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Hormone replacement therapy and the prevention of cardiovascular disease

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Cardiovascular disease (CVD) is the primary killer of both men and women in Western societies. The implementation of preventive strategies has led to a fall in the rate of CVD, but there is still much to be achieved. Proven interventional strategies are largely under-utilized, and the search continues for further promising interventions. HRT appears to reduce CVD in post-menopausal women, based on observational data supported by plethora of evidence for the beneficial cardiovascular effects of estrogen. However, a recent controlled trial in post-menopausal women with established CVD has shown that a specific combined oral HRT regimen did not reduce, and may even contribute to, an early increase in cardiovascular events, suggesting that HRT is inappropriate in secondary prevention. HRT may be useful in the primary prevention of CVD, yet observational data that suggested cardiovascular benefit with HRT also suggests that 80% of CVD in women could be eliminated by lifestyle modification, without the attendant risks of HRT including thrombosis and (potentially) breast cancer. At present, it is arguable that the evidence is inadequate to recommend HRT solely for the purpose of CVD prevention, and that the challenge for the health professional should be appropriate utilization of established preventative therapies, with further research into the potential role of HRT and estrogen-receptor modulators.

Key words: cardiovascular disease/health management/hormone replacement therapy/lifestyle modification/preventative therapy

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Introduction

Cardiovascular disease (CVD) is the number one killer in both men and women in Western society. The disease process is largely attributed to age and lifestyle factors (Stampfer *et al.*, 2000). Effective health management strategies, if appropriately implemented, have the potential for significant impact on both mortality and morbidity. With the emphasis on evidence-based medicine, the efficacy of preventive measures has been proven in numerous well-designed trials (Yusef, 1998). As a result, the implementation of these preventive measures has resulted in a recent fall in the incidence of CVD (Hu *et al.*, 2000).

Lifestyle modifications are well known to reduce CVD with few side effects and many other health benefits. Many pharmacological interventions including anti-platelet, anti-hypertensive and lipid-lowering therapies also reduce cardiovascular morbidity and mortality (Yusef, 1998). Increasing evidence suggests that the targets for cardiovascular risk factor reduction should be more aggressive. Yet it appears that even existing targets are not being met (Mosca *et al*., 1999; Wood, and the Joint European Task Force, 2001). The recent American Heart Association guidelines on preventive cardiology noted that clinicians are missing opportunities for prevention, and that this is especially the case in women (Mosca *et al*., 1999).

Women spend on average 35 years of their lives in a post-menopausal, low-estrogen state. It is during these years that the majority of CVD burden is noted. In this setting, hormone replacement therapy (HRT) may have a role to play in the prevention of CVD. There is overwhelming observational data to support a beneficial effect of HRT on the cardiovascular system. There are, however, limitations in the application of observational data to the clinical setting. A wealth of animal and human interventional data, focusing on isolated mechanisms of estrogen action, has suggested largely beneficial cardiovascular effects. Yet, the translation of animal data to the post-menopausal woman may not be valid. Furthermore, the in-vivo effects of estrogen are ubiquitous and complex. In this setting it is the net effects of

estrogen rather than isolated estrogenic mechanisms that are most relevant clinically (Teede and McGrath, 1999). This can only be assessed in controlled human trials focusing on definitive clinical end-points. In limited controlled data completed to date, the effects of HRT appear to be neutral or even deleterious (Hulley *et al.*, 1998). Estrogen actions depend on dose, type, route of administration and on the co-administered progestin. Therefore, even the results of controlled trials should be interpreted only for the specific HRT preparations used and the populations studied.

In considering the potential role for HRT in CVD prevention, clinicians need to appreciate the difficulties in translating existing data on the vascular effects of HRT into clinical practice. There is also a need to interpret all results in the era of evidence-based medicine and in the setting of inadequate utilization of existing proven preventive therapies (Burger and Teede 2001; Mosca *et al.*, 2001a; Wood, and the Joint European Task Force, 2001). Future research is essential to resolve many important issues prior to establishing definitively the role of HRT and other estrogenic compounds in CVD prevention.

Cardiovascular disease in women, and established risk factors

Coronary heart disease is the leading cause of mortality, and contributes to significant morbidity among women in western society (Mosca *et al*., 1999; Tsang *et al.*, 2000). In fact, in women it causes more deaths than the next 14 most common fatal conditions combined (Tsang *et al.*, 2000). It has been estimated from the prospective Nurses Health Study that the majority of CVD is preventable by modification in lifestyle (Stampfer *et al.*, 2000), and comprehensive risk reduction is a well-established approach to prevent the condition. Proven preventive strategies encompass exercise, nutritional modification, weight loss and cessation of smoking, all of which carry no health risks (Ades, 2001). Additional beneficial strategies include targeted therapy to reduce blood pressure, improve lipid profiles, control diabetes, reduce platelet adhesion and modulate the neurohumoral system (Scandanavian Simvastatin Survival Study Group, 1994; Hennekens *et al*., 1997; Anonymous, 1998; Hansson *et al.*, 1998; Progress Collaborative Group, 2001).

Cessation of smoking is associated with a reduction in coronary events. In subjects who have suffered a myocardial infarction, a 50% reduction in mortality and reinfarction rate has been noted one year after cessation (Wilson *et al.*, 2000). Public health campaigns aimed at smoking cessation have been successful; for example, in Australia intensive public health measures have led to a decline in smoking rates down to 20% of the population, though further effort is still required to minimize the impact of smoking on cardiovascular health.

Adherence to a healthy diet, weight loss and dietary change including modification in dietary fat intake appear to have substantial benefits in CVD prevention (Stampfer *et al.*, 2000; Hooper *et al.*, 2001; Tuomilehto *et al.*, 2001). In a recent Cochrane Database Systematic Review focusing on the cardiovascular effects of dietary fat modification, it was noted that dietary intervention for more than 2 years showed significant reductions in the rate of cardiovascular events (Hooper *et al.*, 2001). Dietary intervention appears to improve lipid profiles, lower blood pressure, reduce insulin resistance and slow progression to diabetes, in those with impaired glucose tolerance (Hu *et al.*, 2000, 2001; Tuomilehto *et al.*, 2001). Unfortunately, obesity is increasing in Western societies, and may be slowing the decline in CVD (Hu *et al*., 2000). This is likely to be contributed to by the sedentary nature of Western lifestyle. Although evidence is largely observational, the role of exercise in the prevention of CVD is also well accepted. The National Heart Foundation of Australia and the American Heart Association recognize physical inactivity as an independent risk factor, and recommend 30 minutes of moderate intensity exercise five times per week. Few people achieve these targets, however (Glassberg and Balady, 1999; Pollock *et al*., 2000).

Improving lipid profiles in those with established CVD has been shown to lead to regression of atherosclerosis and markedly to reduce cardiovascular events (Ades, 2001). In women, low serum high-density lipoprotein (HDL) and raised triglycerides appear more important than low-density lipoprotein (LDL) levels alone. However, lipid-lowering trials have demonstrated that high-risk women benefit from LDL lowering with statins, for both primary and secondary prevention, as much as men (Heart Protection Study Collaboration Group, 2001). Improvement in lipid profiles is achievable through dietary modification, exercise and drug therapy; the latter approach has been proven to be especially effective. In the secondary prevention '4S' trial (Scandinavian Simvastatin Survival Study Group, 1994), cardiovascular events were reduced with statins by 30%. In the secondary prevention 'CARE' trial, even those with lower LDL serum levels had a 24% reduction in coronary events (Sacks *et al*., 1996). In those with low HDL levels, treatment with fibrates resulted in a 22% reduction in relative risk of non-fatal myocardial infarction (Rubins *et al*., 1999). Despite the efficacy of these intervention strategies, recent data have suggested that the ideal cholesterol targets are reached in only one-third of patients with coronary disease (Ades, 2001).

Intensive blood pressure control is well established for the primary and secondary prevention of cardiovascular disease. The 'HOT' study noted that the lower the blood pressure, the lower the risk of vascular events (Hansson *et al*., 1998). The targets for optimal blood pressure control are being lowered progressively. Currently, blood pressures of <140/90 mmHg are being recommended, with <130/85 mmHg in high-risk groups (World Health Organization, 1999). However, despite the overwhelming evidence for blood pressure lowering, the accepted targets are not being met in the majority of subjects (Wood, and the European Joint Task Force, 2001).

Diabetes is an important risk factor in women, with the risk of CVD increased 3- to 7-fold compared with 2- to 3-fold in men. The Nurses Health Study suggested that diabetic women have a 5-fold increase in coronary heart disease compared with non-diabetic women (Manson *et al*., 1991). Diabetic women have more severe CVD, higher CVD-related mortality and poorer prognosis overall compared with non-diabetic women (Lowel *et al*., 2000; Friday, 2001). Although blood glucose control may play a role in the prevention of CVD (Anonymous, 1998), it is accepted that, in diabetic subjects, it is the other risk factors which largely contribute to the burden of macrovascular disease (Friday, 2001). Thus, the presence of diabetes should target this high-risk population for aggressive preventive therapy.

Other important interventions that have a well-established role in the secondary prevention of CVD include aspirin therapy (ISIS Collaborative Group, 1988; Gaziano *et al*., 2000). Inadequate utilization is still problematic however (Rogers *et al*., 1994; Hennekens *et al*., 1997), as even in study settings such as the secondary prevention 'HERS' study, only 78% of women with established CVD received aspirin therapy (Hulley *et al*., 1998). Neurohormonal modulation of the cardiovascular system with beta blockade and angiotensin-converting enzyme inhibitors has also been shown to have a role in the prevention of CVD (Hjalmarson *et al*., 1981; Progress Collaborative Group, 2001).

Despite the established efficacy of these strategies, smoking rates are declining more slowly in women, obesity is increasing, and physical inactivity is escalating. In the USA, 50% of women aged >45 years have hypertension, and 40% aged >55 years have elevated cholesterol (Mosca *et al*., 1999). The under-utilization of preventive strategies may be related to women's misconceptions that they are more likely to die of breast cancer, or that heart disease predominantly affects males (Giardina, 2000; Mosca *et al*., 2000). This highlights the need for further public health measures and education. The attitude and role of the health professional is pivotal in the promotion of both appropriate prevention strategies and treatments for established CVD. Current evidence suggests that neither prevention nor treatment is optimal, especially in women (Rosano *et al*., 1993; Mosca *et al*., 1999).

Estrogen and progestins

Observational studies have long suggested that in post-menopausal women, estrogen combined with progestin may have a role in the prevention of CVD (Wolf *et al*., 1991; Grady *et al*., 1992; Grodstein and Stampfer, 1995). These observations have been supported by interventional studies focusing on the isolated mechanisms of sex steroid action within the vasculature. These mechanisms are ubiquitous and are affected by the dose, type and route of administered estrogen, as well as the co-administered progestin. Appropriate interpretation of the vascular effects of HRT requires an appreciation of the diversity and complexity of sex steroid action.

Pharmacology of estrogens and progestins

Estrogens regulate the growth, differentiation and function of diverse target tissues, both within and outside the reproductive system. There are three primary natural circulating estrogens: estradiol, estriol and estrone sulphate (converted in the periphery from estrone and estradiol) (Stanczyk, 1998). Follicle numbers fall as menopause approaches (Richardson *et al*., 1987), and when follicles are deplete at menopause the estradiol levels fall by around 90% compared with reproductive levels (Teede and Burger, 1998). Estrogen replacement, in the form of HRT, can be used therapeutically in post-menopausal women, and a wide variety of estrogens is available including the natural estrogens (e.g. estradiol), synthetic estrogens (e.g. ethinylestradiol) and conjugated equine estrogens (CEE) (Anderson, 2000). The pharmacokinetics and potency of these estrogens vary significantly based on the type of hormone and the route of administration, potentially contributing to significantly different observed clinical effects (Anderson, 2000).

Progestins regulate the growth, differentiation and function of target tissues primarily within the reproductive system. Progesterone is produced primarily by the dominant follicles following ovulation, with levels falling significantly once the ovarian follicle pool is deplete and menopause occurs (Teede and Burger, 1998). In post-menopausal women, progestins are used therapeutically to oppose the proliferative effects of estrogens on the endometrium and protect against endometrial malignancy (Beresford *et al*., 1997). Progesterone is the only natural form, but its therapeutic use is limited by reduced oral bioavailability. The majority of therapeutic progestins are therefore synthetic progestins; these are subdivided into those related structurally to either progesterone or testosterone (Whitehead, 1994). The different synthetic progestins also have variable pharmacokinetics and biological activity with additional complex and diverse effects outside the reproductive system.

Mechanisms of action: estrogen receptors (ER) and progesterone receptors (PR)

ER and PR are members of a superfamily of related proteins that mediate the nuclear effects of steroid hormones (Baysal and Losordo, 1996; Mendelsohn and Karas, 1999). These intracellular receptors function as ligand-activated transcriptional factors, regulating the synthesis of specific RNAs and proteins (Baysal and Losordo, 1996). They are characterized by a large and complex ligand-binding domain, a DNA-binding domain (which is highly conserved) and a hypervariable region—the amino-terminal region (Katzenellenbogen, 1996).

Estrogen actions are largely mediated by the ER, although non-genomic effects have been documented (Mendelsohn and Karas, 1999). The diversity of action of different estrogenic compounds or ligands can be attributed to the complex ER system. Estrogenic receptor activity is not dependent on steroidal configurations (Davies, 1998; Stanczyk, 1998), with both steroidal and non-steroidal compounds having estrogenic activity. Binding affinity does not directly translate to functional estrogenic activity, so the true 'biological estrogenicity' of a compound cannot be studied directly (Kuiper *et al*., 1998; Anderson, 2000). This complexity of the system reflects many factors including the subtype and distribution of the ER (Kuiper *et al*., 1997; Makela *et al*., 1999; Mendelsohn and Karas, 1999). There are two known ER isoforms, ERα and ERβ (Kuiper *et al*., 1997). ERs combined with different estrogenic compounds can have different ligand-dependent conformational changes. This is by virtue of the ER subtype, the presence of a variety of co-activators and co-repressors, and the cell and tissue type (Mendelsohn and Karas, 1999). It has been hypothesized that the complexity of this system may facilitate selective targeting of ERβ, potentially inducing cardiovascular effects without adverse stimulation of the reproductive tissues (Makela *et al*., 1999).

There are also two isoforms of PR: hPR-A and hPR-B. The PR has important interrelationships with the ER system, and modulates biological responses (Baysal and Losordo, 1996). PRs combined with ligand can suppress ER activity; this is influenced by the PR isoform, the ligand, the promotors and the cell type (Katzenellenbogen, 1996). Cross-talk occurs between ER and PR signalling systems in the modulation of biological responses (Katzenellenbogen, 1996). The complexity of effects of the different progestins are likely to reflect their structural

divergence from natural progestins, rendering their hormonal effects more complex with variable androgenic and estrogenic effects.

The complexity of the sex steroid receptor system and the variety of different estrogenic compounds and progestins used in clinical practice render the interpretation of existing literature very difficult. Ideally, each study needs to be interpreted individually, and the findings attributed only to the specific preparations, combinations, doses and routes of administration used for each given tissue end-point and each species studied.

Hormone replacement therapy (HRT)

HRT has been utilized to treat post-menopausal women for over 50 years. The benefits of HRT include the amelioration of menopausal symptoms, and an improvement in the quality of life. However, HRT has been increasingly prescribed for potential long-term indications including protection against bone loss, urogenital atrophy and prevention of CVD (MacLennan *et al*., 1999). Questions remain about the effectiveness of HRT for long-term indications, especially cardiovascular disease prevention (Mosca, 2000, 2001; Mosca *et al*., 2001a). The clinical advantages of therapy remain to be proven, and the potential disadvantages also need to be clarified, including the indication from epidemiological studies that the incidence of breast cancer may be increased with post-menopausal estrogen use.

HRT and cardiovascular disease

Observational data

A protective role of estrogen in CVD in post-menopausal women has been suggested by the low risk of CVD among premenopausal women and a narrowing of the gender gap after menopause. The suggestion that endogenous estrogen maybe cardioprotective was supported by observational data suggesting lower CVD risk in women on HRT as opposed to non-users (Grady *et al*., 1992). The assumption that exogenous HRT lowers CVD risk is primarily based on observational data, although supportive mechanistic interventional studies suggest beneficial cardiovascular actions of estrogen. In contrast, human controlled trials of HRT focusing on clinical cardiovascular end-points in both men and women have failed to confirm any benefit. Definitively, two large controlled primary prevention studies in post-menopausal women are now underway to address both the risks and benefits of HRT, and these results are awaited with great interest. In the interim, a comprehensive review of the literature is presented herein.

Extensive cohort and case–control studies have focused on the influence of HRT on a range of cardiovascular end-points including death, myocardial infarction, angiographic coronary stenosis grades and angioplasty and bypass grafting rates (Wolf *et al*., 1991; Grodstein and Stampfer, 1995; Grodstein *et al*., 1996, 1997, 2000; Sullivan *et al*., 1997, 1998). The studies vary considerably in their end-points and design, and also in the methods used to eliminate the effects of confounding variables. Most studies have suggested a 40–50% reduction in cardiovascular disease in HRT users compared with non-users (Wolf *et al*., 1991; Grodstein and Stampfer, 1995; Grodstein *et al*., 1996, 1997). Two meta-analyses have estimated the relative risk of CVD in ever-users of HRT compared with non-users at 0.65 [95% confidence interval (CI) 0.59–0.71] (Grady *et al*., 1992) and 0.64 (95% CI 0.59–0.68) (Grodstein and Stampfer, 1995). The relative risk in current users was estimated at 0.5 (95% CI 0.45–0.59) (Grodstein and Stampfer, 1995). Although the observational data are almost unanimous, a recent observational study in women with established vascular disease and unstable angina has suggested a deleterious effect of HRT. In this study, increased events were noted in those initiated on HRT after acute myocardial infarction (Alexander *et al*., 2001).

Inherent in the design of all observational studies is the problem of bias, and there are important caveats that must be applied when interpreting these data. First, most of these studies compared women who had elected to take HRT with women who had either not considered it or elected not to take it (Barrett-Connor, 1991). Even in socioeconomically homogeneous populations, these two groups of women differ. Women who elect to take HRT tend to be better educated, exercise more, have lower blood pressure and better lipid profiles and are more likely to participate in preventive health measures than women who do not take HRT (Barrett-Connor, 1991; Matthews *et al*., 1996). HRT users may therefore be at lower risk of CVD compared with non-users even before starting HRT (the 'healthy user effect'). Adjustment for known confounding variables has little effect on the estimated relative risk of CVD. It does seem likely though that the healthy user effect would not account for all of the 35–50% reduction in CVD risk (Bush *et al*., 1987). Indeed, many of these biases are less notable in the large cohort study, The Nurses Health Study, where 70 533 nurses have been followed for 20 years. Those on HRT still have a lower risk of CVD compared with non-users (Grodstein *et al*., 1996, 2000). Nonetheless, confounding variables (recognized and unrecognized) may have led to an overestimate of the magnitude of HRT reduction in CVD risk.

Second, most women in reported studies were taking unopposed estrogen, rather than combined estrogen-progestin, as is now prescribed for women with an intact uterus (Beresford *et al*., 1997). Progestins may negate some of the cardiovascular effects of estrogens. The apparent opposing effects of progestins has been reviewed (Sitruk-Ware, 2001). In mechanistic studies, modulation of the effects of estrogen on plasma lipids, arterial dilatation, blood flow and ultimately atherosclerosis have all been noted (Adams *et al*., 1997; Sitruk-Ware, 2001). Limited available human observational data based on combined estrogen and progestin use suggest that this effect may not be substantial (Falkeborn *et al*., 1992; Psaty *et al*., 1994; Grodstein *et al*., 1996), although the type and regimen of progestin used may also be relevant (Adams *et al.*, 1990, 1997; Anonymous, 1995).

Proposed mechanisms of the cardioprotective effects of estrogen

Multiple mechanisms have been proposed to account for the apparent protective effects of estrogen against coronary artery disease (Table I).

Lipid effects

The primary proposed mechanisms of beneficial estrogen action on the cardiovascular system are the effects on lipid metabolism (Godsland, 2001). Oral estrogen treatment reduces plasma total and LDL-cholesterol by 5–15%, increases HDL-cholesterol by

Table I. Proposed mechanisms of estrogen action on the cardiovascular system

Effects on lipid metabolism	Increased HDL cholesterol
(Oral estrogen)	Reduced LDL cholesterol
	Reduced Lp(a)
	Reduced oxidation of LDL cholesterol
	Increased triglycerides
Reduced vasomotor tone	Increased nitric oxide generation
	Decreased endothelin production
Smooth muscle effects	Reduced proliferation after endothelial injury
Plaque reduction	Reduction in vessel wall thickness
	Reduced atherosclerotic plaque
Additional effects	Reduced insulin resistance
	Reduced homocysteine
	Increased C-reactive protein
	Reduced cellular adhesion molecules
Effects on the haemostatic system	Increased venous thrombosis
	Reduced endogenous anticoagulants
	Increased coagulation activation
	Increased fibrinolysis

10%, and reduces lipoprotein Lp(a) concentrations (resistant to conventional lipid-lowering therapy) (Anonymous, 1995; Darling *et al*., 1997; Godsland, 2001). The effect on HDL may be especially important, as low plasma HDL is a strong predictor of cardiovascular mortality in women. Estrogen also inhibits oxidation of LDL (Sack *et al*., 1994), which may render them less atherogenic. A possible unfavourable effect of estrogen is an increase (20–25%) in plasma triglyceride concentrations (Anonymous, 1995; Darling *et al*., 1997; Godsland, 2001), as high triglyceride levels are also predictors of cardiovascular mortality in women. In contrast to oral estrogens, the effects of transdermal estrogen preparations on serum lipids are either minimal (Crook *et al*., 1992) or absent (Lufkin *et al*., 1992; Modena *et al*., 1999; Teede *et al*., 2001d). This is thought to be related to the fact that it is not orally absorbed and therefore does not cause an acute increase in concentrations within the liver—the hepatic 'first-pass' effect.

It has been estimated that lipid changes resulting from oral HRT use may only account for 25–50% of the reduction in CVD in observational studies (Bush *et al*., 1987). Unlike statins, controlled trials are limited and the clinical significance of HRT-induced lipid changes is not well characterized. In the secondary prevention HERS trial, lipid benefits were observed, yet reduction in CVD events was not noted (Hulley *et al*., 1998).

The clinical relevance of the HRT-induced lowering of Lp(a) also needs clarification, as the pathophysiological significance of this complex polymorphic lipoprotein particle remains unclear (Marcova and Koschinsky, 1999; Godsland, 2001). Interestingly, a recent HERS study subgroup analysis suggested that Lp(a) was an independent risk factor for recurrent coronary heart disease in post-menopausal women, and that treatment with estrogen and progestin lowered Lp(a) levels. It also demonstrated that among women with a high baseline Lp(a) level, those on HRT had a lower rate of cardiovascular events compared with those with lower baseline Lp(a) (Shlipak *et al*., 2000).

Progestins tend to lower HDL concentrations, and this partially antagonizes the favourable effects of estrogen (Anonymous, 1994, 1995). The more androgenic progestins have a greater deleterious effect on HDL (Crook *et al*., 1992; Anonymous, 1995; Hart *et al*., 1998; Godsland, 2001). The least deleterious effects are observed with dydrogesterone, progesterone and cyproterone acetate (Godsland, 2001). Pending further clarification of the clinical relevance of combined estrogen and progestin-induced lipid changes, statins should be first-line therapy for hypercholesterolaemic women. However, women who require estrogen for other indications may derive further lipid benefits, and in theory the selection of less androgenic progestins may be more appropriate (Godsland, 2001).

Vasomotor tone

The changes in vasoreactivity observed with estrogen have been partly attributed to increased nitric oxide (NO) production. Animal studies have noted gender-specific differences in the NO pathway, with NO release being greater in females, whilst a lower basal vasomotor tone and less responsiveness to vasoconstrictors has been noted compared with males (Hayashi *et al*., 1992; Binko *et al*., 1998). Exogenous estrogen increases the expression of nitric oxide synthase (NOS) in both animals (Weiner *et al*., 1994) and humans (Hishikawa *et al*., 1995). Furthermore, estrogen produces relaxation of vascular smooth muscle by a NO-dependent process (Darkow *et al*., 1997). Interestingly though, estrogen does not activate downstream mediators in the NO pathway, including the primary effector molecule protein kinase G (Teede *et al*., 2001b). However, production of the potent vasodilator NO may be cardioprotective as it not only regulates blood flow but also inhibits platelet aggregation at the level of the endothelium (Ignarro, 1989).

Endothelin-1 is the most potent of the vasoconstrictor hormones. Females have lower endothelin levels, especially when pregnant. Trans-sexuals receiving estrogen have been noted to have a fall in endothelin-1 levels (Polderman *et al*., 1993). A recent review based on randomized, controlled studies of HRT on the cardiovascular system in post-menopausal women concluded that HRT reduced endothelin levels (van Baal *et al*., 2000). Potentially, this reduction may also improve vascular tone following estrogen treatment.

The direct assessment of the effects of sex steroids on arterial blood flow, arterial resistance and vessel diameter has been studied extensively (Reis *et al*., 1994). Most studies have determined endothelial dependence of the artery or vascular territory in question by examining responses to acetylcholine. Invasive vascular reactivity studies have demonstrated that atherosclerotic arteries exhibit a reduced response to acetylcholine administration, which is reversed by estrogen addition in the monkey model (Williams *et al*., 1990). Non-invasive studies of endothelial function using ultrasound techniques including flow-mediated vasodilation have been contradictory. Observational and short-term estrogen studies have suggested benefit, whilst long-term controlled trials have not necessarily supported these results (McCrohon *et al*., 1996; Sorensen *et al*., 1997; Teede *et al*., 2001d,e).

Studies in post-menopausal women have suggested that estrogen therapy may have anti-ischaemic effects in women with established CVD. A controlled trial in 74 post-menopausal

women with stable angina showed that HRT increased the time to ST depression and total exercise duration compared with placebo therapy (Sanderson *et al*., 2001). This finding supported the results of a previous uncontrolled trial (Rosano *et al*., 1997).

Atherosclerosis and vascular structural changes

The effect of HRT on atherosclerosis is perhaps best appreciated from an extensive series of experiments in the cynomolgus monkey atherosclerosis model (Clarkson, 1994; Clarkson *et al*., 1995, 2001). Monkeys were oophorectomized, and then randomized to placebo, oral or transdermal estrogen alone, combined HRT with continuous or cyclic progestin or tibolone. They were fed an atherogenic diet for 2 years, after which (at necropsy) a comprehensive assessment of coronary atherosclerosis was undertaken (Clarkson, 1994; Clarkson *et al*., 1995, 2001). Estrogen alone reduced the cholesteryl ester content (Wagner *et al*., 1997) and the atherosclerotic plaque by either 50% (transdermal therapy) or 70% (oral therapy) compared with those animals receiving placebo (Clarkson *et al*., 1995). Studies in atherosclerosis models in other species, including rabbits, have also suggested that estrogen provides protection against atherosclerosis (Brehme *et al*., 1999). It should be noted, however, that debate persists as to whether animal models of atheroma, induced solely by an atherogenic diet, actually provide a true model of human atheroma.

The effects of additional progestins on atherosclerosis in animal models are less clear. In the cynomolgus monkey, natural progesterone or cyclic medroxyprogesterone acetate (MPA) did not appear to influence the beneficial effects of estrogen on atherosclerosis (Adams *et al*., 1990). However, the effects of continuous MPA in this model were conflicting. Some studies suggested that continuous MPA negated the beneficial effects of estrogen, including reduced atherosclerosis, aortic connective tissue remodelling after lipid lowering and a reduction in dobutamine-induced myocardial ischaemia (Adams *et al*., 1997; Register *et al*., 1998; Williams *et al*., 2002). However, in a recent study in the same monkey model, continuous MPA did not affect the beneficial reduction in atherosclerosis seen with estrogen (Clarkson *et al*., 2001). In rabbits, coronary flow rates were increased with estrogen therapy—an effect not observed when estrogen was combined with several types of progestin, including MPA (Gorodeski *et al*., 1998). The atherosclerotic effects of combining specific progestins with estrogen are not yet clarified, and require further investigation.

Interestingly, it was noted (Hanke *et al*., 1999) that estrogen could inhibit the progression of atherosclerosis in rabbits when only mild or moderate vessel wall abnormalities were present, but appeared unable to do so once severe atherosclerotic disease was established. The anti-atherosclerotic effects of estrogen were apparently mediated by the endothelium, which becomes dysfunctional once atherosclerosis develops. This is supported by studies in the monkey model where the beneficial effect of estrogen on acetylcholine-mediated vasodilation only occurred in segments of the coronary artery where mild atherosclerosis was present. No effects were noted in arterial segments with severe atherosclerosis, suggesting that estrogen may not be useful in secondary prevention (Clarkson, 1994).

In a rat model of carotid injury, vascular ERβ receptors were up-regulated, and this was accompanied by a dose-dependent, estrogen-mediated inhibition of the migration and replication of smooth muscle cells *in vitro* (Makela *et al*., 1999). In the rabbit, rat and mouse, intimal thickening after mechanical carotid balloon injury appears to be reduced by estrogen pretreatment, this being mediated by inhibition of vascular smooth muscle proliferation (Foegh *et al*., 1994; Sullivan *et al*., 1995; Oparil *et al*., 1997). Interestingly, progestin (MPA) blocked the effects of estrogen in this model (Oparil *et al*., 1997). When given alone, MPA enhanced the neointimal response to balloon injury in intact females, presumably by blocking production—and thus the vasoprotective effect—of endogenous estrogen. However, when given in combination with exogenous estrogen, MPA negated the beneficial reduction in neointimal proliferation with estrogen alone (Oparil *et al*., 1997). Estrogen has also been shown to facilitate the re-endothelialization of the carotid artery after balloon injury (White *et al*., 1997). Furthermore, *in vitro* estrogen inhibits vascular smooth muscle cell proliferation—an effect that is directly mediated by the ER (Baysal and Losordo, 1996).

Haemostatic and inflammatory effects

Thrombosis is an important component of arterial vascular events as well as venous thromboembolic events (VTE). Previously, arterial cardiovascular events were attributed to progressive obstruction from atherosclerotic plaques, though this concept has been revised as it has become clear that cardiovascular events are characterized by an acute obstructive process superimposed on a previously mild to moderate single atherosclerotic lesion (Theroux and Cairns, 1998). Subsequent morphological analysis has revealed plaque disruption and intraluminal thrombi (Ross, 1993). Indeed, coronary thrombosis was noted on underlying ruptured plaque in 95% of those suffering sudden cardiovascular death (Davies and Thomas, 1984). Accordingly, the mainstay of acute treatment of acute myocardial infarction is now thrombolytic therapy aimed at dissolving the occluding thrombus and reperfusing the ischaemic myocardium (ISIS Collaborative Group, 1992). Moreover, simple measures including aspirin and heparin therapy have proven useful in both the treatment and prevention of clinical events (Hansson *et al*., 1998; Gaziano *et al*., 2000). Furthermore, inflammatory changes within atherosclerotic plaque appear to increase vulnerability to rupture, leading to thrombus formation, myocardial ischaemia and clinically overt disease. In this setting an intervention which is pro-inflammatory and procoagulant, potentially may increase arterial thrombosis and therefore also clinical cardiovascular events.

Concerns have been raised about the prothrombotic effects of estrogen, with an increase in both VTE and potentially also arterial events (Coronary Drug Project Research Group, 1970; Hulley *et al*., 1998; Hoibraaten *et al*., 2000, 2001; Teede *et al*., 2000a; Alexander *et al*., 2001; Peverill *et al*., 2001). In controlled trials, HRT increased the risk of VTE by 3- to 4-fold, with the increase noted in the early 1–2 years of treatment (Figure 1) (Hulley *et al*., 1998; Hoibraaten *et al*., 2000). This increase appeared to be independent of, and multiplicative with, other prothrombotic risk factors (Lowe *et al*., 2000).

It has also been proposed that the prothrombotic effect of HRT may have been responsible for the early increase in thrombotic arterial cardiovascular events observed in the secondary prevention of cardiovascular disease, the HERS study (Hulley *et al*., 1998), though this has yet to be clarified. A population-based

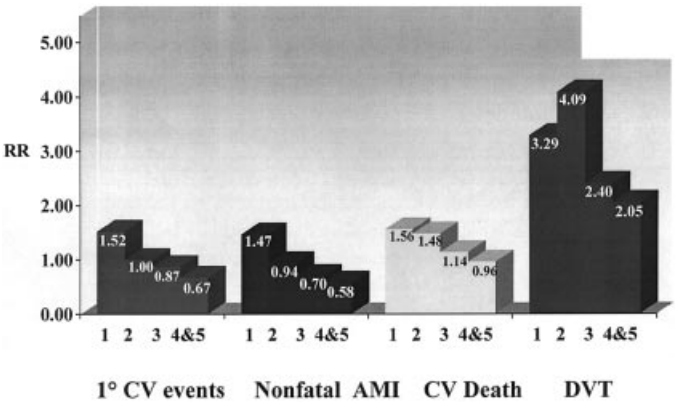

Figure 1. Cardiovascular (CV) outcomes by treatment group and year since randomization in the 'HERS' study (Hulley *et al*., 1998). AMI = acute myocardial infarction; DVT = deep vein thrombosis.

case–control study has demonstrated that in hypertensive women with a first non-fatal acute myocardial infarction (AMI), there was a positive association between genetic thrombophilia, HRT use and AMI (Psaty *et al*., 2001). Overall, the concern is that the procoagulant effects of HRT may negate the cardiovascular benefits of HRT.

In mechanistic studies based on the use of accurate immunodiagnostic haemostatic markers, HRT appears primarily to activate coagulation, and although it increases fibrinolytic potential this may only be a secondary physiological response to the up-regulation noted in coagulation (Teede *et al*., 2000; Hoibraaten *et al*., 2001). Although the mechanism of estrogen-induced up-regulation of coagulation remains unknown, in controlled trials HRT has been shown to reduce the vital tissue factor pathway inhibitor (TFPI), along with a reduction in other endogenous anticoagulants (Peverill *et al*., 2001; Hoibraaten *et al*., 2001). Potentially, this may allow up-regulation of the coagulation cascade. Tissue factor is the principal initiator of the extrinsic coagulation pathway, and the fall in TFPI has been directly correlated to the degree of coagulation activation noted with HRT (Hoibraaten *et al*., 2001). The effects on platelets may be neutral (Teede *et al*., 2001), though further research is required in this area.

As noted, the pathophysiology of an acute arterial ischaemic event includes plaque rupture and subsequent thrombosis. At present, there is no available test that will predict which plaques are vulnerable to rupture, occurring in the setting of an inflammatory process within the plaque. C-reactive protein (CRP) has been noted to be an independent cardiovascular risk factor (Koenig, 2001), though as yet it has not been ascertained whether CRP is a marker or is truly pathogenic in the inflammatory process, or whether modulation of CRP will alter the disease process (Koenig, 2001). HRT has been documented to increase CRP plasma levels (Ridker *et al*., 1999; Walsh *et al*., 2000), potentially increasing the risk of plaque rupture. In contrast to these results, reductions in circulating levels of the inflammatory markers E-selectin, vascular cell adhesion molecule (V-CAM) and intracellular adhesion molecule (I-CAM), which may also reflect plaque stability, have been noted with HRT (van Baal *et al*., 1999, 2000). However, again the net clinical significance of these findings has yet to be established.

Additional effects

A plethora of additional mechanisms of HRT action on the vascular system has been demonstrated, including effects on ion channels, membrane receptor aggregation and changes in protein phosphorylation status (Mendelsohn and Karas, 1999; Pines *et al*., 1999; Stefano *et al*., 2000). Other estrogen effects include changes in the renin–angiotensin system (Proudler *et al*., 1995) and beneficial changes in carbohydrate metabolism and body fat distribution (Barrett-Connor *et al*., 1989). A recent review based on randomized, controlled studies of HRT on the cardiovascular system in post-menopausal women also concluded that HRT reduced homocysteine levels (van Baal *et al*., 2000).

Whilst individual mechanisms of estrogen action are of interest, the mosaic of data available on the cardiovascular effects of sex hormones is complex and influenced by a variety of factors, including natural versus synthetic hormones, species, age, hormonal status, which vessel is studied, baseline endothelial function, arterial structure, lipid and coagulation factors. Furthermore, it remains controversial as to whether there is a true animal model for atherosclerosis as the models used to date have atherosclerosis induced by very high levels of cholesterol intake without other co-existent risk factors. Therefore, despite this wealth of data it remains difficult to assess the effect of HRT on the vascular system (Teede and McGrath, 1999).

Randomized controlled human interventional data

Although the extensive data on the influence of HRT on vascular disease in both human and animal studies appear positive, the picture portrayed in the randomized trials on the effects of HRT on cardiovascular disease suggests that HRT may not be beneficial, but rather have a neutral effect or even increase the incidence of cardiovascular events.

Early data were available from the 1960s with the randomized Coronary Drug Project (CDP) involving over 7500 male subjects with documented myocardial infarction (Coronary Drug Project Research Group, 1970). This project was stopped prematurely as no benefits of estrogen were observed. However, a higher rate of venous thrombosis was observed, and in a recent reanalysis of the CDP data a significant increase in coronary heart disease within the first 4 months of treatment was demonstrated (Mosca, 2001).

Recently, a substantial human study on the effects of HRT on vascular disease in women, the 'HERS' study, was reported (Hulley *et al*., 1998). This was a well-designed, double-blind, placebo-controlled, randomized study of combined continuous oral HRT (0.625 mg conjugated equine estrogen + 5 mg medroxy progesterone acetate) use in the secondary prevention of vascular disease, in 2763 post-menopausal women of mean age 66.7 years, with pre-existing coronary atherosclerotic disease over 4.1 years. The study failed to demonstrate any overall difference in vascular events, including myocardial infarction, coronary revascularization, unstable angina, congestive cardiac failure, stroke, transient ischaemic attack or peripheral arterial disease, between the placebo and active treatment groups. This was despite an improvement in lipid parameters in those patients receiving HRT (Hulley *et al*., 1998). The arterial vascular event rate in the first year was actually significantly increased [relative risk (RR) = 1.52], falling over time to a RR of 0.67 by the fourth

year (Figure 1). The trend towards a fall in the cardiovascular event rate over the 4 years was significant, yet overall, no differences were noted between the active and placebo groups (Hulley *et al*., 1998).

On the basis of previously published data, these results were unexpected. Clinically significant effects of HRT on the haemostatic system were confirmed, with an increase in venous thrombosis similar to that seen with the oral contraceptive pill (Hulley *et al*., 1998). A two-edged sword effect of HRT was proposed, with the authors theorising that the prothrombotic effects of estrogen may negate any possible atherosclerotic benefits in women with established cardiovascular disease and pre-existing plaques, which are prone to rupture, though this has yet to be confirmed.

The criticisms of the HERS study were related primarily to the progestin regimens used (continuous medroxy progesterone acetate, shown in some animal studies to negate the beneficial effects of estrogen, yet not in others) (Adams *et al*., 1997; Clarkson *et al*., 2001), although this progestin regimen is common in clinical practice. Otherwise, it was a well-designed and well-executed study, and the only human controlled trial based on clinical disease end-points to date. It is a landmark study, which has sounded an important note of caution for practitioners prescribing HRT in women with known cardiovascular disease. The most significant limitation is that the results apply only to the specific HRT preparation used and the population studied. The findings are not applicable to those without established vascular disease; nor can they be extrapolated to encompass the effects of other estrogen doses and routes of administration or other progestin regimens.

The 'ERA' trial, which was published in 2000, compared 3.2 years of treatment with estrogen ($n = 100$), combined estrogen + progestin ($n = 104$) or placebo ($n = 105$) in post-menopausal women aged 42–80 years with pre-existing coronary disease (Herrington *et al*., 2000). There was no significant difference in the rate of progression of coronary atherosclerosis between the three groups. Coronary atherosclerosis based on angiographic data does not necessarily predict the risk of subsequent clinical events, and is not a hard clinical end-point. As with all currently available surrogate markers of vascular disease, it does not reflect plaque stability and therefore cannot predict those most likely to rupture and suffer acute cardiovascular events. However, it does provide a human study similar to the original monkey data focusing on atherosclerosis development, and the results of this study are important for two primary reasons. First, an estrogen-alone arm was included and did not demonstrate benefits over combined therapy; second, this study suggested that previous controlled animal trials focusing on coronary atherosclerosis may not be representative of responses in humans.

This is also supported by the recent double-blind, placebo-controlled trial in 664 post-menopausal women with ischaemic stroke or transient ischaemic attack. Treatment with 1 mg of oral 17β-estradiol daily did not alter the risk of subsequent events, with a total of 99 strokes or deaths in the active and 93 in the placebo groups over 2.8 years. The RR was 1.1 (95% CI 0.8–1.4) (Viscoli *et al*., 2001).

Further randomized controlled studies focusing on another surrogate end-point, carotid artery intimal medial thickness (IMT), have also examined the effect of HRT. The first study compared estradiol + monthly gestodene, estradiol + 3-monthly gestodene, or a control group on no HRT. In 321 healthy post-menopausal women with increased IMT (representing early subclinical atherosclerosis), one year of HRT did not slow IMT progression despite falls in LDL-cholesterol levels (Angerer *et al*., 2001). However, a more recent study in 222 post-menopausal women without pre-existing CVD compared unopposed estrogen with placebo therapy with and without lipid-lowering agents over 2 years. The rate of progression of subclinical atherosclerosis (IMT) was lower in those patients randomized to unopposed estradiol compared with placebo, although a significant effect of estradiol was only noted in those women not receiving lipid-lowering therapy (Hodis *et al*., 2001). The women in the latter study did not have pre-existing CVD, and were studied for longer compared with the previously described investigation (Angerer *et al*., 2001).

A pooled study which was published in 2000, analysed data from 28 randomized controlled trials that compared HRT ($n = 2206$) with another agent ($n = 1278$) for up to 3 years (Hemminki and McPherson, 2000). The focus was to compare the rate of cardiovascular events in these controlled trials to the reduced cardiovascular event rate in those on HRT noted previously in observational trials (Barrett-Connor and Grady, 1998). The pooled data demonstrated an increased cardiovascular event rate in those on HRT (OR 1.78, 95% CI 0.7–4.52) that was not statistically significant. The findings do not support a beneficial effect of HRT on cardiovascular event rate seen in observational studies.

Other controlled trials focusing on surrogate outcomes are ongoing. The Women's Angiographic Vitamin and Estrogen (WAVE) trial, the Women's Estrogen/ Progestin and Lipid-Lowering Hormone Atherosclerosis Regression Trial (WELL-HART) and the Estrogen and Graft Atherosclerosis Research (EAGAR) trial based on angiography in women with bypass grafts, are all due for completion in the near future should add further to our knowledge base. These studies, along with the HERS and ERA studies, involve women with established vascular disease. Animal data suggest that estrogen may not have beneficial cardiovascular effects in women once atherosclerosis is established and endothelial damage has occurred (Honore *et al*., 1996; Hanke *et al*., 1999). Both human observational data (Herrington *et al*., 2001) and interventional data suggest that this may be the case (Haines *et al*., 2001), thus highlighting the need for further investigation into the role of HRT in primary prevention.

The Women's Health Initiative trial, a primary prevention trial, is an ongoing study comparing estrogen + progestin to estrogen alone in healthy post-menopausal women aged 55–79 years, with a target enrolment of 27 500 cases. In April 2000, the investigators informed the participants that 'during the first 2 years there was a small increase in the number of heart attacks, strokes and blood clots in women taking active hormones compared to inactive pills'. Over time, these differences appeared to diminish. The overall event rate was low, occurring in <1% of women, and the difference between the groups did not reach statistical significance. Yet, the effects of HRT would be consistent with the HERS study, and given that they are seen in healthy women, even infrequent events are concerning. A report

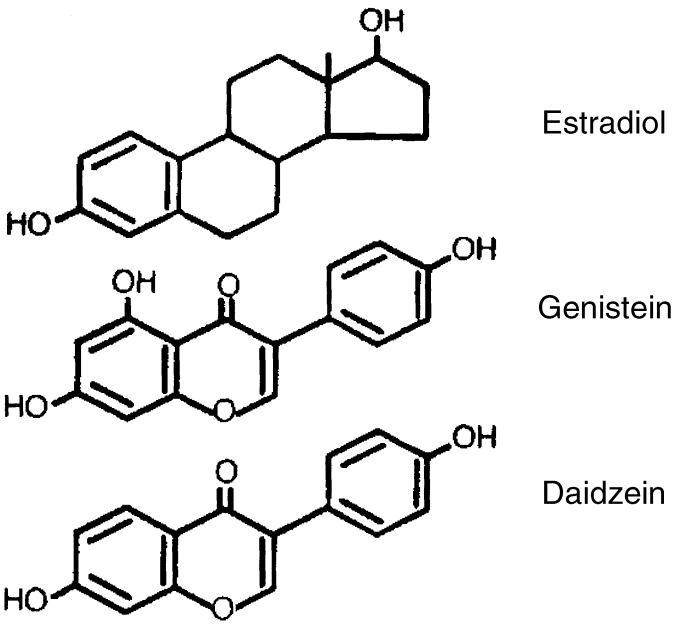

Figure 2. The chemical structures of 17β-estradiol, genestein and daidzein.

scheduled for 2005 will provide more definitive data on the effects of HRT on CVD.

Newer strategies

Low-dose HRT

Lower-dose regimens (0.3 mg CEE or equivalent) have been the focus of much recent research into HRT (Ettinger, 1999). Studies have suggested that low-dose HRT can relieve menopausal symptoms and vaginal atrophy (Utian *et al*., 2001), prevent bone loss when combined with calcium (Genant *et al*., 1997; Recker *et al*., 1999) and improve lipid profiles without inducing endometrial hyperplasia (Genant *et al*., 1997; Lobo *et al*., 2001). The primary advantage of low-dose regimens is the improved side-effect profile and better long-term compliance. A recent analysis from the 20-year cohort study, The Nurses' Health Study in 70 533 women, suggested that the cardioprotective effect of 0.3 mg of CEE was equivalent to that of the higher dose of 0.625 mg (Grodstein *et al*., 2000). This finding is encouraging, but is based on observational data, and once again further clinical trials are required to clarify the role of low-dose HRT regimens in the prevention of disease.

Tibolone

The estro-progestogen tibolone is a steroid hormone with a progestin-like structure that is converted to estrogenic and androgenic derivatives *in vivo*. It has been shown to improve menopausal symptoms and to improve bone density (although fracture data are still lacking), and it potentially has fewer side effects than conventional HRT (Hammar *et al*., 1998; Crook, 2001). Inadequate human observational data exist on the cardiovascular effects of tibolone. Human studies have provided mixed results with reductions in HDL, but also reductions in triglycerides and Lp(a) levels, with less effect than conventional HRT on LDL levels (Godsland, 2001). Animal studies suggested a reduction in vascular adhesion molecules by endothelial cells—a theoretically beneficial effect (Simoncini and Genazzani, 2000). Rabbit studies have suggested a reduction in atherosclerosis development (Zandberg *et al*., 1998), although a comparative study in the cynomolgus monkey atherosclerosis model demonstrated improved lipids and reduced atherosclerosis with CEE, but not with tibolone. Tibolone reduced HDL, but had no impact—either adverse or beneficial—on coronary artery atherosclerosis (Clarkson *et al*., 2001). A small trial in 10 post-menopausal women with angina has demonstrated increased exercise tolerance similar to that observed with estrogen use (Lloyd *et al*., 1998), whilst a randomized trial based on the effects of tibolone on the surrogate marker, carotid IMT, is pending. There is a suggestion that tibolone may not increase thrombotic risk, although the data are inadequate to draw any such conclusions at present (Winkler *et al*., 2000). The net clinical significance of these changes in surrogate endpoints remains unknown, and we must await data from controlled trials focusing on definitive clinical end-points to establish the role of tibolone in CVD prevention.

Selective ER modulators

Selective ER modulators (SERMS) are non-steroidal estrogenic compounds with both estrogenic and non-estrogenic actions. They are established for the prevention of breast cancer and for the treatment of osteoporosis (Bush *et al*., 2001). The data on the cardiovascular effects of SERMS are based on in-vivo animal and human studies, as well as limited data from controlled interventional trials on clinical cardiovascular end-points.

The data on the vascular effects of raloxifene have been extensively reviewed (Moscarelli and Cox, 2000). Clinical trials have demonstrated that SERMs do not change HDL or triglyceride levels, but do significantly improve Lp(a) and LDL levels (Walsh *et al*., 1998; Godsland, 2001). Other circulating markers (including homocysteine levels) each fell, but CRP levels were not changed with raloxifene, compared with falls in homocysteine and a rise in CRP seen with HRT (Walsh *et al*., 2000).

Vascular structural and functional effects have been noted with animal data which are suggestive of up-regulation of NO with vasorelaxation and reduced arterial intimal thickening in response to injury. Raloxifene inhibited atherosclerosis in the cholesterol-fed rabbit model (Bjarnason *et al*., 1997), but not in the monkey model, where no reduction in atherosclerosis was observed (Clarkson *et al*., 1998).

Human interventional data have been completed with tamoxifen in a randomized controlled clinical trial in 13 388 women focusing on breast cancer prevention (Reis *et al*., 2001). No effect was noted on CVD end-points. Even in a subsequent subgroup analysis for those at high baseline risk akin to the HERS study population, no effect of tamoxifen on cardiovascular events was noted (Reis *et al*., 2001). The ongoing double-blind, placebo-controlled Raloxifene Use for the Heart (RUTH) study, focusing on the effects of raloxifene in 10 101 post-menopausal women aged >55 years and with established vascular disease or multiple risk factors, should significantly contribute to our understanding of the net clinical cardiovascular effects of raloxifene (Mosca *et al*., 2001b). Also, the increase in risk of venous thromboembolic disease appears to be equivalent to that seen with estrogen

Table II. The American Heart Association guidelines on HRT and the prevention of cardiovascular disease

Secondary prevention
- HRT should not be initiated for the secondary prevention of CVD.
- The decision to continue or to stop HRT in women with CVD who have been undergoing long-term HRT should be based on established non-coronary benefits and risks and patient preference.
- If a woman develops an acute CVD event or is immobilized while undergoing HRT, it is prudent to consider discontinuance of the HRT, or to consider VTE prophylaxis while she is hospitalized to minimize risk of VTE associated with immobilization. Reinstitution of HRT should be based on established non-coronary benefits and risks, as well as patient preference.

Primary prevention
- Firm clinical recommendations for primary prevention await the results of ongoing randomized clinical trials.
- There are insufficient data to suggest that HRT should be initiated for the sole purpose of primary prevention of CVD.
- Initiation and continuation of HRT should be based on established non-coronary benefits and risks, possible coronary benefits and risks, and patient preference.

From Mosca *et al*. and the American Heart Association (2001a). Hormone replacement therapy and cardiovascular disease. *Circulation*, **104**, 499–503.

(Ettinger *et al*., 1999). Given the established prothrombotic risks of the currently available SERMS, the lack of knowledge on the clinical relevance of changes in surrogate CVD risk factors, and the apparent neutral cardiovascular effects of tamoxifen in clinical trials, these agents cannot be presently considered as cardioprotective (Bush *et al*., 2001).

Phytoestrogens

Characteristics and estrogenicity

Phytoestrogens are a diverse group of plant-derived compounds, similar to estrogenic steroids (Figure 2) (Davies, 1998). Isoflavones are the most common phytoestrogens (Davis *et al*., 1999), with genistein and diadzein being the most estrogenically active and found in greatest concentrations in soybean (Price and Fenwick, 1985). Phytoestrogens have been reported to have both agonist and antagonist estrogenic effects (Kuiper *et al*., 1998), which are not necessarily parallel to those of estrogen. More recently however, the relative binding affinities of several phytoestrogens to ERα and ERβ have been shown to be significant, especially for ERβ. ERβ is highly expressed in vascular endothelium and smooth muscle cells; therefore, in theory phytoestrogens may trigger many of the biological responses that are evoked by physiological estrogens (Kuiper *et al*., 1998). However, whilst the affinity of phytoestrogens for ER has been established, ligand–ER interactions are inherently complex, and our understanding of the biological potencies of the phytoestrogens remains limited.

The observed estrogenic effects of phytoestrogens have been reviewed extensively (Davis *et al*., 1999; Murkies *et al*., 2000). In animals, they include reproductive dysfunction, selective neuroendocrine effects and effects on sexual development (Davis *et al*., 1999; Murkies *et al*., 2000). In premenopausal women, menstrual disturbances have been noted, whilst in post-menopausal women improvement in the vaginal maturation index has been noted (Bickoff *et al*., 1961; Dalais *et al*., 1998). Yet more studies have failed to show estrogenic effects of dietary soybean in both animals and humans (Baird *et al*., 1995; Tansey *et al*., 1998; Teede *et al*., 2001c). Controlled trials have suggested that neither soybean nor isolated sources of phytoestrogens reduce menopausal symptoms over placebo therapy (Baber *et al*., 1999; Kotsopoulos *et al*., 1999; Murkies *et al*., 2000). In the setting of the apparent selectivity and complexity of the endocrine effects of phytoestrogens, it has been suggested that individual effects need to be characterized for each putative estrogenic compound on each estrogenic end-point (Hughes *et al*., 1991).

Effects on the cardiovascular system

Diets rich in phytoestrogens may reduce cardiovascular disease. Specifically, high soy intake is associated with a lower incidence of cardiovascular disease, and the ingestion of vegetable protein—particularly soy—is associated with a reduced risk of coronary heart disease and improved risk factor status (Aldercreutz, 1998; Davis *et al*., 1999; Teede *et al*., 2001c). Interventional studies indicate that soy has favourable effects on lipid profiles in both primates and humans, and on blood pressure in humans (Anderson *et al*., 1995; Davis *et al*., 1999; Teede *et al*., 2001c). A meta-analysis of controlled human clinical trials noted that soy protein consumption significantly reduced total cholesterol (9.3% decrease, 95% CI 0.35–0.85 mmol/l), LDL-cholesterol (12.9% decrease, 95% CI 0.30–0.82 mmol/l) and triglycerides (10.5% decrease, 95% CI 0.003–0.29 mmol/l), with little change in HDL (Anderson *et al*., 1995). Responses were related to pretreatment plasma cholesterol levels. Most research has focused on phytoestrogen-rich whole foods or protein isolates, with little information on concentrated phytoestrogen subfractions in tablet form. However, limited data suggest that isolated phytoestrogens are less effective at improving cardiovascular risk factors (Hodgson *et al*., 1998). Soy supplementation has also been associated with reduced atherosclerosis in animals and improved vascular function in female monkeys, but not in humans (Honore *et al*., 1996; Teede *et al*., 2001c). The active components of soy are not yet established, with potential contributors including vegetable protein, antioxidants and phytoestrogens.

Despite observational data on humans and animal and human interventional data focusing on surrogate end-points, once again the net clinical effects of either soy or isolated phytoestrogens on the cardiovascular system remain unknown. Although ongoing

studies with combinations of phytoestrogens, antioxidants and micronutrients are awaited with interest, there are currently insufficient data available to recommend the consumption of isolated supplements for the prevention of CVD. In contrast, the consumption of diverse and balanced diets, which are rich in foods containing many nutrients, including antioxidants and phytoestrogens, can be safely recommended (Teede and Kritharides, 2000).

Conclusion

CVD is the number one killer of both men and women in Western countries. Prevention strategies are very effective in reducing the burden of CVD. Well-established prevention strategies including lifestyle modifications and specifically targeted pharmacological treatments remain under-utilized, especially in women. Considering the controversy surrounding the role of HRT in the prevention of CVD, it is arguable that although this remains a potentially exciting area for further research, the issue of appropriate clinical use of HRT for CVD prevention is yet to be clarified. There is a need to appreciate the limitations of existing observational and interventional data on the vascular effects of HRT. Ongoing research in this area is important. The role of lower-dose HRT needs to be clarified, but potentially most exciting is the prospect of selective modulation of the estrogen receptor to activate the beneficial cardiovascular effects of HRT, without adverse procoagulant and reproductive effects. Whilst the observational data need not be discarded and the potential for HRT to provide primary CVD prevention has still to be considered, the recent American Heart Association guidelines suggest that there is no current indication to commence HRT for the prevention of CVD alone (Table II). Essentially, these guidelines recommend that HRT should be instituted based on other accepted indications, with caution applied to those with established CVD. Furthermore, HRT should not preclude—and cannot replace—specific targeted therapies proven to reduce the risk of CVD.

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