Molecular Action of Oestrogens in the Prevention of Cardiovascular Disease

Introduction

Oestrogens protect against coronary artery disease (CAD) [1–3]. An estimated 25–50% of this protective effect can be attributed to changes in fasting plasma lipids [4]. Low-density lipoprotein (LDL) cholesterol concentrations are reduced [5,6] as the result of enhanced clearance of atherogenic LDL particles by upregulated LDL receptors [7,8]. Furthermore the oxidizability of LDL particles is reduced [10]. Beneficial non-lipid effects are a direct relaxing effect on the vessel wall [11] and the beneficial promotion of angiogenesis [12] in the repair of damage to the endothelium.

We hypothesized that oestrogens can also improve postprandial lipid metabolism. This could be important since humans are in the postprandial state during most of their lives.

Postprandial lipid metabolism

In the postprandial state the intestine produces chylomicron particles which contain dietary triacylglycerols (TAGs) and cholesterol. The structural protein of these TAG-rich particles is apolipoprotein B48. The liver produces very-low-density lipoprotein (VLDL) particles, which transport endogenous TAGs and cholesterol. The structural protein of these TAG-rich particles is apolipoprotein B100. Chylomicrons and VLDL particles are hydrolysed by the same enzyme, lipoprotein lipase (LPL), bound to proteoglycans on the endothelium, in the common lipolytic pathway [13]. This results in TAG depletion and the formation of relatively cholesterol-enriched and atherogenic remnant particles, VLDL remnants or intermediate-density lipoprotein particles (IDL) and chylomicron remnants. Chylomicron remnants are cleared by the hepatic remnant receptor (the LDL-receptor-related protein, recognizing apolipoprotein E) [14,15], but also by the LDL receptor (B100-E receptor) [7,16]. IDL particles are taken up preferentially by the LDL receptor. IDL particles can also be further hydrolysed to LDL particles. In the postprandial state there is competition for removal (lipolysis and receptor-mediated hepatic uptake) of lipoprotein particles of intestinal and hepatic origin, and this can lead to accumulation of remnant particles. The cholesterol content and thus the atherogenicity of remnants is further increased through cholesterol ester transfer. Cholesterol is transferred from HDL particles to remnants, thereby decreasing the HDL cholest-
terol concentration and increasing the remnant cholesterol concentration.

Remnants are atherogenic particles [17,18], and can be taken up by the arterial wall and macrophages [19]. Patients with familial dysbeta-lipoproteinaemia, a disease characterized by elevated remnant concentrations resulting from a defect in remnant clearance, have premature atherosclerosis. Furthermore a relationship between CAD and high concentrations of remnants has been demonstrated in men [20–22]. Chylomicron remnants can be studied using an oral fat load, to which vitamin A [retinyl palmitate (RP)] is added. RP is incorporated into the core of the particles. RP concentrations, measured by HPLC, reflect the mass of newly synthesized chylomicrons and their remnants [23,24]. A chylomicron or chylomicron remnant particle contains one apolipoprotein B48 as structural protein. Measurement of apolipoprotein B48 concentrations, which reflect the number of chylomicrons and their remnants, is another way to describe chylomicron (remnant) metabolism [25,26]. A rapid ultracentrifugation method [27] can separate plasma into a fraction with a Svedberg flotation unit greater than 1000 (Sf>1000), which is rich in chylomicrons and large chylomicron remnants, and the infranatant or Sf<1000 fraction, which contains the other lipoproteins (small chylomicron remnants, VLDL, IDL, LDL and HDL particles). Another way to separate lipoproteins is density-gradient ultracentrifugation, separating plasma into a VLDL fraction (density<1.006), which contains VLDL, chylomicrons and large chylomicron remnants, an IDL fraction (density 1.006–1.019), which contains IDL and small chylomicron remnants, an LDL fraction (density 1.019–1.063) and an HDL fraction (density>1.063) [28].

CAD and postprandial lipid metabolism

The relationship between CAD and impaired postprandial lipid metabolism has not only been found in hyperlipidaemic men, but also in normolipidaemic (defined as normal fasting plasma concentrations of TAGs and cholesterol) men [20,29]. Recently, the postprandial lipid response was described in a large group of normolipidaemic men and women combined [30]. Angiography was used to verify CAD in all 85 cases (63 men and 22 women), and to exclude CAD in the matched controls. Increased concentrations of chylomicron remnants, measured as RP in Sf<1000 fractions after an oral fat load, were found in patients with CAD compared with controls. No separate analysis was made for the subgroup of women. Another study found no relation between abnormal postprandial lipid metabolism and CAD in women, but CAD was diagnosed as exercise-induced ischaemia [31]. We studied postprandial lipid metabolism in normolipidaemic women with CAD, proven by angiography [32]. Twelve women with a greater than 70% stenosis in at least one coronary artery (CAD) participated in the study. The women were 60 ± 2 years of age (n = 10 postmenopausal) and normolipidaemic [cholesterol 5.7 ± 0.1 (SE) mmol/l, TAG 1.35 ± 0.10 mmol/l]. Twelve CAD control women were individually matched for age, lipids and other risk factors. After an overnight fast, the subjects received an oral fat load, consisting of 50 g/m² fat as cream, with 60000 IU of RP/m². Blood samples were taken before ingestion of the fat load and at hourly intervals up to 12 h thereafter, for determination of RP, apolipoprotein B48 and TAG concentrations. The most striking finding was the higher postprandial apolipoprotein B48 concentrations in the fraction containing the smaller chylomicron remnants (IDL fraction) (Figure 1). Since there is one apolipoprotein B48 molecule per remnant particle, this finding implies that the number of small chylomicron remnants is increased in CAD women. This could result from decreased clearance. A study using stable isotopes showed that increased intestinal secretion of smaller chylomicron remnants is another possibility [33].

Figure 1

Postprandial response of apolipoprotein B48 in the IDL fraction in 12 normolipidaemic CAD women (■) and in 12 CAD controls (○). The CAD cases had significantly higher postprandial apolipoprotein B48 concentrations than the CAD controls. Apolipoprotein B48 is expressed in arbitrary units (means ± S.E.M.). Reprinted from [32] with permission from Elsevier Science Ireland, Shannon.
The conclusion is that an abnormal postprandial lipid response can identify subjects at risk for atherosclerotic disease, women as well as men. It remains to be determined whether improvement in postprandial lipids can decrease risk for CAD. The abnormal postprandial lipid response in women with angiographically proven CAD is in agreement with the observation from the Framingham study that fasting remnants, or IDL particles, are risk factors for CAD [34].

Oestrogens and chylomicron remnants
Animal studies showed that oestrogens can improve the clearance of chylomicrons and remnant particles [35,36]. This oestrogen effect has also been described in humans. Young (age 32 ± 3 years) normolipidaemic women have reduced postprandial lipaemia, compared with older (age 64 ± 3 years) normolipidaemic women, suggesting beneficial effects of endogenous oestrogens on postprandial lipids [37]. This observation is in agreement with preliminary results from an ongoing study, which shows that normolipidaemic postmenopausal women have higher postprandial TAG responses than age-matched premenopausal women (A. van Beek, F. A. C. de Ruijter-Heijstek, D. W. Erkelens and T. W. A. de Bruin, unpublished work). Exogenous oestrogens can also improve the postprandial lipid response. Treatment of premenopausal women with oral contraceptives, a combination of oestrogens and progestagens, accelerated the clearance of chylomicron remnants [39]. The elimination of remnant particles was also improved in patients with familial dysbetalipoproteinaemia [40]. We studied the influence of oestrogen-replacement therapy on chylomicron remnant metabolism in healthy normolipidaemic postmenopausal women [41]. Six normolipidaemic (cholesterol 5.6 ± 0.8 mmol/l, TAG 1.47 ± 0.7 mmol/l) postmenopausal (amenorrhoea > 1 year) women received an oral fat load. Blood samples were taken at 0–8 h for determination of RP concentrations. The test was repeated after 6 weeks of treatment with 2 mg of 17β-oestradiol, a natural oestrogen compound in a dosage frequently used for postmenopausal replacement therapy. The plasma RP areas under the 0–8 h curve (Figure 2), reflecting the total amount of chylomicrons and chylomicron remnants in plasma, decreased significantly by 41% from 27.1 ± 15.9 to 16.6 ± 13.2 mg·h per litre (P = 0.01). Fasting remnants, reflected by IDL cholesterol concentrations, also decreased significantly from 0.62 ± 0.36 to 0.47 ± 0.27 mmol/l. Fasting TAG, LDL cholesterol and HDL cholesterol did not change significantly. The conclusion is that oestrogen-replacement therapy improves the clearance of chylomicron remnant particles and IDLs in healthy normolipidaemic postmenopausal women, even when fasting TAG, cholesterol and HDL cholesterol did not change.

The most likely mechanism of the reduced postprandial chylomicron remnant response is increased remnant clearance by LDL receptors [16,36]. Upregulation of LDL receptors [7,8,16,36] with subsequent lowering of LDL cholesterol concentrations [5,6] are known oestrogen effects, although in the above-mentioned study [41] no reduction in LDL cholesterol was found. Studies on the effect of oestrogens on the remnant receptor are contradictory. Oestrogens are reported not to influence remnant receptor expression [42,43] or to decrease remnant receptor expression [16]. Oestrogens do not influence the activity of the enzyme LPL [6,41].

Oestrogens and postprandial HDL metabolism
In women HDL cholesterol is a major lipid factor that is associated with protection against CAD [4]. The protective effect of HDL is attributed to its function in reverse cholesterol transport [44] and its ability to prevent oxidation of LDL particles [45] and to protect the endothelium against cytotoxic agents derived from remnants of TAG-rich lipoproteins [46]. Fur-
thermore HDL cholesterol can be a good marker for the metabolism of atherogenic remnants of TAG-rich lipoproteins [27,47]. Studies in men have shown that HDL cholesterol concentrations decrease by 35% in the postprandial state [27]. We investigated postprandial HDL metabolism in women [48]. Sixteen normolipidaemic (cholesterol $5.36\pm 0.68$ mmol/l, TAG $1.24\pm 0.55$ mmol/l $\times 27,47$) postmenopausal women received an oral fat load (50 g of fat/m$^2$ as cream, to which 60000 IU of RP/m$^2$ were added). Venous blood samples were taken before ingestion of the fat load and at hourly intervals thereafter up to 8 h. HDL-containing fractions were prepared by precipitation of apolipoprotein B-containing lipoproteins. A reduction in HDL cholesterol was observed from 3 to 8 h after ingestion of the fat load. The minimal postprandial HDL cholesterol concentration was 31.7% ($P = 0.04$) lower than the fasting HDL cholesterol concentration. The decrease in postprandial HDL cholesterol was expressed as area under the decremental HDL cholesterol curve (the fasting HDL cholesterol concentration was subtracted from the HDL cholesterol concentrations at time points 0–8 h, and the integrated area under this curve was calculated) and was attenuated by 66% after 6 weeks of treatment with 2 mg of 17$\beta$-oestradiol ($P = 0.020$). Fasting HDL cholesterol concentrations did not change significantly. An explanation for the improvement in the postprandial reduction in HDL by oestrogens could be the oestrogen-induced decrease in hepatic lipase activity, the enzyme that promotes hepatic uptake of cholesterol from HDL particles. Hepatic lipase determines the HDL particle size, and HDL size is known to be a determinant of HDL catabolism. A decrease in hepatic lipase activity is a known oestrogen effect [41]. Another explanation could be that oestrogens reduce the amount of chylomicron remnants [41], and thus diminish the transfer of cholesterol from HDL to remnant particles. Increased hepatic synthesis of HDL particles [9] can also play a role.

**Conclusion**

Oestrogen-replacement therapy can improve the clearance of chylomicron remnants and attenuate the postprandial decrease in HDL cholesterol. Reduced concentrations of atherogenic chylomicron remnants and increased concentrations of antiatherogenic HDL particles turn the postprandial state into a less atherogenic condition. These all contribute to the protective effect of oestrogens against CAD.


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