Hormones, genetic factors, and gender differences in cardiovascular disease

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1. Gender differences in age of onset of coronary heart disease

On average, women develop heart disease some 10–15 years later than men. This raises the question of whether there is some aspect of ‘femaleness’ which reduces risk, or whether there is some aspect of ‘maleness’ that raises risk. To date, most attention has been focused on the hypothesis that endogenous estrogen is cardioprotective in women [1]. Rising rates of coronary heart disease (CHD) after the menopause, and after oophorectomy, are among the strands of evidence in humans that endogenous estrogen may prevent CHD [2]. However, upon closer examination this evidence is not persuasive, and in fact the evidence is amenable to alternative explanations.

During the first 3 decades of adult life, low-density lipoprotein (LDL) cholesterol levels are lower in women than men, and this may have contribute to the delayed onset of CHD in women. A more widely held explanation for the later onset of CHD in women is their higher high-density lipoprotein (HDL) cholesterol levels, attributed to higher endogenous estrogen levels in women. However, the difference in HDL cholesterol between women and men is an androgen effect, not an estrogen effect. Up to puberty, young men and women have similar HDL cholesterol levels. At puberty, concurrent with the rise in endogenous testosterone levels, the HDL cholesterol levels in young men decline to the adult level [3,4]. A 20% difference in HDL cholesterol levels predicts at least a 20% difference in CHD rates in the short term, and may predict even larger differences in CHD rates over a lifetime [5]. Thus, the entire gender difference in CHD risk may indeed be due to the lifelong difference in HDL cholesterol levels; however, this difference is a consequence of having the Y chromosome. During fetal development, the Y chromosome directs the formation of testes rather than ovaries, and the testes in turn produce testosterone and dihydrotestosterone rather than estrone and estradiol as the primary sex steroids [6]. The high levels of male sex hormones drive down the HDL cholesterol levels, with consequent higher early risk of CHD. Thus, the gender difference in CHD may well be due the most basic genetic difference between men and women, which is the presence of the Y chromosome.

Some of the observational studies in adult males suggest that higher levels of testosterone are associated with lower (rather than lower) levels of HDL cholesterol, and with lower risk of CHD [7]. Intracoronary infusion of testosterone induces coronary artery dilatation and increases coronary blood flow in men with coronary artery disease [8]. These observations do not fit the concept that testosterone is harmful to the male cardiovascular system. On the other hand, androgen deprivation in men is associated with enhanced endothelium-dependent dilatation in men successfully treated for prostate cancer, while dilatation is reduced in genetic females taking high dose androgens [9,10]. In the laboratory, androgen receptor expression is greater in macrophages from male than from female donors, and lipid loading of male (but not female) macrophages was increased by dihydrotestosterone [11]. Dihydrotestosterone also increases the adhesion of monocytes to endothelial cells, increases the expression of endothelial vascular cell adhesion molecule-1, increases platelet expression of thromboxane receptors, and increases platelet aggregation [12,13]. However, there are
even more gaps in our knowledge about the real effects of testosterone than there are for estrogen. As in the case of estrogen, clinical trials with hard endpoints using androgens, androgen antagonists, or selective androgen receptor modulators will ultimately be needed to resolve this issue, but it is difficult to anticipate that such trials will be done in the near future.

The statement that CHD rates in women rise steeply after the age of menopause, and the corollary that this is due to lower levels of estrogen after that age, is also open to question. In actuality, there is no evidence for an increase in the year-on-year rate of increase in CHD around the age of menopause. The linear relationship between age and CHD incidence as seen on a semilogarithmic plot shows that there is a constant proportional increase in CHD incidence with age, with no inflection upward at the average age of menopause [14]. This is evidence for an age effect, and evidence against an effect of menopause. The Nurses’ Health Study investigators have reported that, after controlling for age and smoking status, the natural menopause is not associated with an increased risk for CHD [2]. The same investigators have reported that, in contrast to the natural menopause, bilateral oophorectomy is associated with an increased risk for CHD in women who had never taken estrogen after menopause. However, the study had very few cases of CHD in women with oophorectomy, and the increased risk was no longer significant in the multivariate analysis. Nonetheless, the use of estrogens appeared to eliminate this increased risk. It is possible that women who have a hysterectomy and bilateral oophorectomy (often done for menorrhagia/metrorrhagia associated with endometrial hyperplasia) are at higher risk for CHD because of the co-existence of metabolic risk factors such as central obesity, high blood pressure, lipid disorders, and glucose intolerance. The finding of an apparent lower risk in women who subsequently used estrogen would be subject to the biases discussed below. In summary, the post-menopausal increase in risk is most likely due to age and not the menopause, and the increase in risk in women after oophorectomy may be due to confounding by other risk factors.

2. Observational studies of estrogen users

By far the most persuasive evidence in favor of a protective effect for estrogen comes from the large number of cohort studies comparing CHD risk in postmenopausal women currently using estrogen to never-users. These studies have shown consistently that CHD risk is 35–50% lower in estrogen users, after adjusting for other risk factors [15,16]. The lower risk has been found in studies of estrogen alone, as well as in studies of estrogen in combination with a progestin [16]. For healthy women, the lower risk is found in those who have recently started estrogen as well as in long-term users [16].

These findings from observational epidemiology provide the rationale for clinical trials testing whether and to what degree current use of postmenopausal hormone therapy prevents a first heart attack. However, the observational epidemiology is not sufficient to prove the case, because even the best studies may be subject to a variety of systematic biases that could lead to an overestimation of benefit and an underestimation of harm from hormone therapy, hence the need for an unbiased estimate from clinical trials. These biases in observational studies include healthy user selection bias, compliance bias, surveillance bias, and survivor bias [14,17].

In combination, these biases will lead to a systematic overestimation of benefit, and an underestimation of risk in observational studies. Adjusting for baseline differences in risk factors will mitigate healthy user selection bias, but will not correct for compliance, surveillance, or survivor bias. Thus, the real benefit for CHD may be much less than predicted by the observational studies, or there may be no benefit at all [14]. The clinical trials to date have failed to show overall benefit for CHD over the short term (ongoing trials will ascertain the long term effects particularly in women without prevalent CHD) [18].

3. Biological mechanisms for cardioprotection by estrogen

The possibility that estrogen may reduce CHD risk has stimulated a wide variety of studies that attempt to explain the presumed benefit. Because it was unexpected, fewer studies have been done to explain the apparent excess risk early in the course of treatment, found in recent clinical trials (see below). Effects of estrogen that may predict benefit include: lowered LDL cholesterol and lipoprotein (a) levels, raised HDL cholesterol levels, reduced fibrinogen levels and enhanced fibrinolysis (reduced plasminogen activator inhibitor-1 (PAI-1) and increased D-dimer levels), reduced homocysteine levels, antioxidant properties, and improved endothelial function (e.g. reduced E-selectin levels and enhanced flow-mediated dilatation) [1]. Estrogen and progesterone reduce lipid accumulation in macrophages from female, but not male, donors [19]. On the other hand, several mechanisms that might increase risk after estrogen administration have been found: triglycerides increase, some coagulation markers increase (e.g. Factor VII, prothrombin fragments 1+2, activated protein C resistance), and the inflammatory marker C-reactive protein increases [20–23]. Some observational studies have suggested that certain of the lipid markers changed by estrogen administration are associated with higher relative risks for CHD in women than in men, including HDL cholesterol and triglycerides [23,24]. How-
never, particularly in respect of coagulation and inflammation, laboratory measurements do not predict whether the predominant effect will be favorable or unfavorable. Studies of venous thromboembolism (including clinical trials) provide unequivocal evidence that the overall effect is indeed procoagulant [25,26].

Compounding this difficulty in interpreting laboratory measurements is the fact that progestins counteract some of the estrogen effects, and that the clinical expression of metabolic changes may be time-dependent. The early excess risk for arterial disease observed in the Heart and Estrogen/Progestin Replacement Study (HERS) may have been due to an initial procoagulant or inflammatory effect on susceptible plaques, while the favorable effects in the survivors may be due to the later assertion of the generally favorable lipid effects [27]. In HERS, participants with higher levels at baseline had the largest decrease in lipoprotein (a) on treatment, and had a more favorable clinical outcome than participants with lower baseline levels [28]. The role of estrogen’s direct vascular effect is unclear, since impaired endothelial function has not yet been established as a risk factor for CHD. Interestingly, current estrogen users do not appear to have a lower risk for angina, as one would expect if direct vascular effects were important [16]. On the other hand, in a clinical trial of sublingual estrogen relieved exercise-induced angina [29]. Overall, the studies of mechanisms have not resolved the core issue of whether estrogens protect against CHD.

It is important to realize that almost all the studies of mechanism used oral estrogen preparations, and may turn out to have little relevance towards explaining the gender difference in CHD. Ovarian estrogen directly enters the systemic circulation through the inferior vena cava, unlike oral estrogens, which enter the portal vein and undergo first pass hepatic circulation. Because of extensive metabolism in the liver, in order to achieve similar blood levels the dose of oral estrogen needs to be approximately ten times that of non-oral (e.g. transdermal) estrogen. These doses of estrogens profoundly influence the hepatic metabolism of a variety of proteins, including lipid apoproteins, coagulation proteins, and (probably) C-reactive protein [20–22,30–34]. The large effects on lipids and coagulation proteins described for oral estrogens are greatly attenuated, absent, or in the opposite direction with non-oral estrogens. Non-oral estrogens have very modest effects on lowering LDL-cholesterol and lipoprotein (a), have no effect or reduce triglycerides, have no effect on HDL-cholesterol, and have a modest or no effect on levels of coagulation proteins [30–34]. Non-oral estrogens retain the ability to improve endothelial function [35]. Additional mechanistic studies, as well as epidemiologic studies and clinical trials, that focus on the role of non-oral estrogen preparations are needed. Of interest, epidemiologic studies in postmenopausal women have not shown an association of endogenous estrogen levels with CHD [36,37].

4. Genetic mechanisms for cardioprotection by estrogen

The role of the major monogenic disorders such as familial hypercholesterolemia and familial hyperhomocysteinemia in arterial disease is well established. Even within kindreds with familial hypercholesterolemia, affected females develop CHD later than in males, presumably because the same (unknown) factors that delay onset in non-affected females continue to operate in affected females [38]. Though estrogen administration lowers LDL cholesterol in women with familial hypercholesterolemia [39], this pharmacologic property does not necessarily mean that endogenous estrogen has the same effect. There is no sex difference in the elevated LDL cholesterol levels of individuals with familial hypercholesterolemia [38]. Rather, as in non-affected individuals, factors that blunt the impact of a given level of LDL cholesterol in females (or accelerate it in males) need to be sought.

The importance to arterial disease of polymorphisms in the genes that code for coagulation proteins and markers of inflammation is not clear. For example, a variety of polymorphisms in the fibrinogen gene are known to affect circulating fibrinogen levels, and fibrinogen levels have been associated with arterial disease [40]. However, studies seeking to quantify the association of a fibrinogen gene polymorphism with arterial disease have generally yielded disappointing results. This may be because any individual polymorphism has a modest influence on fibrinogen levels, and the levels are simultaneously affected by both other genetic factors, and importantly, by environmental factors [40]. Hence, it is difficult to discern the direct effect of any individual polymorphism on clinical disease. In addition to fibrinogen, polymorphisms in the genes for FVII, FXIII, FX, prothrombin, thrombomodulin, tissue plasminogen activator, PAI-1, and platelet-membrane glycoproteins have been described [40]. Except for fibrinogen, the relationships of the phenotypic factors affected by these polymorphisms with arterial disease are uncertain. A possible interaction of gender with polymorphisms in the FVII gene has been described, in that plasma activity of FVII in males varies markedly according to the presence of three of these polymorphisms, but in females these polymorphisms are associated with much smaller changes in FVII activity [41]. Estrogen affects many of these phenotypic factors [20–22,32–35], but in general it is not known whether there is a protective or harmful interaction of estrogen with the polymorphisms as risk factors for arterial disease.

Both males and females have α- and β-estrogen receptors in the vascular endothelium, smooth muscle, and myocardium, though receptor numbers may be higher in females because estrogen induces their expression [1,42]. One group of investigators found a gender difference for
the non-genomic effects of estrogen on the response of coronary arteries to acetylcholine. Males did not show the reversal of acetylcholine-induced vasoconstriction in coronary arteries after acute estrogen administration found in females [43]. On the other hand, genetic males receiving long-term estrogen therapy show enhanced flow-mediated dilatation [44]. The presence of higher numbers of functional estrogen receptors in females may relate to the inhibition of injury-induced vascular intimal thickening by estrogen in female arteries, and the lower prevalence of left ventricular hypertrophy in women compared to men [1,45,46]. Estrogen receptor numbers are lower in female atherosclerotic arteries [46]. There is some potential for polymorphisms of the estrogen receptors themselves to affect the clinical outcome of estrogen administration. For example, the common ER Pvu II and XbaI genotypes are not associated with differences in HDL cholesterol levels, but after estrogen administration women who are heterozygous for the genotype have a greater elevation of HDL cholesterol [47]. The clinical relevance of this finding is unknown.

5. Evidence of early harm from clinical trials

The evidence emerging from trials of hormone replacement therapy (HRT) to prevent coronary heart disease (CHD) continues to confound the belief that HRT is cardioprotective. On the contrary, a fairly consistent pattern of early harm is emerging from secondary and, to a lesser extent, from primary prevention trials. Long term effects of HRT on the cardiovascular system, and also on the overall health of postmenopausal women, are being evaluated in the ongoing primary prevention trials [18].

The findings from four clinical trials of HRT in women are consistent with an early excess of arterial cardiovascular events: HERS, the Papworth HRT and Atherosclerosis Survival Enquiry (PHASE), the Women’s Estrogen for Stroke Trial (WEST), and the Women’s Health Initiative (WHI) [27,48–50]. In addition, a combined analysis of short-term trials (typically lasting a year) in healthy women showed a non-significant 39% excess risk for CHD [51]. Support for short-term harm comes also from the early trials in men, and several observational studies of new users of HRT among women with prior CHD [52–55] and one of healthy women in the Group Health Cooperative study [56]. The evidence comes from secondary and primary prevention trials, trials using conjugated equine estrogens (CEE) with and without medroxyprogesterone (MPA), and trials of estradiol (oral and transdermal). Many of the trials have weaknesses (and in the case of WHI the data have not been published); individually their findings can be rationalized away, but in aggregate the findings are persuasive. Therefore, the question is no longer whether the early harm is real, but rather what is causing the harm. It remains possible that the lipid changes induced by HRT may result in long-term benefit for women who survive the first years. This question remains unanswered at this point, but for many women the answer may be moot if ways of avoiding the early harm are not found.

6. Mechanisms for explaining an increased risk for arterial disease shortly after commencing HRT

Hypotheses for explaining early harm center around a potential interaction of HRT with coagulation and/or inflammation mechanisms in a subset of women with vulnerable plaques. According to these hypotheses, exposure to HRT for certain women with a susceptibility factor in the form of an elevated blood or tissue level, or with an augmented response to HRT perhaps due to a genetic mutation, may result in plaque destabilization and thrombosis.

HRT induces a wide variety of changes in coagulation factors in healthy women. Some of these are likely to be procoagulant, while others are likely to profibrinolytic, hence the clinical effect cannot be predicted from laboratory studies. Because of an emphasis on a few factors, in particular reductions in levels of fibrinogen and PAI-1, many scientists believed that HRT exerted a favorable effect on coagulation. As noted above, clinical trials and epidemiologic studies confirm that the net effect of HRT is to promote coagulation in the venous system [25,26]. There is a growing suspicion that coagulation factors beyond platelets may be more important in arterial disease than previously appreciated. Among the coagulation mechanisms the Factor V Leiden (FVL) mutation and the prothrombin G20210A mutation are currently of most interest for both venous and arterial disease.

FVL produces a coagulation factor V which resists the action of the major natural anticoagulant, activated protein C. Hence, the phenotypic expression can be measured as activated protein C (APC) resistance. The prevalence of heterozygous FVL in whites is about 4.8% (range 3–7%), with a much lower prevalence of 0.05% in Blacks and Asians [57]. FVL is associated with venous thromboembolism (VTE), and is found in about 18% of unselected cases of VTE and about 40% of selected cases with presumed familial thrombophilia. The risk for VTE associated with FVL is amplified by exposure to oral contraceptives (OCs) and the risk associated with APC resistance is amplified by HRT use [57–59]. In contrast, the association of FVL with CHD has not been found in men, and in women it has been inconsistent [60–63]. In a case–control study of younger women, FVL was associated with MI in those with other risk factors for CHD, particularly smoking [62]. However, two other case–control studies in women failed to show an association with CHD, or an interaction with HRT use.
In one cross-sectional study of women referred to a lipid clinic for management of dyslipidemia, HRT use in the absence of FVL was associated with a reduced risk for atherothrombotic arterial disease, while HRT use in the presence of FVL was associated with an increased risk [63]. Thus, the status of FVL as a risk factor for CHD is much less certain than for VTE, and only one study suggested an interaction with HRT. On the other hand, there is insufficient data to exclude a possible role for FVL in CHD in women, particularly in the context of CHD associated with HRT use.

The prothrombin G20210A mutation is procoagulant because it increases prothrombin levels. The prevalence is lower than FVL, being found in about 2.7% of whites (range 1–4%). As for FVL, the prevalence is much lower in Blacks and Asians (0.06%) [57]. It is associated with VTE, being found in 7% of unselected cases and 16% of selected cases of VTE. In women, but not in men, the mutation has been found to be associated with CHD [64–67]. A cross-sectional analysis of women with dyslipidemia found that the HRT use was associated with decreased risk for atherothrombotic disease in the absence of the G20210A mutation, and increased risk in its presence [66]. A case–control study of non-fatal MI in women with hypertension (a parallel study of women without hypertension was negative) found that presence of the mutation alone was associated with an odds ratio for MI of 1.5 (95% CI 0.6–1.4) and current use of HRT alone was associated with an odds ratio of 0.9 (0.3–7.7) [64]. However, the presence of both the mutation and use of HRT was associated with an odds ratio of 10.9 (2.2–55.2, P=0.002). This supra-multiplicative effect of the combined factors represents true synergism. If confirmed, a large synergistic effect between HRT and a prothrombotic factor in the range of 3–5% could explain the pattern of early harm and late benefit seen in the HERS trial [64].

HRT also affects several markers of inflammation, some in a potentially unfavorable direction and some in a potentially favorable direction. HRT increases circulating levels of C-reactive protein and matrix metalloproteinases including matrix metalloproteinase-9 (MMP-9) [22,68,69]. On the other hand, HRT decreases circulating levels of cell adhesion molecules E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1, and in the short term improves endothelial function [70,71]. An intriguing hypothesis put forward by Cannon et al. centers on the effects of estrogen on MMP-9 [68]. According to this hypothesis, acute administration may be harmful, and chronic administration may be beneficial. Estrogen decreases PAI-1 levels and increases plasmin activity, which in turn increases the expression of MMP-9. Local elevations of matrix metalloproteinases in the vulnerable plaque are associated with weakening and rupture of the thin fibrous cap, which is thought to precipitate the thrombotic occlusion of the vessel [69]. Thus, the acute effect of HRT might be unfavorable in women with vulnerable plaques. On the other hand, for women who survive the acute phase or who do not have vulnerable plaques, the long-term effect of elevated levels of MMP-9 might be favorable, since the proteinases might prevent the accumulation of matrix proteins and improve arterial compliance.

These intriguing observations and hypotheses have great potential relevance to women, as they affect the future use of exogenous estrogens in the form of HRT. It is unknown whether endogenous estrogens affect these markers of inflammation and coagulation, and therefore unknown whether these factors have a role in explaining the gender difference in cardiovascular disease. If anything, the known and suspected interactions of estrogen with these markers go against the observation of delayed onset in women, since they raise the risk for arterial disease in women. Younger women may have higher rates of VTE events than men, but it is not known whether endogenous estrogen or use of oral contraceptives explain that observation [72].

7. Conclusions

The gender difference in the age of onset of CHD is as yet unexplained. A natural starting point is that the biologic effects of the XX and XY chromosomes are expressed through sex hormones, and that these explain the gender difference in CHD. However, the respective roles of female and male sex hormones in this regard remain unclear. To date, research has focused overwhelmingly on estrogen. The substantial evidence in favor of a protective effect of estrogen in women is being called into question by recent clinical trial data indicating that HRT is not protective, and at least in women with existing heart disease may initially increase risk. Because of the increased risk in secondary prevention trials, and the absence of published clinical trial data for primary prevention, HRT is no longer being recommended for prevention of CHD [73]. It remains possible that HRT given for many years may be cardioprotective.

Oral estrogen has pronounced effects on blood markers and cell function, including endothelial cells and monocyte-derived macrophages. Some of these effects may be favorable, others unfavorable, and thus laboratory studies cannot predict clinical outcomes. Clinical trials indicate that the net effect of estrogen is prothrombotic. The metabolic effects of oral estrogen are very different from those of non-oral (e.g. transdermal) estrogen; the latter is more likely to mimic the effects of endogenous estrogen, and thus may be more informative about the mechanisms underlying the gender difference in CHD. More studies on the effects of non-oral estrogens are needed, including trials with clinical outcomes.

It is possible, but unproven, that in part the gender difference may be due to adverse effects of androgens in men, and more studies of androgens, antiandrogens, and
selective androgen receptor modulators are needed. Males and females not only differ in their levels of sex hormones, the effects of those sex hormones also differ by gender. It appear likely that more research will reveal that the gender difference has male as well as female components.

In addition to effects of the sex chromosomes, it is possible that autosomal mutations may influence the risk for CHD. By definition, autosomal mutations are as frequent in males as in females, hence the search should be focused on mutations that modify risk and interact with sex hormones. This research is in its infancy, but a good example of its potential is the interaction between prothrombotic mutations and estrogen (in oral contraceptives and HRT formulations) that underlie a substantial proportion of VTE in families with thrombophilia, and also in unrelated individuals with VTE. The most pressing scientific challenge of the moment is to find ways of identifying the women who are at increased risk for CHD when they commence HRT. The prothrombotic mutations are being investigated in this regard, as are several other markers of coagulation and inflammation. If it becomes possible to identify susceptible women, they could be counselled against HRT, while the non-susceptible women would have a higher chance of experiencing benefit from HRT.

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