human reproduction update

The route of administration, timing, duration and dose of postmenopausal hormone therapy and cardiovascular outcomes in women: a systematic review

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BACKGROUND: The effect of postmenopausal hormone therapy (HT) on cardiovascular disease (CVD) risk remains controversial.

OBJECTIVE AND RATIONALE: We aimed to systematically review the evidence regarding the role of dose, route of hormone administration, timing of initiation and duration of HT on cardiovascular risk among postmenopausal women.

SEARCH METHODS: The electronic databases Medline Ovid, Web of Science and Cochrane Central were systematically searched to identify studies published before 30 January 2018. Reference lists, using Elsevier's Scopus, of the included studies were searched for further identification of relevant studies. Clinical trials and observational studies that assessed clinical and subclinical cardiovascular outcomes in relation to dose, route of administration, duration of use, or timing of HT initiation among postmenopausal women were included. Data were extracted by independent reviewers using a pre-designed data collection form. The Cochrane Collaboration's tool and the Newcastle-Ottawa Scale were used by two independent investigators to assess the risk of bias in RCTs and in prospective observational studies, respectively.

OUTCOMES: In total, 33 unique studies (6 trials and 27 prospective observational studies) were identified, including a total of 2 588 327 women. The synthesis of the existing knowledge on this topic was challenging due to inconsistent findings between some studies, caused by substantial diversity in scientific rigor and quality across the available literature. Overall, the evidence did not support the concerns that oral or transdermal HT increases heart disease risk. Contrary, observational data showed that a beneficial cardioprotective effect can be observed even with use of low doses of oral HT (effect of 0.3 mg/day of oral conjugated equine estrogen was similar to that seen with the standard dose of 0.625 mg/day), but clinical trials to support a cardioprotective benefit of HT in primary prevention have not been identified. Furthermore, the current data suggested that oral and transdermal HT, in dose-dependent manner and irrespective of HT formulation, may increase thromboembolic risk, as well as risk of stroke. However, transdermal estrogen with <50 μg/day of estrogen combined with micronized progesterone appears to be the safer choice with respect to thrombotic and stroke risk. Also, vaginal HT administration may play a role in myocardial infarction and stroke risk prevention, but this is based on limited evidence and requires further investigation. The timing of HT initiation and duration may be important factors to consider when prescribing HT especially in women with adverse cardiometabolic profile and pre-existing conditions such as coronary/carotid atherosclerosis, which are at risk of developing, and thus progressing to CVD. The quality of evidence was generally low or moderate and the findings were based mostly on observational data.

WIDER IMPLICATIONS: Use of low-dose oral and transdermal HT appears to be safe with regard to CVD risk in women in menopausal transition and within the first years (e.g. 10 years) after menopause onset. In women with increased baseline thromboembolic risk, alternative non-hormonal medications are suggested as first-line treatment and transdermal estradiol alone or with micronized progesterone only should be considered when these options are not effective. When HT is initiated >10 years since the menopause onset (>60 years old), due to greater absolute risks of coronary heart disease, stroke and venous thromboembolism, HT should be used for the shortest time possible and in lowest possible dose and preferably transdermal administration should be recommended. However, an individualized treatment approach including baseline CVD risk assessment should be applied when prescribing HT. The majority of studies included in the current review are from North American and European populations, which might limit the generalizability of the findings of this review to the other populations. Finally, the quality of evidence included in this review was generally low or moderate, highlighting a need for more rigorous research to help us better understand HT and cardiovascular health.

Key words: menopause / hormone therapy / cardiovascular risk / stroke / myocardial infarction / coronary heart disease / venous thromboembolism

Introduction

Menopause, climacteric symptoms and hormone therapy

Menopause is considered the end of a woman's reproductive life and is generally defined by cessation of menstrual periods for 12 consecutive months (Stampfer *et al.*, 1991; Jaspers *et al.*, 2015). Menopausal transition may start several years before and is characterized by irregular menstrual cycles and the presence of menopausal symptoms (Greendale *et al.*, 1999). The most challenging climacteric symptoms are vaginal dryness and vasomotor symptoms with 50.3–82.1% of menopausal women reporting hot flashes or night sweats (Canonico *et al.*, 2007; Renoux *et al.*, 2010b). The duration and intensity of menopausal symptoms varies considerably among women, although most women report that they last between 6 months and years (Marjoribanks *et al.*, 2018). Also, symptoms could be of different

severity, with up to 42% of women aged 60–65 years experiencing moderate to severe vasomotor symptoms (Gartoulla *et al.*, 2015). Certainly, vasomotor symptoms are the main indication for hormone therapy (HT) use. Estrogen products are proven to be efficient in the reduction of hot flashes and are superior to other non-hormonal therapies (Lobo, 2017). However, the effectiveness of HT greatly varies with HT characteristics and currently there are no arbitrary limits regarding the dose and duration of use of HT. While most women will no longer have symptoms after 5 years of treatment, some women may experience long-term hot flashes, in extreme cases even lifelong (Neves *et al.*, 2015). Also, women with premature ovarian failure might need a higher dose of estrogen to control vasomotor symptoms than their older counterparts (Neves *et al.*, 2015).

HT formulations can include either estrogen alone (estrogen-only HT)—mainly indicated for women who have hysterectomy (surgical removal of uterus), or estrogen combined with progestogen

(combined HT)—which is mainly indicated for women with a uterus (Marjoribanks *et al.*, 2018). HT is used in a variety of formulations and doses and can be taken orally and as an implant, skin patch or cream (trans-dermally and vaginally). The clinical effects vary according to the type of HT and the duration of its use (Marjoribanks *et al.*, 2018). The most commonly prescribed is oral HT, and the most common estrogens used are conjugated equine estrogen (CEE), synthetic conjugated estrogens, micronized 17b-estradiol and ethinyl estradiol, while commonly used progestins are medroxyprogesterone acetate (MPA), norethindrone acetate and native progesterone (The NAMS, 2017 Hormone Therapy Position Statement Advisory Panel, 2017). Though MPA is mainly given orally, levonorgestrel and norethisterone are available in transdermal patches combined with estradiol; and levonorgestrel can be delivered directly to the uterus with an intrauterine device (Neves *et al.*, 2015).

For decades HT has been crucial for achieving menopausal symptom relief and improving the quality of women's lives. However, HT has been accompanied by specific cardiovascular health concerns, which could depend on HT preparations and dosages (Manson *et al.*, 2013).

Endogenous versus exogenous estrogen and CVD risk: the conundrum

Premenopausal women have a lower cardiovascular disease (CVD) risk compared to age-matched men; however, this sex-advantage for women gradually disappears after menopause (Yang and Reckelhoff, 2011). This increase in CVD risk after menopause has been attributed to the sharp decline of estrogen levels, suggesting a potential cardioprotective effect of endogenous estrogen in women before the menopause (Yang and Reckelhoff, 2011). Various potential cardioprotective effects of endogenous estrogen have been suggested. Estradiol has beneficial effects on key elements in the pathogenesis of CVD: inflammation (Xing *et al.*, 2009), endothelial function (Baruscotti *et al.*, 2010) and lipid profile (Mumford *et al.*, 2010). When HT was introduced it was hypothesized to reduce CVD risk. Although observational data have suggested that HT decreases the risk of CVD and reduces mortality in postmenopausal women with heart disease (Paganini-Hill *et al.*, 1988; Henderson *et al.*, 1991; Grady *et al.*, 1992; Grodstein *et al.*, 2000), large-scale clinical trials [the Women's Health Initiative (WHI) and the Heart and Estrogen/Progestin Replacement Study (HERS) I and II] indicated an unfavorable HT effect on CVD risk (Xing *et al.*, 2009). WHI reported a 30–40% elevated risk of stroke for women given estrogen combined with progestin or estrogen alone (Wassertheil-Smoller *et al.*, 2003; Hendrix *et al.*, 2006). In line with this, the Nurses' Health Study reported 35% increased risk of stroke with current use of HT (Grodstein *et al.*, 2000). Yet, the latest update from WHI showed that HT with CEE + MPA or with CEE alone was not associated with risk of all-cause, cardiovascular or cancer mortality during a cumulative follow-up of 18 years (Manson *et al.*, 2017). Although consistent evidence suggests estrogen therapy may be cardioprotective if started around the menopause onset and harmful if started in later stages of menopause (>10 years), the evidence was inconsistent with combined HT, suggesting a potential attenuation of the coronary benefit with using a continuous progestogen (Baber *et al.*, 2016). The current evidence on HT and CVD risk is conflicting, with HT being reported

to cause both beneficial and detrimental effects. Many potential factors have been suggested to contribute to the adverse outcomes: the dose, route, the type of HT given (CEE with progestin), the timing of HT initiation/the age of women, a history of CVD/increased CVD risk and the thromboembolic properties of estrogen and progestin. To date, despite the widespread use of HT, there is no comprehensive review on how CVD risk differs by dose, duration, route and timing of initiation of HT treatment.

We aimed to systematically review and summarize the available evidence on the association between HT and CVD risk in postmenopausal women and whether these effects differed by timing of initiation, route of administration, duration and dose of HT.

Methods

Data sources and search strategy

This review was conducted using a predefined protocol and in accordance with PRISMA and MOOSE guidelines. Three electronic databases (Medline Ovid, Web of Science and Cochrane Central) were searched until 30 January 2018 without language restriction. The computer-based searches combined terms related to the menopause (e.g. 'menopausal') in humans; HT and the factors relevant to this review (e.g. 'timing', 'duration', 'dose' and 'administration') and cardiovascular outcomes ('atherosclerosis', 'peripheral arterial disease', 'carotid intima-media thickness', 'stroke', 'transient ischemic attack', 'heart failure', 'coronary heart disease', 'angina', 'chest pain' and 'venous thromboembolism'). Details of the search strategy are found in Supplementary Table I.

Two independent reviewers screened the titles and abstracts of all studies initially identified according to the selection criteria (below), and any disagreement was resolved through consensus or consultation with a third independent reviewer. Full texts were retrieved from studies that satisfied all selection criteria. Reference lists of the included studies and relevant reviews, as well as studies that have cited these articles, were hand-searched and searched with Elsevier's *Scopus*, the largest abstract and citation database.

Study selection and eligibility criteria

Intervention studies were eligible if they: were randomized controlled trials (RCTs), non-RCTs, or prospective observational studies; assessed the effects of the timing, duration, dose or route of administration of HT in menopausal, or postmenopausal women compared to a placebo or no treatment; and collected subclinical or clinical cardiovascular endpoints. To maintain consistency and due to difficulty in interpreting results, head-to-head trials that compared non-hormonal therapies with estrogen or with other medications were excluded. No restrictions on length of follow-up were applied.

Data extraction

Two authors independently extracted data and a consensus was reached in case of any inconsistency with involvement of an additional author. A pre-designed electronic data abstraction form was used to extract relevant information. This included questions on: baseline population; location; age at baseline; study design; number of participants; type and dose of intervention; duration of treatment or follow-up; timing of intervention; route of administration; comparisons; outcome measures; and results for each outcome (odds ratios [OR], risk ratios [RR], hazard ratios (HR) or mm/year for subclinical measurements). Additionally, for intervention studies, allocation concealment and blinding were also recorded. In case

of multiple publications, the most up-to-date or comprehensive information was extracted.

Assessing the risk of bias

The Cochrane Collaboration's tool (Christie *et al.*, 2010) and the Newcastle-Ottawa Scale (Lewis *et al.*, 2006) were used by two independent investigators to assess the risk of bias in RCTs and in prospective observational studies, respectively. The Cochrane Collaboration's tool evaluates seven possible sources of bias: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other bias. The Newcastle-Ottawa Scale uses a star system (with maximum of nine stars) to evaluate three domains: selection of participants; comparability of study groups; and the ascertainment of outcomes and exposures of interest. Studies that received a score of nine stars were judged to be at low risk of bias; a score of seven or eight stars was medium risk; those that scored six or less were considered at high risk of bias. Furthermore, we applied the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach to score the quality of evidence included in current review. The GRADE approach judges the quality of evidence on two key concepts: magnitude of effect and quality of evidence (considering the risk of bias, study design, consistency and directness of findings). The evidence is graded: high, moderate, low or very low. RCTs start as high quality and observational studies start as low quality. Limitations in study quality, important inconsistency of results, or uncertainty about the directness of the evidence can lower the grade of evidence. Also, certain factors such as evidence of a dose response gradient or strong evidence of association based on consistent evidence from two or more observational studies with no plausible confounders may increase the grade (Schünemann *et al.*, 2013). The evaluation was performed independently by two reviewers, while any disagreement was resolved by discussion between the two parties or by including a third reviewer.

Results

Study identification and selection

In total, we identified 11 591 relevant citations, of which 3982 were duplicates. After screening based on titles and abstracts 7, 480 studies were excluded and 129 articles were selected for detailed evaluation of their full texts. Of those, 54 articles, based on 33 unique studies, met the inclusion criteria and were included in the review: 16 studies examined the dose of HT, 12 studies examined the route of administration, eight studies examined the role of timing of HT initiation and 30 examined the duration of HT use (Fig. 1).

## Characteristics of included studies

Among the 33 included studies, six were clinical trials and 27 were prospective observational studies. In aggregate, the studies reported results for 2 588 327 women (2 541 092 from observational studies and 47 235 from RCTs). Seventeen studies were based in Europe; 16 in North America; and none in South America, Australia, Asia and Africa. The baseline age of participants ranged from 30 to 94 years. For trials, the duration of the interventions ranged from 0.5 to 7.2 years, while for prospective observational studies it ranged from 1 to 28 years.

Dose of HT and CVD risk

Sixteen studies, one RCT and 15 observational studies (Paganini-Hill *et al.*, 1988; Hernandez Avila *et al.*, 1990; Rosenberg *et al.*, 1993; Jick *et al.*, 1996; Grodstein *et al.*, 1999, 2000, 2006; Varas-Lorenzo *et al.*, 2000; Angerer *et al.*, 2001; Lemaitre *et al.*, 2002; Ferrara *et al.*, 2003; Arana *et al.*, 2006; Lokkegaard *et al.*, 2008; Renoux *et al.*, 2010a; Shufelt *et al.*, 2014; Canonico *et al.*, 2016) examined the association between HT dose and various CVD outcomes. Findings are summarized in Table I and detailed study characteristics are provided in Supplementary Table II.

Nine observational studies reported the association between HT dose and heart disease risk, reporting in general no association or cardioprotective effect. In the Nurses' Health Study, among women with no history of heart disease (during 488 801 person-years of follow-up), the risk for coronary events was similarly reduced in those currently taking 0.625 mg of oral CEE daily (RR 0.54, 95% CI, 0.44–0.67) and those taking 0.3 mg of oral CEE daily (RR 0.58, CI 95%CI 0. 37–0.92) compared with never users (Grodstein *et al.*, 2000). The latest publication from the same study, investigating estrogen-only and combined HT in women with history of heart disease, reported a 30% lower risk of coronary heart disease (CHD) for women using estrogen alone or combined HT compared with postmenopausal women who never used hormones. Although findings were similar across various doses of oral conjugated estrogen, only the medium estrogen dose (0.625 mg/day combined with progestin) was significantly associated with reduced CHD risk, RR 0.70 (95% CI 0.59–0.83)(Grodstein *et al.*, 2006). In WHI, in a subset of 1246 women and during a median of 10.4 years of follow-up, women who used oral low-dose CEE (<0.625 mg/day) had non-significantly lower rates of CHD, total CVD and CVD mortality comparing to women who used oral conventional-dose CEE (0.625 mg/day) (Shufelt *et al.*, 2014). A study of 635 women reported a decreased myocardial infarction (MI) risk with a medium dose of oral/transdermal HT; the corresponding ORs for low, medium and high doses were 0.96 (95% CI 0.55–1.65), 0.59 (95% CI 0.42–0.82) and 0.75 (95% CI 0.48–1.19) respectively (Varas-Lorenzo *et al.*, 2000). Similarly, a larger study of 24 420 women showed a decreased risk of MI with oral low and medium dose of estrogen, but there was no evidence of decreased MI risk with high estrogen dose (Ferrara *et al.*, 2003). A cohort study among 9236 Swedish women reported reduced risk of developing MI with medium estrogen dose (0.625 mg/day of CEE or 2 mg/day of estradiol) as compared to low-dose HT (RR 0.75, 95% CI 0.56–0.99) (Grodstein *et al.*, 1999). In addition, we found three observational studies that showed no evidence that MI risk varied with HT dose (Hernandez Avila *et al.*, 1990; Rosenberg *et al.*, 1993; Lokkegaard *et al.*, 2008). In the single RCT we included in our review, in 321 healthy postmenopausal women at increased CVD risk, neither of the combined HT regimens (with standard and low progestin) slowed carotid intima-media thickness (CIMT) (progression within 1 year of follow-up (Angerer *et al.*, 2001).

Two observational studies reported an increased risk of particular vascular events, such as venous thromboembolism (VTE) and transient ischemic attack (TIA), with increasing HT dose—one study showed an association of VTE with estrogen-only and combined oral and transdermal HT (Arana *et al.*, 2006) and another demonstrated

Figure 1 Flow diagram of the study selection process for the systematic review.
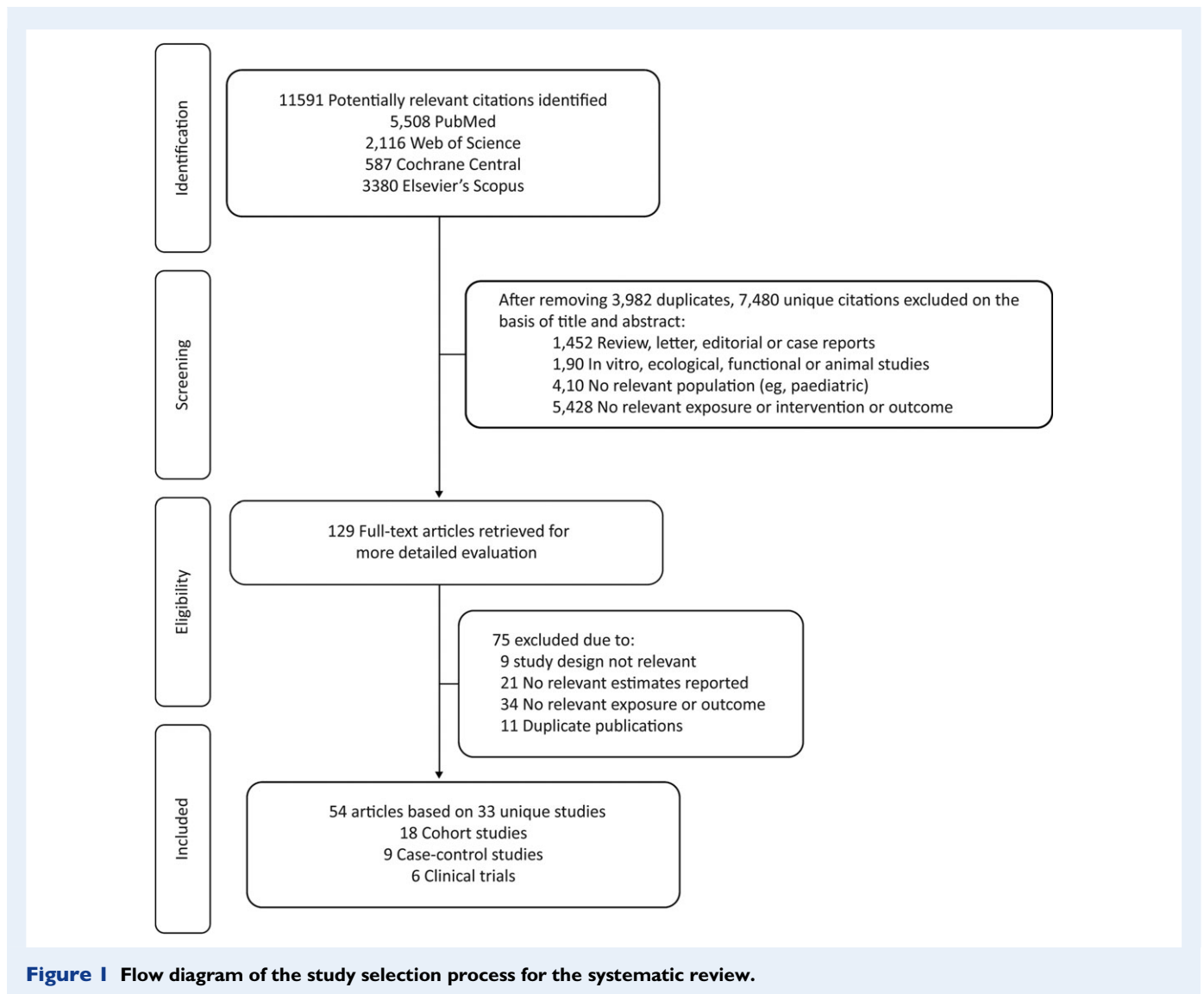
increased risk of TIA associated with oral and transdermal estrogen-only HT (Jick et al., 1996).

We identified seven studies reporting the association between HT dose and stroke risk, with conflicting results. A matched case–control study including >70 000 women reported a dose-dependent relationship between transdermal estrogen and stroke risk, with no increased stroke risk with ≤50 µg of transdermal estrogen, and an 1.89-fold increased stroke risk with >50 µg of transdermal estrogen. However, among women using oral estrogen-only and combine HT regimes the stroke risk was increased from 1.25- to 1.48-fold in both HT regimes (≤0.625 or ≤2 mg/day of estradiol and >0.625 mg/day of estrogen or >2 mg of estradiol) as compared to non-users (Renoux et al., 2010a). Canonico et al. reported increasing-dose-dependent ischemic stroke risk with oral estrogen—the risk was borderline significant with low to medium estrogen dose (<1 mg/day) (OR 1.39, 95% CI 1.00–1.99) and the greatest in those using high (>1 mg/day) estrogen doses (OR 2.41, 95% CI 1.43–4.07); however, in contrast to the findings of Renoux et al. (2010a), stroke risk was not increased with increasing doses of transdermal estrogens (Canonico et al., 2016).

Another study (in >15 000 women) reported dose-dependent stroke risk with no increased stroke risk with low oral estrogens and increased risk with medium and high dose (≥0.625 mg/day) (Grodstein et al., 2000). Lemaitre et al. found no evidence of an increased ischemic stroke risk in users of medium (0.625 mg) compared to low (0.3 mg) estrogen dose (among 864 women), however, when comparing high (>0.625 mg) with low estrogen use, a 2.41-fold increased ischemic stroke risk was observed (Lemaitre et al., 2002). In contrast to this, the WHI did not find a significant difference in stroke risk when comparing low (≤0.625 mg) and medium oral CEE dose (>0.625 mg), RR 1.07, 95% CI 0.76–1.49 (Shufelt et al., 2014). Two observational studies investigated the risk of hemorrhagic stroke, and there was no significant association observed (Grodstein et al., 2000; Arana et al., 2006). A study that investigated all routes of estrogen HT administration reported a protective effect of HT against death due to stroke, yet there was no difference in regard to HT dose (Paganini-Hill et al., 1988). The inconsistent findings on stroke risk may be the consequence of different HT regimes investigated across different studies. Indeed, increased stroke risk was

Table I Summary of findings on association between hormone therapy dose and cardiovascular risk in women.

	Quality of evidence
Low HT dose	
• In observational studies, a cardioprotective effect has been observed even with low HT doses (effect of 0.3 mg/day of oral CEE was similar to that seen with the standard dose of 0.625 mg/day)	B
• The RCTs to support cardioprotective benefit of HT in primary prevention have not been identified	D
High HT dose	
• VTE and stroke risk increases in dose-dependent manner with higher estrogen dose in estrogen alone or combined HT formulations; caution is needed with >0.625 mg/day of estrogen in oral formulations and >50 μg/day in transdermal formulations	B
• Thrombotic risk was significantly higher with preparations containing MPA	B
General conclusion	
• HT should be used in the lowest effective dose to avoid adverse cardiovascular effects, with advancing age HT dose should be reduced.	B

*Findings are based on sixteen studies, one randomized controlled trial (RCT) and 15 observational studies examined the effect of HT dose on various cardiovascular disease (CVD) outcomes; Low dose: 0.3 mg–0.625 mg; Medium dose: 0.625–1.25 mg; High dose: ≥1.25 mg
HT: hormone therapy; VTE: venous thromboembolism; CEE: conjugated equine estrogen; MPA: medroxyprogesterone acetate; B: moderate quality of evidence (We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different); C: low quality of evidence (our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect)

observed with oral estrogen irrespective of the dosage (Renoux et al., 2010a; Canonico et al., 2016) and with high dosages (>0.625 mg/day) or with combined oral HT (Grodstein et al., 2000, 2008). Transdermal estrogens either did not increase stroke risk (Canonico et al., 2016) or increased the risk in regimes with high dosage of estrogen (>50 μg/day) (Renoux et al., 2010a). No association was found between ischemic stroke and use of progesterone, pregnanes and nortestosterones, however ischemic stroke risk was increased with norpregnanes (OR, 2.25; 95% CI, 1.05–4.81) (Canonico et al., 2016). Also, the greatest VTE risk was observed with HT formulations containing MPA (RR 2.67, 95% CI 2.25–3.17) (Sweetland et al., 2012).

Route of HT administration and CVD risk

Twelve studies, one RCT and 11 observational studies (Chilvers et al., 2003; de Vries et al., 2006; Canonico et al., 2007, 2016; Lokkegaard et al., 2008, 2017; Renoux et al., 2010a; Bretler et al., 2012; Sweetland et al., 2012; Harman et al., 2014; Shufelt et al., 2014; Simon et al., 2016), investigated the association between route of HT administration and CVD risk. Findings are summarized in Table II and detailed study characteristics are provided in Supplementary Table III.

Findings on the association between route of HT administration and heart disease were reported in five studies and in general indicated a protective or no effect. In a matched-cohort study involving 5102 women, transdermal estrogen therapy was associated with a 19% lower incidence of CVD events compared with oral estrogen therapy use (incidence rate ratio, IRR 0.81, 95% CI 0.67–0.99) and the observed association was driven mainly by a lower incidence of congestive heart failure and VTE (Simon et al., 2016). Similarly, in a larger study of 93 676 women, transdermal estrogen was associated with a lower risk of CHD compared to oral CEE (HR, 0.63; 95% CI, 0.37–1.06), albeit non-significantly (Shufelt et al., 2014). Three studies investigated the association between route of HT administration and

risk of MI in comparison to never users and reported beneficial or no effect with oral and transdermal HT on MI risk. de Vries et al. (2006), in a study of 9390 women, showed decreased age-adjusted risk of MI with both use of oral and transdermal estrogen-only and combined HT. Similarly, in a case–control study of 1533 women, Chilvers reported a reduced risk of MI with oral HT, but not with transdermal HT (Chilvers et al., 2003). In the large study that followed >400 000 women (during >2 million women-years) Lokkegaard et al. (2008) reported no associations of oral estrogen-only HT with MI, no association of oral nor transdermal combined HT with risk of MI and a decreased MI risk with estrogen-only transdermal HT (RR 0.62, 95% CI 0.42–0.93). Additionally, the vaginal route of HT administration was associated with decreased MI risk (RR 0.56, 95% CI 0.44–0.71) (Lokkegaard et al., 2008).

Four studies reported on stroke risk and one trial investigated CIMT in regard to route of HT administration. In a 4-year double blind RCT (the Kronos Early Estrogen Prevention Study, including 727 recently postmenopausal women at low risk of CVD), low-dose oral or transdermal estrogen with cyclic oral progesterone favorably altered certain CVD risk factors (lipid levels with oral CEE and insulin resistance with transdermal estrogen) and there was no adverse effect of either form of estrogen on either systolic or diastolic blood pressure (Harman et al., 2014). However, the effect of HT on carotid atherosclerosis was neutral regardless of the route of HT administration (Harman et al., 2014). Findings from the WHI, in a subset of 314 women, reported lower (but not statistically significant) stroke risk with transdermal compared to oral conventional-dose CEE (RR 0.87, 95% CI 0.55–1.38) (Shufelt et al., 2014). In a French medical database (including 15 305 women), route of estrogen administration and type of progestogens were shown to be important determinants of ischemic stroke risk. While oral estrogens significantly increased the risk of ischemic stroke (OR 1.58, 95% CI 1.01–2.49) in a dose-dependent manner, transdermal estrogens showed no association (OR 0.83, 95% CI 0.56–1.24). Although there was no significant association of

Table II Summary of findings on association between route of HT administration and cardiovascular risk.

	Quality of evidence
Oral HT administration	
• Does not increase heart disease risk and may be cardioprotective	B
• Increases thromboembolic risk and may increase risk of stroke	B
Transdermal HT administration	
• Is safe with regard to CHD risk	B
• Is safer with regard to thrombotic risk as compared to oral HT administration	B
General conclusions	
• Transdermal estrogen preparation may be safe with regard to CHD and thrombotic risk, and limited evidence indicates no increased risk of stroke associated with use of transdermal estrogen in formulations with <50 μg of estradiol per day	C
• Besides the route of administration, in combined HT the risk from HT may vary with progestin type used	B
• Vaginal HT administration may play a role in myocardial infarction and stroke risk prevention, but data are limited	C

*Findings are based on twelve studies, one RCT and eleven observational studies.
CHD: coronary heart disease. B: moderate quality of evidence; C: low quality of evidence

ischemic stroke with progesterone, pregnane derivatives and nortestosterone derivatives, norpregnane derivatives were found to increase ischemic stroke risk (Canonico et al., 2016). In a large cohort study, including 980 003 women, oral unopposed estrogen or estrogen/progestin treatment was associated with an increased risk of ischemic stroke, whereas there was no increased stroke risk with transdermal application, while vaginal route of administration was associated with decreased stroke risk (RR 0.65; 95% CI, 0.59–0.70) (Lokkegaard et al., 2017). Four observational studies reported the risk of VTE in regard to the route of HT administration. Three studies reported 1.52- to 4.2-fold increased risk of VTE with oral HT and no association between transdermal HT and VTE risk (Canonico et al., 2007; Renoux et al., 2010a; Sweetland et al., 2012). A retrospective matched-cohort study (among 5102 women) reported lower VTE risk with transdermal estrogen-only as compared to oral estrogen-only HT, IRR 0.42 (95% CI 0.19–0.96) (Simon et al., 2016). A large population-based study among more than a million women reported variations in RR of VTE with regard to HT formulation and time since initiation. The risk of VTE varied considerably by HT formulation: greater VTE risk was observed with oral estrogen-progestin HT (RR 2.07, 95% CI, 1.86–2.31) than with oral estrogen-only therapy (RR 1.42, 95% CI 1.21–1.66), with no increased risk with transdermal estrogen-only therapy. The greatest risk increase was observed with HT formulations containing MPA (RR 2.67, 95% CI 2.5–3.17). Also, current users of oral HT had twice the risk of VTE in the first 2 years after starting HT compared to subsequent years (Sweetland et al., 2012). In line with this, Renoux et al. reported increased VTE risk with oral estrogen and estrogen–progestogen therapy that increased with estrogen dose and no increased VTE risk with transdermal estrogen alone or combined with progestogen. The risk of VTE with oral HT formulations was particularly elevated during the first year of use but disappeared 4 months after discontinuation (Renoux et al., 2010b).

One study evaluated the effects of route of administration on atrial fibrillation risk—while overall HT was associated with 9–37% decrease in risk of atrial fibrillation in the first year after MI, the lowest risk of atrial fibrillation was observed in women ≥80 years old for use of overall HT

and vaginal estrogen compared to non-users (HR 0.63, CI 0.42–0.94, and HR 0.58, CI 0.34–0.99, respectively (Bretler et al., 2012).

The timing of HT initiation and CVD risk

Eight studies, two RCTs and six observational (Grodstein et al., 2006, 2008; Prentice et al., 2009; Stram et al., 2011; Manson et al., 2013; Carrasquilla et al., 2015, 2017; Hodis et al., 2016) examined the role of the timing of HT initiation on CVD risk. Different studies looked at different lengths of time between menopause onset and HT initiation: two studies reported on HT initiation in the first 4 years after menopause, three studies at 5 years since menopause, two studies at 10 years since menopause and one study reported on HT initiation 6 years after menopause. Findings are summarized in Table III, and detailed study characteristics are found in Supplementary Table IV.

An intervention trial among 643 women that evaluated subclinical atherosclerotic measures in relation to timing of HT onset reported that oral estradiol therapy, with or without progesterone, was associated with less progression of subclinical atherosclerosis (measured as CIMT) than was placebo when therapy was initiated within 6 years after menopause but not when it was initiated 10 or more years after menopause (Hodis et al., 2016).

Five studies investigated the risk of VTE and stroke with regard to timing of HT initiation. The stroke risk during intervention phase in the WHI was increased by 37% with CEE/MPA and by 35% with CEE, reflecting increased ischemic, but not hemorrhagic, stroke risk. However, in stratified analysis by 10-year age groups, risk of stroke was elevated but non-significantly in both intervention groups. CEE/MPA was observed to significantly increase risk of MI among women >20 years past menopause onset (Manson et al., 2013). Prentice et al. combined both WHI clinical trial data and observational study data to investigate HT initiation <5 and ≥5 years after menopause. Findings indicated increasing VTE risk with CEE with increasing years from menopause to first use of HT and strong early VTE risk elevations with CEE/MPA among recently postmenopausal women without prior HT (Prentice et al., 2009). However, the risk of stroke did

Table III The timing of HT initiation and cardiovascular risk.

	Quality of evidence
Early HT initiation	
• In healthy recently postmenopausal women (<60 years old or who are within 10 years of menopause), the current evidence suggests that use of HT is associated with reduced CHD and mortality risk and no increased stroke risk	B
• There is indication of increased VTE risk even when HT starts near menopause onset, yet, the risk might be minimized using low-dose estrogen-only transdermal/vaginal therapy or combined HT with proper choice of progesterone (e.g. micronized progesterone)	B
• HT initiation 0–5 years after menopause onset was associated with reduced or null risk of future stroke	B
Late HT initiation	
• Observational studies reported no evidence of increased risk CHD/MI risk with later HT initiation (10+ years after the menopause onset)	C
• Observational studies reported increased thromboembolic and stroke risk albeit non-significant	C
General conclusions	
• Late HT initiation (10+ years after menopause onset) should be followed with the HT duration for the shortest time possible	B

*Findings are based on eight studies, six observational and two RCTs; early HT initiation: within 10 years since menopause onset; late HT initiation: 10+ years since menopause onset.
MI: myocardial infarction; B: moderate quality of evidence; C: low quality of evidence

not depend significantly on a gap time from menopause to first use of CEE and CEE/MPA HT. However, it is important to note that it was not possible to calculate RR within the first 5 years since menopause due to small number of events with CEE, therefore, results should be taken with caution. Findings from the Nurses' Health Study indicated a 30–40% increased risk of stroke for women currently taking HT, either estrogen alone or combined with progestin and no difference in the relation of HT to stroke for women initiating therapy near to menopause (<4 years) versus 10+ years after menopause (Grodstein *et al.*, 2008). The latest findings based on pooled individual participant data from >88,000 postmenopausal women from five population-based Swedish cohort studies showed that HT initiated early in relation to menopause onset was not associated with increased risk of incident stroke, regardless of the route of administration, type of HT, active ingredient and duration. Also, while HT initiation 0–5 years after menopause onset, as compared to never use, was associated with a decreased risk of stroke (and hemorrhagic stroke), late HT initiation was associated with elevated risks of stroke and hemorrhagic stroke when CEE was used as single therapy and, furthermore, late initiation of combined HT was associated with increased hemorrhagic stroke risk (Carrasquilla *et al.*, 2017).

Additionally, we identified four observational studies that evaluated the risk of CHD or MI and none of studies reported an increased risk with later HT initiation regardless of HT formulation (Grodstein *et al.*, 2006; Prentice *et al.*, 2009; Stram *et al.*, 2011; Carrasquilla *et al.*, 2015).

Duration of HT use and CVD risk

Thirty studies, three RCTs and 27 observational studies (Henderson *et al.*, 1988; Paganini-Hill *et al.*, 1988; Rosenberg *et al.*, 1993; Jick *et al.*, 1996; Cauley *et al.*, 1997; Heckbert *et al.*, 1997; Sidney *et al.*, 1997; Hulley *et al.*, 1998; Høibraaten *et al.*, 1999; Westendorp *et al.*, 1999; Grodstein *et al.*, 2000; Lemaitre *et al.*, 2002; Chilvers *et al.*,

2003; Le Gal *et al.*, 2003; Tavani *et al.*, 2005; Arana *et al.*, 2006; de Vries *et al.*, 2006; Hsia *et al.*, 2006; Pentti *et al.*, 2006; Somunkiran *et al.*, 2006; Corrao *et al.*, 2007; Lokkegaard *et al.*, 2008; Prentice *et al.*, 2009; Schneider *et al.*, 2009; Stram *et al.*, 2011; Carrasquilla *et al.*, 2015; Tuomikoski *et al.*, 2015, 2016; Renoux *et al.*, 2010a, b), examined the effect of duration of HT use on CVD risk. The findings are summarized in Table IV, and detailed study characteristics are found in Supplementary Table V.

We found four studies that investigated atherosclerotic changes in regard to HT duration. A population-based study among 3784 postmenopausal women showed decreased CIMT in the common carotid artery in women who had used HT for ≥1 year compared with never users, while the use of HT for <1 year was not associated with a change in CIMT (Westendorp *et al.*, 1999). Yet, a small 6-month RCT with 2.5 mg/day of tibolone showed no significant effects of tibolone on either intima-media thickness or blood flow resistance in the carotid arteries in postmenopausal women (Somunkiran *et al.*, 2006). In a longitudinal study among 815 women, oral and transdermal (estrogen-only and combined) HT had a protective effect on carotid atherosclerotic plaque occurrence after 4+ years of use, but not in the group that used HT for <4 years. When stratified by HT regime, this relationship was observed only in oral combined HT and not in the estrogen-only group (Le Gal *et al.*, 2003). However, analysis of 10 739 women from the WHI showed an increased risk of peripheral arterial disease with unopposed oral CEE after 6 years of follow-up (Hsia *et al.*, 2006).

We found 14 studies that investigated VTE and stroke risk with regard to HT duration, and the results were inconsistent. In a population-based cohort study, HT with estradiol was associated with a threefold increased risk of VTE, but this increased risk was restricted to the first year of use, crude OR (<1 year of HT use) was 3.54 (95% CI 1.54–8.2) while crude OR after first year of use was 0.66 (95% CI 0.39–1.10) (Høibraaten *et al.*, 1999). The findings from the HERS trial are in line with the findings of Høibraaten *et al.* (1999),

Table IV The duration of HT and cardiovascular risk.

	Quality of evidence
Short duration	
• HT duration is important predictor of future VTE events irrespective of HT formulation and route of administration indicating high risk even with short HT duration (<1 year)	A
• HT was associated with reduced or null risk of future stroke if initiated relatively soon after the onset of menopause, yet, optimal duration of HT with respect to stroke risk remains to be determined	B
Long duration	
• Evidence from observational studies on HT and MI/CHD and CHD mortality with long term use (5+) years is conflicting	C
• Long HT duration (5+ years) is associated with increased thromboembolic and stroke risk	B
General conclusion	
• Individual CVD risk factors evaluation before HT initiation is strongly advised	C
• Age-related pre-existing conditions (coronary/carotid atherosclerosis, even subclinical) at the time of HT initiation may have a profound impact on the effect of HT on CVD outcomes	C

*Findings are based on 30 studies (3 RCTs and 27 observational studies); short duration <5 years; long duration 5+ years.
A: high quality of evidence (we are very confident that the true effect lies close to that of the estimate of the effect.); B: moderate quality of evidence; C: low quality of evidence.

in that oral estrogen-progestin HT was associated with a 3.29-fold increased VTE risk within the first year of HT use, while the risk was not observed with longer HT duration (Hulley et al., 1998). In a large case–control study, 23 505 cases of VTE were matched with 231 562 controls and the risk of VTE was increased up to twofold in users of oral estrogen-only and combined HT compared to non-users, irrespective of HT duration (≤1 and >1 year) (Renoux et al., 2010b). Similarly, in a small case–control study of 210 women, estrogen-only therapy was associated with increased VTE risk and there was also as suggestion of a duration effect (Jick et al., 1996). In a case–control study of 15 710 stroke cases matched with 59 958 controls, oral estrogen-only HT was associated with a 1.35-fold increased stroke risk with >1 year of HT duration and not with a shorter duration (≤1 year). There was no association between estrogen-only transdermal therapy and stroke risk irrespective of HT duration (Renoux et al., 2010a). Prentice et al. (2009) combined observational study data and clinical trial WHI data and reported no increased VTE (HR 0.84, 95% CI 0.47–1.51) risk but increased stroke risk with longer HT duration (5+ years) in the intervention arm with oral CEE (HR 1.68, 95% CI 1.06–2.66), as compared to never users, while there was no association between oral CEE and stroke risk with 0–4 years of HT use. Yet, in the CEE/MPA intervention arm, there was no significant association between oral CEE/MPA and stroke risk, while increased risk of VTE was observed across all subgroups by HT duration, with highest risk within the first 2 years of HT use (HR 5.30, 95% CI 2.58–10.89) (Prentice et al., 2009). In a case–control study among 864 women, the risk of both types of stroke was transiently increased after initiation of oral estrogen HT. Compared with current HT use initiated earlier (≥0.5 years), a twofold increase in the risk of ischemic stroke (OR, 2.16; 95% CI, 1.04–4.49) and hemorrhagic stroke (OR, 2.20; 95% CI, 0.83–5.81) was seen (Lemaitre et al., 2002). In a large population-based study among 76 875 women, a 35% reduction in incidence of hospitalization for CVD among women who took oral and transdermal HT for >3 years as compared to those treated for <6 months was observed. When comparing route

of HT administration, the reduction in hospitalization was observed in those treated long term with transdermal HT, but not for those who took oral HT (Corrao et al., 2007). One study investigated stroke mortality in pre (1995–2001) and post-WHI (2002–2009) and found no measurable changes in mortality in estrogen HT users, however longer HT duration (1–8 years) was associated with stronger decrease in stroke morality than with shorter duration of HT use (<1 year) (Tuomikoski et al., 2015). In contrast, two studies did not report a beneficial effect of estrogen-only HT on stroke mortality, irrespective of HT duration (<8 and 8+ years and 1 and >10 years) (Paganini-Hill et al., 1988; Cauley et al., 1997). In a nested case–control study of 9429 relatively healthy postmenopausal women, current use of unopposed and opposed estrogen was associated with a 34% increase in the risk of cerebrovascular events. No clear treatment duration (≤1 and >1 year) pattern was observed for ischemic and hemorrhagic stroke, and subgroup analysis by HT duration was not statistically significant, yet, the OR of TIA was significantly increased in HT users and was higher in the first year of HT as compared to longer duration (Arana et al., 2006). Similarly, in the Nurses' Health Study, during more than half a million of years of follow-up, subgroup analysis by oral HT duration did not show variation in stroke risk with increased HT duration and results remained non-significant across the strata (Grodstein et al., 2000). Findings from a nested case–control study including 30 048 women showed no variation in stroke risk with duration of HT (all regimes except estradiol and dydrogesterone), while current short-term (1 year), mid-term (2–4 years) and long-term (5+ years) users had an increased relative risk of developing a VTE as compared to non-users (Schneider et al., 2009).

Sixteen studies reported on heart disease risk in relation to HT duration. In general, existing literature suggested no association between HT duration and CHD risk or suggested a protective effect on MI and CHD with longer duration. The HERS was the first large-scale RCT designed to test the efficacy of HT in the prevention of CHD in women with history of CHD, coronary revascularisation or

MI. After an average of 4.1 years of follow-up, there was no difference in nonfatal MI and coronary death between the hormone (CEE 0.625 mg/day plus MPA 2.5 mg/day) and placebo arms. A *post hoc* time-trend analysis revealed a significant 52% increase in cardiovascular events (42.5/1000 person-years vs. 28.0/1000 person-years) in the first year in the HT group compared with placebo, with a non-significant trend toward fewer events in the treatment arm compared with placebo in later years (23.0/1000 person-years versus 34.4/1000 person-years) (Hulley et al., 1998). Findings from the WHI trial suggest no association between CEE and CEE/MPA oral HT and CHD risk, irrespective of HT duration (Prentice et al., 2009). In line with this, findings from observational studies suggest no association between oral and combined HT duration and risk of MI (Rosenberg et al., 1993; Sidney et al., 1997; Tavani et al., 2005; Lokkegaard et al., 2008; Carrasquilla et al., 2015).

In a population-based study with more than a half million of person-years of follow-up, MI risk was decreased across all HT duration subgroups, with a beneficial effect observed even with short-term HT use (<1 year) (Grodstein et al., 2000). In another population-based study with 4537 cases of MI during 2.62 million person-years of follow-up, a decreased MI risk was observed with longer overall HT duration (>1 and 5+ years) while there was no association with <1 year of HT, yet, due to small number of MI cases, this might be the consequence of underpowered analysis (de Vries et al., 2006). In a case–control study, among 864 women there was a trend for decreased risk of MI with an increased duration of overall HT use, with a significant decrease observed with 5+ years of HT use (trend Chi^2 = 28.6, $P < 0.001$) (Chilvers et al., 2003). Similarly, two observational studies reported decreased MI risk with a long (8+ years) estrogen HT (Heckbert et al., 1997) and decreased CHD/CVD risk with long (3+ years) HT use in general (all regimes) (Corrao et al., 2007).

Additionally, five observational studies investigated CVD related mortality in regard to HT duration and one study reported a HT duration-independent decreased MI mortality (Henderson et al., 1988). Two studies observed a decrease in CHD risk after 10 years of HT (Cauley et al., 1997) and 5 years of HT use (Tuomikoski et al., 2015), while two studies reported no association between HT duration and CHD mortality (Pentti et al., 2006; Stram et al., 2011).

Study quality and between-study heterogeneity

Four of the six included trials demonstrated a medium or high risk of bias within one or more areas of study quality, as evaluated using the Cochrane Collaboration tool (Supplementary Table VI). Only one observational study was considered to be at low risk of bias, with 16 of the 48 studies considered to be at high risk of bias (Supplementary Tables VII and VIII). The variety of available studies (baseline characteristics of study populations, heterogeneity in exposure, outcome and follow-up duration) precluded our ability to quantitatively estimate risk for all cardiovascular outcomes. After assessing the study quality, we applied the GRADE approach to determine the quality of the evidence considering the risk of bias, study design, consistency and directness of findings. Although the study population and the outcomes of each study showed good generalizability and similar results, there was some heterogeneity across HT formulations used and

baseline study characteristics of the participants. Also, the evidence was mostly based on observational data: 27 prospective observational studies and six clinical trials were included in this review. The grading of each statement based on the current review is indicated in Tables I–IV and Fig. 2.

Discussion

Summary of the findings

This review based on data from >2.5 million menopausal women gives an important overview of the current knowledge on the cardiovascular risk related to HT use. In general, oral HT was not associated with increased risk of heart disease, but contrary, beneficial cardioprotective effects may be observed with low doses of oral and transdermal HT. Also, there were some indications that vaginal HT may decrease MI and stroke risk, but the evidence is limited and requires further investigation. However, oral HT might be associated with increased risk of VTE and stroke. VTE and stroke risk increased in a dose-dependent manner with estrogen dose in oral and transdermal HT composed of estrogen alone or in a combination formulation. In women with increased baseline thromboembolic risk, transdermal estradiol alone or with micronized progesterone appears to be safer with regards to CVD risk. Also, due to greater absolute risks of CHD, stroke and VTE, late HT initiation (10 years after the menopause onset or >60 years old) shall be recommended for the shortest time possible and in lowest possible dose and preferably transdermal low-dose HT (<50 μg/day of estrogen) should be advised.

Biological mechanisms underlying the controversial findings on HT and CVD risk

In the first 10 years after menopause onset, the estrogen levels decrease by 60–80% as compared to premenopausal women (Ober et al., 2008). However, in both premenopausal and postmenopausal women, higher levels of estrogen are reported to be associated with adverse cardiometabolic outcomes. Early exposure to estrogen (i.e. an early age at menarche) (Elks et al., 2013; Charalampopoulos et al., 2014) and pregnancy (which is characterized by high endogenous estrogen levels) in premenopausal women (Wu et al., 2017; Li et al., 2018) and a high endogenous estradiol in postmenopausal women (Muka et al., 2017) have been linked with insulin resistance and an increased risk of type 2 diabetes, as well as adverse cardiovascular health and increased risk of CVD (Jaspers et al., 2016). While on the contrary, in a population-based study, women with premature ovarian failure compared to premenopausal women, showed lower estrogen levels, but also a lower mean CIMT and decreased odds of plaque presence (Daan et al., 2016). Therefore, exogenous factors such as HT that alter serum levels of estradiol could play a role in cardiometabolic risk and this role may depend on the extent that HT alters estradiol levels and, thus, may vary by the dose of HT. Indeed, the conventional estrogen HT doses (0.625 mg/day) may increase plasma estradiol concentrations in postmenopausal women, affecting the CVD risk (Smiley and Khalil, 2009). Contrary, a lower dose of estrogen replacement, which alters estradiol levels to a lesser extent, has been found to improve cardiac function and remodeling in murine models of MI, while at increased doses that raised plasma estrogen
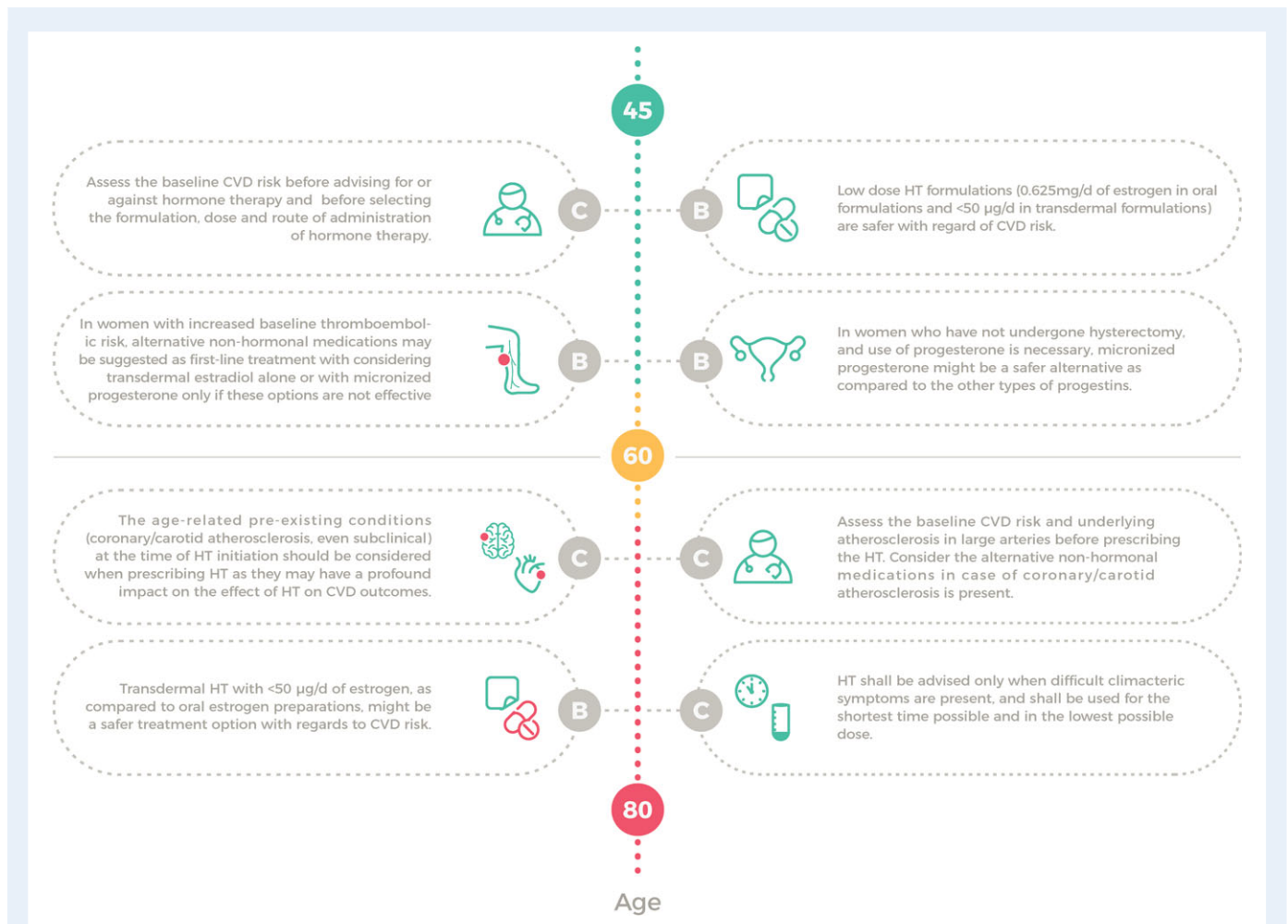
Figure 2 Clinical implications of findings on HT and cardiovascular risk in women. Vertical dotted line refers to women's age (in years). Age from 40 to 60 years refers to menopausal transition and early menopause, while age above 60 years old refers to late menopause. CVD: cardiovascular disease; HT: hormone therapy. Quality of evidence assessed using Grading of Recommendations Assessment, Development and Evaluation: B: moderate quality of evidence (We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different); C: low quality of evidence (Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect).

far beyond the physiological level, estrogen was detrimental to the heart (Zhan *et al.*, 2008; Yang and Reckelhoff, 2011). Also, low doses of CEE in monkeys were associated with a reduction in coronary atherosclerotic plaque extent (Appt *et al.*, 2006). These observed beneficial effects may be due to the improved endothelial function, lipid profile and restoration of plasma estradiol to biological levels that is found when low-dose estrogen is administered. In contrast, greater increases in plasma estradiol of two- to threefold might lead to endometrial hyperplasia (Heckbert *et al.*, 1997; Appt *et al.*, 2006; Zhan *et al.*, 2008). This finding could support the increased ischemic stroke risk with greater HT doses noted in this review and also the greater impact of orally administered HT than transdermal patches. Oral estrogen therapy undergoes the first pass metabolism in the liver, which is associated with a number of adverse hemostatic effects (decreased low-density lipoprotein [LDL] particle size, increased triglycerides/C-reactive protein, increased production of certain coagulation factors), whereas transdermal administration of estrogen

therapy largely avoids these effects (Mohammed *et al.*, 2015). Also, the formulation of HT, especially the type of progestogens in combined HT, could be an important determinant of thrombotic risk. Progestins downregulate estrogen receptors and, via progestin receptor activation, they may oppose the actions of estrogen and MPA may cause this effect to a greater extent than other progestins (Hulley *et al.*, 1998). Findings from RCTs showed that norpregnane derivatives increased markers of blood coagulation activation and induced activated protein C resistance, an established risk factor for VTE (Canonico *et al.*, 2010) and that combined transdermal HT with MPA increased prothrombin fragment 1+2 concentration (Callejon *et al.*, 2005). Yet, nortestosterone derivatives used in transdermal estrogen therapy did not cause changes in matrix metalloproteinase-2 or in LDL particle size (Stevenson *et al.*, 2004), or have beneficial effects on hemostatic parameters (Brosnan *et al.*, 2007). Also, there is evidence that oral (not transdermal) estrogens activated blood coagulation and induced activated protein C resistance (Oger *et al.*, 2003; Post *et al.*, 2003).

Recently, a large population-based study has indicated that in women with carotid atherosclerosis, endogenous estradiol may play a role in the development of vulnerable carotid plaque composition and increase the risk of stroke (Glisic *et al.*, 2017). Similarly, endogenous estradiol in postmenopausal women was associated with increased risk of developing type 2 diabetes, a major risk factor for coronary artery atherosclerosis, stroke and overall CVD risk (Muka *et al.*, 2017). Findings from monkey models support the hypothesis that estrogen therapy may have a cardiovascular benefit when initiated early after the onset of menopause. Based on monkey models in premenopause, estradiol may prevent fatty streak deposition and progression of atherosclerotic plaque (Clarkson and Appt, 2005), Also, monkeys starting HT in early menopause showed reduced coronary artery atherosclerosis, by ~50–70% as compared to placebo. In contrast, delaying initiation of HT in these monkeys for ~6 years in human terms diminished this protection (Williams *et al.*, 1995). Coronary artery fatty streaks and small plaques are common in women at the time of perimenopausal transition, whereas advanced atherosclerotic plaques are common in aging women and in women 5–15 years after menopause (Reslan and Khalil, 2012). Endothelium changes related to atherosclerosis progression in elderly women might be another explanation why HT initiated at the complicated plaque stage might have deleterious effects (beyond ≈60 years of age) (Williams *et al.*, 1995). The underlying mechanisms are not fully understood, but the changes in estrogen receptor signaling (Xing *et al.*, 2009; Muka *et al.*, 2016) or age-related hyper-inflammatory state (Lakatta, 2003) might be important factors.

The duration of HT cannot be observed as a single factor affecting CVD risk. Longer duration occurs simultaneously with the natural aging process, and other important factors are time of HT initiation and underlying endothelium characteristics/presence of other CVD risk factors. Long-term estrogen use may have favorable effects on lipid profile and slow down the atherosclerotic process if administered in women with healthy vasculature (Heckbert *et al.*, 1997). Although the majority of observational studies evaluating stroke risk (Grodstein *et al.*, 2000; Lemaitre *et al.*, 2002; Schneider *et al.*, 2009) reported null findings, there was some indication of increased stroke risk after ≥5 years of HT use (Prentice *et al.*, 2009) and increased risk of TIA irrespective of HT duration (Arana *et al.*, 2006). However, this may be a consequence of HT characteristics and also characteristics of the underlying population investigated.

Quality and credibility of the current evidence and directions for future research

The synthesis of the existing knowledge on this topic was challenging due to inconsistent findings between some studies caused by substantial diversity in scientific rigor and quality across the available evidence. The majority of studies included in the current review are from North American and European populations, which might limit the generalizability of the findings of this review to the other populations. Furthermore, the HT formulation used within studies also differed, that is whether they included progestin or the form of estrogen used, for example, 17β-estradiol or CEEs, which may make the interpretation challenging. Other important factors such as differences in underlying CVD risk factors in study populations, differences in age ranges and variability in adjustment levels (confounding

variables adjusted for in statistical models) made the synthesis of the knowledge challenging. The importance of age is clearly seen in the example of the WHI and HERS trials. The first results from WHI (Rossouw *et al.*, 2002) and HERS trials (Blakely, 2000) changed the clinical practice and led to further multiple trials and studies to delineate the elements that explain the conflicting findings on HT risks and benefits. However, women included in those trials were considerably older than the age at which most women enter the menopause with a mean age in the WHI of 63 years and in HERS of 66.7 years, while the mean age of menopause onset is around the age of 50 years (McNagny, 1999). Therefore, the results of the WHI and HERS trials, although very important, might be driven by the age-related changes that occur simultaneously with HT use. However, those two trials were extremely important and from them arose the so called 'timing hypothesis' that suggests different clinical effects depending on whether HT is initiated close to the onset of menopause (<6 years) or several years later (Lobo, 2017).

Our review emphasizes the gaps in the literature and should stimulate future research to investigate: the risk of VTE and stroke with transdermal/vaginal and oral HT containing different types of progestogens and assess the association with coagulation factors; and the role of underlying diseases and genetic traits in CVD risk, among which genetic variance in estrogen receptor, dyslipidaemia, history of gestational diabetes and pre-eclampsia and carotid atherosclerosis might be the most important. To properly investigate the role of timing of HT initiation, it may be more feasible to conduct large population-based studies rather than RCTs. The trials should recruit women that recently entered the menopause or generally those in their 50 s and follow them for a sufficient amount of time (>5 years). However, CVD rates are considerably lower during this period of life, therefore, the sample size needed to detect a potential adverse effect would most probably make this kind of study design costly and non-feasible. Therefore, retrospective large population-based studies using general practitioner registries may be a better approach to address this research question. A good example is a national historical cohort of women established by linking five Danish registries and including 980 003 women and 20 199 stroke cases (Lokkegaard *et al.*, 2017). It is of high importance to focus the future research on better understanding endothelial dysfunction during the perimenopausal transition and in the first 10 years after the menopause onset. The progression of atherosclerosis may lead to a substantial reduction in estrogen receptors and have a profound impact on observed increased CVD risk with later HT initiation.

Conclusions and clinical implications

The current review presents a cutting-edge summary of HT and CVD risks and the recommendations from this article should be interpreted with caution. The quality of evidence included in this review was in general low or moderate, and findings were based mostly on observational data. The most important clinical recommendations based on this review are summarized in Fig. 2. Use of HT should be individualized and not initiated nor discontinued solely based on a woman's age. Before advising HT use, it is necessary to evaluate baseline CVD risk, age and time since menopause onset. For example, women further from the menopause (e.g. >10 years from the menopause) have a more adverse CVD risk profile and are more

prone to CVD as compared to women who are in first years of the menopause; therefore, the use of HT should be recommended at the lowest dose and for the shortest time period possible. In particular, it is crucial to assess age-related pre-existing conditions (clinical and subclinical coronary/carotid atherosclerosis) at the time of HT initiation as they may have a profound impact on the CVD outcomes. Also, it is recommended that medical professionals discuss with their patients which route of administration might be safer for them, as well as the formulation of HT. The evidence so far shows that the use of transdermal estrogen, as compared to oral estrogen preparations, is less likely to lead to thrombotic events and perhaps also to stroke and coronary artery disease and, therefore, might be a better treatment option for women. While different formulations of HT exist, the use of HT should be based also on women's medical history and particularly on the type of menopause women experienced. For instance, in women who have not had a hysterectomy, when the use of progesterone is necessary, micronized progesterone is considered the safer alternative as compared to the other types of progestins.

Overall, the evidence on HT and CVD risk in women is not robust, but supports the role of different factors, such as route of administration, formulation, age and duration since the menopause, as important determinants of CVD risk related to HT.

Supplementary data

Supplementary data are available at *Human Reproduction Update* online.

Authors' roles

O.H.F. and T.M. conceived and designed the study. C.O.W. and M.G analyzed, interpreted the data and drafted the manuscript. S.S., E.B., C.P.B., R.C. and M.C. selected the articles, retrieved the data and revised the manuscript. All the authors approved the final version of the manuscript.

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Conflict of interest

T.M. and O.H.F. work in ErasmusAGE, a center for aging research across the life course funded by Nestlé Nutrition (Nestec Ltd.), Metagenics Inc. and AXA. T.M. reported receiving research support from Metagenics.Inc. O.H.F. reported receiving grants or research support from Metagenics Inc. These funding sources had no role in design and conduct of the study; collection, management, analysis and interpretation of the data and preparation and review or approval of the manuscript. Other authors, such as M.C., C.O.W., S. S., C.P.B., M.C. and R.C., have nothing to disclose.

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