Abstract. – OBJECTIVE: The aim of the study was to systematically review and meta-analyze the available data on changes in the hormonal profile of postmenopausal women treated with hormone replacement therapy (HRT).

MATERIALS AND METHODS: Full-text articles published up to April 30, 2021, were searched through PUBMED, EMBASE, the Cochrane library and Web of Science (WOS) databases and were screened strictly according to inclusion criteria. Randomized clinical trials and case control studies were enrolled. Studies not reporting steroid serum levels or not providing a control group were excluded from the analysis. Studies enrolling women with genetic defects or severe chronic systemic diseases were excluded. Data are expressed as standardized mean differences (SMDs) with 95% confidence intervals (CIs). Random effect models were used for the meta-analysis.

RESULTS: HRT administration increases estradiol (E2) and reduces follicle stimulating hormone (FSH) serum levels compared with pre-treatment. Their changes are evident when oral and transdermal HRT are administered, while vaginal HRT not. No significant effect on E2 and FSH was found between 6 and 12 months, as well as between 12 and 24 months. No significant effect on E2 and FSH was shown between different regimes. No difference was observed between different HRT regarding their effect on lipid profiles, breast pain and vaginal bleeding, but oral estrogen combined synthetic progestin caused a reduction in sex hormone-binding globulin (SHGB). This might be crucial when choosing the best possible treatment for each patient individually taking into consideration if potential benefits outweigh the risks.

Key Words: Hormone replacement therapy, Estrogen, Progestin, Estradiol, Follicle stimulating hormone, Sex hormone-binding globulin.

Introduction

Decreasing levels of estrogens during menopause are associated with the increased incidence of cardiovascular diseases, dementia, Alzheimer’s diseases and osteoporosis, and if not treated, aging is accelerated. Hormone replacement therapy (HRT) is also believed to prevent various symptoms during the menopause, such as hot flushes and night sweats. In addition, loss of estrogen protection is often accompanied by a subsequent change in follicle-stimulating (FSH) levels, which has been widely considered a marker for poor ovarian reserve at whatever age they occur. Major attention was posed to the need to inform women of the sex hormones of different HRT routes (oral, transdermal or vaginal) so that they can make appropriate treatment choices.

HRT formulations can be classified by estrogen [included conjugated equine oestrogen (CEE) or 17β-E2] with or without progestin [included dydrogesterone (D), synthetic or natural progestin].

Previous studies evaluating the HRT influence on hormonal status were mostly conducted in analysis of one or two formulations, without
discriminating between the types of estrogen and progestin. Furthermore, in most observational studies, steroid variables were not the predominant results. Several studies demonstrating the available effect of transdermal and vaginal HRT, which have been summarized in the meta-analysis, have inconsistent results. However, current literature lacks a systematic review and meta-analysis to summarize the effect of hormonal profile changes in women receiving HRT treatments. Therefore, the aim of this article was to evaluate and summarize the existing data about hormonal profile changes in postmenopausal women receiving HRT, considering all available estrogen-progestin formulation and regimens and providing theoretical basis for personalized treatment.

Materials and Methods

Search Strategy

A review of the literature was performed following the PUBMED, EMBASE, the Cochrane library and Web of Science (WOS) databases: (((((((((Hormone) OR estrogen) OR FSH)) AND (((((Climacteric) OR Menopause) OR Menopause, Premature) OR Perimenopause) OR Postmenopause) OR Premenopause) OR Hot Flashes)) AND ((((((Hormone Replacement Therapy) OR Hormone Replacement Therapies) OR Therapy, Hormone Replacement) OR Replacement Therapies, Hormone) OR Therapies, Hormone Replacement) OR Replacement Therapy, Hormone) OR Estrogen Replacement Therapy). All studies published until July 30, 2021, were considered. The study selection process was conducted with a flowchart of Preferred Reporting Items for Systematic Reviews and Meta analyses (PRISMA).11 (Supplementary Table I).

Study Selection and Inclusion Criteria

The following inclusion criteria were searched: (1) all receiving HRT; (2) interventional study design; (3) studies enrolling women with 1-5 years amenorrhea and (4) evaluation of hormone levels. Both randomized clinical trials (RCTs) and case-control studies were eligible for inclusion in this article. However, enrolled participants with specific conditions (Turner syndrome, cancers and severe heart failure) were excluded.

Data Extraction and Quality Assessment

Two authors (LDH and ZSY) performed independently literature search and extracted data from the included studies evaluated for inclusion criteria. With regard to the design of the study, including author, region, study design, laboratory method used to measure the sex hormones, age, body mass index (BMI), diagnosis criteria, HRT types, sample sizes, administration duration and regimen, route, dosages and parameters. LDH, XLZ and ZSY performed quality control checks on extracted data. Data were extracted using steroid serum levels as primary end points, considering E2 and FSH. We recorded the different concentration units (pg/mL, ng/mL and nmol/L). All data were rechecked by LDH and ZSY.

Data Synthesis and Analysis

We classified HRT as any exposure to oral, transdermal or vaginal preparations, which were analyzed separately. Moreover, HRT included estrogen only preparations (CEE and 17β-E2) and combined dydrogesterone, natural progestin or synthetic progestin (medroxyprogesterone acetate, norethisterone acetate and drospirenone). We also analyzed different regimens (cyclical or continuous, estrogen monotherapy or combined therapy). The time was categorized between 3 and 6 months, 6 and 12 months as well as 12 and 24 months. We also assessed the sex hormone-binding globulin (SHGB), lipid profiles [high density lipoprotein (HDL), low density lipoprotein (LDL), total serum cholesterol (TC) and triglycerides]. Our analysis included comparison that are used as oral or transdermal E treatments. In addition, we included two adverse effect results: vaginal bleeding and breast pain.

Quality Assessment

We assessed the quality of the selected studies using the Newcastle-Ottawa Scale (NOS) scoring system.12 Two members independently performed the NOS grade assessment. According to the quality score assessment, the total score ranged from 0 to 9. Studies with a score of 7 or above were considered high-quality, and studies with a score of 4 or below were considered low-quality. Studies with a score between 4 and 7 were considered medium-quality. Any disagreements between the two reviewers were discussed by consensus, or by involving a third reviewer.

Statistical Analysis

Hormone levels were described as mean ± standard deviation (SD) in most studies while extracted as median and range in one study.13 Standardized mean differences (SMDs) with 95% confidence intervals (CIs) were adopted to
Table 1 (continued). Characteristics of the included studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Region</th>
<th>Study design</th>
<th>Laboratory method</th>
<th>Age</th>
<th>BMI</th>
<th>Diagnosis criteria</th>
<th>HRT</th>
<th>N</th>
<th>Duration</th>
<th>Route</th>
<th>Dosages</th>
<th>Parameters</th>
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<tbody>
<tr>
<td>Gupta et al</td>
<td>USA</td>
<td>RCT</td>
<td>RIA</td>
<td>56.9</td>
<td>25.9 (3.3)</td>
<td>2 years of amenorrhea</td>
<td>17β-E2 17β-E2</td>
<td>9</td>
<td>3 months</td>
<td>vaginal per day</td>
<td>14 ug/7.5 ug</td>
<td>E2, FSH</td>
</tr>
<tr>
<td>Raudaskoski et al</td>
<td>Finland</td>
<td>RCT</td>
<td>RIA</td>
<td>51</td>
<td>26.9 ± 1.3</td>
<td>6 months of amenorrhea, FSH &gt; 28 IU/L</td>
<td>17β-E2 17β-E2 + NETA</td>
<td>15</td>
<td>1 year</td>
<td>oral once daily</td>
<td>50 ug/2/1 mg</td>
<td>E2</td>
</tr>
<tr>
<td>Tupikowska et al</td>
<td>Poland</td>
<td>RCT</td>
<td>CLIA</td>
<td>50.6</td>
<td>26.9 ± 2.6</td>
<td>typical of the climacteric syndrome, FSH &gt; 30 IU/L and E2 &lt; 30 pg/mL</td>
<td>17β-E2 17β-E2 + progestin</td>
<td>26</td>
<td>4 months</td>
<td>transdermal per day</td>
<td>50 ug/5/10 mg</td>
<td>E2, FSH</td>
</tr>
<tr>
<td>Soranna et al</td>
<td>Italy</td>
<td>RCT</td>
<td>RIA</td>
<td>47-56</td>
<td>27.0</td>
<td>No HRT for ≥ 6 months, 1 year of amenorrhea, FSH &gt; 35 IU/L and E2 &lt; 92 pmol/L</td>
<td>Placebo 17β-E2 + D</td>
<td>10</td>
<td>3 months</td>
<td>oral once daily</td>
<td>2 mg/2/5 mg/2/10 mg</td>
<td>E2, FSH</td>
</tr>
<tr>
<td>Chiantera et al</td>
<td>Italy</td>
<td>cohort study</td>
<td>/</td>
<td>49.3</td>
<td>24.6</td>
<td>1 year of amenorrhea, FSH &gt; 30 IU/L and E2 &lt; 20 pg/mL</td>
<td>Placebo 17β-E2 + D + control</td>
<td>20</td>
<td>24 months</td>
<td>oral once daily + oral once daily</td>
<td>500 mg/500 mg</td>
<td>E2, FSH</td>
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<tr>
<td>Villa et al</td>
<td>Italy</td>
<td>RCT</td>
<td>RIA</td>
<td>52.6</td>
<td>3.7 ± 1.1 years postmenopausal, FSH &gt; 50 IU/L and E2 &lt; 73 pmol/L</td>
<td>hemihydrate E + D</td>
<td>10</td>
<td>2 months</td>
<td>oral once daily + oral once daily</td>
<td>2/10 mg</td>
<td>FSH, E2</td>
<td></td>
</tr>
<tr>
<td>Sztefko et al</td>
<td>Poland</td>
<td>cohort study</td>
<td>RIA</td>
<td>54.5</td>
<td>27.5</td>
<td>FSH &gt; 30 mU/mL, E2 &lt; 50 pg/mL</td>
<td>17β-E2 + D 17β-E2 + D</td>
<td>25</td>
<td>12 months</td>
<td>transdermal per day</td>
<td>0.05/5 mg/2/10 mg</td>
<td>FSH</td>
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<tr>
<td>Tobias</td>
<td>UK</td>
<td>RCT</td>
<td>RIA</td>
<td>55.2</td>
<td>NS</td>
<td>amenorrhea for &gt; 6 months, E2 &lt; 40 pmol/L</td>
<td>17β-E2 17β-E2 + D</td>
<td>10</td>
<td>12 months</td>
<td>oral once daily</td>
<td>2 mg/2/10 mg</td>
<td>E2</td>
</tr>
<tr>
<td>Rizzo et al</td>
<td>Italy</td>
<td>open label</td>
<td>RIA</td>
<td>50.5</td>
<td>27.7</td>
<td>1 year of amenorrhea, FSH &gt; 30 IU/L, E2 &lt; 20 pg/mL</td>
<td>17β-E2 + D 17β-E2 + DRSP</td>
<td>80</td>
<td>6 months</td>
<td>oral once daily</td>
<td>1/5 mg/1/2 mg</td>
<td>E2</td>
</tr>
<tr>
<td>Nii et al</td>
<td>Japan</td>
<td>open label</td>
<td>RIA</td>
<td>49.0</td>
<td>21.4</td>
<td>a menopausal interval of &lt; 1-year, climacteric symptoms</td>
<td>17β-E2 17β-E2 CEE</td>
<td>15</td>
<td>3 months</td>
<td>oral once daily</td>
<td>50 ug/1 mg/0.625 mg</td>
<td>E2, FSH</td>
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</table>
# Table I. Characteristics of the included studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Region</th>
<th>Study design</th>
<th>Laboratory method</th>
<th>Age</th>
<th>BMI</th>
<th>Diagnosis criteria</th>
<th>HRT</th>
<th>N</th>
<th>Duration</th>
<th>Route</th>
<th>Dosages</th>
<th>Parameters</th>
</tr>
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<tbody>
<tr>
<td>Chen et al</td>
<td>China</td>
<td>RCT</td>
<td>/</td>
<td>50.19 ± 4.11</td>
<td>/</td>
<td>No HRT for ≥ 6 months, non-hysterectomized postmenopausal women</td>
<td>JWSYS (Chinese herb) 17β-E2 + MPA</td>
<td>24</td>
<td>4 months</td>
<td>oral 3 times a day oral once daily</td>
<td>4 g 100 mg</td>
<td>E2, FSH</td>
</tr>
<tr>
<td>Sztefko et al</td>
<td>USA</td>
<td>RCT</td>
<td>RIA</td>
<td>61.50 ± 7.00</td>
<td>29.00 ± 5.50</td>
<td>/</td>
<td>Placebo 17β-E2</td>
<td>109</td>
<td>2 years</td>
<td>oral once daily / oral once daily</td>
<td>/ 0.625/100 mg</td>
<td>E2</td>
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<tr>
<td>Woo et al</td>
<td>China</td>
<td>RCT</td>
<td>ELISA</td>
<td>56.2 ± 4.9</td>
<td>23.8 ± 3.4</td>
<td>1 year of amenorrhea</td>
<td>CEE + MPA Pueraria lobata Control</td>
<td>43</td>
<td>3 months</td>
<td>oral once daily oral once daily</td>
<td>0.625/10 mg</td>
<td>E2, FSH</td>
</tr>
<tr>
<td>Cortellaro et al</td>
<td>Italy</td>
<td>RCT</td>
<td>RIA</td>
<td>50, 42-56</td>
<td>24.8 ± 2.5</td>
<td>typical climacteric syndromes, and a Kupperman index of over 14</td>
<td>17β-E2 + MPA</td>
<td>25</td>
<td>4 months</td>
<td>Transdermal daily oral once daily</td>
<td>0.05/10 mg</td>
<td>E2</td>
</tr>
<tr>
<td>Hofling et al</td>
<td>Sweden</td>
<td>RCT</td>
<td>RIA</td>
<td>57.1</td>
<td>13.8 ± 3.8</td>
<td>1 year of amenorrhea, FSH &gt; 40 IU/L and E2 &gt; 70 pmol/L</td>
<td>17β-E2 + MPA + NETA Tibolone Placebo</td>
<td>48</td>
<td>6 months</td>
<td>oral once daily oral once daily</td>
<td>2/1 mg 2.5 mg</td>
<td>E2</td>
</tr>
<tr>
<td>Erdem et al</td>
<td>Turkey</td>
<td>RCT</td>
<td>RIA</td>
<td>50.5 ± 4.4</td>
<td>28.1 ± 2.73</td>
<td>1 year of amenorrhea</td>
<td>CEE + MPA Control</td>
<td>40</td>
<td>3 months</td>
<td>oral once daily / oral once daily</td>
<td>0.625/5 mg</td>
<td>E2</td>
</tr>
<tr>
<td>Coksuer et al</td>
<td>Turkey</td>
<td>RCT</td>
<td>RIA</td>
<td>50.8 ± 3.05</td>
<td>51.7 ± 1.6</td>
<td>total hysterectomy and bilateral salpingo-oopherectomy</td>
<td>17β-E2 + DRSP</td>
<td>32</td>
<td>6 months</td>
<td>oral once daily</td>
<td>1/2 mg</td>
<td>E2, FSH</td>
</tr>
<tr>
<td>Xia et al</td>
<td>China</td>
<td>RCT</td>
<td>CLIA</td>
<td>50.7 ± 2.2</td>
<td>13.8 ± 3.8</td>
<td>1 year of amenorrhea, FSH &gt; 30 IU/L and E2 &lt; 20 pg/mL</td>
<td>17β-E2</td>
<td>11</td>
<td>3 months</td>
<td>transdermal, changed once per week</td>
<td>2/1 mg 1.5 mg/patch</td>
<td>E2, FSH</td>
</tr>
<tr>
<td>Fernandes et al</td>
<td>Brazil</td>
<td>RCT</td>
<td>RIA, CLIA</td>
<td>56.4 (4.8)</td>
<td>22.7 ± 2.1</td>
<td>postmenopausal women with urogenital atrophy</td>
<td>CEE placebo</td>
<td>18</td>
<td>3 months</td>
<td>vaginal vaginal</td>
<td>0.625 mg 3g</td>
<td>E2, FSH</td>
</tr>
<tr>
<td>Pan et al</td>
<td>China</td>
<td>cohort study</td>
<td>CLIA</td>
<td>50.29 ± 2.87</td>
<td>22.2 ± 2.1</td>
<td>1 year of amenorrhea, FSH &gt; 40 IU/L and E2 &lt; 20 pg/mL</td>
<td>CEE + MPA Placebo</td>
<td>15</td>
<td>3 months</td>
<td>oral once daily oral once daily</td>
<td>0.625/5 mg 200 mg</td>
<td>E2, FSH</td>
</tr>
<tr>
<td>Benencia et al</td>
<td>Argentina</td>
<td>RCT</td>
<td>RIA</td>
<td>51 ± 4.6</td>
<td>17β-E2 + NETA</td>
<td>1-5 years of spontaneous amenorrhea</td>
<td>a year 17β-E2 + promegestone</td>
<td>7</td>
<td>6 months</td>
<td>transdermal, changed 3-4 days</td>
<td>2/2.5 mg</td>
<td>E2</td>
</tr>
<tr>
<td>Sator et al</td>
<td>Austria</td>
<td>RCT</td>
<td>RIA</td>
<td>54.9, 45-68</td>
<td>17β-E2 + progestrone</td>
<td>Dornecol + 17β-E2 + progestrone Control</td>
<td>6</td>
<td>6 months</td>
<td>oral once daily vaginal</td>
<td>50 ug 50 ug/0.4 mg</td>
<td>E2, FSH</td>
<td></td>
</tr>
</tbody>
</table>

*Table continued...*
Table I (continued). Characteristics of the included studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Region</th>
<th>Study design</th>
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<th>Age</th>
<th>BMI</th>
<th>Diagnosis criteria</th>
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<th>N</th>
<th>Duration</th>
<th>Route</th>
<th>Dosages</th>
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<tr>
<td>Nii et al16</td>
<td>Japan</td>
<td>open label</td>
<td>RIA</td>
<td>49.0</td>
<td>21.4</td>
<td>a menopausal interval of &lt; 1-year, climacteric symptoms</td>
<td>17β-E2</td>
<td>15</td>
<td>3 months</td>
<td>transdermal per day</td>
<td>50 ug</td>
<td>E2, FSH</td>
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<tr>
<td>Yumru et al24</td>
<td>Turkey</td>
<td>cohort study</td>
<td>/</td>
<td>58.4 (46-67 years)</td>
<td>/</td>
<td>hysterectomy, climacteric symptoms</td>
<td>17β-E2</td>
<td>35</td>
<td>3 months</td>
<td>vaginal per day</td>
<td>25 ug</td>
<td>E2, FSH</td>
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<tr>
<td>Honisett et al34</td>
<td>Australia</td>
<td>RCT</td>
<td>CLIA</td>
<td>45-60 years</td>
<td>/</td>
<td>1-5 years amenorrhea</td>
<td>17β-E2 + MPA</td>
<td>10</td>
<td>5 months</td>
<td>transdermal per day + oral</td>
<td>50 ug/5 mg</td>
<td>E2, FSH</td>
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<tr>
<td>Villa et al30</td>
<td>Italy</td>
<td>RCT</td>
<td>RIA</td>
<td>52 ± 3.3</td>
<td>/</td>
<td>FSH &gt; 50 IU/L, E2 &lt; 73 pmol/L</td>
<td>17β-E2 + DRSP</td>
<td>17</td>
<td>6 months</td>
<td>oral once daily</td>
<td>1/2 mg</td>
<td>E2, FSH</td>
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<tr>
<td>Appelberg et al39</td>
<td>Finland</td>
<td>cohort study</td>
<td>/</td>
<td>56 (47-70)</td>
<td>/</td>
<td>amenorrhea, FSH &gt; 30 IU/L, E2 &lt; 0.2 nM</td>
<td>17β-E2 + norethisterone</td>
<td>21</td>
<td>6 months</td>
<td>transdermal per day + oral</td>
<td>50/250 ug</td>
<td>FSH</td>
</tr>
<tr>
<td>Matsui et al26</td>
<td>Japan</td>
<td>cohort study</td>
<td>/</td>
<td>50.6 ± 4.9</td>
<td>21.8 ± 2.9</td>
<td>1-year amenorrhoeic, FSH &gt; 40 IU/L, E2 &lt; 20 pg/L</td>
<td>17β-E2 + D</td>
<td>14</td>
<td>12 months</td>
<td>oral once daily</td>
<td>0.5/5 mg</td>
<td>E2, FSH</td>
</tr>
<tr>
<td>Maffei et al36</td>
<td>Italy</td>
<td>cohort study</td>
<td>RIA</td>
<td>51.9 ± 4.6</td>
<td>/</td>
<td>1-year amenorrhoeic, FSH &gt; 30 IU/L, E2 &lt; 25 pg/L</td>
<td>17β-E2 + dihydrogesterone</td>
<td>21</td>
<td>3 months</td>
<td>transdermal patch/gel + oral</td>
<td>50 ug/1 mg + 10 mg</td>
<td>E2, FSH</td>
</tr>
</tbody>
</table>

RCT= randomized clinical trial; E2 = estradiol; FSH = follicle stimulating hormone; BMI = body mass index; N = number. RIA = radioimmunoassay; CLIA = chemiluminescence immunoassay; ELISA = enzyme-linked immuno sorbent assay; RCT = Randomized Controlled Trial. HRT = hormone replacement therapy; CEE = included conjugated equine oestrogen; MPA = medroxyprogesterone acetate; D = dydrogesterone; NETA = norethisterone acetate; DRSP = drospirenone.
calculate the overall estimates\textsuperscript{14}. The odds ratio (OR) provided the measure of HRT efficacy that we analyzed\textsuperscript{14}. Heterogeneity across studies was quantified using the Q statistic and inconsistency index ($I^2$)\textsuperscript{14}. When $I^2 > 50\%$, heterogeneity was considered severe; when $I^2 \leq 25\%$, heterogeneity was considered low. In case of severe heterogeneity, a random-effects model was used. All evaluated papers were further analyzed with regard to the studies reported in the manuscript. Thus, different drug dosages, routes and schedules of administration were separately considered. The analysis was performed comparing patients to controls after treatment. Sensitivity analyses were performed considering the HRT used in the trials, distinguishing among different estrogen and progesterone administration. Moreover, a second sensitivity analysis was performed, considering the assay accuracy. We set the significance level for this study at 5%.

**Results**

We identified 3,593 cases from the literature search between 1991 and 2016. Of the whole cases, 1,661 patients satisfied the inclusion criteria and were assessed for data extraction (Figure 1).
The baseline characteristics, such as author, year of publication, age, body mass index (BMI), sample sizes, laboratory method used to measure the sex hormones, diagnosis criteria, HRT types, administration duration and regimen, dose and duration included in the studies are shown in Table I. The overall quality of these articles was relatively high (NOS score ≥ 6). The specific details are shown in Supplementary Table II.

### Effect of Different Duration Between HRT and Pre-Treatment on E2 Levels Among Postmenopausal Women

25 papers6-10,15-34 reported data on E2 levels comprising a total of 1,661 patients (1,262 treated women vs. 1,413 pre-treated ones). E2 serum levels were significantly higher in oral (SMD: 3.99 pmol/L, 95% CI 2.99, 5.00 pmol/L, p < 0.00001) and transdermal (SMD: 2.12 pmol/L, 95% CI 1.45, 2.79 pmol/L, p < 0.00001) HRT treated patients compared to the pre-treated ones (Figure 2). E2 serum levels did not change between vaginal HRT treated and pre-treated women (SMD: 0.29 pmol/L, 95% CI -0.37, 0.96 pmol/L, p = 0.39).

One paper29 reported data on E2 levels comprising a total of 28 patients (14 6-month treatment vs. 14 3-month treatment) comparing HRT treatment of 3 months and 6 months. Oral 17β-E2 (2 mg/d) was used in the study, combined with norethisterone acetate and promegestone. No significant difference was found between them on E2 levels (SMD: 0.04 pmol/L, 95% CI -0.70, 0.79 pmol/L, p = 0.96) (Supplementary Figure 1).

One paper29 reported data on E2 levels comprising a total of 106 patients (53 12-month treatment vs. 53 6-month treatment) comparing HRT treatment of 6 months and 12 months. Oral 17β-E2 (2 mg/d) was used in the study, combined with norethisterone acetate and promegestone. No significant difference was found between them on E2 levels (SMD: 0.03 pmol/L, 95% CI -0.35, 0.42 pmol/L, p = 0.86) (Supplementary Figure 2).

One paper25 reported data on E2 levels comprising a total of 40 patients (20 24-month treatment vs. 20 12-month treatment) comparing HRT treatment of 12 months and 24 months. Oral 17β-E2 (2 mg) and transdermal 17β-E2 (50 ug) were used in the study (combined with dydrogesterone). No significant difference was found between them on E2 levels (SMD: 0.14 pmol/L, 95% CI -0.30, 0.58 pmol/L, p = 0.93) (Supplementary Figure 3).

### Effect of Different Duration Between HRT and Pre-Treatment on FSH Levels Among Postmenopausal Women

FSH values were reported in 21 studies6,7,9,15-17,19,20,22-28,30,31,33-36. A total of 1,285 subjects were included (637 treated women vs. 648 pre-treated). FSH serum levels were significantly lower in oral (SMD: -1.89 IU/L, 95% CI -2.48, -1.31 IU/L, p < 0.00001) and transdermal (SMD: -1.21 IU/L, 95% CI -1.72, -0.69 IU/L, p < 0.00001) HRT treated patients compared to the pre-treated ones (Figure 3). FSH serum levels did not change between vaginal HRT treated and pre-treated women (SMD: -4.73 IU/L, 95% CI -11.07, 1.60 IU/L, p = 0.17).

Two papers25,35 reported data on FSH levels comprising a total of 144 patients (72 12-month treatments vs. 72 6-month treatments) comparing HRT treatment of 6 months and 12 months. Oral 17β-E2 (2 mg) was used in these two studies, combined with dydrogesterone. Transdermal 17β-E2 (50 ug) was in one (combined with dydrogesterone)25,35. No significant difference was found between them on FSH levels (SMD: 0.07 IU/L, 95% CI -0.26, 0.40 IU/L, p = 0.85) (Supplementary Figure 4).

One paper25 reported data on FSH levels comprising a total of 78 patients (39 24-month treatment vs. 39 12-month treatment) comparing HRT treatment of 12 months and 24 months. Oral 17β-E2 (2 mg) and transdermal 17β-E2 (50 ug) were in the study (combined with dydrogesterone). No significant difference was found between them on FSH levels (SMD: -0.10 IU/L, 95% CI -0.55, 0.34 IU/L, p = 0.97) (Supplementary Figure 5).

### Effect of Different Usages Between Transdermal and Oral HRT on E2 Levels Among Postmenopausal Women

Three studies15,16,22 were found, in which, transdermal 17β-E2 (1.5 mg or 50 ug) was compared with oral estrogen or combined therapy. Regarding the type and dose of estrogen, oral 17β-E2 (1 mg/d) and CEE (0.625 mg/d) were used in two, oral 17β-E2 (2 mg/d) in one. No significant difference was found between transdermal and oral HRT on E2 levels (SMD: 0.14 pmol/L, 95% CI: -0.54 to -0.26 pmol/L, p = 0.45) (Supplementary Figure 6).

Two studies30,22 comparing continuous with cyclical (sequential) HRT were identified on E2 levels. Transdermal 17β-E2 (50 ug) was used in two studies, transdermal 17β-E2 (50 ug) combined with progestin was used in two studies and one
Figure 2. Forest plot comparing estradiol (E2) serum levels between treatment and pre-treatment groups.

Figure continued
used oral 17β-E2 (2 mg/d) combined with proges-
terin. No significant difference was found between
continuous and cyclical HRT on E2 levels (SMD:
-0.37 pmol/L, 95% CI: -0.51 to 0.42 pmol/L, \( p =
0.85 \)) (Supplementary Figure 7).

Three studies\(^ {18,20,21} \) compared the effect on E2
levels caused by estrogen monotherapy with that
cased by combination therapy. Transdermal 17β-
E2 (50 ug) was used in two studies (two compar-
isons with, variously, norethidrone acetate and

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**Figure 2 (continued).** Forest plot comparing estradiol (E2) serum levels between treatment and pre-treatment groups.
### E2 only oral HRT.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental</th>
<th>Control</th>
<th>Std. Mean Difference</th>
<th>IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLR 2016</td>
<td>621.2 22.7</td>
<td>12 83.2 49.7</td>
<td>15 37.4%</td>
<td>-0.60 [-1.38, 0.18]</td>
</tr>
<tr>
<td>Sorensen 2002</td>
<td>21.2 3.8</td>
<td>10 53.1 5.2</td>
<td>10 26.1%</td>
<td>-6.71 [-9.19, -4.23]</td>
</tr>
<tr>
<td>Xia 2009</td>
<td>52.5 20.7</td>
<td>11 84.1 25.3</td>
<td>11 36.6%</td>
<td>-1.32 [-2.25, -0.38]</td>
</tr>
</tbody>
</table>

Total (95% CI) 33 36 100.0% -2.46 [-4.64, -0.27]

Heterogeneity: Tau² = 3.18; CH² = 21.27; df = 2 (P < 0.0001); P = 91%
Test for overall effect: Z = 2.20 (P = 0.03)

### CEE only oral HRT.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental</th>
<th>Control</th>
<th>Std. Mean Difference</th>
<th>IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLR 2016</td>
<td>47.9 21.9</td>
<td>13 102.2 35.3</td>
<td>15 100.0%</td>
<td>-1.76 [-2.66, -0.87]</td>
</tr>
</tbody>
</table>

Total (95% CI) 13 15 100.0% -1.76 [-2.66, -0.87]

Heterogeneity: Not applicable
Test for overall effect: Z = 3.87 (P = 0.0001)

### E2 only transdermal HRT.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental</th>
<th>Control</th>
<th>Std. Mean Difference</th>
<th>IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appel 1998</td>
<td>39.4 23.8</td>
<td>11 66.6 27.9</td>
<td>11 16.9%</td>
<td>-1.01 [-1.91, -0.11]</td>
</tr>
<tr>
<td>Gupta 2008</td>
<td>48.8 17</td>
<td>10 50.8 12</td>
<td>10 17.0%</td>
<td>-0.13 [-1.01, 0.75]</td>
</tr>
<tr>
<td>NLR 2016</td>
<td>35.6 30.5</td>
<td>11 104.2 15.42</td>
<td>15 15.3%</td>
<td>-2.92 [-4.06, -1.76]</td>
</tr>
<tr>
<td>Sato 2001</td>
<td>40.13 14.02</td>
<td>6 45.86 15.42</td>
<td>6 15.4%</td>
<td>-0.37 [-1.51, 0.78]</td>
</tr>
<tr>
<td>Tupkawes 2006</td>
<td>52.5 21.1</td>
<td>26 37.5 37.5</td>
<td>26 18.7%</td>
<td>0.49 [0.07, 0.94]</td>
</tr>
<tr>
<td>Xia 2009</td>
<td>56.6 22.4</td>
<td>11 92.3 31.8</td>
<td>11 16.7%</td>
<td>-1.25 [-2.18, -0.32]</td>
</tr>
</tbody>
</table>

Total (95% CI) 75 79 100.0% -0.81 [-1.74, 0.12]

Heterogeneity: Tau² = 1.12; CH² = 33.24; df = 5 (P < 0.00001); P = 85%
Test for overall effect: Z = 1.71 (P = 0.09)

### E2 only vaginal HRT.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental</th>
<th>Control</th>
<th>Std. Mean Difference</th>
<th>IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gupta 2008</td>
<td>59 16.2</td>
<td>11 53 15.1</td>
<td>11 41.8%</td>
<td>0.37 [0.49, 1.21]</td>
</tr>
<tr>
<td>Yunna 2009</td>
<td>54.45 16.3</td>
<td>35 63.21 17.69</td>
<td>35 58.2%</td>
<td>-0.51 [-0.99, -0.03]</td>
</tr>
</tbody>
</table>

Total (95% CI) 46 46 100.0% -0.14 [-0.99, 0.71]

Heterogeneity: Tau² = 0.26; CH² = 4.15; df = 1 (P = 0.06); P = 68%
Test for overall effect: Z = 0.33 (P = 0.74)

### CEE only vaginal HRT.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental</th>
<th>Control</th>
<th>Std. Mean Difference</th>
<th>IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farandee 2018</td>
<td>73.9 23.2</td>
<td>18 78.4 31.1</td>
<td>20 100.0%</td>
<td>-0.16 [-0.80, 0.48]</td>
</tr>
</tbody>
</table>

Total (95% CI) 18 20 100.0% -0.16 [-0.80, 0.48]

Heterogeneity: Not applicable
Test for overall effect: Z = 0.49 (P = 0.62)

### E2 combined dydrogesterone oral HRT.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental</th>
<th>Control</th>
<th>Std. Mean Difference</th>
<th>IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiarenzo 2003</td>
<td>36.5 14.2</td>
<td>19 68.9 15.1</td>
<td>19 18.2%</td>
<td>-2.16 [-2.98, -1.35]</td>
</tr>
<tr>
<td>Mattat 2014</td>
<td>78.1 26</td>
<td>14 83.4 39.7</td>
<td>14 18.4%</td>
<td>-0.12 [-0.87, 0.62]</td>
</tr>
<tr>
<td>Sorensen 2002</td>
<td>36.9 8.2</td>
<td>12 80.2 9.9</td>
<td>12 15.3%</td>
<td>-4.60 [-6.23, -2.97]</td>
</tr>
<tr>
<td>Sorensen 2002</td>
<td>32.3 3.1</td>
<td>11 82.3 12.4</td>
<td>11 12.9%</td>
<td>-6.29 [-8.51, -4.08]</td>
</tr>
<tr>
<td>Szeto 2005</td>
<td>32.2 19.4</td>
<td>8 63.2 24.4</td>
<td>8 17.3%</td>
<td>-1.33 [-2.44, -0.22]</td>
</tr>
<tr>
<td>villa 2007</td>
<td>35.14 22.58</td>
<td>10 63.1 27.33</td>
<td>10 17.8%</td>
<td>-1.07 [-2.22, -0.12]</td>
</tr>
</tbody>
</table>

Total (95% CI) 74 74 100.0% -2.35 [-3.73, -0.98]

Heterogeneity: Tau² = 2.52; CH² = 46.44; df = 5 (P < 0.00001); P = 90%
Test for overall effect: Z = 3.36 (P = 0.0008)

### E2 combined synthetic progesterone oral HRT.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental</th>
<th>Control</th>
<th>Std. Mean Difference</th>
<th>IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colkwoe 2011</td>
<td>22 5.3</td>
<td>32 57 11.9</td>
<td>32 33.1%</td>
<td>-3.75 [-4.58, -2.92]</td>
</tr>
<tr>
<td>Rizzi 2013</td>
<td>66.2 34.6</td>
<td>90 87 30.8</td>
<td>90 34.5%</td>
<td>-0.63 [-0.85, -0.41]</td>
</tr>
<tr>
<td>Villa 2011</td>
<td>25.1 20.41</td>
<td>17 96.4 27.87</td>
<td>17 32.4%</td>
<td>-2.85 [-3.84, -1.86]</td>
</tr>
</tbody>
</table>

Total (95% CI) 129 129 100.0% -2.38 [-4.56, -0.21]

Heterogeneity: Tau² = 3.55; CH² = 59.30; df = 2 (P < 0.00001); P = 97%
Test for overall effect: Z = 2.15 (P = 0.03)

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**Figure 3.** Forest plot comparing follicle-stimulating hormone (FSH) serum levels between treatment and pre-treatment groups. 
*Figure continued*
progesterone) and oral 17β-E2 (2 mg/d) in one (one comparison with dydrogesterone). No significant difference was found between continuous and cyclical HRT on E2 levels (SMD: 1.10 IU/L, 95% CI: -0.83 to 3.02 IU/L, p = 0.27) (Supplementary Figure 8).
Association between hormone replacement therapy and sex hormones in postmenopausal women

and dose of estrogen, transdermal 17β-E2 (50 ug) was in four (combined therapy), transdermal 17β-E2 (1.5 mg/d) in one, whereas in five studies oral 17β-E2 (1 mg/d or 2 mg/d) and CEE (0.625 mg/d) were used. No significant difference was found between transdermal and oral HRT on FSH levels (SMD: -0.04 IU/L, 95% CI: 0.77 to 0.03 IU/L, p = 0.45) (Supplementary Figure 9).

Three studies comparing continuous with cyclical (sequential) HRT were identified on FSH levels. Transdermal 17β-E2 (50 ug) was used in two studies, transdermal 17β-E2 (50 ug) in three studies was used and two used oral 17β-E2 (2 mg/d)20,22,35. No significant difference was found between continuous and cyclical HRT on FSH levels (SMD: -0.21 IU/L, 95% CI: -0.91 to 0.49 IU/L, p = 0.56) (Supplementary Figure 10).

Analysis SHBG Levels Comparing 17β-E2 Combined Synthetic Progesterone Oral HRT with Pre-Treatment Among Postmenopausal Women

Three studies21,28,32 compared the effect on SHBG levels caused by 17β-E2 combined synthetic progesterone oral HRT, and it showed a subsequent increase in SHBG level. No significant difference was found between them (SMD: 1.38 nmol/L, 95% CI: 1.04 to 1.72 nmol/L, p < 0.00001) (Figure 4).

Analysis of Adverse Reactions Comparing HRT with Pre-Treatment Among Postmenopausal Women

Lipid profiles were seen to have no significant difference between 17β-E2/CEE combined synthetic progesterone oral HRT and pre-treatment. No significant difference was found between them in LDL6,9,27,28,31 (SMD: 0.11, 95% CI: -0.19 to 0.41, p = 0.48) (Supplementary Figure 11), HDL6,9,27,28,31 (SMD: -0.35, 95% CI: -0.65 to -0.04, p = 0.03) (Supplementary Figure 12), TC6,9,27,28,31 (SMD: -0.31, 95% CI: -0.56 to -0.06, p = 0.02) (Supplementary Figure 13) and triglycerides6,8,9,27,28,31 (SMD: -0.19, 95% CI: -0.71 to 0.33, p = 0.48) (Supplementary Figure 14).

Four studies3,18,25,33 reported no significant difference in the incidence of vaginal bleeding between HRT and pre-treatment. No significant difference was found between them (OR = 1.78, 95% CI: 0.21 to 15.23) (Supplementary Figure 15).

Five studies8,18,19,22,25 reported that no significant difference of the incidence of breast cancer between HRT and pre-treatment. No significant difference was found between them (OR = 0.75, 95% CI: 0.18 to 3.09) (Supplementary Figure 15).

Discussion

To the best of our knowledge, no systematic research syntheses have been made on the effect of HRT on serum concentrations of sex steroids in postmenopausal women. Its unique characteristics are that we searched for comparison in order to clarify if and to what extent the effect if HRT is determined by the type of estrogen-progestin administration, the route of estrogen administration (oral, transdermal or vaginal), ratio at different time point of HRT administration and the mode of HRT administration (continuous or cyclical, monotherapy or combined therapy). This study indicated that HRT administration is capable of impacting serum E2 and FSH levels in postmenopausal women, varying with the types of estrogen-progestin compared with pre-treated concentrations after 2, 3, 4, 6, 12 and 24 months. HRT formulations can increase serum levels of E2, while it reduces the FSH levels compared with pre-treatment. However, the effect size is influenced by the HRT combination and routes. In the present meta-analysis, the E2 levels increase and FSH levels decrease were evident in patients treated with oral and transdermal estrogen (17β-E2 and CEE), combined with different progestin. Interestingly, the vaginal HRT
administration made no difference to E2 and FSH serum levels. In our results, we suggest one possible explanation to the effect that the pharmacokinetic results with the oral and transdermal HRT are greater than that with the vaginal one. Moreover, no reduction in E2 and FSH concentrations was observed between 3 and 6 months, 6 and 12 months, as well as between 12- and 24-months HRT treatment. We considered that the longitudinal approach allowed for a more detailed description of postmenopausal hormonal dynamics and has the potential to detect subtle changes that are not observed in cross-sectional studies due to inter-individual differences. Although the variations were not statistically significant, they were maintained within certain levels. These effects are consistent with the production of physiological steroids. It is well known that levels of FSH continue to be high during early menopause and remain elevated through the late stage of post menopause, with isolated high FSH values occurring even earlier sometimes. An increasing proportion of women presenting with elevated FSH values before menopause are accompanied by a significant decline of E2 levels, which is linked to accelerated bone loss. Hence, upon exogenous administration of estrogen, it is rapidly metabolized into its circulating products. As far as the effect of estrogen administration is concerned, the present study did not show any difference in E2 and FSH between transdermal and oral estrogen. Finally, the present study did not show any difference in the decrease in E2 and FSH concentrations according to the type of HRT regimen (continuous or cyclical, monotherapy or estrogen-progestin replacement therapy), which also supports the lack of effect of progestin in cyclical, although the fact that continuous represent greater progestin exposure than cyclical.

In postmenopausal women treated with HRT, with circulating E2 and SHBG altered. Although menopause is characterized by a marked reduction in E2, SHBG levels are only slightly reduced or not at all. Postmenopausal HRT with oral estrogen increases SHBG levels. Consistent with the results, our study showed a significant decline in SHBG levels on 17β-E2 combined with synthetic progestin. From a metabolic perspective, low SHBG has recently emerged as an independent marker of insulin resistance and risk of type 2 diabetes, although the interconnection is yet clear. Low SHBG concentrations in postmenopausal women are significantly related to a more adverse lipid and glucose profile, in spite of no significant change in lipid profiles in the literature.

Considering the differences in the measurement accuracy in steroid hormones between different laboratory techniques, we performed sensitivity analysis which did not change the results of our meta-analysis.

### Limitations

Our study has certain limitations. First, the period of treatment was of relatively short duration (3-6 months). Second, baseline hormone concentrations varied extensively among studies, thus clinical significance of a decrease in normal or relatively low baseline levels is unclear. Nevertheless, the study did not describe that estrogen correlated with overweight and fat mass. In a recent report, Liedtke et al demonstrated a positive correlation between BMI and E2. This is reinforced by the negative correlation between BMI and FSH previously reported. Therefore, BMI is of crucial importance for the hormone level in the post menopause.

### Conclusions

This meta-analysis is not designed to drive conclusions in favor or against HRT in menopause, but to point out the hormonal changes, which follow the hormonal administration. Whatever estrogen and progestin formulation we choose in postmenopausal women, the end results are a rise in E2 serum levels and a decrease in FSH serum levels according to the oral and transdermal combination reagent after 3 months. Moreover, continuous hormone medication can maintain the appropriate concentrations. Oral 17β-E2 combined synthetic progestin tended to raise SHBG level. The effect of oral and transdermal HRT does not depend on the regimen (cyclical or continuous), the route (transdermal or oral) and the type (monotherapy or combined therapy).

### Conflict of Interest

The Authors declare that they have no conflict of interests.

### Acknowledgements

None.

### Funding

No funding was secured or received for writing this systematic review.
**Data Availability**
The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Ethics Approval**
Not applicable.

**Informed Consent**
Not applicable.

**Authors’ Contributions**
LDH and ZSY performed independently literature search and extracted data from the included studies evaluated for inclusion criteria. LDH and XLZ was responsible for the statistical analysis and reviewed the manuscript. LDH and ZSY performed independently literature search and extracted data from the included studies evaluated for inclusion criteria. LDH and XLZ performed quality control checks on extracted data.

**Reference**
20) Bednarek-Tupikowska G, Filius A, Kuliczkowska-Płatasej J, Tupikowski K, Bohdanowicz-Pawlak A, Milewicz A. Serum leptin concentrations in pre-


