with type 2 diabetes transdermal oestradiol (with or without a progestogen) did not adversely affect markers of cardiovascular risk [32]. Despite this, controversy exists regarding HT route and dosage that best improves the metabolic profile basically due to the fact that studies among women with the METS are short-term [33–36]. On the other hand, short term

oral and transdermal HT have different impact on markers of inflammation, coagulation and fibrinolysis which may affect clinical endpoints in post-menopausal women [9,37]. Women treated in this series with transdermal oestradiol and cyclic progesterone displayed higher fasting glucose levels after 10 years of treatment; however, this did not differ when compared to controls or the other HT regimens. Insulin resistance in non HT using post-menopausal women may be explained by lower oestradiol/SHBG levels and higher BMI values [38]. In our series oestradiol/SHBG levels increased after 10 years with no change in BMI, hence explaining higher glucose levels for those using cyclic progesterone is difficult and surely related to the limitations of the retrospective small sample design.

SHBG in post-menopausal obese women positively correlates with HDL-C and negatively with fasting insulin and fasting/postprandial glucose levels [39,40]. Low SHBG levels are inversely strong predictors of type 2 diabetes in both women and men [40]. In addition, women with higher SHBG levels are associated with higher HDL-C and lower LDL-C levels [41]. In post-menopausal women, different HT regimens produce changes in circulating SHBG levels according to formulations and duration of therapy [15,42,43]. In the present series after 10 years all HT using groups presented higher SHBG levels. It could be speculated that higher SHBG levels be related to the increased oestradiol levels achieved during treatment. Supporting this is the fact that progesterone addition in the other regimens conferred no difference.

No changes in BP determinations were found after 10 years in any of the HT using groups, whereas both systolic and diastolic BP significantly increased among non-HT users. Maintenance of BP may depend upon the balance between the hypertensive effect of angiotensin II and the hypotensive effect of bradykinin [44]. The renin angiotensin system also plays an important role in maintaining BP in normotensive post-menopausal women receiving HT. It seems that HT may stabilise and even lower BP, especially when the transdermal route is used, alone or associated to oral progesterone (as used in this series). Our results fit well with the report showing that BP remains stable after 12 months of treatment with transdermal oestradiol combined with oral medroxyprogesterone acetate 2.5 mg/day [45].

Cardiovascular disease risk increases after the menopause, which may be related to the metabolic changes occurring during the female menopausal transition. The METS is a constellation of risk factors related to increased cardiovascular risk [46]. Its definition has been recently unified by different international scientific societies [47], fixing single set values for TGs, HDL-C, glucose and BP while waist

circumference has not been unanimously defined due to ethnic and racial differences. Lera Orsatti et al. [48] have reported that waist circumference > 88 cm significantly correlates with BMI ($r=0.88$). In our series, BMI > 30 kg/m² was included as an indirect measure of obesity to define one cluster of the METS. METS rate was high among all studied groups at baseline in the present series. Despite this, no differences were seen between groups. Interestingly after 10 years no significant increase was observed for any of the studied groups. Concerning morbidity of our series, there were two non-fatal cardiovascular events among those using HT. HT, especially oral oestrogen, has been associated to the risk of venous thrombosis or stroke in post-menopausal women with elevated body weight (obesity) and those with prothrombotic mutations [49–51].

Finally as for the limitations of the present series one can mention its retrospective nature that includes small number of cases. Moreover, we present data of women who continued follow up yet not of those who chose to discontinue HT, perhaps mostly after the WHI study due to mass media information suggesting discontinuation. Analysing only these women may also be seen as a potential selection bias moreover if one considers that they could be considered as healthier. Elevated body weight – either overweight or obesity – may be the single METS risk factor that, when corrected, significantly reduces hypertension, hyperlipidaemia, type 2 diabetes mellitus and cardiovascular risk [52,53]. A high rate of women in the present series presented either overweight or obesity upon HT initiation which remained stable after 10 years. Waist circumference may be a better predictor than BMI in defining the METS since it may be directly related to visceral adiposity and insulin resistance [54,55]. Unfortunately baseline waist circumference values were not available, as this was not relevant 10 years ago.

In conclusion, long-term HT is plausible in selected women without significant risks. As reported here transdermal oestradiol, alone or in combination with natural progesterone, seems a feasible choice for women with elevated body weight, moreover if no major differences in metabolic parameters were observed. Despite this, mentioned limitations do not allow definitive conclusion and calls for the designing of large randomised clinical trials.

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