Summary

This thesis evaluates the relationship between endogenous or exogenous oestrogen or oestrogen-related substances and asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase (NOS) inhibitor and arterial disease risk marker, in middle-aged women. The effect of endogenous oestrogens was investigated in a longitudinal study of women going through the menopausal transition, in a case-control study in which postmenopausal women were compared with age-matched premenopausal women and in a study of women with a surgical menopause. In five randomised clinical trials, the effects of oral and non-oral oestrogen therapies as well as oral oestrogen therapy combined with a progestogen and oestrogen-related substances on ADMA concentrations were investigated. Concentrations of ADMA, arginine and symmetric dimethylarginine (SDMA) were measured by high-performance liquid chromatography in all studies described in this thesis.

Background

Arterial disease is the main cause of death in the industrialised countries. Premenopausal women have lower arterial disease incidence than men of the same age. After the menopause, the risk for arterial disease increases rapidly in women. Possibly, a strong reduction in oestrogen production after the menopause plays an important role in this acceleration of the arterial disease risk in women. Observational studies indicated that the use of oestrogen therapy alone or combined with a progestogen (both will further be referred to as hormone therapy (HT)) could protect postmenopausal women from this increase in arterial disease risk. However, large randomised placebo-controlled trials reported no cardiovascular benefit of long-term oral HT use, and possibly even an early increase of arterial disease risk in late postmenopausal women. The effects of HT in early postmenopausal women as well as the effects of non-orally administered HT on arterial disease risk in postmenopausal women are still unclear.

Studies with large numbers of women are needed to study the influence of endogenous or exogenous oestrogens on hard clinical arterial disease endpoints such as myocardial infarction and stroke. An interesting alternative could be to investigate the influence of oestrogens on arterial disease risk markers instead. Many of these risk markers are related to different processes involved in the pathogenesis of arterial disease, such as coagulation, lipid metabolism, endothelial function, inflammation and oxidation.

Nitric oxide (NO) is a potent vasodilator produced by the endothelium. A reduced NO availability has been suggested to play a role in the development of
arterial disease. ADMA, an endogenously produced methylated form of arginine, inhibits the enzyme NOS, resulting in low NO concentrations. High blood levels of ADMA have been associated with increased risk of cardiovascular events and mortality risk in specific patient populations. Male participants dominated most of these study populations.

From the available literature, a link between oestrogens and ADMA can be inferred. However, the influence of menopause on ADMA concentrations in women is not clear and reports on the effect of HT on ADMA concentrations in postmenopausal women are scarce. Therefore, this thesis addressed the following question: Do menopause, HT and alternatives for HT modify ADMA concentrations in middle-aged women?

**ADMA and menopause**

The changes in ADMA concentrations resulting from a physiological and a surgical menopause are evaluated in Chapter 2. In a longitudinal study, women were examined annually from two years before until two years after physiological menopause, and in a case-control study, postmenopausal women were compared with age-matched premenopausal women. Surgical menopause effects were investigated in women undergoing a prophylactic bilateral salpingo-oophorectomy. The following parameters were measured: serum concentrations of oestradiol, follicle-stimulating hormone (FSH), inhibin A, inhibin B, ADMA, lipids, leptin, homocysteine, C-reactive protein (CRP) and coenzyme Q10. Length and weight were measured as well, and the body mass index was calculated.

After the physiological and surgical menopause, serum oestradiol and inhibin A and B decreased, whereas FSH increased. Serum ADMA, total and low-density lipoprotein (LDL)-cholesterol and leptin concentrations were significantly higher in postmenopausal women compared to premenopausal women and serum homocysteine increased during the menopausal transition. Furthermore, total and LDL-cholesterol increased after the surgical menopause as well. None of the other parameters was influenced statistically significantly by the menopausal transition.

In conclusion, the arterial disease risk profile is affected unfavourably by menopause. Changes in most arterial disease risk markers were small, despite substantial changes in the hormonal parameters studied.

**ADMA and oral oestrogen-only or combined oestrogen-progestogen therapy**

In Chapter 3 the effects of short-term oral HT on ADMA, arginine and SDMA were investigated in a prospective, randomised, placebo-controlled 12-week study. Healthy postmenopausal women received daily placebo or oral 17β-oestradiol, either unopposed or sequentially combined with dydrogesterone or trimegestone. Fasting plasma concentrations of ADMA, arginine and SDMA were measured at baseline and after four and twelve weeks.

ADMA concentrations reduced in all active treatment groups. Compared to baseline and placebo, the largest reduction in ADMA levels was observed after 17β-oestradiol combined with trimegestone (-18.7% and -21.1% at four and twelve weeks, respectively). At four and twelve weeks, this combination significantly reduced arginine concentrations as well (-30.9% and -36.3%, respectively). SDMA
concentrations were significantly lower after 17β-oestradiol combined with dydrogesterone after twelve weeks (-11.6%).

In conclusion, oral 17β-oestradiol, either alone or combined with dydrogesterone or trimegestone, reduced plasma levels of the NOS inhibitor ADMA. The largest reduction was seen after 17β-oestradiol combined with trimegestone. Whether the reduction of the NOS substrate arginine in the group receiving 17β-oestradiol combined with trimegestone counteracts the potentially beneficial effect of ADMA reduction or only reflects increased NO production remains to be investigated.

In Chapter 4, the effects of oral unopposed 17β-oestradiol versus 17β-oestradiol continuously combined with gestodene were investigated in a placebo-controlled, double-blind study. The study duration was 13 (28-day) cycles and fasting plasma concentrations of ADMA, arginine and SDMA were measured at baseline and in treatment cycles four and 13.

Unopposed oral 17β-oestradiol reduced ADMA concentrations with 7.7% and combined therapy reduced ADMA concentrations by 7.5% compared with placebo after 13 cycles. Both treatment regimens significantly reduced arginine concentrations compared to placebo as well. Only unopposed 17β-oestradiol treatment significantly reduced SDMA concentrations. In summary, adding gestodene to oral 17β-oestradiol did not modify the reduction in ADMA concentrations seen with 17β-oestradiol alone therapy.

The effect of an oral combination of 17β-oestradiol and norethisterone acetate on ADMA concentrations was investigated as well (Chapter 5). The oral combination reduced ADMA concentrations with 7%. This decrease was similar to the decrease found with 17β-oestradiol alone (Chapter 3 and 4) and 17β-oestradiol combined with dydrogesterone (Chapter 3) and gestodene (Chapter 4). Both these progestogens did not modify the ADMA-reducing effect of 17β-oestradiol. Possibly, norethisterone acetate also does not change the ADMA-reducing effect of oestrogen as well.

**ADMA and route of HT administration**

In the same study as described in Chapter 4, the effects of transdermal versus oral 17β-oestradiol on ADMA concentrations were also investigated. After oral 17β-oestradiol administration a significantly larger reduction in ADMA concentration was observed than after transdermal administration. Oral, but not transdermal treatment, significantly reduced arginine and SDMA concentrations compared to placebo.

The difference in influence of intranasal versus oral 17β-oestradiol combined with norethisterone (acetate) on ADMA concentrations in postmenopausal women was investigated in a study reported in Chapter 5. In a randomised, double-blind, comparative study, healthy postmenopausal women daily received intranasally or orally administered 17β-oestradiol combined with norethisterone (acetate), in comparable dosages. At baseline, week twelve and 52, fasting plasma concentrations of ADMA, arginine and SDMA were measured.

ADMA concentrations reduced with 7.4% after oral administration, while after intranasal administration no effect (0.8%) was observed after 52 weeks. In both groups, arginine decreased transiently by approximately 6% compared to baseline at week twelve. Only oral administration reduced SDMA concentrations. Therefore,
the two studies of Chapters 4 and 5 provide evidence that both transdermally and intranasally administered HT are not as effective in reducing ADMA and SDMA concentrations as orally administered HT.

**ADMA and alternatives for HT**

Chapter 6 discusses the effects of a supplement containing soy isoflavones and *Actaea racemosa L.* on several arterial disease risk markers in menopausal women. In a randomised, placebo-controlled, double-blind study, menopausal women received daily either placebo or a supplement containing soy isoflavones and *Actaea racemosa L.* for twelve weeks. Fasting concentrations of ADMA, lipids and CRP were measured at baseline and week twelve.

In the supplement group, total cholesterol and LDL-cholesterol showed a small reduction at week twelve (both -0.2 mmol/l). Concentrations of ADMA, arginine, SDMA, triglycerides, lipoprotein(a) and CRP did not change significantly. After the 12-week study period, none of the parameters investigated revealed significant between-group differences.

Therefore, twelve weeks of administration of a supplement containing soy isoflavones and *Actaea racemosa L.* had little or no influence on the arterial disease risk markers studied. This supplement probably has neither protective nor adverse effects on the cardiovascular system, however, large long-term studies are needed to confirm this.

In addition, in Chapter 7 in the same women described in Chapter 6 we investigated the influence of the supplement intervention on menopausal symptoms as well. The modified Kupperman Index, the Greene Climacteric Scale, a visual analogue scale designed to measure quality of life and the daily number and severity of hot flushes, were evaluated at screening and at weeks six and twelve.

At weeks six and twelve, all scores in both groups had improved compared with baseline, though the overall difference in scores between the groups was not statistically significant. Therefore, the supplement containing soy isoflavones and *Actaea racemosa L.* had no additional effect compared with placebo on menopausal symptoms in women experiencing at least five vasomotor symptoms per day.

The short-term effects of three different doses of the SERM HMR 3339 in comparison with placebo and raloxifene on ADMA, arginine and SDMA concentrations are described in Chapter 8. In a randomised, placebo-controlled, double-blind, dose-ranging study, healthy postmenopausal women received daily either placebo, HMR 3339 2.5 mg, HMR 3339 10 mg, HMR 3339 50 mg, or raloxifene 60 mg for twelve weeks. Fasting plasma concentrations of ADMA, arginine and SDMA were measured at baseline and after four and twelve weeks.

HMR 3339 induced a dose-dependent reduction of ADMA and SDMA concentrations, with the largest effects in the HMR 3339 50 mg group compared to baseline and placebo (at twelve weeks: -7.0%, for ADMA and -16.2%, for SDMA). Twelve weeks of raloxifene 60 mg significantly reduced SDMA but not ADMA concentrations. Arginine concentrations were not changed by any treatment. These results suggest that HMR 3339 may have a potentially beneficial effect on the cardiovascular system by reducing the NOS inhibitor ADMA in postmenopausal women.
Conclusion

The results of the present thesis are discussed in Chapter 9. In this same chapter, briefly is speculated upon the implications for the clinical practice of these results and some suggestions are made for future research. Both endogenous and exogenous oestrogens as well as the SERM HMR 3339 reduced ADMA concentrations in healthy middle-aged women. Adding the progestogen trimegestone to oral oestrogen therapy augmented the oestrogen-induced ADMA reduction, whereas adding dydrogesterone or gestodene to oral oestrogen therapy did not modify this reduction in ADMA concentrations. In addition, norethisterone acetate did not seem to modify the oestrogen-induced ADMA reduction. Neither transdermally nor intranasally administered HT was as effective in reducing ADMA and SDMA concentrations as orally administered HT. Also, neither soy isoflavones combined with Actaea racemosa L., nor the SERM raloxifene modified ADMA concentrations.

There is a clear relation between endogenous oestrogen concentrations as well as oestrogen therapy and ADMA concentrations. Higher oestrogen concentrations coincide with lower ADMA concentrations. The clinical implications of this relation between oestrogens or oestrogen-related substances and ADMA concentrations remain unclear and the mechanisms underlying the oestrogen-induced ADMA reductions are not fully understood. Future research should focus on these two aspects of the relationship between oestrogens and ADMA in women.