Introduction

The positive benefit : risk ratio for postmenopausal hormone replacement therapy (HRT) with oral oestrogen is driven by the promise of a 50% reduction in coronary heart disease (CHD) events. This figure is derived not from formal placebo-controlled clinical trials, but rather from the observation that women who use HRT suffer less from CHD than women who do not. Such a beneficial role would be consistent with the protection from atherosclerosis that is seen when oestrogen is given to fat-fed animals and with the effects of HRT on risk factors such as plasma lipoproteins. Because CHD is the major cause of mortality in postmenopausal women, such protection would be a major advance in female health care. If the observational and experimental data could be validated in a placebo-controlled trial of CHD events, then those women who are free from climacteric symptoms or who find them bearable would be strongly advised to consider HRT, simply for the life extension associated with protection from CHD.

As a consequence, the past 30 years have seen a major research effort aimed at unravelling the mechanisms that underlie the low incidence of CHD seen in HRT users. Investigations have focused on their high plasma levels of high-density lipoproteins (HDL) and low levels of both low-density lipoproteins (LDL) and lipoprotein(a). Oral oestrogens can also increase fasting triglyceride levels; this is in theory a detrimental change, but one that is usually considered to be outweighed by the other changes. Other antiatherosclerotic mechanisms, such as involvement in nitric oxide release from the endothelium, have been identified but lipoprotein metabolism remains a key area of interest, not least because such measurements are relatively inexpensive.

Different forms of HRT have traditionally been ranked in terms of their potential influence on CHD according to how closely their metabolic (i.e. lipoprotein) profile compared with that of the ‘gold standard’ of oral oestrogen. By this criterion, postmenopausal therapy with tibolone (Livial®; NV Organon, Oss, The Netherlands) has been considered in some countries to be inferior to that with oestrogen because it does not reduce LDL levels and indeed causes HDL to fall, rather than rise. The effects of tibolone on other aspects of CHD risk, such as fasting triglycerides and lipoprotein(a), are attractive, but concern has often been expressed regarding the fall in HDL.

Recent developments, including publication of long-awaited formal placebo-controlled clinical trials, strongly question the validity of the ‘metabolic risk’ concept for HRT. Against virtually all predictions, these trials failed to show any benefit of HRT over placebo in terms of clinical events, such as myocardial infarction or stroke, or even on ‘anatomical’ surrogates such as coronary angiography. The observation that HRT users suffer less from CHD than do non-users remains true, but the real question is whether this is a drug effect or whether it is due to fundamental health differences at baseline. Differences in lifestyle, social

Tibolone reduces plasma levels of total cholesterol by approximately 5%, triglycerides by approximately 25% and lipoprotein(a) by 20–30%, with little effect on the levels of low-density lipoproteins. In contrast to these theoretical beneficial effects, there is a 20–30% fall in levels of high-density lipoproteins (HDL). The failure of recent placebo-controlled clinical trials of hormone replacement therapy to show a cardiovascular benefit with elevated HDL levels raises the possibility that hormone replacement therapy-induced plasma lipid changes may not accurately predict clinical events.

Advances in our understanding of lipoprotein metabolism, especially resulting from identification of new receptors, challenge the conventional belief that reducing HDL levels will cause cardiovascular disease.

Key Words: Coronary heart disease, high-density lipoprotein, hormone replacement therapy, lipids, tibolone.
class, education, hostility and responsiveness to stress between HRT users and non-users have often been ignored, but now provide a way to reconcile observational findings with new experimental data from placebo-controlled trials. The substantial protection against atherosclerosis seen in placebo-controlled studies of fat-fed animals treated with oestrogen show that this steroid has the potential to reduce atherosclerosis in postmenopausal women, but tells us nothing about the effects on acute disease events such as myocardial infarction. Similarly, it is not yet certain that the intriguing effects of oestrogen on arterial function will translate into a reduction in acute clinical events.

Given this controversy over the cardiovascular benefits of oestrogen-based HRT, a re-evaluation of the risk profile of novel therapies such as tibolone is needed. As with many newer therapies, no prospective placebo-controlled studies of clinical or anatomical CHD end-points in tibolone users have been reported. The drug is profoundly antiatherosclerotic in fat-fed rabbits[4], whereas a study in fat-fed monkeys found an improvement in vascular function but not plaque size[5]. Moreover, tibolone influences fibrinolysis and endothelial function in ways that would be expected to reduce the risk for arterial thrombosis. Do these positive actions outweigh any deleterious effect on plasma lipoprotein metabolism? The present review summarizes our understanding of the influence of tibolone on the plasma lipoprotein system and specifically revisits the issue of the cardiovascular consequences of drug modulation of HDL levels.

The plasma lipoprotein profile of women using tibolone

Over 25 years of study have resulted in at least 30 publications, involving more than 1000 women, on the plasma lipoprotein response to tibolone. The standard of this research has increased steadily over this period, and we now have well controlled, technically proficient comparative data. As with all forms of HRT, these studies rarely exceed 3 years in duration, and it is simply assumed that these effects are maintained over much longer periods of time. Details of many of these studies can be found in review articles[6,7].

Total lipids

Tibolone reduces fasting plasma levels of total cholesterol by approximately 5%. The clinical significance of this is difficult to assess without knowing the underlying effects on metabolically distinct lipoprotein classes.

Triglycerides

Plasma levels of triglycerides are reduced by approximately 25%. Contrasting sharply with the increase observed with some oral oestrogen therapies, the extent of this reduction is similar to that induced by fibrate drugs. Considered in isolation, such an effect would be predicted to reduce CHD risk.

Low-density lipoprotein cholesterol

A few studies in women treated with tibolone reported either an increase or a decrease in LDL-cholesterol levels, with the great majority finding no effect at all. This neutral influence is supported by the unchanged levels of apolipoprotein B, a marker for LDL in healthy women. The ability of oral oestrogen to lower LDL levels has been linked to the low CHD risk in HRT users, and indeed some have argued that postmenopausal therapies must reduce LDL levels. This attitude is now being revised in light of the developing consensus that, if a woman has a ‘problem’ LDL level, then the appropriate intervention is diet and drugs of proven efficacy (such as statins), rather than oestrogen.

In the context of tibolone, the LDL issue has become rather complex. An often ignored aspect of the oestrogen risk factor profile is the conversion to smaller, denser (and in theory more atherogenic) LDL particles. Interestingly, the normal LDL-cholesterol levels seen in tibolone users may hide small shifts to larger, less dense (and in theory less atherogenic) LDL particles[8]. Tibolone also protects LDL from peroxidative damage measured using in vitro assays[8]; in combination with the effects on particle size, this would be predicted to reduce CHD risk, even though LDL-cholesterol levels are unchanged.

Lipoprotein(a)

One of the most striking metabolic effects of tibolone is the reduction in levels of atherogenic lipoprotein(a) by 20–30%. There is as yet no evidence that reducing lipoprotein(a) levels is of clinical benefit, but a recent post hoc analysis of a large clinical trial of HRT[9] suggests a link between CHD, HRT and lipoprotein(a). Such stratifications should be treated with caution, but this observation does suggest that a greater understanding of the influence of tibolone on lipoprotein(a) metabolism would be worthwhile.

High-density lipoprotein cholesterol

The published literature on tibolone has shown theoretical benefits on triglycerides and lipoprotein(a) levels (actions that are shared with androgenic steroids) and improvements in LDL quality (but not quantity). In contrast, the 20–30% fall in plasma levels of HDL-cholesterol has caused concern ever since this was reported approximately 20 years ago[10]. This fall is also seen in plasma levels of apolipoprotein A-I, the major protein component of HDL, and is more evident in larger HDL particles.

Table 1  Functions of high-density lipoprotein

<table>
<thead>
<tr>
<th>Function</th>
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<tbody>
<tr>
<td>Transfer of cellular cholesterol for disposal by the liver</td>
</tr>
<tr>
<td>Regulation of triglyceride metabolism</td>
</tr>
<tr>
<td>Protection of low-density lipoproteins from oxidation</td>
</tr>
<tr>
<td>Regulation of expression of endothelial cell adhesion molecules</td>
</tr>
<tr>
<td>Regulation of prostacyclin metabolism</td>
</tr>
<tr>
<td>Enhancement of the protein C pathway</td>
</tr>
<tr>
<td>Destruction of trypanosome parasites</td>
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</table>

Some have claimed that the fall is transient, that the levels remain within a nominal ‘normal range’, or that HDL is not causally involved in atherosclerosis. However, none of these arguments are particularly robust; recent developments in HDL biochemistry strongly caution against extrapolation of drug-induced changes in plasma levels\[11\].

**High-density lipoprotein and cardiovascular disease**

The term ‘HDL’ refers to a wide spectrum of lipoprotein particles that are differentiated from other classes by their high content of enzymes, activators, inhibitors and other proteins\[12\]. Unlike the triglyceride-rich lipoproteins LDL and lipoprotein(a), HDL has multiple regulatory functions that extend beyond the simple transfer of lipids (Table 1).

Within populations, HDL levels predict risk for CHD; the higher the HDL level, the lower the CHD risk. Furthermore, low HDL levels are a fundamental component of the insulin resistance syndrome, a condition that is associated with premature CHD. Given the epidemiological evidence for a protective effect of HDL, which is consistent with the various antiatherosclerotic and antithrombotic roles ascribed to this lipoprotein, one would predict that any intervention that increases HDL levels would reduce CHD risk and that any intervention that reduces these levels would promote CHD. If this simple relationship holds true, then the long-term health consequences of tibolone therapy would indeed be a concern. Some would argue that a detrimental effect on HDL might be outweighed by other, more positive impacts on risk profile, but the effect of steroids on HDL has so permeated discussions of HRT and cardiovascular health over the past few decades that such a defence would be hard to sustain.

In fact, the literature on the clinical consequences of HDL manipulation is full of inconsistencies and surprises. When animals were engineered to express the transgene for human apolipoprotein A-I, their HDL levels increased and they were protected against atherosclerosis\[13\]; this represents powerful evidence for a direct effect of HDL. When HDL levels were increased by introducing the transgene for the minor HDL protein apolipoprotein A-II, however, atherosclerosis was rampant\[13\]. These data are derived from highly artificial circumstances, but suggest that not all drugs that increase HDL levels will reduce atherosclerosis.

Paradoxical results are seen in HDL knockout mice; HDL levels are severely reduced in apolipoprotein A-I knockouts, but there is no increase in atherosclerosis\[14\]. Indeed, when mice are made transgenic for human hepatic lipase or the lipoprotein receptor scavenger receptor B1, HDL levels fall precipitously but the animals are protected from atherosclerosis\[15\]. The relevance of these experimental studies to postmenopausal health care may seem distant, but they do emphasize the complexity of HDL metabolism and caution against extrapolating from changes in plasma levels to changes in disease event rates.

Are there any lessons to be learnt from the study of humans, rather than experimental animals? A major problem is an absence of drugs that specifically increase or decrease plasma HDL levels. For instance, the most successful class of HDL modulators – the fibrate series of hypolipaemic drugs – also reduce fasting triglyceride levels and influence fibrinogen function. Data are now available from various prospective randomized controlled trials in which HDL levels were raised with fibrate therapy. In the Veterans Affairs High Density Lipoprotein Intervention Trial (VA-HIT), 2531 men with pre-existing CHD were randomly assigned to receive gemfibrozil or placebo for an average of 5 years\[16\]. Consistent with the data from apolipoprotein A-I transgenic mice, fibrate therapy reduced the incidence of CHD in the treated group by 22% as compared with the rate with placebo (Table 2). A similar benefit was evident in a smaller trial that was designed for assessment of angiographic end-points: the Diabetes Atherosclerosis Intervention Study (DAIS)\[17\]. However, the Bezafibrate Infarction Prevention (BIP) study\[18\], which involved over 3000 men, failed to show any benefit of fibrate therapy over placebo, despite increasing HDL-cholesterol levels by 18% (Table 2).

If we put aside this latter finding and accept that fibrate therapy reduced CHD risk in the VA-HIT\[16\], then how do we dissect out the mechanism? Equally good arguments can be made for this reduction being due to a triglyceride-reducing effect and to a HDL-raising effect (Table 2). Indeed, bezafibrate did reduce CHD risk in a subset of hypertriglyceridaemic men in the BIP study\[18\]. Although a post hoc stratification, this strongly suggests that these drugs work through mechanisms other than, or additional to, an effect on HDL. Such a concept is supported by a recent re-analysis of the VA-HIT data in which protection from CHD was limited to those who were diabetic or hyperinsulinaemic at baseline\[19\]. Overall, the evidence from these placebo-controlled trials of ‘hard’ CHD endpoints suggests that fibrates do not protect non-diabetic, normotriglyceridaemic individuals from CHD, despite increasing their HDL levels. This strongly challenges the orthodoxy that oestrogen will protect women from CHD by raising their HDL levels.

Are there similar strands of evidence that might support or refute the concern over tibolone that reducing HDL levels has harmful effects? Probucol is an intriguing drug that lowers LDL levels and protects LDL from oxidation, but also reduces HDL levels by 30%. Despite this effect on HDL, probucol prevents atherosclerosis in spontaneously hypercholesterolaemic rabbits, regresses cholesterol-rich xanthomata in familial hypercholesterolaemia patients and substantially reduces the risk for restenosis after vascular...
Table 2  Effect of fibrate therapy on plasma levels of high-density lipoprotein cholesterol and triglycerides, and the incidence of coronary heart disease

<table>
<thead>
<tr>
<th>Study (n)</th>
<th>Drug</th>
<th>HDL-cholesterol</th>
<th>Triglyceride</th>
<th>CHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>VA-HIT (2531)</td>
<td>Gemfibrozil</td>
<td>+6%</td>
<td>-31%</td>
<td>-22% (P &lt; 0.01)</td>
</tr>
<tr>
<td>DAIS (420)</td>
<td>Fenofibrate</td>
<td>+6%</td>
<td>-10%</td>
<td>-23%</td>
</tr>
<tr>
<td>BIP (3090)</td>
<td>Bezafibrate</td>
<td>+18%</td>
<td>-21%</td>
<td>-9.4%</td>
</tr>
</tbody>
</table>

BIP=Bezafibrate Infarction Prevention Study; CHD=coronary heart disease; DAIS=Diabetes Atherosclerosis Intervention Study; HDL=high-density lipoprotein; VA-HIT=Veterans Affairs High Density Lipoprotein Intervention Trial.

surgery. There are no controlled trials on CHD events, but the Probucol Quantitative Regression Swedish Trial (PQRST) used femoral angiography as a surrogate[20]. A total of 303 men were randomly assigned to receive either probucol or placebo; for ethical reasons it was decided to give both groups a low-fat diet and a lipid-lowering drug (cholestyramine). Disappointingly, there were no differences between the two groups in cumulative angiographic scores over 3 years.

Are there any lessons here for tibolone? A pessimistic view would be that a benefit in terms of angiographic endpoints with probucol would have been expected (because of the effects on LDL) and that the HDL fall must have countered this benefit, and therefore that HDL reduction is dangerous. Femoral angiography is an outdated means of studying CHD, however, and the design of that study (even those on placebo were receiving two cholesterol-lowering interventions) is a cause for concern. The role of probucol in CHD prevention remains an enigma.

Plasma HDL levels are also lowered in response to low-fat diets, especially in women[21]. Nevertheless, these diets continue to be promoted as a first-line strategy for CHD prevention, presumably because the modest effect on LDL is believed to outweigh any risk associated with reducing HDL levels.

New research into the effects of tibolone on high-density lipoprotein

The studies outlined above strongly suggest that reducing HDL levels does not always increase CHD risk. One possible explanation is that a fall in HDL plasma levels does not impair HDL function. Preliminary work carried out in London (U.K.) and Munster (Germany) indicated that tibolone reduces some, but not all, classes of HDL and that the unaffected classes are capable of maintaining reverse cholesterol transport, as measured by the ability of plasma from tibolone users to remove (‘efflux’) cholesterol from cells in culture[22]. In that study, we randomly assigned 68 postmenopausal women to placebo or tibolone 2.5 mg day\(^{-1}\) for 3 months. Tibolone reduced HDL-cholesterol levels by 14%, which is consistent with an increase in hepatic lipase activity. However, the ability of plasma from these women to accept cholesterol from cell membranes in vitro was unaffected by tibolone therapy. Surprising findings also emerged when probucol was shown to lower HDL levels but improve cholesterol efflux[23].

Conclusion

The serious mismatch between the expected cardiovascular benefit of HRT over placebo and the reality of recent clinical trials forces us to question the value of traditional ‘risk marker’ profiling of new HRT formulations. In the case of tibolone, strong arguments can be made that focusing on one specific effect (such as HDL) may not be an appropriate strategy. The clinical significance of altering HDL levels has come under question, and new data challenge the idea that all drugs that reduce HDL will be atherogenic.

References


