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Association between hormone replacement therapy and sex hormones in postmenopausal women: a systematic review and meta-analysis

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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).

CONCLUSIONS: The review suggested oral and transdermal HRT could lead to a rise in E2 serum levels and a decrease in FSH. The types and doses of HRT did not seem to modify the E2 and FSH level. Also, oral estrogen combined synthetic progestin could cause a reduction in 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[1-3]. Hormone replacement therapy (HRT) is also believed to prevent various symptoms during the menopause, such as hot flushes and night sweats[2]. In, addition, loss of estrogen protection is often accompanied by a subsequent change in follicle-stimulating (FSH) levels, which has been widely considered[4,5] 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 dydrogestrone (D), synthetic or natural progestin].

Previous studies[6,7] evaluating the HRT influence on hormonal status were mostly conducted in analysis of one or two formulations, without

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discriminating between the types of estrogen and progestin. Furthermore, in most observational studies[8-10], steroid variables were not the predominant results. Several studies[8-34] 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 wells 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 I *(continued)*. Characteristics of the included studies.

Study	Region	Study design	Laboratory method	Age	BMI	Diagnosis criteria	HRT	N	Duration	Route	Dosages	Parameters
Gupta et al[23]	USA	RCT	RIA	56.9 (4.0)	25.9 (3.3)	2 years of amenorrhea	17β-E2 17β-E2	9 10	3 months	transdermal per day vaginal per day	14 ug 7.5 ug	E2, FSH
Raudaskoski et al[21]	Finland	RCT	RIA	51 (44-59)	26.9 ± 1.3	6 months of amenorrhea, FSH > 28 IU/L	17β-E2 17β-E2 + NETA	15 17	1 year	transdermal per day oral once daily	50 ug 2/1 mg	E2
Tupikowska et al[20]	Poland	RCT	CLIA	50.6 ± 3.0	/	typical of the climacteric syndrome, FSH > 30 IU/L and E2 < 30 pg/mL	17β-E2 17β-E2 + progestin Control	26 54 40	4 months	transdermal per day transdermal per day + oral 12 d (days 19-30) /	50 ug 50 ug/5 mg /	E2, FSH
Soranna et al[17]	Italy	RCT	RIA	47-56	27.0	No HRT for ≥ 6 months, 1 year of amenorrhea, FSH > 35 IU/L and E2 < 92 pmol/L	Placebo 17β-E2 17β-E2+D 17β-E2 + D	10 10 11 12	3 months	oral oral once daily oral once daily oral once daily	/ 2 mg 2/5 mg 2/10 mg	E2, FSH
Chiantera et al[25]	Italy	cohort study	/	49.3	24.6	1 year of amenorrhea, FSH > 30 IU/L and E2 < 20 pg/mL	Placebo 17β-E2 + D 17β-E2 + D	20 19 20	24 months	oral once daily oral once daily + oral 10 d (days 14-28) transdermal per day + transmerdal 10 d (days 14-28)	500 mg 2/10 mg 50 ug/10 mg	E2, FSH
Villa et al[28]	Italy	RCT	RIA	52.6	28.5	3.7 ± 1.1 years postmenopausal, FSH > 50 IU/L and E2 < 73 pmol/L	hemihydrate E + D Placebo	10 8	2 months	oral once daily + oral 10 d (days 14-28) /	2/10 mg /	FSH, E2
Sztefko et al[35]	Poland	cohort study	RIA	54.5 ± 3.34	27.5	FSH > 30 mU/mL, E2 < 50 pg/mL	17β-E2 + D 17β-E2 + D Control	25 8 16	12 months	Transdermal per day + oral once daily oral once daily /	0.05/5 mg 2/10 mg /	FSH
Tobias[18]	UK	RCT	RIA	55.2	NS	amenorrhea for > 6 months, E2 < 40 pmol/L	17β-E2 17β-E2 + D	10 16	12 months	oral once daily oral once daily	2 mg 2/10 mg	E2
Rizzo et al[27]	Italy	open label	RIA	50.5	27.7	1 year of amenorrhea, FSH >30 IU/L, E2 < 20 pg/ml	17β-E2 + D 17β-E2 + DRSP	80 80	6 months	oral once daily oral once daily	1/5 mg 1/2 mg	E2
Nii et al[16]	Japan	open label	RIA	49.0	21.4	a menopausal interval of < 1-year, climacteric symptoms	17β-E2 17β-E2 CEE	15 15 15	3 months	transdermal per day oral once daily oral once daily	50 ug 1 mg 0.625 mg	E2, FSH

table continued

Table I. Characteristics of the included studies.

Study	Region	Study design	Laboratory method	Age	BMI	Diagnosis criteria	HRT	N	Duration	Route	Dosages	Parameters
Chen et al[8]	China	RCT	/	50.19 ± 4.11	/	No HRT for ≥ 6 months, non-hysterectomized postmenopausal women	JWSYS (Chinese herb) 17β-E2 + MPA	24 14	4 months	oral 3 times a day oral once daily	4 g 0.625/2.5 mg	E2, FSH
Sztefko et al[35]	USA	RCT	RIA	61.50 ± 7.00	29.00 ± 5.50	/	Placebo 17β-E2	109 107	2 years	/ oral once daily	/ 1 mg	E2
Woo et al[9]	China	RCT	ELISA	56.2 ± 4.9 56.2 ± 4.9 56.2 ± 4.9	23.8 ± 3.4 23.8 ± 3.4 24.1 ± 3.4	1 year of amenorrhea	CEE + MPA Pueraria lobata Control	43 45 39	3 months	oral once daily oral once daily /	0.625/5 mg 100 mg /	E2, FSH
Cortellaro et al[31]	Italy	RCT	RIA	50, 42-56	/	typical climacteric syndromes, and a Kupperman index of over 14	17β-E2 + MPA CEE + MPA	25 20	4 months	Transdermal daily + oral 8d (days 23-30) oral daily + oral 8d (days 23-30)	0.05/10 mg 0.625/10 mg	E2, FSH
Hofling et a[32]	Sweden	RCT	RIA	57.1 57.5 57.2	24.8 24.5 24.4	1 year of amenorrhea, FSH > 40 IU/L and E2 > 70 pmol/L	17β-E2 + NETA Tibolone Placebo	48 51 55	6 months	oral once daily oral once daily oral once daily	2/1 mg 2.5 mg /	E2
Erdem et al[10]	Turkey	RCT	RIA	50.5 ± 4.4 52.6 ± 4.0	/	1 year of amenorrhea	CEE + MPA Control	40 40	3 months	oral once daily /	0.625/5 mg /	E2
Coksuer et al[6]	Turkey	RCT	RIA	50.8 ± 3.05	28.1 ± 2.73	1 year of amenorrhea, FSH > 30 IU/L and E2 < 20 pg/mL	17β-E2 + DRSP	32	6 months	oral once daily	1/2 mg	E2, FSH
Xia et al[15]	China	RCT	CLIA	50.7 ± 2 51.7 ± 1.6	13.8 ± 3.8 12.1 ± 2.5	total hysterectomy and bilateral salpingo-oopherectomy	17β-E2 17β-E2	11 11	3 months	transdermal, changed once per week oral once daily	1.5 mg/patch 1 mg	E2, FSH
Fernandes et al[7]	Brazil	RCT	RIA, CLIA	56.4 (4.8) 57.7 (4.7)	/	postmenopausal women with urogenital atrophy	CEE placebo	18 20	3 months	vaginal vaginal	0.625 mg 3g	E2, FSH
Pan et al[33]	China	cohort study		50.29 ± 2.87 52.00 ± 1.83		1 year of amenorrhea, FSH > 40 IU/L and E2 < 20 pg/mL	CEE + MPA Placebo	15 15	3 months	oral once daily oral once daily	0.625/5 mg 500 mg	E2, FSH
Benencia et al[29]	Argentina	RCT	RIA	51 ± 4.6	22.7 ± 2.1 22.6 ± 0.5	1-5 years of spontaneous amenorrhea	17β-E2 + NETA 17β-E2 + promegestone	7 7	a year	oral once daily + oral 14d (days 14-28) oral daily + oral 14d (days 14-28)	2/2.5 mg 2/0.5 mg	E2
Sator et al[22]	Austria	RCT	RIA	54.9, 45-68		1 year of amenorrhea, FSH > 30 IU/L and E2 < 45 pg/mL	17β-E2 17β-E2 + progesterone 17β-E2 + progesterone Control	6 7 8 3	6 months	transdermal, changed 3-4 days transdermal, changed 3-4 days + vaginal 10 d (days 21-30) oral once daily + vaginal 10 d (days 21-30) /	50 ug 50 ug/0.4 mg 2/0.4 mg /	E2, FSH

table continued

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

Study	Region	Study design	Laboratory method	Age	BMI	Diagnosis criteria	HRT	N	Duration	Route	Dosages	Parameters
Nii et al[16]	Japan	open label	RIA	49.0	21.4	a menopausal interval of < 1-year, climacteric symptoms	17β-E2 17β-E2 CEE	15 15 15	3 months	transdermal per day oral once daily oral once daily	50 ug 1 mg 0.625 mg	E2, FSH
Yumru et al[24]	Turkey	cohort study	/	58.4 (46-67 years)	/	hysterectomy, climacteric symptoms	17β-E2	35	3 months	vaginal per day	25 ug	E2, FSH
Honisett et al[34]	Australia	RCT	CLIA	45-60 years	/	1-5 years amenorrhea	17β-E2 + MPA Placebo	10 12	5 months	transdermal per day + oral once daily oral/transdermal	50 ug/5 mg /	E2, FSH
Villa et al[30]	Italy	RCT	RIA	52 ± 3.3	/	FSH > 50 IU/L, E2 < 73 pmol/L	17β-E2 + DRSP Placebo	17 15	6 months	oral once daily /	1/2 mg /	E2, FSH
Appelberg et al[19]	Finland	cohort study	/	56 (47-70)	/	amenorrea, FSH > 30 IU/L, E2 < 0.2 nM	17β-E2 + norethisterone 17β-E2	21 11	6 months	transdermal per day transdermal per day	50/250 ug 50 ug	FSH
Matsui et al[26]	Japan	cohort study	/	50.6 ± 4.9	21.8 ± 2.9	1-year amenorrhoeic, FSH > 40 IU/L, E2 < 20 pg/L	17β-E2 + D Control	14 14	12 months	oral once daily /	0.5/5 mg /	E2, FSH
Maffei et al[36]	Italy	cohort study	RIA	51.9 ± 4.6	/	1-year amenorrhoeic, FSH > 30 IU/L, E2 < 25 pg/L	17β-E2 + dihydro-gesterone	21	3 months	transdermal patch/gel + oral 12 d (days 19-30)	50 ug/1 mg + 10 mg	E2, FSH

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.

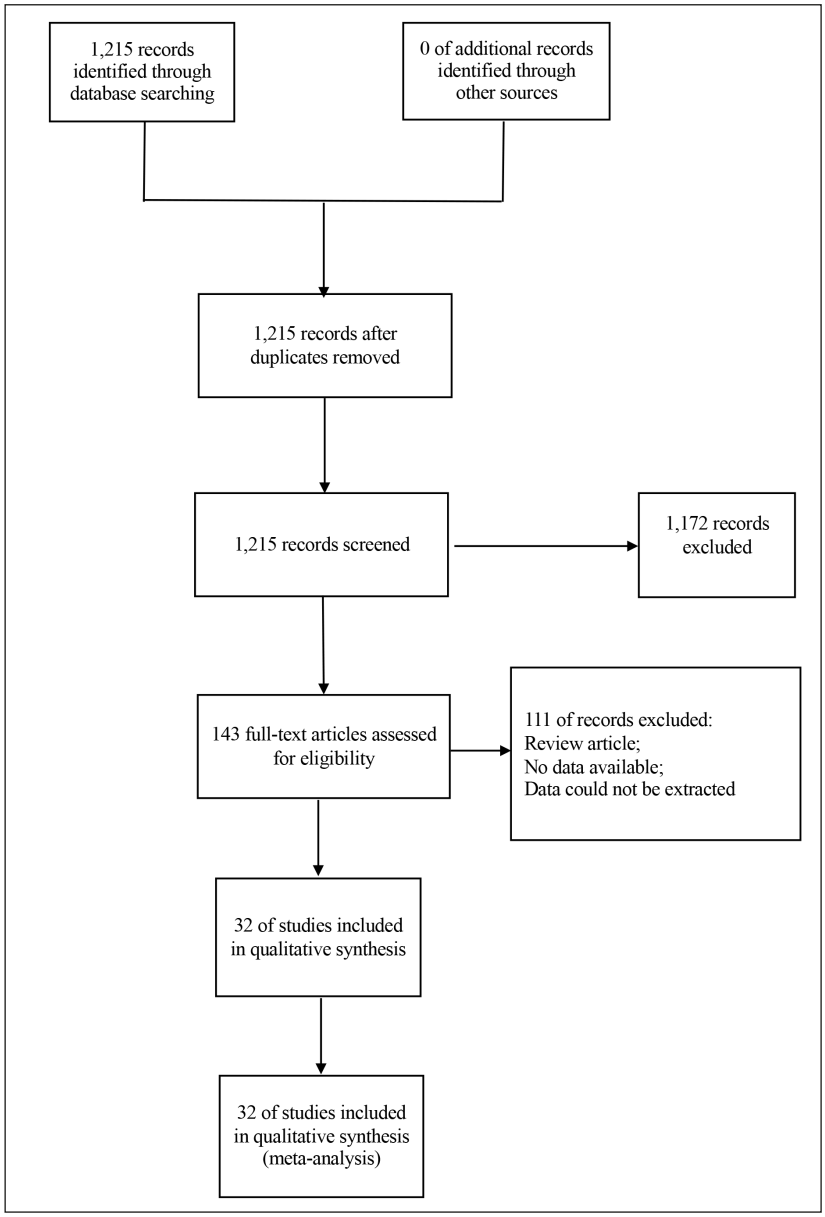

Figure 1. PRISMA flow diagram.

calculate the overall estimates[14]. The odds ratio (OR) provided the measure of HRT efficacy that we analyzed[14]. Heterogeneity across studies was quantified using the Qstatistic and inconsistency index (I^2)[14]. When $I^2 > 50\%$, heterogeneity was considered severe; when $I^2 < 25\%$, 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 papers[6-10,15-34] 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 paper[29] 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 paper[29] 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[29]. Transdermal 17β-E2 (50 ug) was in one (combined with dydrogesterone)[25]. 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 paper[25] 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 studies[6,7,9,15-17,19,20,22-28,30,31,33-36]. 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, $p = 0.17$).

2 papers[25,35] 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 E2 levels (SMD: 0.07 IU/L, 95% CI -0.26, 0.40 IU/L, $p = 0.85$) (**Supplementary Figure 4**).

One paper[25] 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 E2 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 studies[15,16,22] 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 studies[20,22] 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

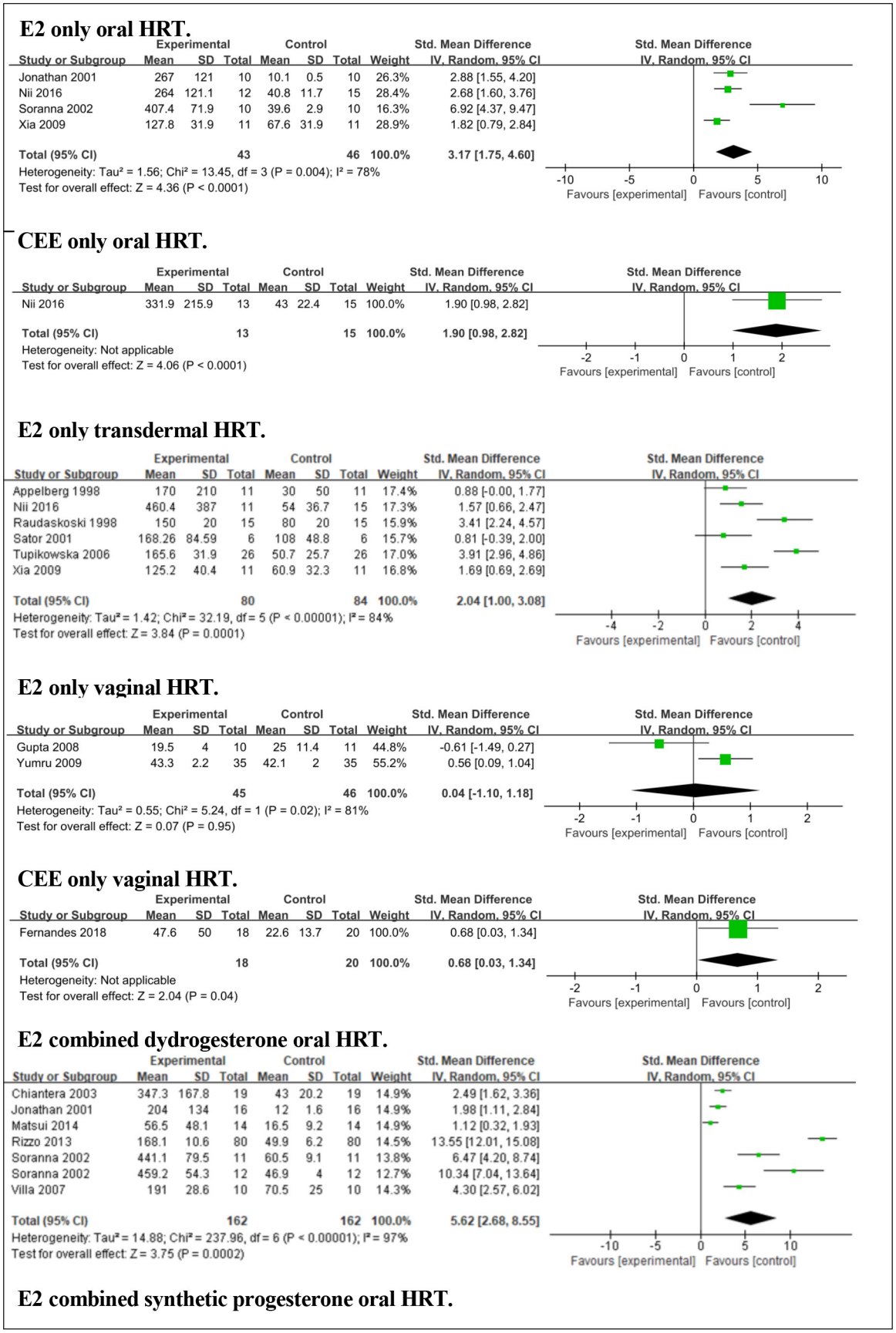

Figure 2. Forest plot comparing estradiol (E2) serum levels between treatment and pre-treatment groups.

Figure continued

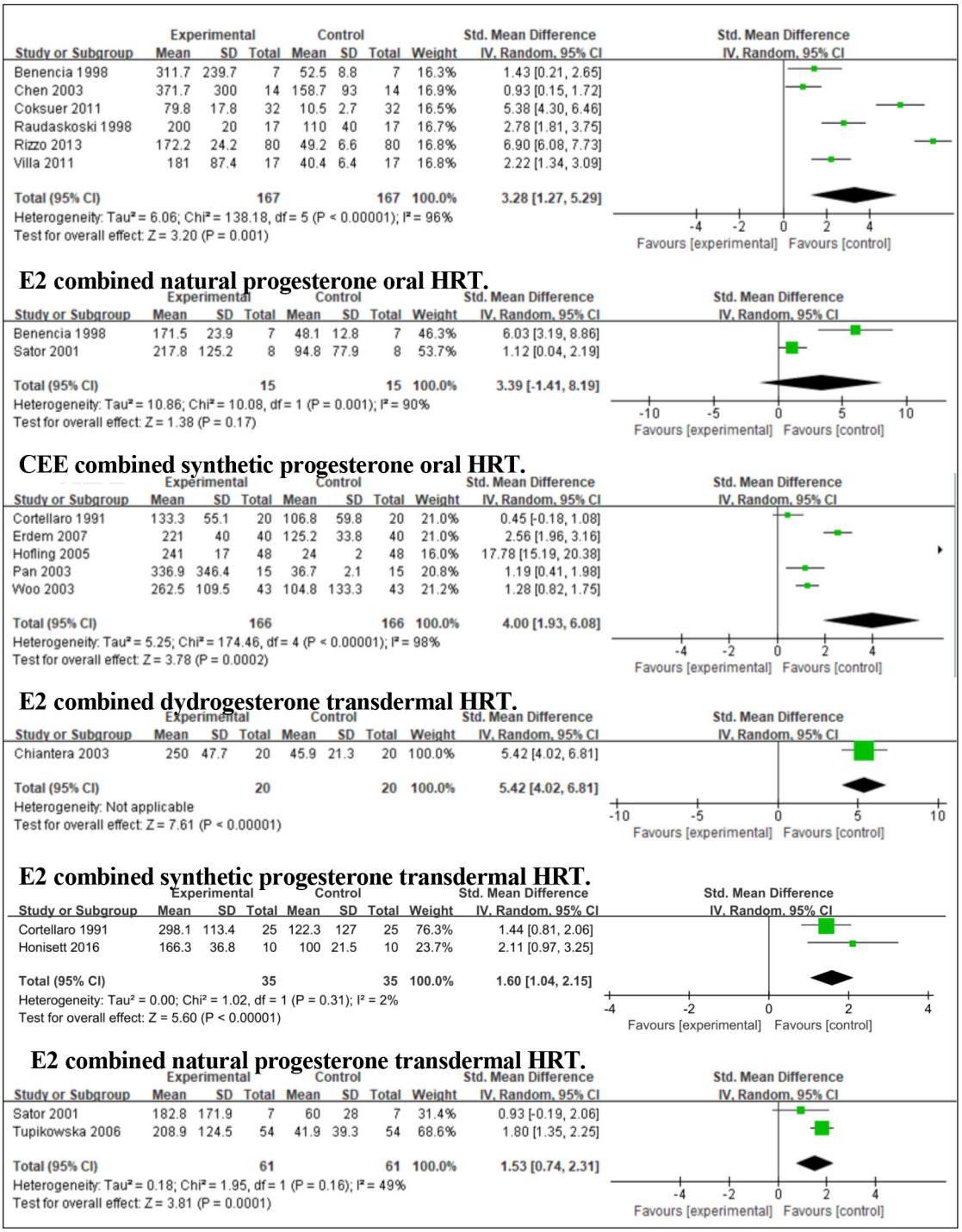

Figure 2 ***(continued).*** Forest plot comparing estradiol (E2) serum levels between treatment and pre-treatment groups.

used oral 17β-E2 (2 mg/d) combined with progestin. 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 caused by combination therapy. Transdermal 17β-E2 (50 ug) was used in two studies (two comparisons with, variously, norethidrone acetate and

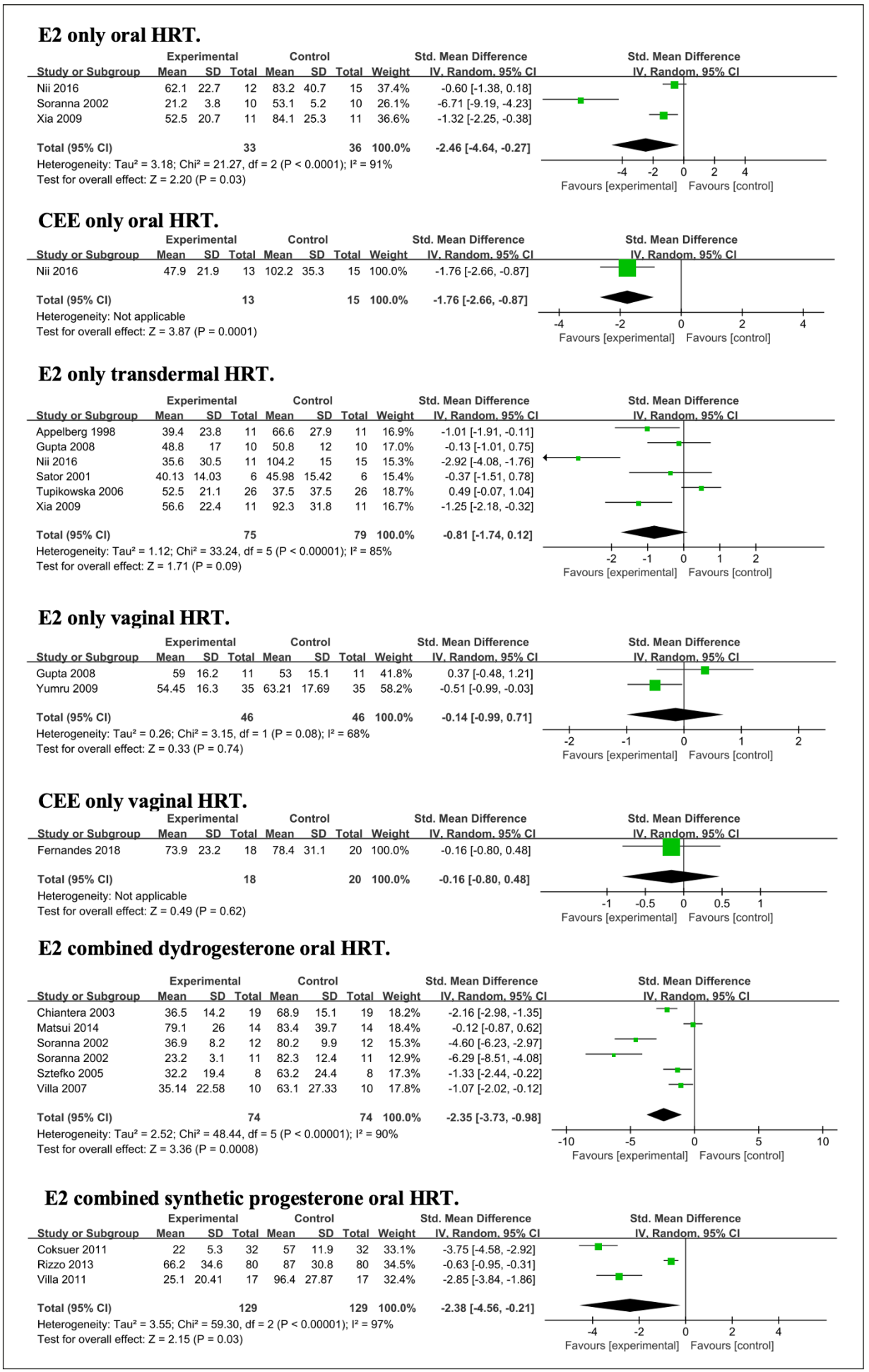

Figure 3. Forest plot comparing follicle-stimulating hormone (FSH) serum levels between treatment and pre-treatment groups.

Figure continued

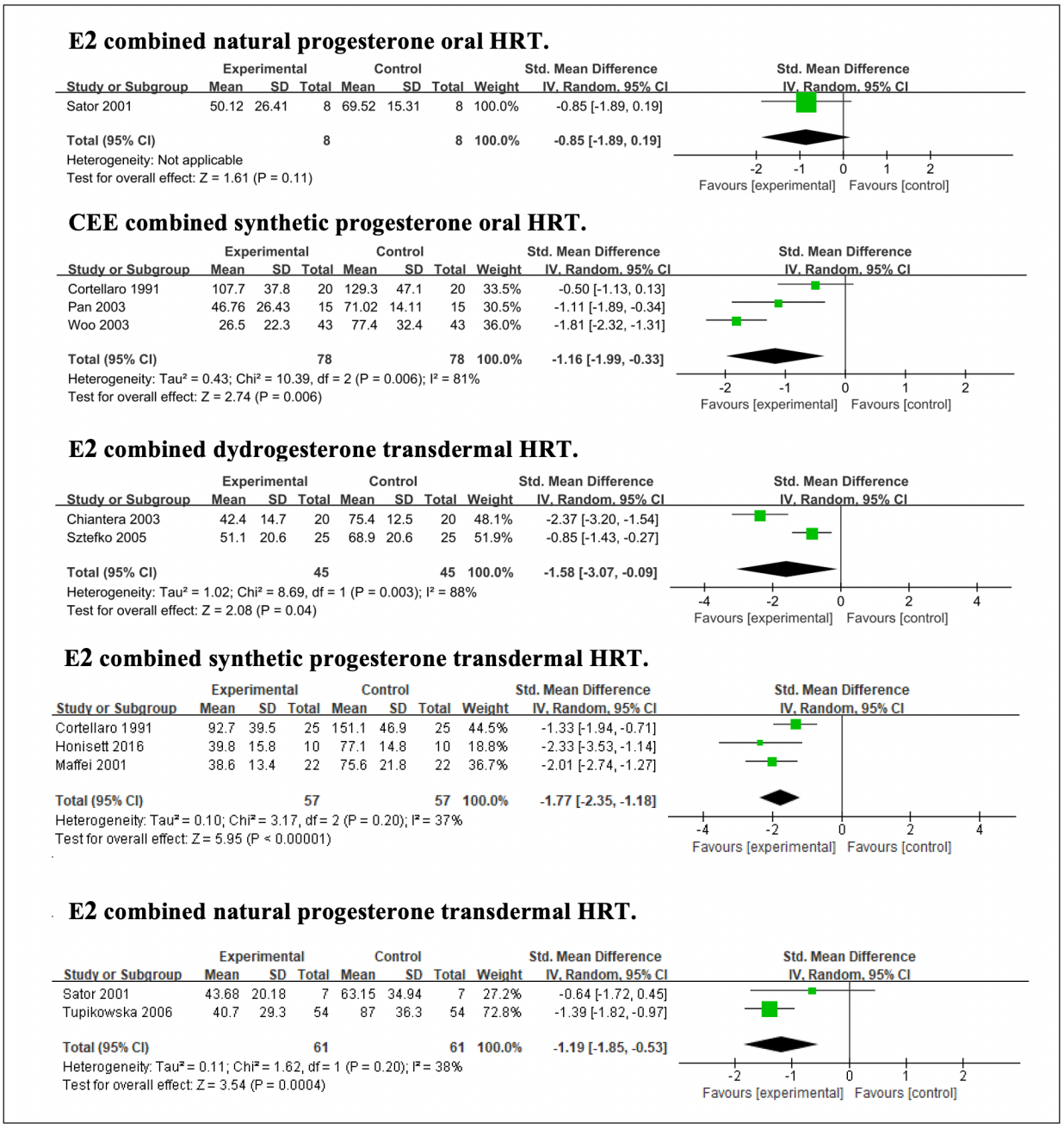

Figure 3 ***(continued).*** Forest plot comparing follicle-stimulating hormone (FSH) serum levels between treatment and pre-treatment groups.

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**).

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

Five studies[15,16,22,25,35] were found, in which, transdermal 17β-E2 or in combination was compared to oral estrogen or in combination. Regarding the type

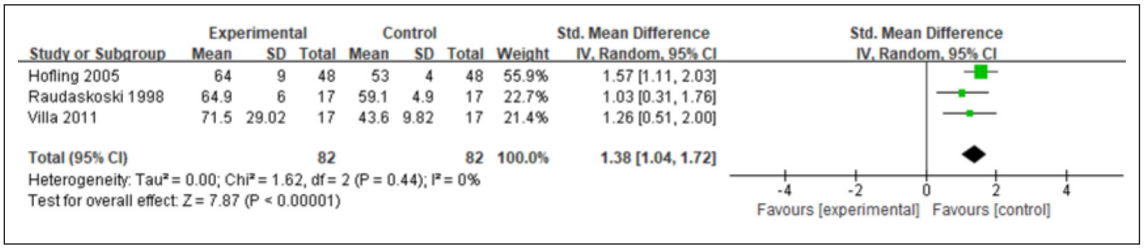

Figure 4. Forest plot of SHBG levels comparing 17β-E2 combined synthetic progesterone oral HRT with pre-treatment.

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 17Ð-E2 Combined Synthetic Progesterone Oral HRT with Pre-Treatment Among Postmenopausal Women

Three studies[21,28,32] 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 LDL[6,9,27,28,31] (SMD: 0.11, 95% CI: -0.19 to 0.41, p = 0.48) (**Supplementary Figure 11**), HDL[6,9,27,28,31] (SMD: -0.35, 95% CI: -0.65 to -0.04, p = 0.03) (**Supplementary Figure 12**), TC[6,8,9,27,28,31] (SMD: -0.31, 95% CI: -0.56 to -0.06, p = 0.02) (**Supplementary Figure 13**) and triglycerides[6,8,9,27,28,31] (SMD: -0.19, 95% CI: -0.71 to 0.33, p = 0.48) (**Supplementary Figure 14**).

Four studies[8,18,25,33] 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 studies[8,18,19,22,25] 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[37]. 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[38-40] 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[41,42]. 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[43]. Low SHBG concentrations in postmenopausal women are significantly related to a more adverse lipid and glucose profile[44,45], 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[46,47]. In a recent report, Liedtke et al[48] demonstrated a positive correlation between BMI and E2. This is reinforced by the negative correlation between BMI and FSH previously reported[49]. 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.

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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 XLZ performed quality control checks on extracted data.

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