MOLECULAR METABOLISM

Sex differences in lipid and lipoprotein metabolism

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ABSTRACT

Background: Endogenous sex hormones are important for metabolic health in men and women. Before menopause, women are protected from atherosclerotic cardiovascular disease (ASCVD) relative to men. Women have fewer cardiovascular complications of obesity compared to men with obesity. Endogenous estrogens have been proposed as a mechanism that lessens ASCVD risk, as risk of glucose and lipid abnormalities increases when endogenous estrogens decline with menopause. While baseline risk is higher in males than females, endogenously produced androgens are also protective against fatty liver, diabetes and ASCVD, as risk goes up with androgen deprivation and with the decline in androgens with age.

Scope of Review: In this review, we discuss evidence of how endogenous sex hormones and hormone treatment approaches impact fatty acid, triglyceride, and cholesterol metabolism to influence metabolic and cardiovascular risk. We also discuss potential reasons for why treatment strategies with estrogens and androgens in older individuals fail to fully recapitulate the effects of endogenous sex hormones.

Major Conclusions: The pathways that confer ASCVD protection for women are of potential therapeutic relevance. Despite protection relative to men, ASCVD is still the major cause of mortality in women. Additionally, diabetic women have similar ASCVD risk as diabetic men, suggesting that the presence of diabetes may offset the protective cardiovascular effects of being female through unknown mechanisms.

1. INTRODUCTION

Prior to menopause, women are protected from myocardial infarction (MI) compared to age-matched men. The age of onset for the first myocardial infarction is ~10 years later for women compared to men. Furthermore, at any given age, women have one-third to one-half of the risk of cardiovascular disease relative to men [1,2]. The mechanisms for this protection include the effects of sex hormones as well as hormone-independent effects of the sex-chromosomes in tissues throughout the body. The protective effect of endogenously produced estrogens with regard to ASCVD is supported in humans by the increase in risk when levels of naturally cycling estrogens and progestins decline with menopause. Despite lower risk than men, ASCVD is still the major cause of mortality in women, causing 1 in 3 deaths in women. Over the past two decades the prevalence of myocardial infarctions has increased in women midlife (ages 35—54 years), despite a decline in similarly aged men [3]. The increased prevalence diabetes in women partially offsets the protection conferred by being female [4—6].

The mechanisms conferring protection from atherosclerotic cardiovascular disease (ASCVD) in non-diabetic women have been of considerable research interest. Estrogens have effects in many organ systems that contribute to cardiovascular risk *vs.* protection, including regulation of liver lipid metabolism and serum lipoprotein levels. The primary movement of lipids between tissues occurs either as free fatty acids released by adipose tissue or in the form of lipoprotein carriers made primarily by the liver and gut (chylomicrons and very low density lipoprotein (VLDL) for triglyceride (TG), and low density lipoprotein (LDL) and high-density lipoprotein (HDL) for cholesterol). Many aspects of hepatic fatty acid, TG, and cholesterol biology are regulated by endogenous estrogens and androgens, but the physiologic control of these effects is distributed among different tissues, primarily adipose and liver, but increasingly it is recognized that CNS effects of sex hormones also contribute. More is known about estrogen control of lipid metabolism than androgen control of lipid metabolism. Estrogens mediate their effects through three receptors, Estrogen Receptor alpha (ERα), Estrogen Receptor beta (ERβ) and G-protein coupled Estrogen Receptor (GPER). The liver is an important site where fatty acids, TG, and cholesterol metabolism are coordinated to meet metabolic needs in normal physiology. Despite the protective effects of endogenous sex hormones in males and females, physicians are confronted with limited ability to recapitulate these physiologic roles with estrogen treatment approaches for women after menopause, or older men with androgen deficiency. Thus, there is an important therapeutic opportunity to

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develop tissue selective and pathway-preferential sex hormones in order to recapitulate this protective physiology in younger adults.

2. MECHANISMS OF ESTROGEN SIGNALING

Many aspects of sex-differences in physiology arise due to the mechanisms of the sex-hormones. Estrogen signaling pathways have pleotropic effects on many tissues and pathways that govern lipid and lipoprotein metabolism, but our understanding of these effects is complicated in that there are numerous endogenous estrogens and they differ between species [7]. 17β estradiol is the predominant endogenous estrogen in humans. It is made by the ovaries and circulates in plasma associated with sex-hormone binding globulin. Estrogens are lipophilic steroid hormones that are thought to passively diffuse through the plasma membrane into the cytoplasm and nucleus where they bind the steroid nuclear hormone receptors, ERα and ERβ [8]. Unliganded ERα and ERβ are kept inactive by association to Heat Shock Protein 90 (Hsp90) complexes. Binding of estrogens to ERα or ERβ promotes dissociation from Hsp90, dimerization, and translocation into the nucleus to activate gene transcription [8]. These genomic sequences are referred to as Estrogen Response Elements (EREs) and are commonly found in the promoter or enhancer regions of genes whose transcription is regulated by estrogens. The liver is a major tissue that impacts lipid metabolism in response to estrogen signaling. Over 1000 human liver genes display a sex-bias in their expression [9]. The top biological pathways are in lipid metabolism and genes related to ASCVD [9,10]. Additionally, chromatin immunoprecipitation assay revealed 43 of the lipid genes are transcriptionally regulated by ERα [11]. In the mouse, scores of liver genes involved in TG and cholesterol metabolism vary with the four-day estrous cycle of the mouse in an ERα-dependent manner [12], demonstrating a tight coordination of liver lipid metabolism with reproductive needs.

Estrogens also regulate liver lipid metabolism by modifying signaling to estrogen receptors localized to the plasma membrane by palmitoylation of a serine residue and association with caveolin-1 [13—16]. This membrane-localized estrogen signaling through ERα and ERβ activates ERK 1/2 and the PI3K pathways [14—16]. The benefits of the ERα agonist propyl-pyrazole-triol (PPT) with regard to liver lipid metabolism are largely accounted for by membrane-localized ERα [17]. Additionally, estrogens can activate the cell surface G-protein coupled Estrogen Receptor (GPER, also called Gpr30), which is expressed in multiple tissues including liver [18—20]. Activation of GPER by estrogens promotes increased cyclic AMP (cAMP) and intracellular Ca^{2+} [19]. Whole-body deletion of GPER promotes atherosclerosis and increases LDL cholesterol levels in mice [21]. The relative contributions of estrogen signaling through ERα, ERβ, or GPER with regard to lipid, lipoprotein metabolism, and ASCVD are not well defined.

3. MECHANISMS OF TESTOSTERONE SIGNALING

Testosterone is the major male sex hormone, dictates male sexual development, and maintains male sexual function throughout life after puberty. Testosterone can alter cell metabolism through effects on gene transcription through the androgen receptor (AR) and through non-genomic signaling mechanisms, similar to estrogen signaling through ERα and ERβ. AR is a classic steroid hormone receptor that enters the nucleus after binding of testosterone or dihydrotestosterone [22,23]. Once in the nucleus, AR regulates gene transcription by classic hormone-receptor signaling to androgen-response elements (AREs) in promoter and enhancer regions of target genes [22,23]. In addition to classic ARE-mediated transcription, AR activates several non-genomic signaling pathways, including the Mapk pathway and the PI3K/Akt pathway [24]. Membrane-associated AR can also mediate signaling via regulation of intracellular calcium [24]. In addition, other plasma membrane associated receptors, including the sex-hormone binding globulin receptor and epidermal growth factor receptor, can mediate effects of testosterone on cell signaling [25]. This modulation of cell signaling by testosterone not only changes intracellular signaling events but also influences transcription by both AR and non-AR transcription factors.

4. SEX-DIFFERENCES IN BODY COMPOSITION ARE DUE TISSUE-DISTRIBUTED ACTIONS OF SEX-HORMONES AND CHROMOSOMAL EFFECTS

A key aspect of sex-differences in lipid and lipoprotein metabolism is the differential ability of subcutaneous fat to expand and store excess nutrient calories as TGs in males vs. females. The concept of women as "pear-shaped", with more subcutaneous fat, and men as "apple-shaped", with more visceral fat, was put forth by Vague in 1947 [26]. Body fat distribution, as measured by waist-to-hip ratio, predicts risk of cardiovascular disease [27,28], and women appear to be at lower risk of cardiovascular disease due to a more favorable body fat distribution in lower body, subcutaneous stores. Two large prospective studies confirmed that body fat distribution does indeed predict risk of future cardiovascular disease [27—29]. Exercise and weight loss can reduce waist to hip ratio and reduce risk of cardiovascular disease, but long-term weight loss in obese patients remains a clinical challenge due to weight regain. Furthermore, a pooled meta-analysis found that waist-to-hip ratio contributed to cardiovascular risk similarly between men and women [30]. In the intra-abdominal compartment, men have larger fat cell sizes than women, whereas in subcutaneous depots, women have larger fat cell sizes than men [31]. Overall, important differences between abdominal wall and subcutaneous adipose tissue sites is apparent and may have physiologic and pathophysiologic implications [32].

There may be racial differences in the distribution of adipose in upper body vs. lower-body compartments. Women of Caucasian background are more likely to gain weight with an upper-body distribution where adipocytes are less responsive to the stimulatory effects of insulin on glucose uptake, and less sensitive to the anti-lipolytic effects of insulin. However, for any abdominal circumference, women of African ancestry have relatively less intra-abdominal vs. subcutaneous fat distribution. Moreover, fat cells from upper body and lower body were equally sensitive to the effects of insulin to regulate nutrient uptake and lipolysis, which the authors state correlated with clinical data that upper-body obesity in Caucasian women but not in African American women is associated with insulin resistance and dyslipidemia [33,34]. Conversely, testosterone seems to promote central adipose storage, as men with hypogonadism store a greater portion of dietary fatty acids in lower body subcutaneous fat (a female-like storage distribution) [35].

The distribution of fat in subcutaneous depots in women is, in part, attributable to sex-hormone signaling in adipocytes. Loss of ovarian hormones with menopause is associated with a relative re-distribution of body fat from a more subcutaneous distribution to a more visceral distribution [36]. Estrogen signaling through ERα in pre-adipocytes drives differentiation of white adipocytes [37]. Female and male mice with selective deletion of ERα in adipocytes have less subcutaneous adiposity [37]. Part of the improved subcutaneous adipose tissue nutrient storage in women is because estrogen promotes insulin sensitivity and adiponectin action, two mediators of subcutaneous fat

storage [38]. Castration of male mice enhances insulin sensitivity and increases lipolytic rates from adipocytes [39].

The physiology of subcutaneous and visceral adipose in women is quite distinct in humans, with over 2800 genes differentially expressed [32]. Using Affymetrix arrays to assess gene expression in four subcutaneous sites, nearly 3000/24,000 transcripts were differentially expressed between all sites. Major differences were found between the hip and flank compared to the lower and upper abdomen, but no genes were significantly different when the lower abdomen was compared to the upper abdomen and the hip to the flank. Genes involved in the complement and coagulation cascades and immune responses showed increased expression in the lower abdomen compared to the flank. Genes involved in basic biochemical metabolism including insulin signaling, the urea cycle, glutamate metabolism, arginine and proline metabolism and amino-sugar metabolism had higher expression in the lower abdomen compared to the hip [32]. Not all of estrogen-control of adiposity occurs at the level of adipocytes. Estrogen signaling in the CNS also contributes to body weight and adiposity, and thus at least indirectly to lipid and lipoprotein metabolism in animal models. Mice with hypothalamic deletions of ERα have hyperphagia and obesity [40,41]. This CNS-mediated ERα effect seems to require lipid sensing through neuronal lipoprotein lipase [42]. Additionally, some of the contribution to body composition is genetic, as genome-wide association meta-analyses of traits related to waist and hip circumferences revealed 19 loci associated with increased hip fat distribution [43], which mirrored the findings of a meta-analysis of genetic studies [44]. Reue and colleagues have developed a mouse model, which they term "the four core genotypes", which allows them to separate the hormonal and chromosomal contributions to biology. They found that the presence of two X chromosomes in females allows for expansion of adipose tissue independent of gonadal hormones [45]. Thus, the distribution of adiposity in men *vs.* women, and in women before *vs.* after menopause represents effects of gene regulation in multiple tissues, as well as hormonal effects of estrogens and androgens.

5. SEX-DIFFERENCES IN FREE FATTY ACID METABOLISM, THE RELATIONSHIP BETWEEN ADIPOSE MUSCLE AND LIVER PHYSIOLOGY

The sex-differences in adiposity distribution and TG storage capacity impact the flux of fatty acids that occurs in fasting and feeding. In response to obesity both men and women have increased fatty acid release into blood. Adipose in the visceral (or splanchnic) compartment has a higher contribution to fatty acid delivery to the liver compared to adipose from subcutaneous leg fat [46]. The liver takes up these fatty acids and assembles them into TGs, which are subsequently packaged into TG-rich VLDL particles for export from the liver. Obesity is associated with increased production of apoB-rich VLDL-TG particles by the liver to a greater degree in men than in women [47,48]. Lower VLDL-TG levels produced by the liver are in part secondary to lower fatty acid delivery to the liver due to enhanced fatty acid clearance by muscle in women [49—51]. It is also known that in response to fatty acid delivery to the liver that women secrete VLDL particles that are more TG-rich [52], which would help the liver export liver TGs and prevent liver fat accumulation with obesity. In response to obesity, women do have increased VLDL production, especially when this obesity is abdominal [53]. Production of more TG-rich VLDL is matched with accelerated VLDL-TG clearance rates in women [54], which collectively contribute to less liver fat and lower plasma VLDL-TG levels with obesity in women.

Not all studies suggest a strong correlation between blood fatty acid levels and BMI. The strongest relationship between high fatty acid levels and BMI is at higher levels of BMI. Expressed per cell, women have higher lipoprotein lipase (LPL) activity than men, greater lipolysis in response to lipolytic stimuli of fasting, but greater suppression of lipolysis by insulin in the fed state [31,39]. However, using tracers for FFA flux, Mittendorfer and colleagues found that males and females had a similar increase in the rate of appearance of free fatty acids (FFA-Ra) with increased adiposity. The FFA-Ra in relationship to fat-free mass (FFM) was greater in women than in men, but not related abdominal fat mass in this study [55]. Some studies show that blood levels of fatty acids are higher in women than men with obesity, but with a less severe impact on insulin resistance (reviewed in [56]). Thus, the appearance of free fatty acids in the blood has a complex association with obesity and appears to depend a great deal on adipocyte function.

Even with increased lipolysis associated with obese states, females are resistant to free fatty acid-induced insulin resistance. In experimental models, infusion of Intralipid plus heparin is a way to acutely elevate serum fatty acid levels and define fatty acid-mediated insulin resistance independent of diet or weight differences between groups. Female rodents are resistant to free fatty acid-induced insulin resistance and maintain normal insulin action in muscle and liver [57]. Female humans are also protected from insulin resistance due to Intralipid, and muscle is an important mediator of this protection [49]. For men, this accumulation of intramuscular TG associates with insulin resistance and impaired glucose disposal by muscle, whereas women are relatively protected from insulin resistance associated with intramuscular TG [58]. Thus, sex-differences fatty acid handling and intramuscular TG metabolism relate to sex-differences in risk for glucose tolerance and type-2 diabetes.

6. SEX DIFFERENCES IN TRIGLYCERIDE METABOLISM

In the fasted state, most TGs circulate in the form of apoB100-containing VLDL made by the liver. As mentioned above, women secrete more TG-rich VLDL particles, which are matched by higher rates of LPL-mediated VLDL-TG clearance, contributing to overall lower blood TG levels in women with obesity. In the fed state, TGs circulate in the form of apoB48-containing chylomicrons. In response to both short and long-term high-fat feeding, women have better clearance of meal-related TGs and increased storage of those nutrients in subcutaneous gluteal fat, rather than abdominal fat, whereas storage is similar in abdominal vs. subcutaneous fat in men [54,59,60]. The increased TG clearance by subcutaneous fat is more pronounced with addition of high-carbohydrates to the diet in women [61]. Increased blood levels of TGs correlate with increased risk of cardiovascular disease, a correlation which is stronger in women in the Framingham Heart Study [62]. Increased ASCVD risk due to TGs is reduced when corrected for low HDL cholesterol in men, but the correlation remains even after correcting for low HDL cholesterol in women [63,64]. The reason that high TGs correlate more strongly with ASCVD risk in women than in men remains unknown and is an extremely important uncertainty to answer.

Some of the sex-differences in VLDL-TG biology and fatty liver are due to liver estrogen signaling, which limits non-alcoholic fatty liver disease (NAFLD) with obesity. Loss of global estrogens after menopause, in experimental models, and due to estrogen antagonists lead to liver fat accumulation. Tamoxifen (TMX) is an anti-estrogen drug used for the treatment of hormone-sensitive breast cancer, which increases NAFLD and steatohepatitis [65,66]. Rates of NAFLD increase as women transition to menopause [67,68]. Aging is a natural risk factor for

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NAFLD, which may confound the impact of menopause on risk of NAFLD. In women undergoing surgical menopause, the risk of NAFLD is increased nearly two-fold [69]. This biology is modeled in animal models in which removal of the ovaries by ovariectomy leads to an accumulation of liver TG content [70–72]. Depletion of endogenous estrogens with 4-vinylcyclohexene diepoxide (VCD) also causes insulin resistance, fatty liver, and dyslipidemia [73]. Thus, absence of ovarian hormones leads to an increased risk of NAFLD that is at least partially reversible with estrogen treatment in rodents and postmenopausal women.

Loss of ovarian hormones also leads to weight gain, confounding definition of effects due to loss of estrogen signaling. To define tissue-specific contributions of estrogen signaling, several groups have created hepatocyte-specific ERα-knock out mice. The ability of estrogens to reduce liver steatosis is lost with deletion of hepatocyte ERα, suggesting that estrogens are acting directly in the liver to reduce TG content through ERα [12,74,75]. Loss of hepatocyte ERα results in loss of estrogen regulation of target genes [74,75], increased expression of lipid synthesis genes [76], and impaired estrogen-regulation of other lipid metabolic target genes [77]. One proposed mechanism for ERα regulation of lipid synthesis targets involves estrogen-ERα regulation of the nuclear receptor Small Heterodimer Partner (SHP), a target gene of ERα [74,78]. Additionally, estrogen-ERα regulation of liver lipid metabolism has been proposed to act *via* microRNA mir-125b [79].

The mechanisms by which estrogen signaling protects against hepatic steatosis includes reductions in *de novo* lipogenesis (hepatic synthesis of fatty acids) as reported by different laboratories. Using a combination of chromatin immunoprecipitation and tiled microarrays (ChIP-on-chip) approach, Gao et al. identified binding regions of ERα to DNA in intact chromatin in the liver [11]. This analysis revealed 19 gene ontology (GO) categories including lipid biosynthesis (GO 0008610) and fatty acid metabolism (GO 0006520) that are significantly enriched for genes that had ERα recruited to their promoter after 2 h of estradiol treatment [11]. Conventional ChIP followed by qPCR shows binding to ERα to promoter regions of lipogenic genes including STAT3 and SHP are consistently increased after treatment with estradiol or ERα agonist [11]. This report is consistent with their previous observation that estradiol treatment promotes ERα binding to STAT3 promoter and STAT3-Tyr phosphorylation, which subsequently suppresses Fasn, Scd1, Acaa1, and Gpam expression in the liver in ob/ob mice [80]. Many of these genes also vary in a tetradian-manner with the mouse's estrus cycle and are lower during the high-estrogen phase of the cycle, which correlates with lower liver fat content [12].

Estradiol treatment suppresses liver lipogenesis by maintaining ACC phosphorylation, which correlates with decreased tracer incorporation into hepatic lipid [75,81–83]. This mechanism likely contributes to the correction of pathway-selective insulin resistance in the liver with estradiol treatment [75]. ACC phosphorylation is regulated by AMPKα phosphorylation in the liver. Estradiol induces signal transduction through ERα, which localizes to both the plasma membrane and nucleus. Activation of estrogen signaling by the ERα agonist PPT promotes AMPK phosphorylation *via* ERα localized exclusively at the plasma membrane, but not in ERα knockout mice. This study demonstrates that signaling changes mediated by membrane-localized ERα result in important metabolic effects independent of nuclear ERα [17]. Additionally, oral conjugated estrogens (CE) and the selective estrogen receptor modulator bazedoxifene (BZA) also promote AMPK phosphorylation *via* ERα in liver after ovariectomy [84]. In this study by Kim et al., oral CE and BZA reduce hepatic FAS expression and FAS activity and are associated with decreased liver TG accumulation in female mice after ovariectomy [84].

Liver estrogen signaling also likely promotes fatty acid oxidation in liver. Levels of mRNA for CPT-1, a protein to transport fatty acid into mitochondrial for β-oxidation are induced with estradiol treatment after ovariectomy. Estradiol treatment after ovariectomy also increases oxygen consumption and liver ATP production along with changes in UCP2 expression in the liver [85]. Additionally, estradiol and CE increase production of FGF21 by the liver which may also increase hepatic fatty acid oxidation [84].

7. SEX-DIFFERENCES IN CHOLESTEROL METABOLISM

There are important sex-differences in cholesterol metabolism which likely contribute to the large sex-differences in rates of ASCVD. Women have a nearly decade-long delay in first myocardial infarction compared to men, which may be largely driven by hormonal effects of estrogens on cholesterol metabolism before menopause [1,2,86]. Even after levels of ovarian hormones decline with menopause, women have lower risk of cardiovascular disease relative to men [1,2,87]. The prevalence of coronary heart disease (CHD) according to 2011–2014 NHANES data is 19.7% in men and 11% in women 60–79 years of age, and similar trends for older individuals [2]. Rates of heart attack are less than half among women in this high-risk age group [2]. It is not well known if these protective effects exhibited by women later in life are conferred by chromosomal effects, such as established by Reue and colleagues, or due to a different trajectory set by the pre-menopausal hormonal environment.

Liver estrogen signaling may contribute to sex-differences in atherosclerosis by promoting the hepatic steps of reverse cholesterol transport (RCT). RCT is the process of cholesterol removal from peripheral tissues culminating in delivery of cholesterol to the liver for conversion to bile acids, which are ultimately delivered into the feces (reviewed in [88]). Estrogen's role in the early steps of the RCT pathway is controversial in humans. The cholesterol efflux capacity of macrophages is enhanced by estradiol-esters present in HDL [89]; however, there were no sex-differences in macrophage to HDL efflux relative to men [90]. In premenopausal women, the concentration of estrogen in plasma is not associated with cholesterol efflux capacity [90]. In premenopausal women with polycystic ovary syndrome (PCOS), which is a state of reduced estrogen and increased androgens, cholesterol efflux capacity is reduced [91]. The estrogen deficiency of menopause, however, increases the cholesterol efflux capacity of HDL relative to premenopausal women, likely because of increased VLDL-TG levels after menopause [92]. In postmenopausal women, hormone treatment with CE-progestin increases cholesterol efflux capacity of HDL [93]. Thus, estrogen signaling pathways have been shown to have some effects on cholesterol efflux capacity, but there is not a consistent relationship between estrogen enhancing or impairing this initial step in RCT based on the literature in humans.

Estrogen signaling pathways have a more established role promoting the hepatic steps of RCT. Liver estrogen signaling through ERα has been shown to regulate hepatic cholesterol uptake and the efflux capacity of HDL from macrophages during the proestrus period when estrogen levels are high [77]. Female mice have increased total-body RCT compared to males fed a western diet [94]. In this study, liver deletion of ERα impaired total body RCT in female mice, suggesting that liver ERα is required for females to enhance total body RCT [94]. Estradiol and PPT treatment of mice both promote liver secretion of cholesterol into bile, a process that is prevented by concurrent treatment with an ERα antagonist [95]. The role of sex and estrogen on later stages in RCT is not well studied in humans.

8. HORMONE TREATMENT AND THE RISK OF CARDIOVASCULAR DISEASE IN POSTMENOPAUSAL WOMEN

Despite decades of studies, treatment approaches with estrogen formulations in women after menopause have largely failed to recapitulate the protective physiology of a true replacement strategy. The results of treatments with estrogens with regard to glucose, lipids, and cardiovascular risk have varied based on the formulation of estrogen used, pairing with progestin, and route of delivery (reviewed in [96]). Two of the early randomized controlled trials of hormone treatment were the Women's Health Initiative (WHI) and the Heart and Estrogen/Progestin Replacement Study (HERS) [97,98]. For both studies, hormone treatment consisted of conjugated estrogens plus a progestin if the women had an intact uterus. After 6.8 years of follow-up the HERS trial showed improvement in blood cholesterol levels and diabetes risk factors, but no improvement in cardiovascular disease [99]. After 5.6 years of follow-up in the WHI trial cardiovascular risk was higher in those with hormone treatment [98]. In the WHI trial where hormone treatment was largely initiated late after natural menopause, ASCVD risk was higher in women treated with hormones over 10 years after the onset of menopause. This effect led to the "timing hypothesis," which suggests that hormone treatment is most beneficial if initiated soon after menopause, and potentially harmful if initiated late (>10 years) in menopause.

The timing hypothesis was tested in the Early versus Late Intervention Trial with Estradiol (ELITE) study, which randomized postmenopausal women to placebo or oral estradiol plus vaginal progesterone for 10 days per cycle [100]. Women were stratified into two groups —early menopause if menopause occurred in the last 6 years, and late menopause if menopause occurred at least 10 years prior to enrollment in the study. Estradiol treatment reduced the progression of carotid intima medial thickness (CIMT) in the early menopause group but failed to delay atherosclerosis in the late menopause group. This result supports the timing hypothesis of estrogen treatment.

Serum TG levels were increased with hormone treatment in the WHI trial. The cause of the TG-rich dyslipidemia with hormone treatment of postmenopausal women has been controversial. Variations in estrogen levels with a woman's menstrual cycle do not impact VLDL-TG or VLDL-apoB kinetics or concentrations [101]. Estrogen treatment, however, seems to increase serum TG in a manner that depends on the route and formulation. Oral delivery of micronized estradiol increased VLDL production rates by 80%, whereas transdermal estradiol had no effect on VLDL production rates in this study [102]. Another study with oral ethinyl estradiol increased VLDL apoB production over 100% [103], which is a similar result found to earlier studies with conjugated equine estrogens [104]. Progestins oppose the effect of estrogens by promoting VLDL clearance in both humans and animals ([105,106] and reviewed in [38]). Transdermal preparations of estradiol have less effects on lowering LDL cholesterol and increasing HDL cholesterol [107—111] and do not seem to increase plasma TGs when compared to oral estrogen formulations [107—112]. In fact, most studies demonstrate that transdermal estradiol reduces plasma TGs [107,108,111]. In a study examining VLDL-TG kinetics transdermal estradiol had no effect on VLDL-TG production but promoted VLDL-TG clearance [113]. The larger effect of oral estrogens on VLDL-TG production suggest that the liver is the primary organ responsible for hypertriglyceridemia with treatment.

9. ARE MALE SEX HORMONES MEDIATORS OF INCREASED ASCVD RISK IN MEN?

Testosterone has been hypothesized to contribute to a man's increased risk of ASCVD. The hypothesis that high testosterone increases ASCVD risk in men is controversial for several reasons. Firstly, the majority of cross-sectional studies examining the relationship between testosterone levels and ASCVD support an inverse relationship between testosterone and risk of cardiovascular disease [114—119]. Certain studies, however, support a neutral [120—123], positive or J-curve [124] relationship between testosterone and cardiovascular disease. In a meta-analysis of testosterone association with cardiovascular disease, testosterone correlated inversely with cardiovascular disease only when men above age 70 were included in the analysis [125]. This suggests that an age-related decline in testosterone [115] may be responsible for the inverse relationship between testosterone levels and risk of cardiovascular disease. Secondly, studies of testosterone deprivation show increased risk of cardiovascular disease [126—128]. This suggests that low testosterone increases cardiovascular disease risk. Thirdly, studies of testosterone therapy have different effects on risk of cardiovascular disease depending on testosterone status prior to treatment. For example, in hypogonadal men, testosterone treatment reduces risk of cardiovascular disease in men [129,130]. In normal men, testosterone therapy seems to increase risk of cardiovascular disease in randomized controlled trials [131—133].

The unexpected conclusion that testosterone lowers risk of cardiovascular disease in men may partly be explained by the impact of low testosterone on risk of metabolic syndrome. Metabolic syndrome is associated with higher risk of cardiovascular disease [134], which may be an important confounder in understanding the cardiovascular disease risk associated with testosterone levels. Reduced testosterone levels are associated with increased fasting glucose, fasting insulin, and type 2 diabetes [135—139]. Testosterone treatment in men with low testosterone improves insulin sensitivity, reduces glucose and insulin, and reduces risk of type 2 diabetes [140—142]. In addition, testosterone treatment in hypogonadal men reduces obesity and improves lean muscle mass, both of which would contribute to reducing risk of type 2 diabetes [140]. Thus, the "benefit" of testosterone may be related more to improvements in muscle glucose metabolism and insulin sensitivity than improvements in cardiovascular disease, especially when considering the impact of testosterone in hypogonadal men.

The development of Androgen Receptor (AR) knockout (ARKO) models has allowed for a more precise definition of the contribution of androgen signaling to sex-differences in lipid metabolism and atherosclerosis. Mice with a global AR knockout (ARKO) had worse atherosclerosis relative to controls on an Apolipoprotein E knockout (ApoE$^{-/-}$) background [143,144]. Global ARKO mice had increased weight gain, increased plasma cholesterol and TGs, increased liver TG content, and impaired glucose metabolism. Additionally, 5α-dihydrotestosterone, a non-aromatizable AR agonist, reduced atherosclerosis, obesity, plasma cholesterol, and plasma insulin liver TG content and reduced atherosclerosis. These data suggest that AR signaling reduces atherosclerosis and improves glucose and lipid risk factors for cardiovascular disease. While these studies are informative about androgen signaling, they don't provide clear mechanisms of the increased risk of cardiovascular disease seen in men.

10. SEX-SPECIFIC CONSIDERATIONS FOR THE TREATMENT OF DYSLIPIDEMIA AND ASCVD

The historically lower risk of cardiovascular disease in women than in men, in some ways has biased physicians and scientists from understanding cardiovascular disease and its treatment in women. In women, death from cardiovascular causes is higher than from breast and ovarian cancers combined. Although most studies suggest that cholesterol lowering approaches with statins are equally effective in men and women, trials from the major statin studies had only 15—30% women [145]. The study with the largest percentage of women was the MEGA study with pravastatin which had 68% women, for which pravastatin did not reach statistical significance compared to diet alone [146]. PCSK9 degrades the LDLR, increasing blood LDL-C levels. In men but not in women, PCSK9 levels correlate with LDL cholesterol [147]. Deletion of PCSK9 in mice produces dramatic upregulation of LDLR only in male mice [148]. However, it is not conclusive if treatment with PCSK9 inhibitors aliorocumab and evolocumab is equally effective in men and women [149]. In a study looking at short-term mortality after myocardial infarction, among patients less than 50 years of age, the mortality rate for the women was more than twice that for the men [150,151]. Women are less likely to be treated with reperfusion therapy and beta blockers [150]. After stent placement women have increased risk of subsequent major adverse cardiovascular events compared to men [152,153]. Compared to men, women receive less cholesterol screening and fewer lipid-lowering therapies. Even for LDL of 160 women are less likely to be treated with a high-potency statin [154,155]. Women are more likely to have myopathy and discontinue statin therapy, a biology likely related to sex-differences in statin metabolism. Older women may have less benefit from angiotensin converting-enzyme inhibitor use than men [156], and potentially less benefit from low-dose aspirin for primary prevention [157], although guidelines still support the use of both in clinically-indicated populations. Thus, in addition to understanding the physiology of sex-difference in lipid metabolism, it is also imperative to understand potential sex-differences in the efficacy of treatment and prevention strategies for ASCVD.

11. CONCLUSIONS AND FUTURE DIRECTIONS

There are major sex differences in lipid and lipoprotein metabolism that contribute to sex-differences in ASCVD risk. The mechanisms for these sex differences are complex and involve hormonal effects that are distributed across tissues, as well as effects mediated by genes on the X-chromosome that escape the process of X-inactivation. It is important to note that the historically lower rates of ASCVD in women have created a false perception that ASCVD is less important in women than in men, which has certainly hindered both clinical and basic science discoveries. The British Heart Foundation states that the number of women living with ASCVD is now roughly the same as the number of men [158]. The lower perceived risk in women is unfortunate, because ASCVD is the major cause of death in women. This oversight is beginning to be corrected. We also don't understand well the interaction between diabetes and ASCVD in women, which may have a heightened contribution to risk compared to men [4—6]. This is of particular importance in certain groups of high-risk women including individuals of African and Hispanic descent for whom risk of diabetes is very high. Lastly, sex hormone treatment of older adults after endogenous hormone levels decline holds to promise to improve many aspects of cardiometabolic health, but physicians are largely unable to recapitulate the physiologic benefits of endogenous estrogens or androgens with treatment approaches in older adults. Much remains to be learned about mechanisms for these sex-differences. Gaining this knowledge would allow us to therapeutically target the relevant protective pathways as well as aid our ability to physiologically replace sex-hormones in older adults.

CONFLICTS OF INTEREST

None declared.

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