Summary

Menopause has been shown to be a turning point in a woman's life with respect to the risk for development of coronary heart disease (CHD). Menopause and not just increasing age has been associated with an elevated CHD risk. This suggests a beneficial effect of endogenous oestrogens on CHD. Although an abundance of observational studies showed a cardioprotective effect for postmenopausal hormone therapy (HT), more recently, randomised clinical trials did not. It is important however, to not consider all HT regimens as being alike. Differences in oestrogens and progestogens used, but also in administration route, have been shown to have differing effects on, for example, a surrogate endpoint such as the lipid profile and a hard clinical endpoint like venous thromboembolism (VTE) risk. Effects of non-oral routes of administration, compared with oral, on a wide range of cardiovascular risk markers were the focus of this thesis.

After the general introduction (Chapter 1) the studies conducted in this thesis are described in two parts: In Part I the effects of transdermal versus oral HT were studied and Part II compared the effects of intranasal versus oral HT. In addition, in Part III, the literature regarding effects of non-oral HT on cardiovascular risk markers has been reviewed systematically, followed by a discussion and conclusion on the findings of this thesis.

This thesis is based on data obtained in two randomised, double-blind clinical trials performed in healthy early postmenopausal women. In the first study (Chapter 2 to 4) 152 hysterectomised women (age 54.6 ± 4.5 years) were assigned to either transdermal 17β-oestradiol 50 μg (tE2), or oral 17β-oestradiol 1 mg (oE2), or oral 17β-oestradiol 1 mg continuously combined with gestodene 25 μg (oE2+G), or to placebo, for 52 weeks, followed by 12 weeks of placebo in each group. The second study (Chapter 5 to 9) included non-hysterectomised women: 233 women (age 55.8 ± 5.2 years) in the study on lipids and lipoproteins, 90 women (age 56.6 ± 4.7 years) in the other studies. Women received daily either intranasal E2 175 μg with norethisterone 275 μg (E2+NET) or oral E2 1 mg with norethisterone acetate 0.5 mg (E2+NETA) for 52 weeks.

The following cardiovascular risk markers have been studied: lipids and lipoproteins, markers of inflammation, markers of vascular dysfunction, resistance to activated protein C, anti- and prothrombotic proteins and fibrinolytic parameters.

Lipids and lipoproteins

A disturbed lipid profile is an important risk for the development of CHD. After menopause, lipids change towards a more atherogenic profile. Previously, oral HT has been shown to lower total and LDL-cholesterol and lipoprotein(a), and to raise HDL-cholesterol and triglycerides.

Chapter 2 studied the effects of transdermal and oral HT compared with placebo on the lipid profile. After one year a decrease was found with both routes of administration in total cholesterol (tE2 -4.7%, oE2 -6.9%, oE2+G -10.5%) and LDL-cholesterol (tE2 -5.8%, oE2 -12.6%, oE2+G -13.6%). No changes were seen in HDL-cholesterol or triglycerides. Lipoprotein(a) was decreased during oral HT (oE2 -6.6%, oE2+G -8.2%), but not during transdermal oestradiol.
Effects of intranasal and oral E₂+NET(A) on the lipid profile are described in Chapter 5. A decrease from baseline was found in total cholesterol (intranasal -8.5%, oral -11.2%), LDL-cholesterol (intranasal -8.5%, oral -13.0%), HDL-cholesterol (intranasal -4.3%, oral -3.5%), triglycerides (intranasal -11.7%, oral -8.9%) and lipoprotein(a) (intranasal -15.7%, oral -24.3%).

In both studies decreases in total cholesterol, LDL-cholesterol and lipoprotein(a) were larger during oral compared with non-oral HT. For triglycerides, the decrease was smaller under oral E₂+NETA, compared with intranasal E₂+NET.

Results of the two studies point towards a possible cardioprotective effect for non-oral HT, with the effect being smaller compared with oral HT.

Markers of inflammation

Inflammation plays an important role in the development of atherosclerotic disease. Indeed, elevated levels of inflammation markers have been associated with an increased CHD risk. Compared with increases in C-reactive protein (CRP) as seen during oral HT, transdermal HT has been reported to have a minor effect. Decreases in cell adhesion molecules have been shown during oral HT; information on the effect of transdermal HT is inconclusive.

Chapter 3 describes the effect of transdermal and oral HT on cell adhesion molecules, when compared with placebo. In the oral, not the transdermal, groups soluble vascular cell adhesion molecule (oE₂ -3.8%, oE₂+G -9.3) and sE-selectin (oE₂+G -11.1%) were reduced, not soluble intercellular adhesion molecule.

Effects of intranasal and oral E₂+NET(A) on markers of inflammation are compared in Chapter 6. Decreases in soluble vascular cell adhesion molecule, soluble intracellular adhesion molecule, and sE-selectin were more pronounced during oral E₂+NETA (-12.9%, -14.7% and -20.7%, respectively) than during intranasal E₂+NET (-6.3%, -7.1% and -5.3%, respectively). CRP levels were increased during oral E₂+NETA, with the largest effect already seen within 12 weeks (+64.9%). Most importantly, CRP levels were not increased during intranasal E₂+NET.

If we might extrapolate our findings to clinical effects, our results suggest that non-oral HT can be considered as safe compared with oral HT with respect to atherosclerotic risk.

Markers of vascular dysfunction

Atherosclerosis is preceded by endothelial dysfunction. Various ultrasonographic parameters, as well as blood markers, can demonstrate changes in vascular function in very early stages of atherosclerosis.

Effects of transdermal and oral HT compared with placebo on ultrasonographic parameters are shown in Chapter 3. Some divergent effects were found for both administration routes in pulsatility index, compliance and distensibility in the main branches of the carotid artery. The pulsatility and resistance index in the retinal and femoral arteries or stiffness parameters of the femoral and brachial arteries remained unchanged during both transdermal and oral HT.
In **Chapter 3** no effect for transdermal and oral oestradiol therapy compared with baseline and placebo was found in endothelin-1 levels. In Chapter 4 and 9 the effects on asymmetric dimethylarginine (ADMA) were studied. **Chapter 4** showed that ADMA was lowered by all active treatments compared with placebo (tE2 -4.0%, oE2 -7.7%, oE2+G -7.5%). In **Chapter 9** a reduction was seen during oral E2+NETA (7.4%), but not during intranasal E2+NET. In both studies the reduction in the oral groups was larger compared with the effect in the non-oral group.

Although effects on ADMA suggest a possible cardioprotective effect, our observations in other vascular dysfunction parameters point in a more neutral direction for non-oral HT.

**Factors associated with venous thromboembolism**

Resistance to activated protein C is strongly associated with an increased VTE risk. Deficiencies in protein S, protein C and antithrombin are other important risk factors for VTE, as are increased levels of prothrombin and factor VIII. In line with clinical findings, prothrombotic changes have been demonstrated for these markers for oral, but less so for transdermal HT.

In **Chapter 7** the effects of intranasal and oral E2+NET(A) on markers associated with VTE were studied. The increase in normalised Activated Protein C sensitivity ratio was much smaller during intranasal E2+NET than during oral E2+NETA (+11.2% and +53.8%, respectively). For protein C changes were similar in both groups (intranasal -11.1%, oral -11.6%). Free protein S was slightly decreased in the intranasal group (-2.2%), not in the oral group. For antithrombin the decrease was less pronounced in the intranasal (-5.7%) than in the oral (-10.2%) group. Both groups did not show a change in factor VIII, whereas prothrombin was equally decreased in both groups (intranasal -5.2%, oral -4.8%).

According to these findings, intranasal HT is likely to be safer with respect to VTE risk, when compared with oral HT.

**Haemostatic markers associated with coronary heart disease risk**

High levels of pro-coagulant proteins, such as fibrinogen and factor VII, and of pro-fibrinolytic parameters, like tissue-type plasminogen activator (tPA) and its inhibitor plasminogen activator inhibitor type-1 (PAI-1), are associated with an adverse cardiovascular outcome. **Chapter 8** investigated the effects of intranasal and oral E2+NET(A) on haemostatic markers associated with CHD risk.

Among the markers of coagulation only factor VII activity was decreased (-14.0%) in the intranasal group. Changes in fibrinogen, factor VII activity and prothrombin fragment 1+2 were smaller in the intranasal than in the oral group which showed changes of -6.5%, -20.3% and +19.0%, respectively. Neither group showed changes from baseline in thrombin-antithrombin (TAT) complex.

Among the fibrinolytic parameters, intranasal E2+NET demonstrated a decrease from baseline in tPA (-10.4%) and PAI-1 activity (-17.0%) and an increase in D-dimer (+16.1%). Changes in tPA and PAI-1 antigen were smaller in the intranasal than in the oral group, showing a -17.8% and a -38.0% decrease respectively. After 52 weeks changes in the oral group in PAI-1 activity (-30.6%), D-dimer (+17.6%) and plasmin-α2-antiplasmin (PAP) complex (+8.9%) did not differ from the intranasal group.
Although end-of-study effects did not differ, increases in week 12 in D-dimer and in PAP were smaller in the intranasal (+29.8% and +31.6%, respectively) than in the oral group (+50.7% and +58.8%, respectively).

Although both groups did not demonstrate any effect on homocysteine after 52 weeks, oral, but not intranasal E2+NET(A) revealed a decrease (-4.9%) in week 12.

Our findings of minor pro-coagulatory effects and a probable pro-fibrinolytic effect might point towards a possible beneficial effect for intranasal E2+NET. This contrasts with the more pronounced, pro-coagulant but also pro-fibrinolytic effects seen with oral E2+NETA.

In Chapter 10, the literature regarding the effects of non-oral HT regimens on markers studied in this thesis was reviewed systematically. We concluded that non-oral HT had minor, if any, effects on the cardiovascular risk markers studied, and that except for CRP and APCr, the effects seen do not largely differ from oral HT. Therefore, the assumption seems to be justifiable that non-oral HT is likely to be safe with respect to CHD and VTE. Finally, Chapter 11 discusses the results of this thesis, as well as the implications for clinical practice and suggestions for further research. As the effect of oral HT on cardiovascular risk in apparently healthy, early menopausal women is still not established, our findings can only with great cautiousness be extrapolated to clinical effects for non-oral HT.

The results of the studies described in this thesis confirm the clinically demonstrated safety of non-oral HT with respect to VTE risk. Effects on markers for CHD risk suggest a less pronounced impact of non-oral HT on both potentially harmful effects and possibly beneficial effects with non-oral HT. Overall, a more neutral effect of transdermal and intranasal HT when compared with oral HT is suggested. To confirm or refute our conclusions, large randomised clinical trials among early menopausal women with hard clinical endpoints are warranted. Such trials could provide the ultimate proof of cardiovascular safety of non-oral HT.