UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS:

ENDOCRINOLOGIA

Terapia hormonal oral vs. não-oral em mulheres na pós-menopausa e o risco de primeiro episódio de tromboembolismo venoso: revisão sistemática e meta-análise

Denise Rovinski

Porto Alegre, agosto de 2017.

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Denise Rovinski

Dissertação apresentada ao Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, como requisito parcial para obtenção do título de Mestre.

Orientadora: Profª. Drª. Poli Mara Spritzer

Porto Alegre, agosto de 2017.

CIP - Catalogação na Publicação

Rovinski, Denise
Terapia hormonal oral vs. não-oral em mulheres na pós-menopausa e o risco de primeiro episódio de tromboembolismo venoso: revisão sistemática e meta-análise / Denise Rovinski. -- 2017.
50 f.
Orientadora: Poli Mara Spritzer.

Dissertação (Mestrado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Medicina, Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, Porto Alegre, BR-RS, 2017.

1. Menopausa. 2. Terapia hormonal. 3. Tromboembolismo venoso . I. Spritzer, Poli Mara, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

AGRADECIMENTOS:

Agradeço primeiramente à minha orientadora, Dra Poli Mara Spritzer, pela confiança em mim depositada e pelos grandes ensinamentos que levarei adiante.

Agradeço, também, aos colegas que tiveram participação direta na realização desse trabalho: Gislaine Casanova, Tayane Fighera e Ramon Bossardi.

Aos demais colegas da Unidade de Endocrinologia Ginecológica do Hospital de Clínicas de Porto Alegre e à Natália Goulart e Miriam Sant´Helena, pela parceria.

Obrigada à minha família: meus pais, Marcos e Sonia, minha irmã Cíntia e meu namorado Rodrigo.

Essa Dissertação de Mestrado segue o formato proposto pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, sendo apresentada em duas partes: Parte I (introdução) e Parte II (artigo original submetido à publicação):

- **Parte I:** Terapia hormonal na menopausa: o risco de tromboembolismo venoso em mulheres usuárias de TH oral e não-oral

- **Parte II:** *Risk of venous thromboembolism events in postmenopausal women using oral versus non-oral hormone therapy: a systematic review and meta-analysis*

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Abreviações:

SWAN: *Study of women's health across the nation*

THM: Terapia hormonal na menopausa

TH: Terapia hormonal

HERS: *Heart and estrogen/ progestin replacement study*

WHI: *Women's health initiative*

CV: cardiovascular

ELITE: *Early vs late intervention trial with estradiol*

TEV: Tromboembolismo venoso

ESTHER: *Estrogen and thromboembolism risk*

NAMS: *North American menopause society*

Parte I:

Terapia hormonal na menopausa: o risco de tromboembolismo venoso em mulheres usuárias de TH oral e não-oral

De acordo com a Organização Mundial da Saúde (OMS) menopausa é definida como a cessação permanente da menstruação devido ao término da atividade ovariana. Os anos que precedem a amenorréia definitiva, que ocorre em média aos 51 anos de idade, são caracterizados por alterações endócrinas que afetam a freqüência e a duração dos ciclos menstruais e, durante essa transição, surgem diferentes sintomas que modificam o estado físico e mental da mulher, afetando a sua qualidade de vida (1, 2).

Com o aumento da expectativa de vida, muitas mulheres viverão por mais de 20 a 30 anos após a menopausa e, embora essa possa cursar de forma assintomática, alguns estudos mostram que até 90% das mulheres apresentam algum sintoma que interfere de forma negativa não apenas fisicamente, mas também psicologicamente e socialmente (3). Os sintomas vasomotores, que se manifestam como uma sensação de calor, rubor e sudorese, são os que mais comumente levam as mulheres a procurar tratamento. Outros sintomas freqüentes incluem cefaléia, fadiga, irritabilidade, depressão e ressecamento vaginal (1, 4).

Os sintomas surgem especialmente na peri- menopausa e na pós-menopausa recente, porém podem persistir por mais de uma década. O estudo SWAN, um estudo observacional que avaliou 3302 mulheres, mostrou que a duração dos sintomas vasomotores é de cerca de 7 anos, o que reforça a necessidade de uma terapia segura que possa ser usada por um tempo prolongado (5).

A terapia hormonal na menopausa (THM) com estrogênio é o tratamento mais eficaz para alívio dos sintomas vasomotores e genito-urinários e há pelo menos cinco décadas vem gerando discussões acerca de seus riscos e benefícios. Inicialmente, a terapia com estrogênio isolada demonstrou complicações uterinas, aumentando o risco de desenvolvimento de câncer e hiperplasia endometrial (6). Posteriormente, na década de 1980, passou-se a associar a terapia estrogênica com os progestágenos, para proteção do endométrio. Nessa época a THM passou a ser amplamente prescrita e os estudos mostravam melhora dos sintomas menopáusicos, bem como superioridade dos benefícios, como redução do risco cardiovascular, osteoporose e de câncer de cólon, sobre os riscos de câncer de mama e de tromboembolismos. Passou-se, então, a recomendar a THM não apenas para a resolução dos sintomas relacionados ao hipoestrogenismo, mas também para a prevenção da osteoporose e de doenças coronarianas (7, 8).

Em 1998, após a publicação do estudo *Heart and Estrogen/ Progestin Replacement Study* (HERS), a THM passou a ter o seu risco-benefício questionado e seu uso caiu drasticamente devido ao fato deste ter apontado um maior risco de eventos coronarianos em mulheres previamente cardiopatas isquêmicas, no grupo em tratamento (9). O estudo *Women's Health Initiative* (WHI), publicado posteriormente, reforçou o receio do uso da THM após ter sido interrompido precocemente por uma incidência significativamente maior de eventos cardiovasculares no grupo tratado do que no grupo placebo (10), principalmente no grupo que recebeu terapia estrogênica com estrogênios conjugados associada à medroxiprogesterona, mostrando efeito adicional do progestágeno sobre o risco CV (11). Estudos recentes mostram o grande declínio que o uso da THM sofreu após a publicação de tais estudos. Segundo Jewerr et al. (2014), a prevalência de mulheres entre 45-69 anos nos EUA em tratamento era de 13,5% em

1999, com queda para 2,7% em 2010 (12). Na Europa também se observou uma queda de pelo menos 50% do uso da terapia combinada de estrogênio e progesterona em dados analisados em 17 países europeus, entre os anos de 2002 e 2010 (13).

A avaliação posterior dos estudos WHI e HERS mostrou que alguns fatos devem ser considerados: ambos haviam incluído mulheres com longo período de menopausa (cerca de 10 anos) e o esquema de terapia hormonal estabelecido era fixo, sem variação das drogas, da via de administração ou de dose das mesmas (7).

O estudo ELITE comprovou que o início do tratamento em mulheres com menos de 60 anos e com até 10 anos de menopausa reduziu os riscos cardiovasculares e a mortalidade, quando comparadas a mulheres com mais idade e com mais tempo em amenorréia (14). No entanto, além da idade de início do tratamento, outros aspectos cruciais da THM que influenciam nos desfechos cardiovasculares, bem como eventos tromboembólicos, são a dose dos hormônios e a via de administração dos mesmos (7, 15).

O tromboembolismo foi desfecho de grande impacto negativo no WHI. Enquanto a elevação do risco para isquemia aguda do miocárdio não fatal foi de 1,32 vezes (HR 1,32; CI 1,02-1,72), o risco de eventos tromboembólicos foi duas vezes maior no grupo em uso da THM em relação ao grupo placebo (HR 2,11; CI 1,58-2,82) (10). Em contrapartida, há evidencias de que a via não oral não interfere no risco tromboembólico (15). Metanálises prévias sugerem que o estrogênio transdérmico não adiciona risco tromboembólico nas mulheres após a menopausa em uso de TH (16, 17).

O TEV é uma patologia comum na população, tendo como principais fatores de risco a idade, obesidade, imobilização, cirurgias, trauma, evento tromboembólico prévio e uso de terapia hormonal, apresentando elevada taxa de mortalidade, principalmente quando associado a tromboembolismo pulmonar (18, 19).

Diferentes mecanismos de ação nas variáveis hemostáticas podem explicar as diferenças no risco de TEV entre o uso da TH oral e transdérmica. A via normal de coagulação representa o balanço entre fatores pró-coagulantes e os mecanismos que inibem a trombogênese (20). A TH com estrogênio oral realiza a primeira passagem hepática, que interfere na biossíntese e clearence de proteínas envolvidas na coagulação (20). Há elevação de marcadores de ativação da trombina e redução da ativação da anti-trombina e do nível da Proteína S plasmática, bem como ocorre elevação da resistência à Proteína C ativada. Assim, o uso do estrogênio por via oral causa um desequilíbrio nos fatores de coagulação, que pode levar a um estado de hipercoagulabilidade. Enquanto a THM oral causa essa elevação do risco de eventos tromboembólicos, a THM transdérmica parece ter pouca ou nenhuma interferência sobre a hemostasia (21).

O estudo ESTHER (*Estrogen and Thromboembolism Risk*) foi o primeiro a estabelecer essa diferença de risco tromboembólico entre as vias de administração do estrogênio, mostrando segurança da administração não oral da TH em relação a tromboembolismos. Além da via de administração, esse estudo avaliou também a associação com diferentes tipos de progestágenos. Enquanto o uso da progesterona micronizada e dos derivados pregnanos mostraram segurança, os derivados norpregnanos mostraram-se trombogênicos (22).

Até o momento não há ensaios clínicos avaliando o risco tromboembólico de THM com estrogênio transdérmico, tanto isolada quanto em associação com diferentes tipos de progestágenos. Com as evidências que temos, os *guidelines* sugerem uma conduta individualizada, avaliando-se o risco e o benefício da THM para cada mulher. Recentemente, a Sociedade Norte-americana de Menopausa (NAMS 2017) questionou a orientação do uso da TH por menor período de tempo e menor dose suficientes para

alívio dos sintomas, sugerindo ser mais adequada a conduta de usar a dose, duração, regime e via de administração apropriados (23).

Em meta-análise recente, de 2015, Mohammed et al. evidenciou aumento de 1,6 vezes no risco de TEV com a TH oral quando comparada diretamente com a TH não-oral (17). No entanto, além do estudo ter incluído artigo com mulheres com eventos tromboembólicos prévios, também foi posteriormente criticado por não ter diferenciado os tratamentos entre estrogênio-terapia isolada ou terapias combinadas com progestágenos, desconsiderando a influência desses no risco tromboembólico.

Nosso estudo de meta-análise objetivou analisar e integrar o que há de evidências científicas até então sobre o risco de eventos tromboembólicos em mulheres submetidas à THM com estrogênio oral ou transdérmico, em associação ou não com progestágeno, sem eventos prévios. Espera-se que o presente estudo contribua com embasamento científico para o tratamento adequado e seguro das mulheres com sintomas da menopausa.

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Parte II: Artigo Original

Risk of first venous thromboembolism event in postmenopausal women using oral versus non-oral hormone therapy: a systematic review and meta-analysis

Risk of first venous thromboembolism event in postmenopausal women using oral versus non-oral hormone therapy: a systematic review and meta-analysis[1]

Running title: Venous thromboembolism and hormone therapy

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Disclosure statement: The authors have nothing to disclose.

[1] Grant support: Brazilian National Institute of Hormones and Women's Health (CNPq/Fapergs 465482/2014-7); Funding for Research of Hospital de Clinicas de Porto Alegre (FIPE 15-0341)

SUMMARY

Hormone therapy (HT) is an effective treatment for climacteric symptoms. Nevertheless, combined oestrogen-progestin therapy and oral route seem to entail higher risk of venous thromboembolism (VTE) than oestrogen-only therapy and transdermal administration. We performed a systematic review of MEDLINE, Cochrane CENTRAL, EMBASE, and ClinicalTrials.gov to investigate the risk of thromboembolic events in postmenopausal women using non-oral oestrogen compared to placebo and to oral oestrogen, and analysed studies comparing the thrombotic impact of oestrogens alone vs. combined oestrogen-progestin therapy. Twenty studies were included in the meta-analysis (9 case-control studies, 9 cohort studies, and 2 randomized controlled trials). All studies included postmenopausal women with no history of VTE. No risk of VTE was detected with non-oral HT (OR 0.91 [0.78-1.06]), non-oral ET-only (OR 0.85 [0.66-1.10]), and non-oral combined HT (OR 0.91 [0.77-1.08] vs. control groups. Conversely, increased risk of VTE was observed as compared with control groups in users of oral HT (OR 1.74 [1.44-2.10], oral ET-only (OR 1.33 [1.04-1.69], and combined oral HT (OR 2.36 [1.80-3.09]). The comparison of non-oral vs. oral HT showed increased VTE risk with oral HT (OR 1.74 [1.43-2.11]. Sensitivity analysis including cohort studies and randomized control trials (excluding case-control studies) showed that higher risk of VTE was maintained for oral HT vs. non-oral HT (OR 1.53 [1.24-1.9]). VTE risk was increased in women without established cardiovascular disease or previous thromboembolic events using oral HT. The transdermal route might be a safer choice than the oral for HT.

Keywords: Menopause; oestrogen replacement therapy; hormone replacement therapy; pulmonary embolism; venous thromboembolism.

INTRODUCTION

Hormone therapy (HT) is the most effective treatment for relieving climacteric symptoms, which affect up to 75% of menopausal women (1). Vasomotor symptoms such as hot flushes and night sweats, which are the main complaints of menopausal women, can be disabling, leading to significant impairment of quality of life (2). For some time, treatment of these symptoms relied mostly on oral oestrogen-progestin use (3). However, in 2002 the results of the Women's Health Initiative (WHI) raised concerns about the risk of cardiovascular disease and venous thromboembolism in association with this combined scheme (4). Since then, further evidence has been produced suggesting that the risk of thromboembolic events might be higher in users of combined oestrogen-progestin therapy than in users of oestrogen-only therapy (5, 6).

In addition, observational studies have shown that the risk of thromboembolic events is lower with transdermal oestrogen therapy (ET) than oral therapy (7, 8). The prothrombotic impact of oral ET is related to first-pass metabolism, which induces undesirable effects such as increased triglyceride levels, decreased low-density lipoprotein particle size, and production of some coagulation factors and C-reactive protein. The fact that these changes are not observed with transdermal therapy may be clinically relevant to patients at high risk of thromboembolic events (9, 10).

In turn, the role of progestins in thromboembolic risk is still uncertain (11). Progestins are used in HT exclusively for endometrial protection in non-hysterectomized women, and determine a decrease in the endometrial risk of hyperplasia and cancer. Different progestins may have distinct pharmacological properties and clinical effects (12), with variable impact on vessel blood flow and prothrombotic state.

Despite these observations, only inconclusive information (13-15) is currently available regarding the prevalence of thromboembolic events in users of HT. In the present study, we reviewed the existing evidence about the risk of thromboembolic events in postmenopausal women using non-oral oestrogen compared to placebo and to oral oestrogen, and analyzed studies comparing the thrombotic impact of oestrogens alone vs. combined oestrogen-progestin therapy.

MATERIALS AND METHODS

This study was performed in accordance with Cochrane Collaboration guidelines and the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (16).

Eligibility criteria, search strategy, and study selection

We gathered data from clinical trials and from case-control and cohort studies designed to assess venous thromboembolism (VTE) (pulmonary embolism and/or deep vein thrombosis) in postmenopausal women using oral or non-oral HT. The control group was defined as placebo or non-users of HT. For clinical trials and cohort studies, only works including postmenopausal women with no established cardiovascular disease or previous venous thromboembolism were selected. For multiple articles on the same sample, we selected the article containing the most complete information. Eligibility assessment was performed independently in an unblended, standardised manner by two reviewers, and inconsistencies were settled by a third reviewer.

MEDLINE, Cochrane Central Register of Controlled Trials (Cochrane CENTRAL accessed through Wiley Science), and EMBASE were searched for studies published until February 2017. We also searched http://ClinicalTrials.gov to retrieve

RCTs with unpublished results. The following medical subjects headings (MeSH) were used in the search: postmenopause OR menopause AND “estrogen replacement therapy” OR “hormone replacement therapy” AND “pulmonary embolism” OR “venous thromboembolism” OR thromboembolism.

Data extraction and quality control assessment

Titles and abstracts were independently evaluated by two investigators (D.R. and T.M.F.), who also selected the articles for inclusion in the analyses. When necessary, these investigators evaluated the full text of articles. Disagreements were resolved by consensus or by consultation to a third reviewer (P.M.S.). If the required data were not located in the published article, authors were contacted to provide the missing information.

The following data were collected: first author and study group, publication year, number of patients, mean age, time since menopause, pre-existing disease, medications, country of study, number of participants, detailed interventions, type of control (placebo or no treatment), duration of follow-up, and outcome.

Two investigators (D.R and R.B.R) used the Newcastle-Ottawa Scale (NOS) to assess the quality of the case control and cohort studies included in the meta-analysis (Table 1). This scale uses a “star system” according to which included studies are judged on three broad perspectives: selection of the study groups; comparability of the groups; and ascertainment of outcome of interest.

Statistical analysis

Statistical analyses were performed using R version 3.3.0 (R Foundation for Statistical Computing, Vienna, Austria. Meta-analyses were run in R using the metafor

package (17). The odds ratio (OR) with 95% confidence intervals (CIs) was used as the effect size estimator. Because of the differences in study design and sample characteristics, considerable heterogeneity was expected between the studies. Therefore, the pooled OR was calculated using the random-effects models with the DerSimionian-Laird estimator (18), which is based on a normal distribution.

Heterogeneity in effect sizes across studies was assessed using the I^2 statistics and Cochran's Q test (with P<0.10 indicating significant heterogeneity) (19). Risk of publication bias was assessed using funnel plot graphics, analysed both visually and with the Egger test. The significance of the intercept was evaluated by t test, with P <0.10 considered indicative of significant publication bias (20).

RESULTS

Study selection

Figure 1 provides details of the study selection. The primary search identified 836 articles. After title and abstract screening and exclusion of duplicates, 43 potentially eligible studies were retrieved for full text review. Of these 43 articles, 23 were excluded: 13 did not report on the outcome of interest (venous thromboembolism) and 10 included women with cardiovascular disease or history of previous VTE. Therefore, 20 studies were included in the meta-analysis (6-8, 21-37). Of these, nine were case-control studies investigating the use of oral and non-oral HT in postmenopausal women with venous thromboembolism events versus healthy control women (6-8, 21-26), nine were cohort studies of postmenopausal women in use or not of oral or non-oral hormone therapy, having venous thromboembolism events as outcome (27, 29-33, 35-37), and two were randomized controlled trials (RCTs) assessing the risk of VTE in oral HT, but not in non-oral HT (28, 34).

Description of the studies

Table 2 summarises the characteristics of the nine case-control studies. Table 3 describes the nine cohort studies and two RCTs included in the meta-analysis.

All 20 studies included postmenopausal women with no history of VTE, with at least one group using oral or non-oral HT. Except for three studies directly comparing oral vs. non-oral HT (32, 36, 37), all others included a control or placebo group. The two RCTs included only women without established CVD. Seven (22, 27-29, 31, 34, 35) of the 20 studies compared oral HT vs. no HT only, and were excluded from non-oral HT analysis.

Even though the type of formulation and the dose of oral oestrogen were not the focus of our analysis, we observed that most studies used CEE, micronized estradiol, or estradiol valerate without stratification for VTE risk. This was also the case for non-oral HT (17β estradiol in all cases): separate analyses were not performed for patches or gel, and risk was not analysed according to dose. Thus, all oral and all non-oral formulation types were included in a single oral or non-oral HT group respectively.

Nine of the 20 studies (6, 7, 22, 24-26, 28, 33, 35) presented data regarding subgroups using or not progestins. Therefore, in addition to oestrogen route, we were also able to analyse the impact of adding progestins to hormone treatment on VTE risk. Eleven studies did not clearly state how many women used ET-only or ET plus progestins.

VTE was defined as any thrombotic outcome (pulmonary embolism and/or deep vein thrombosis) in women without previous events and without increased risk of VTE (malignancy, immobilization, or recent surgery). One study did not inform the age range among the inclusion criteria (6), but the age of participants was similar to that of the

women included in the other 19 studies (mean age ranging from 48 to 65.9 years). Most studies were performed in European countries, Canada, and the US. NOS scores of the included studies ranged from 5 to 8 (Table 1).

Data synthesis and meta-analysis

A meta-analysis was performed to evaluate the risk of any VTE event in postmenopausal women using HT, considering administration route and, when available, information regarding the isolated use of oestrogen or combined use of oestrogen-progestin.

Non-oral HT vs. control group

We performed three analyses with non-oral HT users to assess the risk of VTE. First, we analyzed non-oral HT users compared to placebo or control groups, including users of oestrogens and oestrogens plus progestin therapy (Fig 2A). Data from 10 studies (6-8, 21, 23-26, 30, 33) were available for this analysis, totalling 741,076 control women and 83,337 non-oral HT users. This showed no risk of VTE with non-oral HT (OR 0.91 [0.78-1.06]), with moderate heterogeneity (I^2 44%).

The second analysis was performed with users of non-oral ET-only compared with a control group (Fig 2B). Six studies were included (6, 7, 24-26, 33), totalling 702,813 non-users and 54,984 users of non-oral ET. No risk of VTE was detected in users of non-oral ET-only (OR 0.85 [0.66-1.10]), with moderate heterogeneity (I^2 54%).

The next analysis assessed data from five studies (7, 8, 24-26) describing VTE risk in users of non-oral combined HT (oestrogen plus progestin) (Fig. 2C), totalling 1,501 users vs. 226,146 non-users of HT. Again, no risk of VTE events was observed (OR 0.91 [0.77-1.08], and heterogeneity was very low (I^2 0.01%).

Oral HT vs. control group

The same analyses were performed to assess risk of VTE with oral HT. Seventeen studies (6-8, 21-31, 33-35) were included, comparing users of oral HT (oestrogen-only or oestrogen plus progestin therapy), totalling 334,596 women receiving oral HT and 855,337 women without HT (Fig. 3A). Increased risk of VTE was detected (OR 1.74 [1.44-2.10]. There was high heterogeneity among the studies (I^2 90%).

Considering women using oral ET-only, nine studies were included in the analysis (6, 7, 22, 24-26, 28, 33, 35), with 93,777 ET-only users and 778,459 non-users (Fig. 3B). Again, an increased risk of VTE was detected in ET-only users (OR 1.33 [1.04-1.69], I^2 75%).

The analysis of combined oral HT vs. control group (Fig. 3C) included data from 10 studies (6-8, 22, 24-26, 28, 33, 35), with 778,989 control women and 212,042 combined oral HT users. An increase greater than twofold in the risk of VTE events was found in combined oral HT users (OR 2.36 [1.80-3.09]), with high heterogeneity among the studies (I^2 93%).

Non-oral HT vs. oral HT

The risk of VTE was assessed comparing users of non-oral vs. oral HT. The first analysis comprised 13 studies (6-8, 21, 23-26, 30, 32, 33, 36, 37), including case-control studies, cohort studies, and RCTs (Fig. 4A), for a total of 115,963 women using non-oral HT and 366,635 using oral HT. VTE risk was increased with oral HT (OR 1.74 [1.43-2.11], I^2 64%).

The next step was a sensitivity analysis, which excluded case-control studies. A total of 111,061 users of non-oral HT and 348,945 users of oral HT from five cohort studies were considered (30, 32, 33, 36, 37) (Fig. 4B). In this analysis, the higher risk of VTE was maintained for oral HT users vs. users of non-oral HT (OR 1.53 [1.24-1.9], and heterogeneity was lower than that observed in the previous analysis (I^2 49%).

No publication bias was found in the analysis of control group vs. non-oral treatment (Figure 5 A, B, C). This was the case for both analysis of non-oral HT vs. oral HT (figure 5 - G, H) and control group vs. oral ET-only (Figure 5 D, E, F) ($p = >0.10$ for all comparisons). Publication bias may have occurred in the analyses of control vs. oral HT or vs. oral combined HT ($p < 0.10$).

DISCUSSION

The present meta-analysis including 20 studies (case-control and cohort studies as well as RCTs) detected increased risk of VTE associated with oral ET, especially when combined with progestins. In contrast, the risk of VTE events was similar in patients using transdermal HT and in non-users of HT. Indeed, while oral HT was associated with a 1.7-fold increase in the risk of VTE compared to not using HT, non-oral HT did not significantly influence thrombotic events. These findings were supported by the results of our meta-analyses comparing non-oral vs. oral HT, which also showed an increased risk of VTE with oral HT. To the best of our knowledge, this is the first meta-analysis about VTE risk in postmenopausal women using HT that strictly included studies performed with previously healthy women and providing a sensitivity analysis considering cohort studies only.

In a previous meta-analysis, Canonico et al. assessed eight observational studies and nine RCTs comparing users of oral or non-oral HT vs. non-users of HT. Those

authors concluded that the risk of VTE was substantially increased in relation to oral oestrogen use, whereas no additional risk was detected with transdermal oestrogen use (13). However, in that meta-analysis, only four studies assessed the risk of VTE in relation to non-oral oestrogen therapy (6, 8, 21, 23). At the same time, Sare et al., evaluating the risk of arterial and venous cardiovascular events, analysed 16 RCTs that assessed the effects of oral HT on VTE events (14). Those authors detected higher risk of VTE in oral HT users. Nevertheless, it should be noted that studies enrolling women with previous cardiovascular events, such as the HERS study, were also included among the 16 RCTs.

Recently, Mohammed et al. meta-analyzed 15 observational studies and clinical trials aiming to determine the risk of vascular events in HT users (15). Of these, nine studies compared oral vs. non-oral HT regarding the risk of VTE. That study reported a 1.63-fold increase in risk of VTE with oral HT when compared to non-oral HT. Again, one of the studies in the meta-analysis included women with recurrent VTE events (38) – a study which was actually excluded from the present report (38). In addition, five new studies directly comparing the impact of oral vs. non-oral HT on VTE events were added to our meta-analysis (7, 23, 26, 36, 37).

The reason why we only included studies with apparently healthy post-menopausal women was that current evidence suggests that HT is not safe for women with prior thrombotic events or any other established cardiovascular disease. Supporting our exclusion criterion, the EVTET study, designed to evaluate the recurrence of VTE in postmenopausal users of HT, was prematurely finished based on circumstantial evidence emerging during the trial, showing an incidence of new thrombotic events of 10.7% in women with previous VTE under HT vs. 2.3% in the placebo group (39). In contrast, Olié et al. (38) concluded, after 6.5 years of follow-up, that this increased risk

was not found with non-oral HT in women with previous VTE. Although recent guidelines also suggest that non-oral HT might have less deleterious metabolic effects than oral HT, the safety of these preparations in patients at high cardiovascular risk has not been evaluated in RCTs, and reliable evidence is steel needed (40, 41).

The Endocrine Society Clinical Practice Guideline considers history of VTE due to pregnancy, oral contraceptives, unknown aetiology, or blood clotting disorders a contraindication for any ET, whereas VTE due to past immobility, surgery, or bone fracture would be a contraindication for oral but not necessarily transdermal therapy (40). NICE guidelines recommend special care with women who experienced previous VTE events (41). The decision whether to offer or not HT to these women is complex and therefore the involvement of a haematologist is recommended for assessment of thrombophilia risk before considering HT, unless anticoagulant therapy is already in use. Other risk factors should be identified, such as age, genetic abnormalities, obesity, smoking, and inherited thrombophilia, and transdermal rather than oral HT should be considered for women at high risk of VTE (41).

Considering that not all of the 20 studies included in the present meta-analysis provided details about the main risk factors for VTE events, we were not able to stratify the risk of VTE according to smoking, age, or obesity, which may explain the high heterogeneity detected in some of our analyses. In this sense, Canonico et al. reported an analysis from the ESTHER Study evaluating the impact of HT route in association with increased BMI: the combination of oral HT and overweight or obesity elevated the risk of VTE, while transdermal oestrogen did not confer additional risk in this subgroup of women. Therefore, transdermal oestrogen might be safe regarding thrombotic risk, particularly among obese women; however, the safety of transdermal oestrogen has to be confirmed in randomized trials (42).

VTE is a common disease, with an annual incidence rate of more than 1 per 1,000. The mortality rate of VTE is high, particularly when associated with pulmonary embolism (43). Established risk factors for VTE include age, a prior thrombotic event, surgery, trauma, immobilization, prothrombotic mutations, obesity, and HT (44). Data from both observational studies and RCTs consistently demonstrate increased risk of VTE with oral HT (4, 45). In the WHI trials, when the entire cohort was analysed, there were 18 additional VTE events per 10,000 women per year of oestrogen-progestin therapy and 7 additional VTE events per 10,000 women per year of oestrogen-only therapy (44). It should be noted that these randomized studies have been criticized in relation to patient age (patients were on average 10 years older than the age at which HT is usually recommended) and hormone regimen (a fixed regimen was employed, without variation in terms of drugs, dose, and administration route) (46). As previously reported, there is biological evidence to support the difference in thrombotic risk according to route of oestrogen administration (30). Full-dose oral oestrogen therapy has been associated with an increase in plasma inflammatory markers such as C-reactive protein (CRP) and fibrinogen, while low doses have not been consistently associated with changes in circulating markers of endothelial function (47). The contrasting effects of oral vs. transdermal HT on CRP and hepatic proteins such as IGF-1, SHBG, TBG, and CBG have been attributed to the high local concentration of oestrogens in the portal circulation after oral administration and their subsequent first-pass metabolism in the liver (48).

In the present meta-analysis, VTE risk was not increased in non-oral HT groups, regardless of the addition of progestins. However, the combination of progestins and oral ET was associated with additional increases in the risk of VTE. The mechanisms involved in the impact of combining progestins and oral ET on the risk of VTE are still

poorly studied. The different progestin molecules and the various HT combinations for treating menopausal women contribute to this uncertainty. Sare et al. compared the risk of VTE linked to ET-only or combined oestrogen-progestin HT in postmenopausal women and concluded that the association of a progestin increased VTE risk. However, additional sub-analyses for transdermal therapy and progestogen types were not available (14). Recent data have shown that nor-pregnane derivatives, but not micronized progesterone, increase the risk of VTE among transdermal oestrogen users (49). Our present data suggest oral oestrogen administration is linked to increased risk of VTE independently of progestin use. It should be noted that a limitation of the present meta-analysis is that we were unable to determine whether specific progestins could alter the risk of VTE, since few studies are available addressing this information. Other limitations include the small number of studies available in literature, not allowing us to analyze the effects of different estradiol doses on VTE risk. However, different doses of HT were evaluated in a previous meta-analysis performed by our group regarding cardiovascular risk factors. In that study, the effect of low-dose HT did not differ from that of placebo or conventional-dose HT regarding weight, BMI, blood pressure, CRP, or HDL-C. In contrast, low-dose HT was associated with better lipid profile vs. placebo, and induced higher total and LDL-C and lower triglycerides vs. conventional-dose HT (50).

CONCLUSION

Considering only women without established CVD or previous VTE events, VTE risk was increased in oral HT users when compared to non-users, while non-oral HT did not significantly affect this risk. This finding supports the preference for transdermal route for HT, especially in women with other risk factors for vascular

events. The present findings are supported by a sensitivity analysis including only cohort studies and directly comparing non-oral HT vs. oral HT. Further clinical trials with larger populations and longer follow-up periods are needed to sort out the impact of different types of progestins, and in particular micronized progesterone, and different doses of oestrogen on VTE risk.

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Legend of Figures and Tables

Figure. 1. Flow diagram of the study selection process.

Figure 2. Forest plot showing venous thromboembolism events in postmenopausal women using or not using non-oral HT. (A) Oestrogen-only and combined oestrogen and progestin therapies; (B) non-oral estrogen therapy only; and (C) combined non-oral estrogen plus progestin therapy.

Figure 3. Forest plot showing venous thromboembolism events in postmenopausal women using or not using oral HT. (A) Oral oestrogen-only and combined oestrogen and progestin therapies; (B) oral oestrogen-only; and (C) combined oral oestrogen plus progestin therapy.

Figure 4. Forest plot showing venous thromboembolism events in postmenopausal women using oral or non-oral HT. (A) Analysis with case-control and cohort studies and (B) sensitivity analysis including only cohort studies.

Figure 5**.** Funnel plots for risk of publication bias for: (A) control vs. non-oral HT, (B) control vs. non-oral ET-only, (C) control vs. non-oral combined HT, (D) control vs. oral HT, (E) control vs. oral ET-only, (F) control vs. oral combined HT, (G) non-oral HT vs. oral HT, and (H) non-oral HT vs. oral HT in cohort studies.

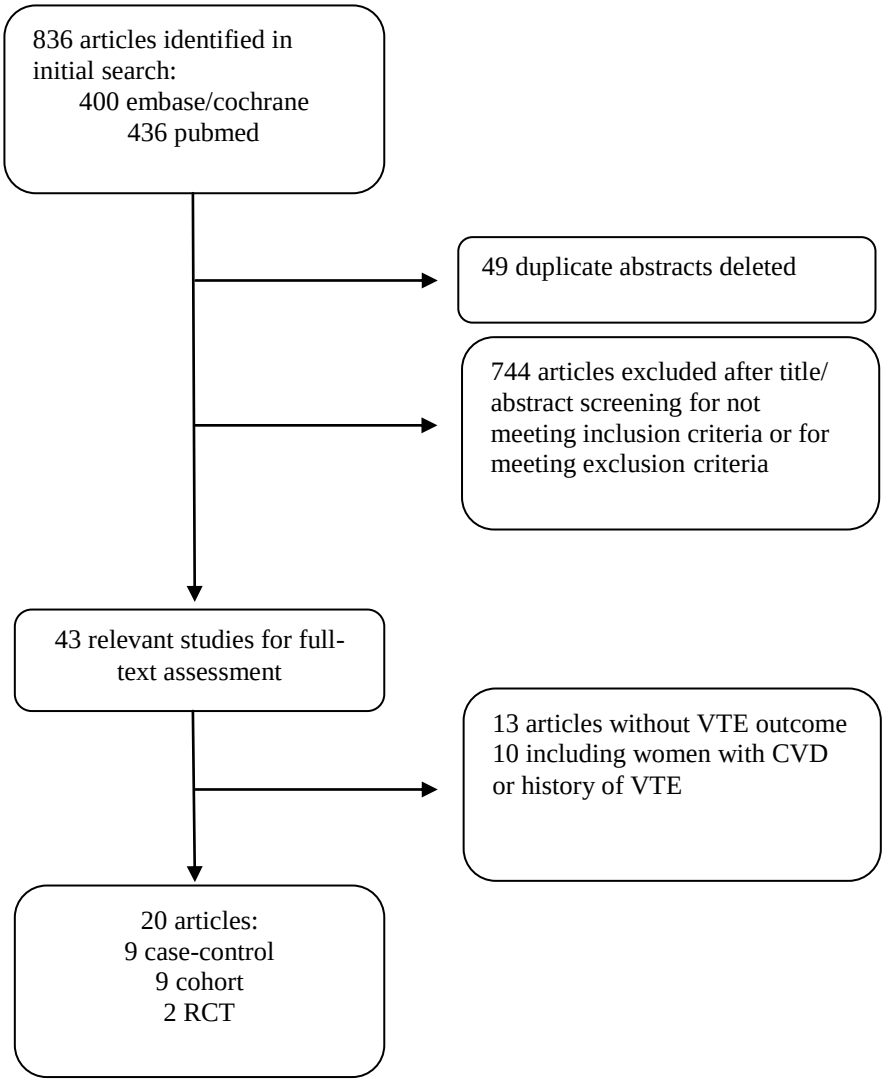
Table 1. Newcastle-Ottawa Scale (NOS) and quality of the studies included in the meta-analysis

Table 2. Characteristics of case-control studies including patients with venous thromboembolism using or not HT

Table 3. Characteristics of cohort studies and randomized controlled trials

What is known about this topic	• Hormone therapy is the most effective treatment for relieving climacteric symptoms, which affect up to 75% of menopausal women and compromise quality of life. • The risk of thromboembolic events might be higher in users of combined oestrogen-progestin therapy than in users of oestrogen-only therapy. • The risk of thromboembolic events may be lower with transdermal oestrogen therapy than oral therapy.
What this paper adds	• In women without established cardiovascular disease or previous thromboembolic events, the risk of venous thromboembolism (pulmonary embolism and/or deep vein thrombosis) was increased in users of oral hormone therapy when compared to non-users. • Non-oral hormone therapy did not significantly affect the risk of venous thromboembolism. • The transdermal route might be a safer choice of hormone therapy, especially in women with other risk factors for vascular events.

Figure. 1. Flow diagram of the study selection process



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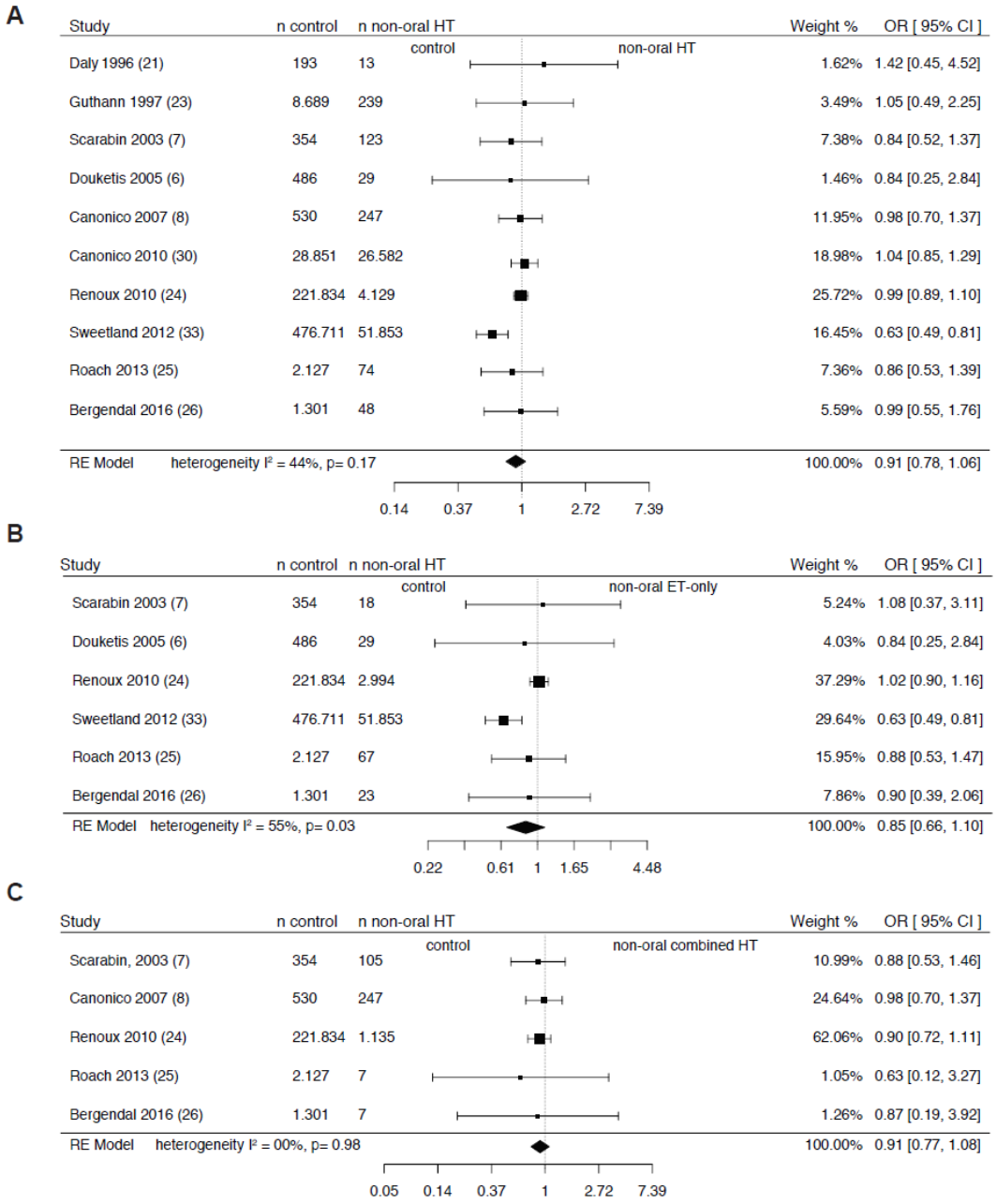
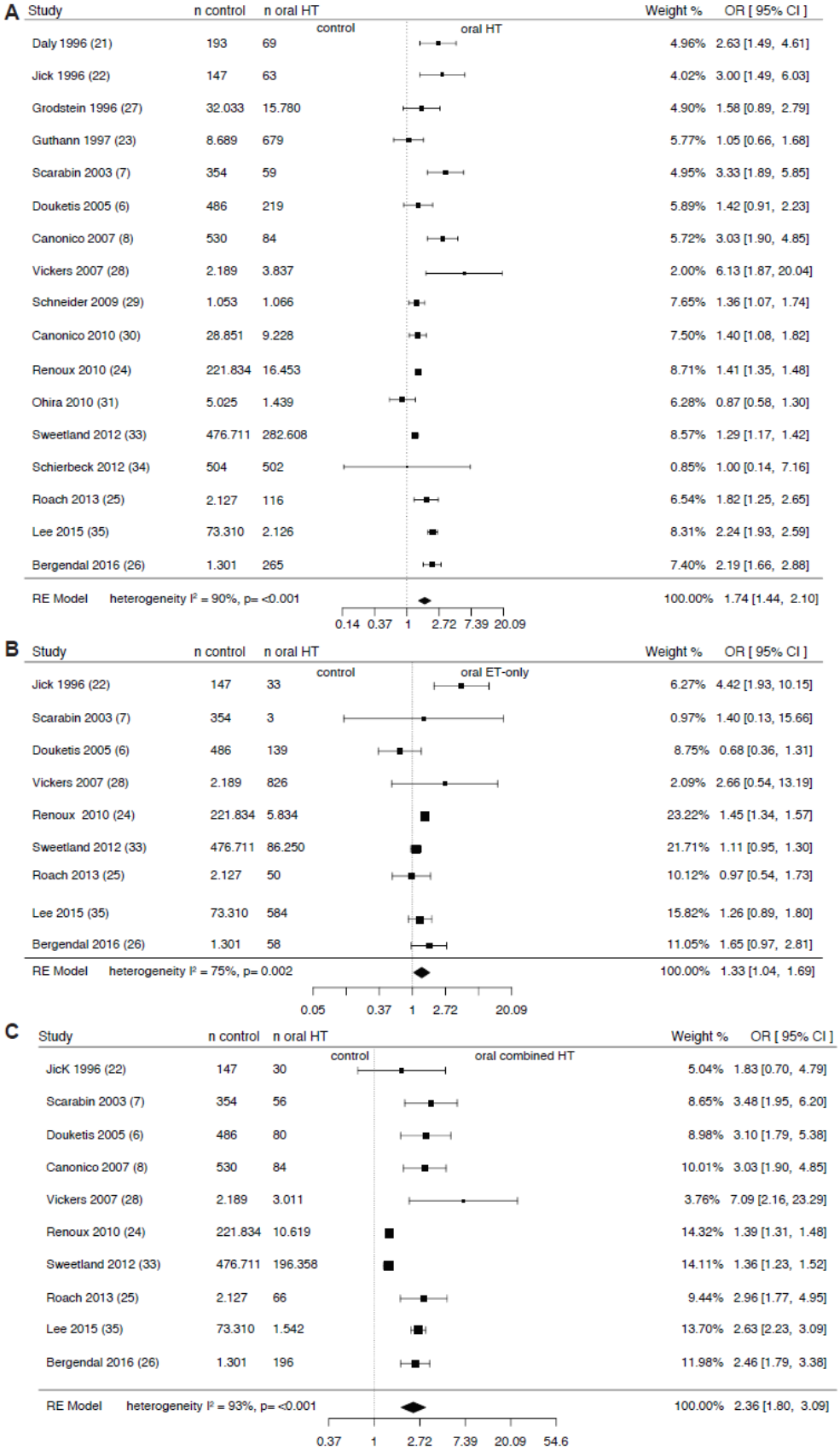


Figure 3. Forest plot showing venous thromboembolism events in postmenopausal women using or not using oral HT. (A) Oral oestrogen-only and combined oestrogen and progestin therapies; (B) oral oestrogen-only; and (C) combined oral oestrogen plus progestin therapy.



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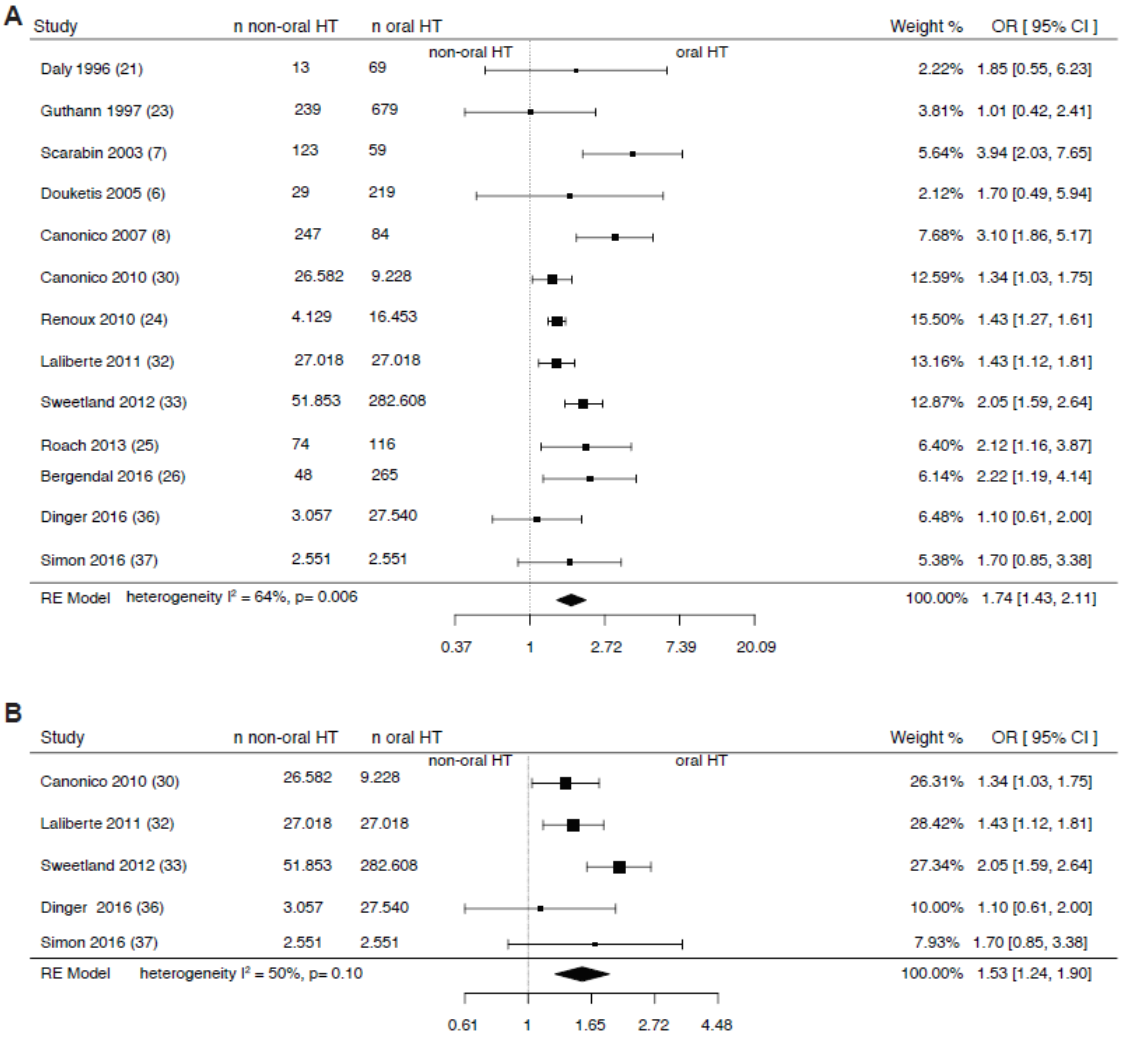

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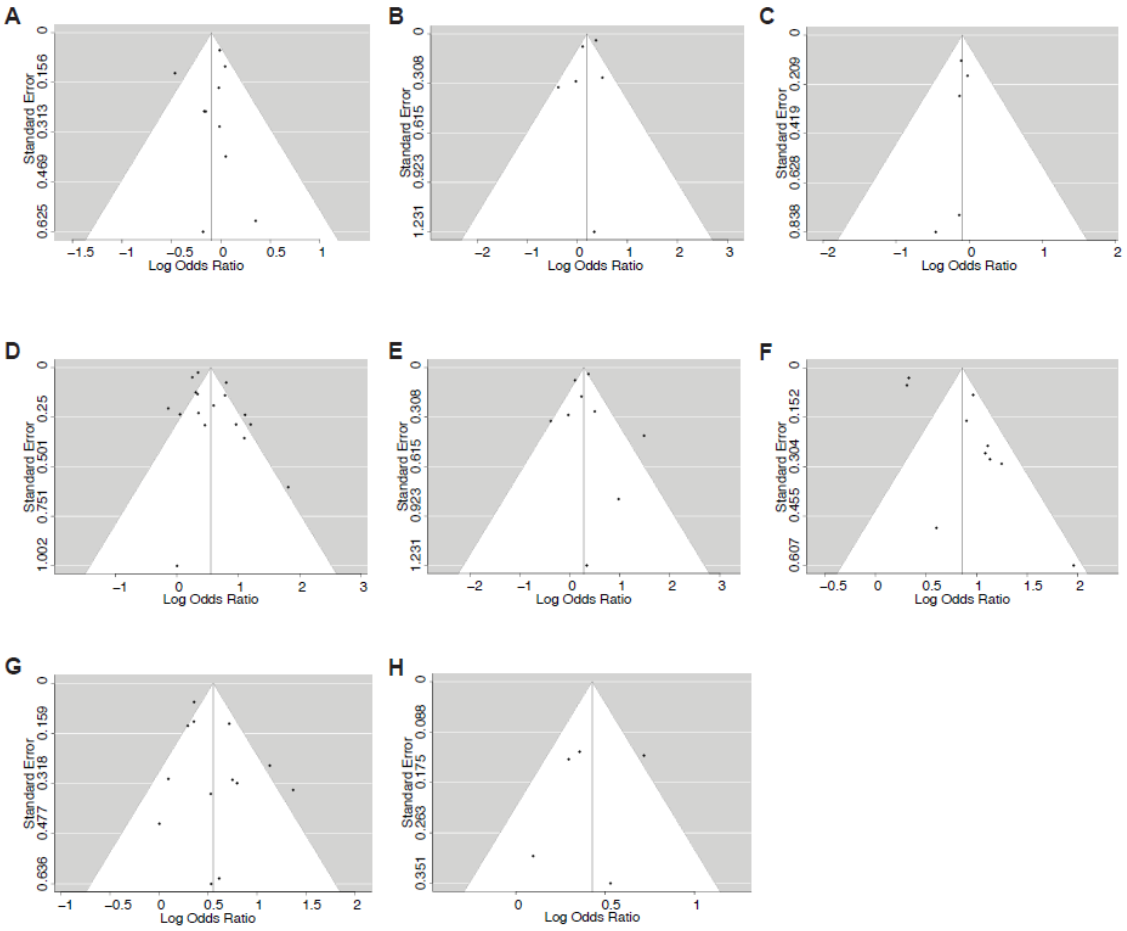

Table 1. Newcastle-Ottawa Scale (NOS) and quality of the studies included in the meta-analysis[a]

Study	Selection	Comparability	Exposure
Daly, 1996(21)	**	*	***
Jick, 1996 (22)	**	*	***
Grodstein, 1996 (27)	*	*	***
Gutthann, 1997 (23)	****	*	***
Scarabin, 2003 (7)	***	*	***
Douketis, 2005 (6)	**	*	***
Canonico, 2007 (8)	**	*	***
Vickers, 2007 (28)	****	*	***
Schneider, 2009 (29)	****	*	***
Ohira, 2010 (31)	****	*	***
Canonico, 2010 (30)	****	*	***
Renoux, 2010 (24)	****	*	***
Laliberte, 2011 (32)	***	*	***
Sweetland, 2012 (33)	****	*	***
Schierbeck, 2012 (34)	****	*	***
Roach, 2013 (25)	****	*	***
Lee, 2015 (35)	****	*	***
Bergendal, 2016 (26)	****	*	***
Dinger, 2016 (36)	**	*	***
Simon, 2016 (37)	**	*	***

[a]Quality of selection (minimum 1 – maximum 4 stars); comparability (minimum 0– maximum 1star); exposure (minimum 1 – maximum 3 stars).

Table2. Caracterirtics of case-control studies including patients with venous thromboembolism using or not HT						
Study	Country	Inclusion criteria	Exclusion criteria	Study duration (years)	Oral HT	Non-oral HT
Daly 1996 (21)	UK	45-64 years Case: PE, DVT, or both (mean age 53.9 ± 5.9 years) Control: no VTE (mean age 53.9 ± 5.6 years)	History of PE, DVT, or MI and history of surgery in the previous 6 weeks or illness necessitating bed rest for longer than 1 week, history of cancer of the breast, ovary, endometrium or other recent or active cancer, serious heart disease or use of anticoagulants	1.8	Low dose: CEE 0.625mg, 1mg 17B Estradiol or 1.5mg piperazine oestrone sulphate High dose: CEE 2.5mg or 2mg 17B E2	Low dose: Transdermal preparations delivering 50mcg 17B E2 High dose: 100mcg 17B E2
Jick 1996 (22)	USA	50-74 years Case: VTE (n=42) Control: no VTE (n=168) (mean age not informed; 50% of the population between 60-69 years)	Trauma or surgery in the previous 6 months, epilepsy, stroke, cancer, renal failure or coronary artery disease	14	CEE 0.325mg, 0.625mg or >1.25mg/day	-
Gutthann 1997 (23)	UK	50-79 years Case: VTE Control: no VTE (mean age not informed; 80% of the population between 50-70 years)	History of VTE or risk factors for VTE	3.7	Low dose: CEE 0.625mg High dose: CEE 1.25mg	Low dose: Transdermal E2 25-50mcg High dose: transdermal E2 100mcg
Scarabin 2003 (7)	France	45-70 years Case: VTE (mean age 62.1 ± 6.8 years) Control: no VTE (mean age 62 ± 6.8 years)	Previous episode of VTE or predisposing factor for VTE	3	Low dose: CEE 0.625mg, 1mg E2 or 1mg estradiol valerate High dose: CEE 1.25mg, 1.5-2mg E2, or 2mg estradiol valerate	Low dose: <50mcg E2 High dose: 50-100mcg E2

Douketis 2005 (6)	Canada, Italy, and the Netherlands	Postmenopausal women, any age Case: DVT Control: no DVT (mean age not informed; 80% of the population between 50-79 years)	PE, ovarian failure, language barrier or cognitive impairment	3	Oral oestrogen only or oestrogen plus progestin	Transdermal oestrogen
Canonico 2007 (8)	France	Postmenopausal women, any age Case: VTE (mean age 61.6 ± 6.7 years) Control: no VTE (mean age 61.5 ± 6.6 years)	History of VTE, contraindication for HT or predisposing factor for VTE	6	Oral oestrogen only or associated with progestin (most frequently 17B E2 0.5-2mg/day)	Transdermal oestrogen alone or associated with progestin (mostly <50mcg/day)
Renoux 2010 (24)	UK	Postmenopausal women, 50-79 years Cases: VTE (mean age 65.9 ± 8.5 years) Control: no VTE (mean age 65.8 ± 8.5 years)	History of VTE	20.2	Oral oestrogen alone or with progestogen Low dose: CEE<0.625mg or E2<2mg High dose: CEE 0.625mg or E2 2mg	Transdermal oestrogen alone or with progestogen Low dose: <=50mcg High dose: >50mcg
Roach 2013 (25)	Nether-lands	Postmenopausal women, 50-70 years (mean 59 years) Case: VTE Control: no VTE	Severe psychiatric problems and not speaking Dutch	5.5	Micronized E2 alone, CEE 0.625mg + MPA and Micronized E2 (1mg or 2mg) + NETA	Transdermal patches containing micronized E2
Bergendal 2016 (26)	Sweden	40-64 years not using combined hormonal contraception Case: 1st DVT episode (mean age 54.6 ± 6.5 years) Control: no DVT (mean age 54.5 ± 6.5 years)	Not speaking Swedish, previous thrombosis or current malignancy	6	17B E2 alone or with NETA or MDX	Transdermal oestrogen alone or with progestogen

PE: pulmonary embolism; DVT: deep vein thromboembolism; VTE: venous thromboembolism; MI: myocardial infarction; CEE: conjugated equine oestrogen; MPA: medroxyprogesterone acetate; HT: hormone therapy; NETA: norethisterone acetate; DRSP: drospirenone; E2: estradiol; CVD: cardiovascular disease.

Table 3. Characteristics of cohort studies and randomized controlled trials							
Study	Country	Inclusion criteria and age of participants	Exclusion criteria	Follow-up (years)	Oral HT	Non-oral HT	Outcome
Grodstein 1996 (27)	USA	40-55 years, postmenopausal women (mean age not informed)	History of previous PE, cancer (except non-melanoma skin cancer), angina, MI, stroke or other cardiovascular disease	12	Most HT consisted of oestrogen alone, without added progesterone (CEE 0.3,0mg, 0.625mg or >1.25mg/daily)	-	Pulmonary embolism
WISDOM 2007 (28) (RCT)	UK, Australia and New Zealand	WISDOM study, 50-69 years Oral Combined HT: mean age 61.7 ± 5.1 years and 63.3 ± 4.7 years ET: mean age 61.9 ± 5.1 years) Control: mean age 63.3 ± 4.6 years)	Breast cancer, any other cancer in the past 10 years (except basal or squamous cell skin), endometriosis or endometrial hyperplasia, VTE, gall bladder disease, MI, unstable angina, cerebrovascular accident, subarachnoid haemorrhage, transient ischemic attack, or use of HT in previous 6 months	1	CEE 0.625mg isolated or combined with medroxyprogesterone acetate 2.5/5.0 mg orally daily	-	VTE (secondary outcome)
Schneider 2009 (29)	UK	UK-based General Practice Research Database, postmenopausal women aged < 70 years (mean age not informed; 54% of the population between 50-59 years)	Cancer, stroke, MI, VTE	6.0	Group 1: at least one prescription for any dosage form of estradiol/ dydrogesterone Group 2: at least one prescription for oral CEE plus norgestrel, oral estradiol (valerate) plus norethisterone (acetate) or oral CEE plus MPA Group 3: had never received any prescription for HT	-	VTE (secondary outcome)
E3N 2010 (30)	France	Postmenopausal women from E3N	History of VTE and cancer other than basal	10.1	Mostly 17B E2, associated or not with micronized progesterone,	Mostly 17B E2, associated or not with	VTE

		prospective cohort study (mean age 54 years)	cell carcinoma		pregnane derivatives, norpregnane derivatives or nortestosterone derivatives	micronized progesterone, pregnane derivatives, norpregnane derivatives or nortestosterone derivatives	
Ohira 2010 (31)	USA	Post-menopausal women Cases: VTE (mean age 64 years) Control: no VTE (mean age 61 years)	Women who were not white or black or were scarcely represented in some field centres, prior VTE or cancer, warfarin users	11.8	Oral HT formulations	-	VTE
Laliberte 2011 (32)	Canada	Postmenopausal women aged ≥ 35 years, recent users of HT, with 2 or more prescription refills (mean age: 48.9 ± 7.1 years)	History of VTE	10.1 (±4.6)	Oral oestrogen only (Cenestin, Estrace, Premarin)	Transdermal oestrogen only (E2 transdermal system, Vivelle-Dot)	VTE, DVT and PE
Sweetland 2012 (33)	UK	Postmenopausal women, 50-64 years (mean age: 56.7 ±4.5 years)	Pre or perimenopausal, history of cancer or clotting problem, surgery in the 12 weeks prior to recruitment or unknown use of HT	3.1	CEE or E2 isolated or associated with MPA, NETA or norgestrel	Patch or gel formulation of oestrogen with or without a progestin	VTE
Schierbeck 2012 (34) (RCT)	Denmark	Recently postmenopausal white women, 45-58 years, last menstrual bleeding 3-24 months before study entry or perimenopausal symptoms in combination with	History of bone disease, uncontrolled chronic disease, previous or current cancer or thromboembolic disease, current or past treatment with glucocorticoids for	10.1 during HT + 5.7 post-treatment	Triphasic estradiol and norethisterone acetate (intact uterus) or estradiol 2mg/day (who undergone hysterectomy)	-	VTE (secondary outcome)

		recorded postmenopausal FSH levels HT: mean age 49.5 ±2.7 years Control: mean age 50 ± 2.8 years	more than six months, current or past use of HT in the past 3 months, and alcohol or drug dependence				
Lee 2015 (35)	Taiwan	50-79 years (randomly selected from National Health Insurance database: women who had a prescription for HT or medical service for a post-menopausal condition; or had neither prescription for HT nor medical service for a postmenopausal condition) HT: mean age 60.7 ± 8.1 years Control: mean age 59.5 ± 7.6 years	Prior VTE, who were ever prescribed HT in the past 3 years, hysterectomy	2	A list of all medications containing oestrogens and/or progestogens recommended for HT and available in Taiwan during the study period was extracted from the database. In Taiwan, there were no pharmaceutical products for transdermal HT, tibolone or estradiol implant during the study years.	-	VTE
Dinger 2016 (36)	7 European countries	HRT starters (first-ever users), HRT switchers or HRT restarters (mean age: 54 ± 7.3 years)		8.5	DRSP 2mg + E2 1mg, other oral continuous combined HRT preparations containing a progestin other than DRSP or other oral HRT preparations	Non-oral oestrogen preparations	VTE, DVT or PE
Simon 2016 (37)	USA	Postmenopausal women, 50 years or more, at least 2 HT prescription refills (mean age: 55 years)	Other type of HT, CVD, cancer, thrombophilia or liver disease	10.6	Oral oestrogen alone	Isolated transdermal oestrogen	VTE, DVT or PE

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