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Vascular system and protocols of postmenopausal HRT administration

Epidemiological evidence suggests that estrogen replacement therapy has a protective effect against cardiovascular disease in postmenopausal women. The mechanisms of this effect are not completely understood. It is well documented that estrogen favorably influences plasma lipoprotein levels but, in a recent epidemiological evaluation, it was estimated that lipid factors explained only 25 to 50 % of the reduced incidence of cardiovascular diseases observed in estrogen-treated women. Since hormone receptors are present on arterial endothelial and smooth muscle cells, a direct action of hormone replacement therapy (HRT) on the arterial wall is a possibility.

The aim of this review was to evaluate the clinical effects of HRT on the vascular system of postmenopausal women, following various protocols of administration. Analysis of HRT-induced effects on the vascular system was based on data obtained in recent clinical studies of vasodilation, endothelial function and atherosclerosis. Some *in vitro* data are also presented on mechanisms of action.

Clinical studies of HRT effects on vasodilation

Estrogens exert many direct effects on the cardiovascular system that are independent of metabolic changes. However, few clinical markers for these direct arterial effects have been studied. Currently, the pulsatility index (PI) which is thought to represent impedance to blood flow distal to the point of sampling, the resistance index (RI) and vascular responses to acetylcholine are considered to be the main markers.

Endometrial perfusion

In a controlled study involving asymptomatic postmenopausal women aged 55-60 years (Achiron et al., 1995), the responses of the endometrial arteries to HRT (conjugated estrogen (Premarin® 0.625 mg/day) combined with 5 mg medroxyprogesterone acetate) and tamoxifen were analyzed (table 7.I). The study population was divided into three groups : 10 women using cyclic replacement therapy, 10 women receiving tamoxifen (20 mg/day) as adjuvant

Table 7.I : Clinical effects of HRT on vasodilation

Authors	N Age (years)	HRT	Duration	Arteries	Effects	
Achiron et al. (1995)	30 55-60	• CE (0.625mg, p.o.) + MPA (5mg)	2 years	endometrial perfusion		RI decrease (26 %)
Bonilla-Musoles et al. (1995)	203	• E2 (0.05mg - transdermal), n=58 • E2 (0.05 mg - transdermal) + MPA (2.5mg), n=25 - (5 mg), n=35 or + P (100mg) n=20 • E2 (0.05mg - transdermal) + MPA (10 mg), n=30 or + P (200mg), n=30	1 year	uterine	PI decrease (35%)	RI decrease (13%)
Ziegler et al. (1991)	6 27-36	E2 (0.1 to 0.4 mg - transdermal) + P (300 mg, vaginal)	28 days	uterine	PI decrease (71%) days 13-14, 26-27	
Hillard et al. (1992)	12 48-60	E2 (0.05 mg - transdermal) + norethindrone acetate (0.7 mg, n=7) or MPA (10 mg, n=4)	2 cycles	uterine	PI decrease (47%) day 12 PI decrease (34%) day 26	
Volterrani et al. (1995)	11 40-63	• E2 (1mg - sublingual)	40 min	forearm		RI decrease (40 %)
Belfort et al. (1995)	10 61±5	• E2 (2mg, p.o.)	2 months	retinal ophtalmic		RI decrease (9.5%) RI no change
Gangar et al. (1991)	12 47-59	E2 (0.05 mg -transdermal) + MPA (10 mg, weeks 10 to 21))	22 weeks	internal carotid	PI decrease (11%) weeks 9 and 22	
Penotti et al. (1993)	25 51.7±3.3	• E2 (0.05mg - transdermal) + MPA (10mg)	24 weeks	internal carotid middle cerebral	PI decrease (25%) weeks 22 and 24 PI decrease (25%) weeks 22 and 24	
Megnien et al. (1993)	9	E2 (0.1 to 0.4 mg - transdermal) + P (300 mg, vaginal)	28 days	brachial	Diameter increase (7%) day 28	Resistance decrease (40%) day 28

Table 7.I : Clinical effects of HRT on vasodilation *(continued)*

Authors	N Age (years)	HRT	Duration	Arteries	Effects	
Collins et al. (1995)	9 59±3	E2 (2.5 µg, intracoronary)	20 min	coronary – epicardial – resistance	 Diameter : no change Blood flow : no change	
Gilligan et al. (1994)	20 61±7	E2 (75 ng/ml, intracoronary)	20 min	coronary – epicardial – resistance	 Diameter : no change Blood flow : no change	 Resistance : no change
Reis et al. (1994)	33 64±2.1	EE (35 µg, intravenous)	15 min	coronary – epicardial – resistance	 Cross sectional area increase Blood flow : no increase	 Resistance : decrease
Giraud et al. (1996)	26 55.8±4.2 57.4±3.8	 CE (0.625 mg, per os) or CE (0.625 mg per os) + MPA (2.5 mg)	3 months	aorta (ascending and descending)	Cross sectional area Increase (6-7%) or No change	Compliance no change no change

treatment for metastatic breast cancer at different stages and 10 women free of hormonal treatment. The durations of treatment were matched in the two groups, with an average of 24 months. A transvaginal color flow imaging system was used to explore endometrial and subendometrial blood flow. Quantitative data from areas of color were evaluated by pulsed Doppler spectrum analysis. Resistance indices (RI) were measured as indicators of endometrial perfusion : RI=(S-D)/S (S, systolic peak ; D, diastolic peak). All examinations were performed by the same investigator with an intra-observer error of no more than 6 %. Arterial waveform analysis in the women on HRT showed the lowest impedance to diastolic flow, as reflected by the RI. RI values in untreated women were higher (mean 0.89) than in those on tamoxifen or HRT. The lowest RI was obtained in women taking HRT (mean 0.66, $p<0.05$). This response was found to correlate with plasma estrogen levels. Blood pressure values were not reported. These data suggest that HRT enhances endometrial blood perfusion through vasodilation.

Uterine artery

The effect of HRT on the uterine artery was analyzed in three studies. A study (Ziegler et al., 1991 ; table 7.I) was performed on young women deprived of ovarian function to clarify the role played by physiological levels of ovarian hormones on vascular resistance of uterine arteries. Six women (27-36 years) were included in this study and received transdermal estradiol-17β (0.1 to 0.4 mg/day, Estraderm TTS® 100) and 3×100 mg tablets of micronized progesterone (Utrogestan) vaginally from days 15 to 28. Vascular resistance of uterine arteries was evaluated before treatment and on days 13 to 14 and on days 26 to 27 using transvaginal pulsed Doppler combined with real-time imaging. PI was calculated. In the absence of ovarian hormone, uterine arteries had a higher degree of vascular resistance expressed by high PI values. On cycle days 13 to 14, PI showed a marked decrease (71 %) and on cycle days 26 to 27, PI values were similar to those obtained after exposure to estradiol only. These data show that transdermal administration of estradiol-17β in quantities which duplicate the ovarian production of estradiol seen during the menstrual cycle (288 ± 33 pg/mL on day 13) decrease the vascular resistance of uterine arteries. Progesterone did not interfere markedly with the vasodilatory effect of estradiol-17β on uterine arteries.

A study (Hillard et al., 1992 ; table 7.I) was performed on natural postmenopausal women receiving estrogen replacement therapy to establish the effect of progestogen addition on arterial tone in the uterine artery. Twelve postmenopausal women (52.5 years) who had all undergone natural menopause were treated with continuous transdermal estradiol-17β (0.05 mg/day, Estraderm TTS® 0.05) and either norethindrone acetate (0.7 mg/day, n=7) or medroxyprogesterone acetate (10 mg/day, n=4) added sequentially from day 17 to day 28 of each 28-day cycle. The treatment was administered for 2 consecutive cycles. Assessments of the PI in the uterine arteries (transvaginal

Doppler with color flow imaging) were performed pretreatment and on day 12 (estradiol-17β-only phase) and day 26 (combined estradiol/progestogen phase) of both treatment cycles. There was a significant correlation between baseline PI and time since menopause. In every patient, transdermal estradiol lowered the PI and this effect was partially antagonized when a progestagen was added. There were no differences in effect between the 2 progestagens. In the first cycle, the mean PI fell to 53 % of its pretreatment value within 12 days of commencing transdermal estradiol and to 66 % of the pretreatment value in the combined estradiol/progestogen phase. This difference between the estradiol-only and the estradiol/progestogen phase of treatment was significant. A similar pattern was observed in cycle 2 and the difference between the estradiol-only and the estradiol/progestogen phases remained significant. Patients with the longest intervals since menopause had the highest pretreatment values and exhibited the greatest reductions in the PI. These results suggest that estrogens rapidly have profound effects on arterial tone in uterine arteries in postmenopausal women. The addition of progestogens partially antagonized the response to transdermal estradiol but these data suggest that any potential adverse effect of progestogen on the estradiol-induced change in PI is partial and of short duration.

The effects of HRT on uterine arterial blood flow were also studied in 203 postmenopausal women (Bonilla-Musoles et al., 1995). One hundred and five postmenopausal women who had never been on HRT were evaluated by transvaginal color Doppler sonography prior to starting HRT, after 26 days of HRT and every 3 months for 1 year. Ninety-eight women already on HRT remained on their HRT regimen and were evaluated by transvaginal color Doppler sonography every three months for 1 year. All women had undergone natural menopause. All investigations were performed by the same two investigators. The women were divided in three groups according to the HRT regimen : 58 were on unopposed estrogen therapy with continuous transdermal estradiol-17β patches (Estraderm TTS®, 0.05 mg/day) ; 85 women were taking continuous combined estrogen (Estraderm® patches) and medroxyprogesterone acetate (2.5 mg, n=25 ; 5.0 mg, n=35), or natural progesterone (100 mg, n=20) ; 60 women were on a regimen of combined sequential estrogen (Estraderm®) and estrogen-progestogen (medroxyprogesterone acetate (10 mg/day, n=30) or natural progesterone (200 mg/day, n=30). In this last group, the progestogen was added to the estrogen regimen from day 17 to day 28 of each month and the first examination was done on day 13 and the second on day 26 of the first month. There was a 35 % reduction in mean PI and a 13 % reduction in mean RI during the first month of therapy. The fall in PI and RI observed during the first examinations remained stable in all subsequent examinations. No significant differences in PI and RI values were found with the different HRT regimens. The addition of a progestogen did not alter the effect of estrogen alone. No difference in response occurred between the two progestogen regimens studied. Blood pressure values on HRT

treatment were not reported. The decrease in PI was significantly greater in women who had been postmenopausal for longer. These data suggest that HRT induces rapid vasodilation and an increase in flow in the uterine artery. The beneficial effects on arterial flow were unaffected by the addition of progesterone and were maintained for at least 1 year of estrogen therapy (see also the above presented experimental study from Magness and Rosenfeld, 1989).

In different experimental conditions, progesterone was shown to cause a dose-dependent endothelium-independent relaxation of human placental arteries and veins. This relaxation seems to be mediated by a receptor-activated cAMP-mediated mechanism (Omar et al., 1995).

Forearm

In a double-blind, randomized, placebo-controlled study (Volterrani et al., 1995), performed in 11 normotensive, non hyperlipidemic, postmenopausal women (53 ± 6 years), the acute response to sublingual estradiol-17§ was studied on the forearm resistance vessels. Six women were in natural menopause and five had hysterectomy (table 7.I). Subjects were given, in double blind, randomized fashion, a sublingual placebo tablet on 1 day and sublingual estradiol on the other. Blood flow was measured by venous occlusion plethysmography ; mean blood pressure was measured and mean peripheral vascular resistance was calculated. After administration of sublingual estradiol (1 mg), plasma concentrations of estradiol-17β increased from 112 ± 38 to 3 234 ± 411 pmol/L. There was a significant increase in flow (>50 %) 40 min after estradiol administration, in comparison with baseline resting blood flow and placebo administration. Forearm vascular resistance was significantly decreased (40 %) at this time. There was a small but significant decrease in mean blood pressure 20 min after estradiol compared with placebo (88 ± 1.7 mmHg versus 94.9 ± 2.2 mmHg), but blood pressure was not different 40 min after the administration of estradiol compared to placebo. These results show that the acute effects of sublingual estradiol include an increase in blood flow associated with a reduction in vascular resistance in the peripheral vascular bed of the forearm. The lack of effect on resting blood pressure would indicate an increase in cardiac output. The mechanisms responsible for the acute blood flow response to estrogen are controversial (direct effects on vascular myocytes and indirect effects via the endothelium).

Brachial artery

Megnien et al. (1993) measured brachial artery diameter, blood velocity and flow by two-dimensional pulsed Doppler before treatment and on days 14 and 28 in 9 young women (27-37 years) with inactive ovaries who received transdermal estradiol-17β (0.1 to 0.4 mg/day, Estraderm TTS® 100) and vaginal progesterone (300 mg/day) from day 15 to day 28. Blood pressure did not

change. An increase (6.8 %) in brachial diameter began at day 14 and became significant at day 28 (3.91 ± 0.10 mm versus 3.66 ± 0.11 mm before treatment). Blood velocity and flow increased significantly at day 28. The decrease in resistance began at day 14 and was significant at day 28 (–40 %). Brachial vasoconstriction during hand exclusion, in response to low flow state, disappeared at day 14. These results suggest that, in young women deprived of ovarian function, HRT dilates small and large arteries, whereas estrogen alone attenuates the large-artery vasoconstriction in an acute response to a low flow state.

Cerebral arteries

Blood velocity and vascular resistance were studied in the cerebral microcirculation (central retinal and ophthalmic arteries) according to the phases of reproductive life (Belfort et al., 1995 ; table 7.I). Color flow Doppler ultrasonography was used to determine systolic, diastolic and mean velocity as well as the RI in 10 nonpregnant women (29 ± 5 years), in 10 third-trimester pregnant women (26 ± 5 years) and in 10 postmenopausal women (61 ± 5 years). The postmenopausal women had all undergone hysterectomy. These women were studied before beginning estrogen replacement therapy with daily oral micronized estradiol-17β (2 mg, Estrace®) and again 2 months after starting the medication. The postmenopausal women, who were significantly older, had higher mean blood pressure and a lower heart rate than both the pregnant and premenopausal nonpregnant patients. Pregnant women had a significantly higher central retinal artery diastolic velocity (4.2 ± 0.8 cm/sec) and a significantly lower central retinal artery RI (0.56 ± 0.05) than both the premenopausal non pregnant women (diastolic velocity 2.8 ± 0.8 cm/sec, RI 0.68 ± 0.1) and the postmenopausal women (diastolic velocity 2.6 ± 0.9 cm/sec, RI 0.73 ± 0.08). There were no significant differences in any of the ophthalmic artery Doppler parameters studied in the three groups of women. Estradiol therapy in the postmenopausal patients was associated with a reduction in central retinal artery vascular resistance (diastolic velocity 4.1 ± 1.6 cm/sec, RI 0.66 ± 0.1), with no significant change in the ophthalmic artery Doppler parameters. Blood pressure values after estradiol therapy were not reported. These data suggest that the reactivity of the cerebral microvasculature changes in response to a woman's hormonal status. These effects were observed in small-diameter cerebral vessels (retinal artery, 100 mm) but not in a larger vessel (ophthalmic artery, 1 mm). The hormones involved are not yet clearly defined, but estrogens may play an important role in the dilation of smaller-caliber cerebral vessels.

Doppler ultrasound was used to assess blood flow characteristics in the internal carotid arteries of healthy postmenopausal women (Gangar et al., 1991 ; table 7.I). Twelve postmenopausal women (50.5 years, range 47-59) were prescribed transdermal estradiol (Estraderm TTS® 50) continuously but withdrew from therapy between weeks 7 and 9. After restarting treatment at week

10, medroxyprogesterone acetate (MPA, 10 mg/day) was added for 12 days in each month. MPA was omitted during the month in which the final assessment was made (week 22). Patients were assessed before treatment and at weeks 4, 6, 9 and 22. The PI was measured from the flow velocity waveforms. Baseline PI in the cerebral circulation of postmenopausal women correlated with time since menopause. A significant reduction in PI (–11 %) was observed at week 9 and was maintained until week 22. In all patients, there was a significant negative correlation between the change in PI between baseline and week 22 (during estradiol therapy) and the mean of baseline and final (week 22) PI values.

In 25 early postmenopausal women, PI variations in the internal carotid artery and middle cerebral artery were measured by means of a bidirectional Doppler ultrasonographic system, during a 6-month period of HRT (Penotti et al., 1993 ; table 7.I). All the women (51.68 ± 3.30 years) were within 6 months and 2 years from the last menses and were given continuous estradiol transdermal supplementation (Estraderm TTS®, 50 mg/day) with medroxyprogesterone acetate (10 mg/day) in the first 12 days of every second month. In both arteries, a highly significant reduction in PI was observed at 6 weeks, reflecting a blood flow increase in cerebral arteries. A further significant reduction in PI was measured in the internal carotid artery at the end of the first cyclic progestogen supplementation. At the end of the study, a 25 % reduction in PI values was measured in both arteries. No variation of the estrogen-induced PI reduction was measured at the 24th week, at the end of the cyclic progestogen supplementation. These results show that in early postmenopausal women, HRT causes a rapid and durable reduction in PI in brain arteries and suggest that progestogen supplementation does not worsen the favorable vascular effects of estrogen. However, the effect of estrogen on the PI might be more intense and rapid without cyclic progestogen supplementation. Thus, further double-blind, cross-over studies are needed to conclude that progestogens do not reduce the estrogen-mediated effect on the arterial wall.

In a study performed on 23 women, the effect of the suspension of HRT was investigated on cerebral vessel reactivity (Penotti et al., 1996). Doppler ultrasonography was used to measure the PI of the internal carotid and middle cerebral arteries, at the start of the 12-month period and then every 3 months, and during therapy in the estrogen-only phase. The patients (all without a history of significant illness) were all receiving continuous estradiol transdermal supplementation (Estraderm TTS®, 50 mg/day) and 12-day courses of medroxyprogesterone acetate (10 mg/day) every second month. Patients were studied by the same investigator. The women were randomly divided into two groups : the 11 women in the first group continued therapy for the first 6 months and then suspended it for the remaining 6 months and the 12 women in the second group received no HRT for the first six months and resumed treatment in the second 6-month period. HRT improves the reactivity of the internal carotid and middle cerebral arteries in postmeno-

pausal women (see above). The suspension of HRT reversed the favorable therapy-induced reduction in the PI of both the internal carotid artery and the middle cerebral artery. This effect was rapidly evident : after the suspension of therapy, the PI of both arteries was significantly higher (9 to 13 %, 3 months, and 16 to 19 % 6 months after the suspension of HRT) than in the subjects who continued HRT. The PI remained stable in women receiving HRT. After the crossover at 6 months, the PI rapidly dropped to values similar to those at baseline in the patients who resumed HRT and increased in those who suspended therapy. These results show that resistance to blood flow in cerebral vessels of postmenopausal women rapidly changes after HRT suspension. In addition, the fact that both the increase in PI resulting from the suspension of therapy and the decrease from the resumption of therapy were progressive suggests a multiplicity of biological mechanisms by which HRT affects vessel reactivity.

Coronary arteries

Several studies have evaluated the effect of short-term administration of estrogen on coronary blood flow and coronary vascular resistance in postmenopausal women and are detailed in « Endothelial function » (Gilligan al., 1994b ; Collins et al., 1995 ; Reis et al., 1994 ; table 7.I).

HRT appears to result in hemodynamic changes with an improvement of some Doppler-derived parameters of aortic blood flow (Pines et al., 1992) and a reduction in PI and RI in all the vascular beds studied (brachial, uterine and cerebral arteries). The effects of HRT on coronary arteries have not, however, been studied after chronic administration. In addition, acute administration of estrogen induced controversial effects on coronary arteries : estradiol-17β did not affect basal coronary artery diameter, blood flow or resistance, whereas ethinyl estradiol induced coronary resistance and conductance vessel dilation.

Other markers should be studied to determine the vascular effects of HRT in postmenopausal women. As reported by Giraud et al. (1996) (table 7.I), the aorta and its branches become less compliant or stiffen with age. After menopause, this stiffening process may progress more rapidly, and acceleration of atherosclerosis and decreased aortic compliance may contribute to the development of HTA. Furthermore, the reduction in aortic compliance coupled with increasing systemic vascular resistance with advancing age increases the systolic load of the heart. Estrogen receptors have been found in aortic tissue, thus the aorta could be a potential target for an estrogen effect. Aortic size and compliance were assessed as markers of estrogen-induced vascular effects. In Giraud's study, performed on 26 healthy postmenopausal women, aortic size was assessed by measuring cross-sectional area of the ascending and descending aorta by magnetic resonance imaging before and after 3 months of HRT. Compliance was defined as the slope of the area-pressure relationship. Women were randomized to receive either conjugated estrogens

(0.625 mg/day) (55.8 ± 4.2 years) or conjugated estrogens (0.625 mg/day) and medroxyprogesterone (2.5 mg/day) (57.4 ± 3.8 years). Estrogen therapy was associated with a significant decrease in both systolic and diastolic blood pressure and the combination of estrogen and progestin was associated with a decrease in diastolic but not systolic blood pressure. After 3 months of estrogen therapy alone, both the ascending and descending aortic cross-sectional area was larger (within the physiologic pressure range) in the estrogen-treated group. In contrast, the combination of estrogen and progestin therapy did not modify aortic size in postmenopausal women. No change in aortic compliance was detected. Further long-term studies are needed to determine the effects of HRT on these markers.

Clinical studies of HRT effects on endothelial function

The endothelium plays a primary role in the local regulation of vascular activity by synthesizing vasodilating and vasoconstricting substances. In humans, impaired endothelium-dependent vasodilation is associated with diseases such as essential or secondary HTA, hypercholesterolemia, atherosclerosis or advancing age, all of which are well-documented cardiovascular risk factors. Menopause was shown to affect endothelium-dependent but not endothelium-independent vasodilation in both normotensive and hypertensive women. Aging is associated with progressive endothelial dysfunction in normal subjects, and this appears to occur earlier in men than in women. In women, however, a steep decline commences at around the time of the menopause (Celermajer et al., 1994).

Taddei et al. (1996) reported that in normotensive women up to the end of the fifth decade, advancing age only slightly affected endothelium-dependent vasodilation, whereas in normotensive men, endothelial responses started to decline in the third decade. After the age of 49 years, endothelium-dependent vasodilation showed a decline in women that was steeper than in men. The sex-related difference in endothelium-dependent vasodilation was no longer statistically significant after 60 years. These results seem to indicate that although, in normotensive men, endothelial dysfunction associated with increasing age is a consistent and uniform event, in normotensive women menopause could be a crucial time characterized by a remarkable deterioration of endothelial function. After the fifth decade, the extent of impairment of endothelium-dependent vasodilation is lower in men than in postmenopausal women. Such a mechanism protecting endothelial function also seems to be present in hypertensive premenopausal women. However, as in normotensive women, in hypertensive women older than 60 years, aging-related endothelial dysfunction is similar to that in men of the same age.

As proposed by these authors, endothelial dysfunction associated with menopause could be one explanation for increased cardiovascular morbidity and mortality in postmenopausal women. Studies in a variety of experimental

models have evaluated the effect of estrogens on endothelial function. Some recent studies are detailed to illustrate the estrogen-induced improvement in endothelial function in postmenopausal women.

Brachial artery

In a controlled study performed on 135 healthy women taking no regular cardiovascular medication, brachial artery diameter was measured at rest and in response to reactive hyperemia (flow-mediated dilation), using high resolution external vascular ultrasound (McCrohon et al., 1996 ; table 7.II). There were 40 premenopausal women (26 ± 6 years) and 95 postmenopausal women (50 ± 65 years). Forty postmenopausal women (58 ± 3 years) were at least 2 years postmenopausal and had never taken any form of HRT. Fifty-five postmenopausal women (57 ± 4 years) were taking regular HRT at the time of the study and had been prescribed HRT continuously for 2 years or more, starting HRT within 2 years of the menopause. In this last group, 40 women had not had hysterectomy and were prescribed combined HRT : oral HRT in 36 with estrone (0.625-1.25 mg, n=19), oral estradiol (1-2mg/day, n=10) or conjugated equine estrogen (0.625-1.25 mg/d, n=7) and topical estrogen in 4 (transdermal estradiol, 10-25 mg/day) with oral progesterone. The progesterone component was medroxyprogesterone (n=32), norethisterone (n=7) or didrogesterone (n=1). The other 15 women had all had hysterectomy and had been taking continuous unopposed estrogen with conjugated equine estrogen (0.625-1.25 mg/day, n=19), estrone (0.625-1.25 mg/day, n=2), ethinyl estradiol (0.02 mg/day, n=2) or oral estradiol (2mg/day, n=1) and topical estradiol (25 mg/day, n=1). Arterial reactivity was significantly impaired in the postmenopausal women taking no HRT. Both flow-mediated and glyceryl trinitrate-induced dilation were significantly reduced compared to premenopausal women. On multivariate analysis, only flow-mediated dilation was significantly related to postmenopausal status. Flow-mediated dilation was significantly better in women on HRT (« class-effect ») than in women without HRT. Flow-mediated dilation was similar in women taking estrogen alone and in those on combined HRT. These data are consistent with a decline in arterial endothelial function after the menopause and suggest that long-term HRT is associated with improved endothelial function in healthy postmenopausal women.

In 40 postmenopausal women (60 ± 8 years), the forearm vascular responses (plethysmography) to the endothelium-dependent vasodilator acetylcholine were studied before and during infusion of estradiol-17§ into the ipsilateral brachial artery (Gilligan et al., 1994a ; table 7.II). Twenty women (59 ± 8 years) had no evidence of cardiovascular disease and the other 20 (61 ± 8 years) had one or more conditions known to be associated with impaired endothelium-mediated vasodilation : coronary artery disease (n=6), HTA (n=13), high cholesterol levels (n=9) or diabetes mellitus (n=5). Forearm blood flow was measured during intra-arterial infusion of acetylcholine

chloride at 7.5, 15 and 30 mg/min and sodium nitroprusside at 0.8, 1.6 and 3.2 mg/min for 5 min : estradiol (20 ng/ml) was infused at 1ml/min for 20 min. The forearm vasodilation induced by acetylcholine or sodium nitroprusside was less marked in the women with risk factors for vascular dysfunction compared with the healthy women. Infusion of estradiol increased forearm venous estradiol levels in all women to levels typical of reproductive-age women at midcycle (318 ± 188 pg/ml), but caused no vasodilation. Both groups of women showed potentiation of the acetylcholine vasodilator responses after estradiol infusion (18 ± 30 % in women with risk factors and 14 ± 23 % in healthy women). This augmentation of acetylcholine-induced vasodilation probably reflects a potentiation of endothelium-dependent vasodilation rather than a nonspecific effect of estradiol in women with risk factors for cardiovascular disease and evidence of vascular dysfunction. Additionally, estradiol potentiated endothelium-independent vasodilation.

In a randomized, double-blind, placebo-controlled, cross-over trial (Lieberman et al., 1994 ; table 7.II), the effect of estrogen replacement on endothelium-dependent vasodilation in the brachial artery was assessed in postmenopausal women with hypercholesterolemia. Thirteen postmenopausal women (55 ± 7 years) were randomly assigned to 1 of 3 treatment groups : placebo, oral estradiol-17β (1 mg/day, Estrace) or oral estradiol-17β (2 mg/day, Estrace). At the conclusion of each 9-week treatment period, the patients were given progesterone (10 mg/day) for 10 days. HRT was discontinued for 3 weeks before patients crossed over to the next treatment regimen. All patients received the placebo and the 2 doses of estrogen in random order. Vascular function (high resolution ultrasonography) studies were done during the 8th or the 9th week of each treatment period. Endothelium-dependent vasodilation was determined by measuring the change in the caliber of the brachial artery during reactive hyperemia (flow-mediated vasodilation). Basal forearm resistance was similar during all 3 treatment phases. Flow-mediated endothelium-dependent vasodilation of the brachial artery was significantly greater when patients received estradiol treatment (13.5% and 11.6% for 1 and 2 mg respectively) than when they received placebo (6.8%). There was no difference between the 2 estrogen doses studied. Estrogen administration had no effect on endothelium-independent vasodilation. These data show that sustained (9 weeks) estrogen replacement therapy improves endothelium-dependent vasomotion on a peripheral artery in postmenopausal women with mild hypercholesterolemia.

Coronary arteries

In 20 postmenopausal women (61 ± 7 years), coronary artery diameters were measured by quantitative coronary angiography and blood flow velocity was measured with a Doppler wire placed in a proximal left coronary artery segment (Gilligan et al., 1994b ; table 7.II). Seven women had angiographic evidence of atherosclerosis of the left coronary artery segment and 85 % had

Table 7.II : Effects of HRT on endothelial function

Authors	N Age (years)	HRT	Duration	Arteries	Effects
McCrohon et al. (1996)	95 50±6.5	oral E (E1, E2, CE, EE) or transdermal estrogen (n=5) unopposed (n=15) or + progestogens (n=40)	> 2 years	brachial	increase in flow-mediated vasodilation (estrogen alone or combined HRT)
Gilligan et al. (1994)	40 60±8	E2 (20ng/ml/20min, intraarterial)	20 min	forearm	increase in acetylcholine (Ach) -induced vasodilation
Lieberman et al. (1994)	13 55±7	E2 (1mg, p.o) E2 (2mg, p.o)	9 weeks	brachial	increase in flow-mediated vasodilation (1mg ≡ 2 mg)
Gilligan et al. (1995)	33 59±7	E2 (intraarterial) E2 (0.1mg, transdermal)	20 min 3 weeks	forearm	increase in Ach-induced vasodilation no change in Ach-induced vasodilation
Gilligan et al. (1994)	20 61±7	E2 (75 ng/ml, intracoronary)	20 min	coronary – epicardial – microvascular	 increase in Ach-induced vasodilation
Collins et al. (1995)	9 59±3	E2 (2.5 μg, intracoronary)	20 min	coronary – atherosclerotic artery – microvascular	 conversion from Ach-induced vasoconstriction to Ach-induced vasodilation increase in the coronary flow response to Ach
Reis et al. (1994)	33 64±2.1	EE (35 μg, intravenous)	15 min	coronary – epicardial (normal) – epicardial (endothelial dysfunction) – resistance (normal) – resistance (endothelial dysfunction)	 no change in Ach-induced increase in cross sectional area inhibition of Ach-induced decrease in cross sectional area no change in Ach-induced increase in flow and decrease in resistance inhibition of Ach-induced decrease in flow and increase in resistance

one or more risk factors for atherosclerosis and vascular dysfunction (HTA, hypercholesterolemia or diabetes). Acute intracoronary administration (75 ng/ml) of estradiol, achieving physiological levels (282 ± 121 pg/ml) in the coronary sinus, improved responses of the coronary circulation to the endothelium-dependent vasodilator acetylcholine. At the large-vessel level (epicardial coronary artery), the vasoconstriction produced by acetylcholine was prevented by estradiol, and estradiol augmented the fall in coronary resistance induced by acetylcholine, a change associated with a significant increase in coronary blood flow, suggesting that an effect of estradiol also occurred at the level of the small intramyocardial resistance vessels. Estradiol did not affect basal coronary artery diameter, blood flow or resistance. It remains to be determined whether the same vascular effects would be observed at a lower dose than those used in this study and with estrogens other than estradiol-17β. Further long-term studies are needed to determine whether short-term replacement effects are observed with long-term therapy.

In a placebo-controlled study (Collins et al., 1995 ; table 7.II), the effect of estradiol-17β on the coronary circulation was assessed in postmenopausal women and men with coronary artery disease. The study population consisted of 9 female patients (59 ± 3 years) and 7 male patients (52 ± 4 years) with angiographically proven coronary artery disease. None of the women were receiving or had received hormone therapy. Six postmenopausal women (55 ± 3 years) and 6 male patients (56 ± 3 years) with angiographically proven coronary artery disease served as control patients. Patients underwent measurement of coronary artery diameter (quantitative angiography) and coronary blood flow (intracoronary Doppler catheter) after intracoronary infusion of acetylcholine before and 20 min after intracoronary administration of 2.5 mg of estradiol-17β into atherosclerotic, nonstenotic coronary arteries. There was no difference in coronary artery diameter or coronary blood flow from baseline and at 10 or 20 min after the infusion of estradiol in postmenopausal women. In control patients, infusion of intracoronary placebo did not change coronary diameter responses or coronary blood flow responses to acetylcholine. In postmenopausal female patients with documented coronary artery disease, the vascular responses of atherosclerotic coronary arteries to acetylcholine were converted from net vasoconstriction (8 ± 5 % decrease in coronary artery diameter) to net dilation (9 ± 3 % increase in coronary artery diameter) within 20 min of exposure of the coronary arteries to estradiol-17β. This effect was not apparent in men of a similar age. In postmenopausal women, estradiol-17β also enhanced the coronary flow response to acetylcholine (126 ± 37 % increase before and 248 ± 89 % increase after estradiol administration) suggesting an additional effect on the coronary resistance vessels (microvascular coronary arterial bed). In men, estradiol did not change the acetylcholine-induced increase in blood flow. These data suggest that acute estrogen administration may modulate coronary endothelium-mediated dilation in women but not in men.

In an other placebo-controlled study (Reis et al., 1994 ; table 7.II), the effect of estrogen administration on acetylcholine-induced changes in coronary circulation was determined before and 15 min after intravenous administration of ethinyl estradiol (35 mg) in postmenopausal women with clinically indicated coronary angiography. Intracoronary Doppler ultrasonography and quantitative coronary angiography were used to assess the coronary artery cross-sectional area, flow and resistance responses to a single dose of estrogen. Thirty-three postmenopausal women were studied : 22 (64 ± 2.1 years) received intravenous estradiol and 11 (64 ± 3 years) received an intravenous placebo. Intravenous administration of ethinyl estradiol was not associated with changes in blood pressure or heart rate. Ethinyl estradiol increased coronary blood flow (23 ± 4.5 %), decreased coronary resistance (–15 ± 3.2 %) and increased epicardial cross-sectional area (20 ± 6.5 %), suggesting that this synthetic estrogen induced coronary resistance and conductance vessel dilation. The placebo was not associated with changes in coronary blood flow or coronary vascular resistance. In 8 women with normal coronary resistance vessel endothelial function, estradiol administration did not modify the acetylcholine-induced increase in coronary flow, decrease in resistance or increase in epicardial cross-sectional area. In 7 women with coronary irregularities, acetylcholine induced a paradoxical decrease in coronary flow (–33.5 ± 12.3 %), an increase in coronary resistance (38.9 ± 14.1 %) and a decrease in epicardial cross-sectional area (–14.2 ± 4.4 %). This abnormal response was inhibited by ethinyl estradiol. Fifteen min after estrogen administration, there were no acetylcholine-induced changes in coronary flow, resistance or epicardial cross-sectional area. Normal coronary responses to acetylcholine were not affected by ethinyl estradiol administration. These data suggest that, in postmenopausal women, ethinyl estradiol, at a dose identical to that used in commonly prescribed oral contraceptive pills, has *in vivo* coronary vasoactive properties that decrease basal coronary resistance and conductance vessel vasomotor tone and attenuate abnormal coronary hemodynamic responses to acetylcholine of both resistance and conductance coronary arteries with dysfunctional endothelium.

The data of the above mentioned studies show that estrogens, although having little direct effect, acutely enhance the coronary vasodilatory response to the endothelium-dependent vasodilator acetylcholine and inhibit the paradoxical vasoconstrictor response to acetylcholine in postmenopausal women. In addition, acute intraarterial estradiol-17β administration enhances endothelium-dependent vasodilation in the forearm of postmenopausal women, including those with evidence of preexisting vascular dysfunction. Based on these results with acute administration of estradiol, it has been postulated that sustained improvement in the endothelial regulation of vascular tone may be related to the beneficial effects of chronic estrogen therapy.

However, in a recent study (Gilligan et al., 1995 ; table 7.II), the forearm vasodilatory response to acetylcholine after 3 weeks of estradiol administra-

tion was not improved. In addition, the response to sodium nitroprusside (endothelium-independent), which was improved marginally during the acute study after intraarterial estradiol, was unchanged chronically. Thirty-three postmenopausal women (59 ± 7 years) were enrolled in this study. Fifteen women had no evidence of cardiovascular disease and the remaining 18 women had at least one condition known to be associated with impaired vascular function (coronary artery disease, HTA, hypercholesterolemia or diabetes mellitus). The effect of estradiol-17β on the forearm vascular responses (venous occlusion plethysmography) to acetylcholine and sodium nitroprusside were studied during intraarterial infusion of estradiol and after 3 weeks (1 cycle) of transdermal estradiol (100 mg/day, Estraderm® 0.1). As reported previously, acute intraarterial infusion of estradiol potentiated forearm vasodilation induced by both acetylcholine (endothelium-dependent, 49 ± 67 %) and nitroprusside (endothelium-independent, 5 ± 31 %). However, after 3 weeks, the vasodilator responses to acetylcholine and to sodium nitroprusside were unchanged from initial measurements. The difference observed between acute and chronic estradiol administration could be related to the lower plasma levels achieved with chronic administration (120 ± 57 pg/mL versus 345 ± 202 pg/mL after intraarterial administration). It is also possible that chronic estradiol administration acts, in postmenopausal women, through different cellular mechanisms, including regulation of gene expression, which oppose the acute potentiation of endothelium-dependent vasodilation. If higher doses of estrogen are needed to produce chronic enhancement of endothelial function, it is unlikely that they could be used because of the potential risks of high dose estrogen replacement therapy.

Further studies are needed to determine whether other estrogen preparations may have different chronic effects on endothelial function and whether different vascular beds (coronary circulation, for example) respond differently to chronic HRT.

In acute experiments, estrogens have been demonstrated to potentiate endothelium-dependent vasodilation in both conductance and resistance vessels. There is a growing body of evidence linking the NO pathway to estrogens. The estrogen-induced potentiation of the endothelium-dependent vasodilation could be related to enhanced release of relaxing factors from the endothelium and/or to potentiation of the effect of NO on smooth muscle cells.

A recent randomized (Cicinelli et al., 1997), placebo-controlled study was designed to investigate the acute effects of transdermal estradiol administration on NO production in postmenopausal women. NO production was assessed by monitoring plasma levels of nitrite and nitrate, the 2 stable oxidation products of NO metabolism. Twenty women (52 to 60 years) in spontaneous menopause were enrolled in this study and were randomized to receive transdermal estradiol patches (100 mg/day, Estraderm® 0.1) or a non-

 medicated patch. Plasma concentrations of stable NO oxidation products and

serum concentrations of estradiol were assessed before and 24 hours after placing the patch. In the group treated with estrogen, the mean concentration of NO metabolites 24 hours after patch application was significantly higher (37.31 ± 7.62 mmol/L) than at baseline (21.04 ± 5.71 mmol/L) and in the control group (23.50 ± 4.03 mmol/L). The correlation between the mean percent increase in NO metabolites and absolute estradiol concentrations 24 hours after estrogen administration was statistically significant. However, in the estrogen group, the percent increase in the concentration of NO metabolites showed wide interpatient variability, ranging from 26 to 206 % (90.40 ± 61.25 %). These data show that acute transdermal administration of estradiol to healthy postmenopausal women induces an increase in plasma levels of NO. The long term effects of repeated oral or transdermal combined HRT administration must be evaluated.

In another randomized, controlled study (Roselly et al., 1995), the effect of long term HRT on NO release was studied in healthy postmenopausal women. Twenty-six postmenopausal women were randomly assigned to receive either HRT (transdermal estradiol-17β, Estraderm TTS® 50) and norethisterone (NETA) orally from days 1 through 12 of each month, n=13) or no HRT (n=13). Serum levels of nitrite/nitrate (which reflect endogenous NO production) were determined at baseline and during the 6th, 12th and 24th months of the study. Serum estradiol-17β levels were similar regardless of whether HRT-treated women were or were not taking NETA (133 ± 30 versus 130 ± 26 pmol/L). The results show that administration of estrogen increased serum nitrite/nitrate levels. With estradiol treatment, levels of circulating nitrite/nitrate returned approximately to those observed in premenopausal women. The increases in serum nitrite/nitrate levels in samples collected after 6, 12 and 24 months of estradiol administration did not vary significantly. Estradiol-induced NO synthesis was inhibited when women were taking oral NETA. No significant increases in serum nitrite/nitrate levels were observed when estradiol was coadministered with NETA.

In vitro studies of HRT effects on endothelium-independent relaxation

The effects of estradiol-17β on human coronary arteries and its mechanisms of action remain unknown. In particular the effect of sex and the endothelium have not been fully evaluated.

A study (Chester et al., 1995) examined *in vitro* the possibility that estrogen could relax human coronary arteries directly and investigated some of the possible mechanisms of this relaxing effect. Atherosclerosis-free epicardial arteries from men (n=7, 26-51 years) and women (n=7, 43 ± 1 years) were removed from patients undergoing heart or combined heart and lung transplantation. None of the female patients was receiving HRT. Changes in

isometric tension from ring segments placed in organ baths were measured. Estradiol-17β (10^{-10} to 10^{-5} mol/L) caused significant concentration-dependent relaxation of coronary segments pre-contracted with a thromboxane A_2 analog. The response was slow in onset, taking 7-15 min to reach a maximum at each concentration. There was a significant increase in the response in arteries from female patients compared with those from male patients. There was no difference between groups with or without endothelium, and nitric oxide synthase or cyclo-oxygenase inhibition did not modify the estradiol-induced relaxation. These data suggest that estradiol-17β induces endothelium-independent relaxation in isolated human coronary preparations and that female coronary arteries from premenopausal women are more sensitive than male coronary arteries.

Acute and, in some studies, long-term HRT are associated with improved arterial endothelial function in healthy postmenopausal women and in women with impaired vascular endothelial function. The effects of long-term HRT on endothelial function remain, however, controversial and need further studies. This benefit was observed in both the combined HRT and unopposed estrogen therapy groups. A NO-related mechanism and a possible endothelium-independent effect may contribute to the cardiovascular protective effect of estrogens in postmenopausal women. Further studies on coronary artery preparations from postmenopausal women are needed to conclude that endothelium-independent relaxation may also be involved in the protective effect of estrogens on the risk of coronary heart disease.

Clinical studies of HRT effects on carotid intima-media thickness in atherosclerosis

Two epidemiological, one cross-sectional and one long-term studies have reported the association between estrogen-use in postmenopausal women and intima-media thickness of the carotid arteries, an indicator of atherosclerosis. However, in a more recent study, no such association was found (table 7.III).

The relations between estrogen use and subclinical disease were examined in a large sample of older women in the Cardiovascular Health Study, a study of risk factors for coronary heart disease and stroke in the elderly (Manolio et al., 1993). Present and past estrogen use was ascertained in 2 955 women (>65 years). Present estrogen users were defined as women with prescriptions for oral estrogen recorded by medication inventory, regardless of self-reported past use. Past estrogen users were women responding positively to ever having taken conjugated equine estrogens (Premarin®) or other estrogens for hot flashes or other symptoms of menopause and not having a current prescription. Of 2 955 women interviewed, 12 % were currently using oral estrogen or combined estrogen/progesterone and 79 % of these women were taken unopposed estrogen. An additional 26.5 % reported past use of hormones,

Table 7.III : Effects of HRT in atherosclerosis or hypercholesterolemia

Authors	N Age (years)	HRT	Duration	Artery	Effects
Manolio et al. (1993)	2 955 >65	oral E CE/P	current users	carotid	decrease in intima-media thickness
Espeland et al. (1995)	186 61.3±5.9	no information « class effect »	current users	carotid	inhibition or reversion of the 3-year progression in intima-media thickness
Nabulsi et al. (1996)	5 436 45-54	no information « class effect »	current users	carotid	no effect in intima-media thickness
Akkad et al. (1996)	17 66.7	E2 (600 mg), estriol (270 mg), E1 (1.4 mg) p.o	6 months	carotid	reduction in plaque length and thickness no effect in intimal thickness
Wilcox et al. (1997)	18 53.4±4.9	E1 (0.625 mg, p.o.) E2 (0.05 mg, transdermal)	30 days	–	decrease in plasma endothelin-1 levels

making a total of 39 % of women who were ever users of HRT. Estrogen use was more common in younger women and in those with prior hysterectomy, for both present and past use. Near and far wall maximal intima-media thickness of the carotid arteries were measured and averaged as an indicator of atherosclerosis : separate measurements were made for the common and internal carotid arteries. Estrogen users (« class effect », past or present) had lower LDL cholesterol, fibrinogen, albumin, fasting insulin and glucose, obesity, and subclinical disease, as measured by ECG left ventricular mass, carotid stenosis grade and carotid intima-media thickness. After ajustment for other factors, estrogen use was associated with decreased carotid wall thickness. Further adjustment for HDL and LDL cholesterol reduced the magnitude and significance of this association, such that only ever versus never and present versus never use retained bordeline associations with wall thickness. These results suggest that HRT (oral estrogen) in older women is associated with lower measures of subclinical disease. Other important data are the fact that excluding lipid levels from these models demonstrated strong relations between present estrogen use and carotid wall thickness independently of other nonlipid factors. There was a considerable difference in mean duration of use between present (18 years) and past (3 years) users. Duration was not, however, significantly associated with wall thickness in multivariate models. The fact that past users differed little from never users suggests that present use or factors associated with it are more strongly related to wall thickness than are past use and factors that accompany it.

In 186 postmenopausal women in ACAPS (Asymptomatic Carotid Atherosclerosis Progression Study), the effect of HRT on 3-years changes in carotid intima-media thickness was explored using serial B-mode ultrasound measurements (Espeland et al., 1995). Women were classified into three groups ; 1/ current HRT users at baseline, 2/ new users during the trial, 3/ never users. Information on the specific type, route or dose of estrogen was not collected in standardized fashion and neither were data related to the use of progestational agents. Eligibility included increased intima-media thickness and elevated LDL cholesterol. 34 % of the 186 postmenopausal women randomized to receive either placebo or lovastatin reported current use of HRT. Users tended to be younger (61.3 ± 5.9 years) than non users (65 ± 6.9 years), to be more likely to have had hysterectomy and to have more favourable HDL and LDL cholesterol levels. Cross-sectional intima-media thickness was similar among HRT users and nonusers. In the placebo group, intima-media thickness tended to progress among HRT nonusers but to regress among HRT users : mean covariate-adjusted progression rates were significantly different in HRT users and nonusers. HRT may halt, and even reverse, the progression of carotid wall intima-media thickness among women not otherwise receiving lipid therapy. The magnitude of this effect appeared to be nearly equal to that of lovastatin. The use of HRT appeared not to alter the efficacy of lovastatin in lowering LDL cholesterol. When combined with lovastatin, HRT appeared to have little additional impact on intima-media progression.

However, in a recent cross-sectional study examining the association of menopausal status, years since last menstruation and HRT status with carotid artery intima-media thickness determined by B-mode ultrasound, present and past use of HRT was not associated with intima-media thickness in postmenopausal women aged 55 to 64 years (Nabulsi et al., 1996). Female participants (n=5436, aged 45 to 64 years) in the Atherosclerosis Risk in Communities Study, without a history of symptomatic cardiovascular disease were included in the analyses. Details on hormone formulations and dose were not available and if associations existed for a particular formulation or dose, then the inability to stratify by formulation or dose may have obscured the true associations. After adjustment for age, race, years of smoking, body mass index, sport index, systolic blood pressure, use of blood pressure medications, drinking status, diabetes and education level, no overall association between menopausal status and carotid intima-media thickness was found in women aged 45-54 years. These data are in accordance with some others suggesting that, in general, there is no strong evidence that natural menopause is associated with increased cardiovascular heart disease or stroke risk. However, the evidence is stronger that surgical menopause is associated with an increased risk of cardiovascular heart disease. The current analysis revealed no negative association between HRT and carotid intima-media thickness. Among current estrogen users, women who had used oral contraceptives had greater intima-media thickness than those who had never used oral contraceptives. This suggests that women who use oral contraceptives may derive less benefit from HRT. This potential interaction merits further investigation. Women in this study were younger than in Manolio's study (see above), which might explain, in part, the different data. In addition, it is also possible that a longer duration of HRT than in this study (3 years for past users and 9 years for current users) may be necessary to affect carotid intima-media thickness.

Additional data from randomized clinical trials are needed to assess the impact of HRT (in terms of class and according different routes of administration) on atherosclerosis and its clinical manifestations.

The presence of an estrogen receptor in human coronary artery endothelial cells was recently shown. No sex differences in estrogen receptor expression could be identified (Kim-Schulze et al., 1996). However, the mechanism by which estrogen receptor binding influences endothelial cell behavior and, subsequently, vascular function remains to be elucidated. A strong association between estrogen receptors and the absence of coronary atherosclerosis was demonstrated in premenopausal women (Losordo et al., 1994). The absence of this relationship in postmenopausal women could be explained by the estrogen-deficient state of the subjects, making the presence of the receptor alone insufficient to exert an atheroprotective effect (Baysal and Losordo, 1996). When compared to premenopausal women, the atherosclerotic risk is 3.4 times higher in women after natural menopause and up to 5.5 times greater after bilateral oophorectomy (Witteman et al., 1989).

In a pilot study (Akkad et al., 1996 ; table 7.III), the effect of estrogen replacement therapy on atheromatous plaques was studied in carotid arteries of naturally or surgically menopausal women. Seventeen postmenopausal women with known carotid disease (mean age 66.7 years) were treated with unopposed oral estrogen (Hormonin, 600 mg micronised estradiol-17β, 270 mg estriol and 1.4 mg estrone, one tablet daily) for 6 months. Two women were oophorectomised, the remaining 15 had undergone a natural menopause and their mean time since menopause was 13.6 years. Carotid intimal thickness, plaque length and plaque thickness were measured using a Diasonics VST Masters Duplex Ultrasound scanner, prior to treatment and subsequently following 3 or 6 months of estrogen replacement, by one operator. Intra-observer variability was 8 to 10 % for the three markers studied. A non statistically significant reduction in mean intimal thickness was observed at 3 (–5 %, 95 % CI –17 to 7) and 6 months (–6 %, 95 % CI –26 to 14). Plaque length was significantly reduced at 3 (–8 %, 95 % CI –15 to –2) and 6 months (–28 %, 95 % CI –42 to –13). Plaque thickness was also significantly reduced, but only at 6 months (–18 %, 95 % CI –30 to –6) of treatment. These data suggest significant plaque regression during estrogen replacement. Further studies are needed to confirm these data and to determine the effect of HRT on carotid plaque regression.

Atherosclerosis is characterized by endothelial injury and subsequent intimal smooth muscle proliferation. Patients with atherosclerosis have elevated levels of endothelin-1, thereby suggesting a possible etiologic role in the pathogenesis of the disease. However, it is not clear whether endothelin-1 is an active participant in the atherosclerotic process or is released from endothelial cells damaged by other causes.

Wilcox et al. (1997) (table 7.III) in a prospective randomized study enrolled 18 postmenopausal women (53.4 ± 4.9 years) and 10 premenopausal women (31.3 ± 4.3 years) to establish levels of plasma endothelin-1 in postmenopausal women with increased cardiovascular risk as compared with healthy premenopausal women and to measure the effects of different forms of estrogen replacement therapy on plasma endothelin-1. The postmenopausal women were recruited on the basis of hypercholesterolemia (260.7 ± 11.8 mg/dL) and 8 had essential hypertension (SAP > 140 mmHg). After obtaining baseline blood samples, the 18 postmenopausal women were randomized to receive for 30 days either 0.625 mg/day oral estrone sulfate (n=6), 0.05 mg transdermal estradiol patches (n=6) or placebo transdermal patches (n=6). Baseline plasma endothelin-1 levels were significantly higher in postmenopausal women than in premenopausal women (4.48 ± 0.46 and 2.80 ± 0.41 pg/mL). Plasma endothelin-1 levels decreased with estrone sulfate (–13.7 %) and with transdermal estradiol (–10.2 %) and were unchanged in the placebo group. There were no significant changes in endothelin-1 levels with the estrogens tested and the 2 routes of estrogen administration. The decrease in endothelin-1 with estrogens was statistically significant for the

entire group. Hypertensive women had the largest decremental change in endothelin-1 levels after estrogen therapy (–23.9 %). Mean blood pressure did not change significantly after estrogen treatment and did not correlate with changes in endothelin-1 levels. Baseline plasma endothelin-1 levels correlated positively to plasma cholesterol levels. These data provide another potential mechanism explaining the cardioprotective effects of HRT. However, the sample size was too small to compare routes of administration and further studies are needed to determine the role of HRT (combined) in the modulation of endothelin-1 levels in postmenopausal women and the clinical effects of such a modulation.

In vitro studies in atherosclerosis

The early stage of atherosclerosis has a proliferative component which includes vascular smooth muscle cell proliferation and its migration into the intima. Cell proliferation and migration are believed to be critical for vascular structural changes occurring in atherosclerosis and restenosis or venous graft disease.

In a study (Suzuki et al., 1996) performed on 2 cell lines of human female aortic smooth muscle cells, the presence of estrogen receptors was examined by Western and Nothern blot analyses and the role of estrogen and progesterone was investigated on the growth factor-induced proliferation and migration of these cells *in vitro*. The results showed the presence of both estrogen receptor protein and its mRNA in aortic smooth muscle cells. Among 3 major endogenous estrogens tested (estrone, estradiol-17β and estriol), estradiol-17β at its physiological concentration (10^{-9} M) inhibited both the mitogen-induced proliferation and migration of aortic smooth muscle cells. These effects were dose-dependent. The combination of progesterone (10^{-9} to 10^{-6}M) did not show any effect on the inhibitory effects of 10^{-7} M estradiol-17β, but preincubation of aortic smooth muscle cells with the anti-estrogenic agent tamoxifen significantly antagonized the inhibitory effect of estradiol-17β. The authors noted that the concentration of estradiol-17β in postmenopausal women on HRT is much lower than that of the physiological level of reproductive-age women in the middle of the menstrual cycle (10^{-9} M) and concluded that these data are insufficient to explain the beneficial effects of HRT on vascular smooth muscle cells.

A study (Dai-Do et al., 1996) was designed to investigate a possible gender difference in the effects of estradiol-17β on smooth muscle cell proliferation and migration in cells obtained from postmenopausal women and age-matched men. Vascular smooth muscle cells were isolated from saphenous veins of 8 postmenopausal women without HRT (57-72 years) and 12 age-matched men (55-71 years) undergoing coronary bypass surgery by the explant technique. In unstimulated cultured vascular smooth muscle cells,

estradiol-17β alone exerted no significant effect. However, estradiol-17β (10^{-8} to 10^{-6} M) inhibited proliferation and migration of vascular smooth muscle cells of saphenous veins stimulated by growth factors such as platelet-derived growth factor and basic fibroblast growth factor. These antiproliferative and antimigratory effect of estradiol-17β occurred in vascular smooth muscle cells obtained from postmenopausal women as well as from age-matched men with coronary artery disease. These effects may, together with the effects of estrogen on risk factors and endothelial cells, account for the reduction of cardiovascular events seen with HRT. However, as in Suzuki's study, the concentrations of estradiol used in this study were much larger than that obtained with HRT. Therefore further studies are needed to clarify the vascular effects of HRT.

As suggested by Manolio et al. (1993) and Nabulsi et al. (1996), it is possible that the reduced risk of atherosclerotic coronary disease associated with HRT, at least in the first two decades after menopause, may be attributable more to the acute physiological effects of HRT than to prevention of atherogenesis itself. Proposed mechanisms of estrogen-mediated cardiovascular protection, mainly obtained in animal studies (reviewed by Samaan et al., 1995) and some also suggested in women, are : beneficial alteration of the lipid profile, antioxidant effect, reduction in serum fibrinogen, reduction in platelet aggregation, reduction in monocyte adherence, inhibition of myointimal proliferation, inhibition of smooth muscle cell collagen biosynthesis, potentiation of endothelium-derived relaxing factors, calcium channel blocking effect, alpha2-inhibition, increased prostacyclin biosynthesis and reduced endothelin-1 levels.

To conclude, the presence of estrogen receptors in vascular endothelial and smooth muscle cells suggests that these cells are targets for this steroid. Estrogen has been shown, in experimental studies, to regulate contractility, proliferation and matrix formation and composition. However, few data are available on clinical effects of HRT on the vascular system according to the estrogen formulation, route of administration and combination with a progestin.

The most typical reaction to acute and long-term administration of HRT is arterial vasodilation with an increase in blood flow to various sites such as the uterine, carotid, cerebral and brachial arteries and, in some experiments, coronary arteries. Estrogens may have endothelium-dependent and endothelium-independent effects in normal arteries or when endothelial function is altered (in atherosclerosis, HTA or hypercholesterolemia). No marked differences according to estrogen regimen and duration of treatment appear in the decrease in pulsatility and resistance index. However, the question remains on whether the HRT-induced increase in blood flow and decrease in vascular resistance have any clinical or prognostic relevance. Rosano et al. (1993) reported a beneficial effect of acute, sublingual estradiol-17β (1mg) adminis-

tration on myocardial ischemia in postmenopausal women with angiographically documented coronary artery disease. This effect (increased the time to a 1 mm ST depression and increased total exercise time) was observed 40 min after administration and could be related to a direct coronary relaxing effect or to peripheral vasodilation. Further studies will be required to determine whether HRT is beneficial in the long-term treatment of postmenopausal women with angina. In addition, in a randomized, controlled study of postmenopausal women with borderline to mild hypertension, Pines et al. (1996) reported that with oral HRT (conjugated estrogens or estradiol) during 6 to 9 months, there was an improvement in cardiac function both at rest and during exercise, an effect partially related to a possible increased inotropic state of the myocardium. More work is needed to describe the hemodynamic effects (total resistance, heart rate, cardiac output...) of acute and long-term HRT and to determine their association with HRT-induced vasodilation.

The arterial wall represents a pharmacological and therapeutic target for cardiovascular drugs and it is suggested that long-term HRT should be evaluated on parameters known to be associated with increased cardiovascular risk. The main arterial surrogate markers could be pulse pressure, arterial distensibility, arterial wave reflections and arterial wall geometry.

Analytical studies of arterial function treat blood pressure as a periodic phenomenon that can be divided into two components ; a steady component (mean arterial pressure) which is determined exclusively by cardiac output and vascular resistances, and a pulsatile component which represents the variations of the pressure curve around the mean and is defined by pulse pressure. Pulse pressure is influenced by haemodynamic mechanisms other than those of mean arterial pressure : changes in ventricular ejection, large artery compliance and timing of reflected waves.

Investigating HRT-induced modifications in pulse pressure might be relevant, since pulsatile pressure has independent detrimental consequences on the arterial wall and the development of cardiac hypertrophy in hypertensive patients and is an independent risk factor for coronary mortality in postmenopausal women.

Estrogens have been studied in terms of changes in systolic, diastolic and mean pressures. Pulse pressure may be modified independently from these parameters as a consequence of specific changes in mechanical properties of the large arteries and in the timing of incident and reflected pressure waves.

Early arterial wave reflections contribute significantly to ventricular afterload and are associated with the development of left ventricular hypertrophy.

Decreased aortic distensibility has also been found to be associated with the extent of left ventricular hypertrophy. Decreased arterial distensibility could follow to functional and/or structural modifications of the arterial wall. This wall stiffness is associated with increased arterial pulsatility.

As menopause was recently shown to be associated with increased pulse pressure, decreased arterial distensibility and increased arterial wave reflections, the clinical evaluation of HRT-induced effects on these arterial parameters could afford further arguments to support the hypothesis that estrogen replacement therapy significantly reduces the risk of cardiovascular morbidity and mortality.

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