Introduction

The potential importance of oestrogens as antioxidant cardioprotectants is currently attracting considerable attention. Oestrogen is recognized to have a protective effect against coronary heart disease and its antioxidant action could contribute to the cardiovascular benefits observed on oestrogen administration in postmenopausal women [1,2]. This antioxidant-mediated cardioprotective action is likely to be independent of the favourable influence of oestrogen on risk factors such as the plasma lipid profile [1–3]. Furthermore oestrogen improves lipid metabolism in the postprandial state: it makes this state less atherogenic by improving the clearance of chylomicron remnants and by attenuating the postprandial decrease in levels of beneficial high-density lipoprotein (HDL) cholesterol [4]. Other beneficial effects of oestrogen include the lowering of plasma homocysteine levels [5] and serum levels of angiotensin-converting enzyme [6], both of which are risk factors for cardiovascular disease. Oestrogen may be able to enhance beneficial relaxation of the vasculature by modulating the synthesis of vasodilators such as the free radical, NO [7,8], and smooth-muscle Ca²⁺ signalling [8]. However, the role of NO as a beneficial mediator of cardiovascular function is clearly one of balance. This is because it can also react with other free radicals such as superoxide to form potentially damaging species such as peroxynitrite, which is implicated in low-density lipoprotein (LDL) oxidation [9].

Antioxidant properties in vitro have been shown for endogenous oestrogens such as 17β-oestradiol [10–13], synthetic exogenous oestrogens such as 17α-ethynylestradiol (used in oral contraceptives) [14] and the dietary phytosterogens (possess weak oestrogenic properties) such as the isoflavonoid genistein [15,16]. Genistein is found predominantly in soya products and has been suggested to have cardioprotective properties [17,18]. Genistein has a number of
other properties that suggest its potential as an antiatherogenic agent including its ability to inhibit the endothelial cell proliferation required for the angiogenesis involved in lesion formation, although this may be by a mechanism not directly related to its inhibitory effect on tyrosine kinase [19].  

17β-Oestradiol is also a good membrane antioxidant when introduced into liposomal membranes during their preparation [20]. It has a high cholesterol coefficient value, demonstrating its superiority to the natural membrane-stabilizing antioxidant cholesterol [21]. Of considerable interest is the finding that 17β-oestradiol inhibits the oxidation of isolated human LDL in vitro [22,23] and has been shown to inhibit the oxidation of LDL after its administration to postmenopausal women [24]. Oxidative damage to LDL has been implicated in atherogenesis [25,26] and the antioxidant properties of endogenous, synthetic and dietary oestrogens (structures for some of these compounds are shown in Figure 1) will be evaluated and compared with the antioxidant cardioprotective drug tamoxifen (antioestrogen/partial oestrogen antagonist) [27,28]. In addition to its antioxidant action, tamoxifen also beneficially modulates risk factors including lipid profiles [29] and homocysteine levels [30] in a similar manner to oestrogen and also induces the negative growth factor, transforming growth factor β, which is thought to play an important role in inhibiting the smooth-muscle cell proliferation involved in atherogenesis [31].

Membrane antioxidant action of oestrogens

Initial investigations of the antioxidant action of oestrogens in vitro, in particular 17β-oestradiol, mostly concerned the ability of oestrogens to inhibit lipid peroxidation in a range of membrane systems including microsomes and liposomes [12,13,27,28,32]. Studies on the antioxidant properties of 17β-oestradiol in membrane systems have now been expanded to include synthetic oestrogens and dietary phytoestrogens and also include investigations of oestrogens as protectants of LDL against oxidative damage.

Lipid peroxidation is a free-radical-mediated chain reaction, which can be initiated by the hydroxyl radical, that attacks polyunsaturated fatty acids in membranes and plasma lipoprotein particles resulting in oxidative damage [33,34]. In addition, the lipid hydroperoxides formed are readily decomposed by traces of transition metal ions to produce the free radical intermediates of lipid peroxidation capable of propagating the chain reaction [33,34]. Membrane lipid peroxidation is frequently measured by the spectrophotometric thiobarbituric acid (TBA) test. The test sample is heated with TBA at low pH, and the absorbance of a pink chromogen presumed to be a (TBA)₂-malondialdehyde adduct (although the term thiobarbituric acid-reactive substance is frequently used) is measured at 532 nm [33,35]. It should be noted that none of the oestrogenic compounds discussed here interfered with the development of colour in the TBA test: no inhibition was observed when the compounds were added to peroxidizing microsomes or liposomes at the same time as the TBA reagents instead of at the beginning of the incubation. Although the TBA test is adequate for measuring lipid peroxidation in defined membrane systems such as microsomes and liposomes, its application to
body fluids has many problems relating to its lack of specificity, and for such samples a modified HPLC-based assay that separates the authentic (TBA)_2-MDA adduct from other chromogens absorbing at 532 nm should be used [35].

17β-Oestradiol has been found to be a good inhibitor of lipid peroxidation in microsomal and preformed liposomal systems and more effective as an inhibitor of lipid peroxidation than tamoxifen, droloxifene (3-hydroxytamoxifen citrate) [32,36], nafoxidine (structurally related to tamoxifen) and the pure oestrogen antagonist ICI 164, 384 [13]. However, 4-hydroxytamoxifen was found to be a better inhibitor than 17β-oestradiol of microsomal and liposomal peroxidation [12,13,32]. Interestingly, the synthetic oestrogen 17α-ethynylestradiol was more effective as an inhibitor of lipid peroxidation than either 17β-oestradiol or 4-hydroxytamoxifen in the microsomal test systems [14]. It is possible that the ethynyl group possessed by 17α-ethynylestradiol, but not by 17β-oestradiol, makes it a more effective antioxidant than 17β-oestradiol and this may reflect an increase in its lipophilic nature compared with 17β-oestradiol [14].

Time course studies showed that 17β-oestradiol, 17α-ethynylestradiol, tamoxifen and related compounds (all at their IC50 concentrations) inhibited microsomal and liposomal lipid peroxidation throughout the incubation period and there was no clear evidence of a lag period followed by an acceleration of peroxidation to the control rate [12-14]. This suggests that these compounds are unlikely to be classical chain-breaking antioxidants, even though hydroxy groups with potentially donatable hydrogen atoms are present in many of these compounds (although tamoxifen is an exception here). A liposome system termed the introduced-into-liposome system (test compounds are introduced into the liposomes during liposomal preparation rather than being added to preformed liposomes) was used to compare with cholesterol the ability of the test compounds to stabilize the membrane against lipid peroxidation [20,27,32]. Cholesterol has no effect in the preformed liposomal system because it cannot enter the liposomal membranes once they are formed [20]. 17β-Oestradiol and 4-hydroxytamoxifen were approximately equipotent and both were more effective than tamoxifen, which in turn was more effective than cholesterol. These results indicate the superiority of 17β-oestradiol, tamoxifen and related compounds over the natural membrane compo-
brane fluidity), as demonstrated for tamoxifen and oestrogens [37,38].

Protection against LDL oxidation by oestrogens

The ability of oestrogen to protect LDL against oxidative damage in vitro [21–23] could contribute to the cardiovascular benefits observed on oestrogen administration in postmenopausal women [1,2], independently of a favourable alteration of the plasma lipid profile. Studies in monkeys and rabbits have shown the antiatherogenic effect of hormonal replacement to be independent of variations in lipid profiles. In oophorectomized female monkeys or an atherogenic diet, hormonal replacement with 17β-oestradiol, either alone or with cyclical progesterone for a period of 30 months, significantly decreased the development of coronary artery atherosclerosis independently of lipid profile changes [44]. Female cholesterol-fed rabbits treated with oestradiol for 33 weeks developed less atheroma in arterial tissue than controls, even though no differences in total cholesterol or lipoproteins were observed [45].

Oxidative damage to LDL (particularly to the apoprotein B molecule) is considered to be an important stage in the development of atherosclerosis; it is a prerequisite for macrophage uptake and cellular accumulation of cholesterol leading to the formation of the atheromal fatty streak [25,26]. Lipid peroxidation is thought to start in the polyunsaturated fatty acids of the phospholipids on the surface of LDL and then propagate to core lipids resulting in modification of the cholesterol, phospholipids and the apolipoprotein B molecule, in addition to the polyunsaturated fatty acids [25,26]. Protection by oestrogens against this oxidative damage to LDL thus may be an important contributing factor to their cardioprotective effect.

In a study on the action of 17β-oestradiol, tamoxifen and related compounds on oxidative damage to LDL, isolated human LDL was stimulated to undergo lipid peroxidation by the addition of Cu(II) ions [23]. This is a widely used experimental system [46] that is relevant to events occurring within the atherosclerotic lesion. 17β-Oestradiol was more effective as an inhibitor of Cu(II) ion-dependent lipid peroxidation than tamoxifen although less so than 4-hydroxytamoxifen. 17β-Oestradiol also prevented peroxidation-induced modifications in the surface charge of the LDL, although to a lesser extent than 4-hydroxytamoxifen [23]. These alterations in the surface charge of LDL are of great importance because they are associated with its recognition and uptake by macrophages in atherosclerotic lesions [25,26].

The membrane antioxidant properties of isoflavonoid phytoestrogens suggest that they may protect LDL against oxidative damage in a similar manner to oestrogen [23] and indeed genistein and daidzein have been found to be effective inhibitors of oxidative damage to LDL; they were both more effective than tamoxifen although less effective than 17β-oestradiol (J. O’Reilly and H. Wiseman, unpublished work). Equol was again approximately equipotent to 17β-oestradiol as found in the membrane systems (J. O’Reilly and H. Wiseman, unpublished work). 17β-Oestradiol, genistein, daidzein, equol, tamoxifen and 4-hydroxytamoxifen (see Figure 1) may stabilize LDL against lipid peroxidation by interactions between their hydrophobic rings and the polyunsaturated residues of the phospholipid layer of LDL; this possible mechanism is supported by the inhibition of lipid peroxidation arising from similar interactions in liposomal membranes [27,28].

17β-Oestradiol has been shown to inhibit the oxidation of LDL in postmenopausal women [24]. Acute intra-arterial infusion of 17β-oestradiol increased serum oestradiol levels from typical postmenopausal levels to physiological concentrations for reproductive-aged women at mid-cycle [24] and significantly prolonged the lag time of the LDL compared with baseline levels, indicating a decrease in susceptibility to oxidation. This was measured by the time of the onset of LDL oxidation, i.e. the lag time in the presence of Cu(II) ions. One month after discontinuation of treatment the LDL lag time had returned to baseline levels [24], suggesting that prolonged hormonal replacement therapy would be required to maintain the cardioprotective benefits. However, oestrogen hormonal replacement therapy has been indicated to carry similar risks of endometrial cancer to tamoxifen prophylaxis and requires careful monitoring [27,28].

Oestrogens as antioxidant cardioprotectants: the future

It may be of importance that the daidzein metabolite equol appeared to confer a protective ability against oxidative membrane damage that approached that of 17β-oestradiol. However, the likely antioxidant cardioprotective contribution of
equol in individuals is unclear at the present time because studies have shown that only around 33% of subjects are equol producers [47]. Plasma levels of equol would therefore be expected to vary widely and it is possible that populations that are very effective at producing equol might derive antioxidant cardioprotective benefits, from soya consumption, approaching that of oestrogen. This would support the use of isoflavonoids as an alternative/supplement to hormone replacement therapy in individuals who are identified equol producers in heterologous populations worldwide.

The synergistic interaction of equol with the dietary flavonoid quercetin [43] may confer additional cardioprotective benefits, as might the additive interaction of quercetin with 17β-oestradiol [43]. This suggests that the interaction of dietary antioxidants with endogenous oestrogens, and also with phytoestrogen metabolites, is another area worthy of further study in terms of assessing modulation of cardiovascular risk by dietary components in particular populations.

The improvement of the antioxidant properties of phloretin by its biotransformation to 3-hydroxyphloretin [41,42], which could enhance its cardioprotective benefits, is clearly one future trend for this rapidly expanding subject area. Indeed, the whole prospect of the production and use of dietary oestrogens with improved cardioprotective properties for use as functional foods (nutraceuticals), food additives and pharmaceuticals shows great promise [48].

It would appear likely that there are interactions between the various reported beneficial effects of oestrogen on the cardiovascular system and the different oestrogens that we are exposed to: as further mechanisms of cardioprotective action are elucidated for oestrogens, and reliable estimates of dietary exposure are made, we will be in a much better position to predict their exact benefit/risk ratio for an individual.

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Introduction

Today, it is increasingly appreciated that diet can play a fundamentally important role in the prevention of cardiovascular disease [1]. Similarly, the cardioprotective benefits of oestrogens, ranging from endogenous oestrogens to the pharmaceutical oestrogens used in hormone-replacement therapy [2], are attracting attention. Oestrogenic compounds are thought to exert their cardioprotective effects by a number of mechanisms including beneficial alteration of lipid profiles [2,3] and protection of low-density lipoproteins against oxidative damage [4]. Some plants (and plant-derived foodstuffs) contain oestrogenic substances. There is epidemiological evidence that these compounds, collectively termed phytoestrogens, could account for the cardioprotective properties of certain foods. Phytoestrogens include isoflavonoids [5] and saponins [6]; however, most recent attention has focused on the isoflavonoids, such as genistein, found in soya (Glycine max) products [7].

To take full advantage of the potential health benefits of phytoestrogens would require dietary modification, particularly in the West where soya isoflavonoid consumption (and thus overall flavonoid consumption) is comparatively low [8]. This may be achieved by the development of new food products, new food additives and the genetic engineering of crop plants. This would take into account current cultural–culinary habits and not be dependent on radical dietary change.

There is also potential for the improvement of the efficacy of phytoestrogens in terms of structural modifications and alterations to specific mechanisms of action. The antioxidant function of monophenolic flavonoid-type phytoestrogens may be improved by o-diphenol formation [9] (although oestrogenic receptor-based functionality may be lost). Phytoestrogens and modified phytoestrogens could be described as

Prospects for the production and use of new improved dietary oestrogens for cardioprotection

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