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Sex-biased and sex hormone-dependent regulation of apolipoprotein A1

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Abstract

Pronounced sex differences in the development and outcome of cardiovascular diseases (CVD) exist. Apolipoprotein A1 (APOA1), the basic structural protein of high-density lipoprotein (HDL), is involved in key metabolic processes. However, its role in the pathogenesis of CVD is incompletely understood. The effects of biological sex on factors influencing the APOA1-lipid balance and the underlying mechanisms are also poorly understood. Here, we summarize evidence supporting sex-biased and sex hormone-dependent regulation of APOA1. In particular, we discuss sex-biased *APOA1* genetic variation, sex differences in APOA1 regulation and cardiovascular physiology, and sex hormone-dependent regulation of APOA1 in cis- and transgender individuals. We put forward that studying the effects of biological sex will contribute to a better understanding of the role of APOA1 in cardiovascular physiology and its sex-biased association with CVD. Importantly, in situations of sex hormone therapy or inhibition, more sex-stratified data are required to inform clinical management of APOA1-related cardiovascular risk in a sex-dependent manner.

Keywords: estrogen; lipoprotein; sex; testosterone; transgender

Abbreviations

APOA1, apolipoprotein A1

CVD, cardiovascular diseases

HDL, high-density lipoprotein

HDL-P, HDL-particles

SNPs, single nucleotide polymorphisms

Introduction

Cardiovascular diseases (CVD) are one of the leading causes of death globally [1]. Notably, there are pronounced sex differences in the development and outcome of CVD, as well as the response to pharmacological therapies [2-9]. Genetic and epigenetic mechanisms, fibrotic, inflammatory and metabolic processes, as well as sex steroid hormones and their receptors are expected to account for sex-biased CVD development and outcome [8, 10-17]. In addition, socio-cultural differences in access to care, intensity of and compliance to treatment, as well as other similar elements also contribute to differences in outcomes between the sexes. However, for the purpose of this article we focus on the biological basis of sex differences in pathophysiology.

Obesity, insulin resistance and type 2 diabetes mellitus, atherosclerosis and hypertension are on the rise. Altered glucose and lipid metabolism contribute to the development of these disorders, which are associated with CVD-related morbidity and mortality. Alterations in the levels of sex hormones are markedly associated with lipid profile variations, abdominal fat accumulation, insulin resistance and blood pressure, thereby leading to metabolic and adipocyte physiology disturbances contributing to increased risk for CVD [18, 19].

Apolipoprotein A1 (APOA1) is the basic structural protein of high-density lipoprotein (HDL) and is therefore involved in lipid metabolism [20-22], in glucose metabolism [23, 24] and in spermatozoa motility in men [25]. In addition, *APOA1* single nucleotide polymorphisms (SNPs) can modulate various disorders, such as obesity, metabolic syndrome, atherosclerosis and cardiac dysfunction [26-30]. APOA1 levels may also be affected by a number of environmental factors, such as diet [31, 32], toxins [33, 34], as well as exercise [35, 36]. In addition, APOA1 is regulated differently by sex hormones, i.e. estrogen and testosterone (discussed below).

The activity of APOA1 in all these situations is incompletely understood, including its specific function in atherogenesis and protection from pathological cardio- and cerebrovascular events, or its dissociation processes from one lipoprotein particle to another. In addition, the effects of biological sex on factors that influence the APOA1 and lipid balance, along with the underlying mechanisms, are also poorly understood. A better understanding of the role of biological sex in APOA1 regulation and HDL subgroups may help to resolve present contradictions in CVD risk and development. We therefore summarize here current knowledge regarding sex-biased and sex hormone-dependent regulation of APOA1.

Production of apolipoprotein A1

APOA1 is produced in the liver (70%) and small intestine (30%) [37]. After its production, it forms homodimers and binds lipids (phospholipids, cholesterol, sphingolipids and ceramides) mediated by

the ATP-binding cassette A1 (ABCA1) [38]. APOA1 is a cofactor of Lecithin-Cholesterol-Acyltransferase (LCAT) to esterify cholesterol [39]. The transporters ABCA1, ATP-binding cassette G1 (ABCG1) and the scavenger receptor type B1 (SR-B1) change composition, morphology and size of the new HDL-particles (HDL-P) [39]. HDL-P are a heterogeneous group of lipoprotein particles that differ in size, density, composition of proteins and lipids and show species-, sex-, ethnic- and age-related differences [40, 41]. Instead of the previously frequently used differentiation into small, medium, large and very large HDL-P, the current differentiation is according to protein composition. In fact, 15 different HDL subgroups have been identified and are categorized according to protein composition. These subgroups show different concentrations of APOA1 and different relative cardiovascular risk, e.g. HDL-P with APOA1/APOA2 and APOC1, or APOA1/APOA2 and APOE are associated with a lower relative risk. In contrast, HDL-P containing APOA1/APOA2 and α 2 macroglobulin, or complement C3, or haptoglobin, or plasminogen are associated with a higher relative risk of cardiovascular events [41]. Recently, there has been a change in paradigms from HDL quantity to quality and function [42]. APOA1 plays an important role in this, because it is known that HDL of high quality (larger particles with spheric morphology) contains more APOA1 than those of low quality (smaller HDL-P with unspecified morphology) [43].

The regulation of APOA1 transcription and translation, as well as post-transcriptional modifications, is not yet fully understood. Environmental factors, such as diet, have a considerable effect on the concentration of APOA1, which is also subjected to epigenetic factors. Along this line, studies with experimental animals have shown that the gut microbiome of mice fed a high-fat diet can increase APOA1 production in the hepatocytes, mediated by the Toll-like receptor 5 (TLR5) [44]. Diet effects are also present in humans, whereby the replacement of carbohydrate or protein with fat promotes increases in HDL cholesterol and APOA1 levels [45-47]. Long non-coding RNA can also regulate the apolipoprotein gene cluster, which includes APOA1 [48]. However, the underlying mechanisms are poorly understood. Similarly, physical exercise has been demonstrated to increase APOA1 fractions in both men and women [49]. Consequently, in addition to sex, gender-mediated mechanisms, i.e. masculine and feminine psychosocial roles and health behaviors, may also contribute to the regulation of APOA1.

Genetic variation

SNPs in *APOA1* play an important role in lipid metabolism, blood pressure, as well as the development and severity of CVD, and there are sex-biased effects of the SNPs on lipid balance [50-52]. A common gene variant of *APOA1* (-75bpG>A) was assessed in homozygous and heterozygous carriers and it was discovered that different genotypes affect plasma lipid levels

differently between men and women. In men, the three possible alleles (AA, GG and AG) were associated with differences in triglycerides, while in women they were associated with differences in HDL-C plasma levels [53]. SNPs in the *APOA1/C3/A4/A5-ZPR1-BUD13* gene cluster affect cholesterol balance. Certain SNPs (rs5072, rs5128 and rs651821) are associated with hypertriglyceridemia, and others (rs5104 and rs651821) with low HDL-cholesterolemia, with rs5072 leading to hypertriglyceridemia only in women [52]. These results show that *APOA1* SNPs exert different mechanisms of action between men and women and suggest that *APOA1* in its wild-type form may also play at least partially different roles in male and female cholesterol management.

Sex differences in apolipoprotein A1 regulation and cardiovascular physiology

Significant sex-related differences in lipid composition, particularly in HDL-APOA1, were described in the Framingham Offspring study. Women have a two-fold higher concentration of large HDL-P and more APOA1 than men in plasma [54]. How this is mediated is unclear. However, a contributing mechanism could be sex hormones regulating the levels of APOA1 in an opposite fashion; estrogen increases while testosterone decreases APOA1 plasma levels [55].

The influence of APOA1 on cardiovascular physiology may also be sex-dependent. Increasing arterial stiffness in men was significantly associated with higher plasma APOA1 levels, but there was no such association in women, even though women had higher APOA1 plasma levels than men [56]. This indicates that APOA1 may exert sex-dependent actions. Similarly, in the ATTICA study, APOA1 levels have been shown to be inversely associated with 10-year cardiovascular risk in women but not men, whereby each 10 mg/dL rise in APOA1 reduced the risk of developing CVD by 19% [57].

Interestingly, there are 3 of 15 subgroups of HDL-P recently described that differ between men and women. Women have significantly larger amounts of subtypes containing complement C3, ceruloplasmin and APOA4 [41]. Mechanistically, estrogen might be playing an important role in a potentially sex-biased regulation of *APOA1* expression, as there are Estrogen Response Elements (ERE) in *APOA1*; one at position 20250772 (AGGTCACGCTGTCCC) and another at position 20248958 (GGCTCACTGTGACCT) [58]. Interestingly, interactions between common polymorphisms in estrogen receptor α (*ESR1*) and *APOA1* have been observed, resulting in sex-biased responses to statin therapy [59].

Estrogen-dependent regulation of apolipoprotein A1

Exogenous estrogen may modulate APOA1 concentrations. Administration of the oral contraceptive pill, containing estrogen and progestin, promotes increased HDL-APOA1 production rate and pool size [60]. Similarly, oral estrogen has been shown to increase APOA1 in postmenopausal women receiving hormone therapy [61]. Interestingly, the menopausal status has not been shown to alter levels or kinetics of APOA1 in cis-gender women. This contradicts the abovementioned findings of an increase in APOA1 levels with the administration of exogenous estrogen. In fact, it would be expected that the decline in endogenous estrogen following menopause would have an impact on the levels of APOA1 and HDL-C. However, such analyses may be confounded by the influence of age and adiposity [62]. Certainly, further research is required to better understand the impact on APOA1 levels and the regulatory mechanisms involved.

Influence of exogenous sex hormones on apolipoprotein A1 in transgender individuals

The potential regulatory role of sex steroids on APOA1 is further supported by studies in transgender individuals (Figure). In particular, testosterone was shown to change the composition of plasma lipoprotein particles in male transgender adolescents [63]. Compared with cis-gender female individuals, male transgender adolescents have lower plasma levels of HDL and higher atherogenic low-density lipoprotein (LDL). In contrast, cis-gender and transgender men have similar APOA1 blood profiles. Therefore, testosterone is considered a major contributor of plasma lipoprotein composition [63]. However, the sample size was relatively small and only young transgender men receiving testosterone were included, while transgender women receiving estrogen were not included in the study. It should be noted, though, that in androgen-deficient cis-gender women, the administration of transdermal testosterone, albeit at much lower concentrations, did not alter APOA1 levels [64].

Lipid profiles have also been compared in young cis- and transgender men and women [40]. In children before puberty, there are no sex differences in lipid profiles. However, after puberty they change as follows: in young men very-LDL (VLDL), cholesterol, cholesterol ester, phospholipids and triglyceride increase significantly, while young women have more HDL, APOA1, polyunsaturated fatty acid (PUFA) and docosahexaenoic acid (DHA). In transgender individuals treated with puberty blockers (i.e. gonadotrophin-releasing hormone agonists) and sex hormones (i.e. estrogen in trans-women, testosterone in trans-men), trans-women have an increase in APOA1 and DHA (profile similar to young cis-women), while trans-men have a decrease in HDL, APOA1, DHA and increase in APOB:APOA1 ratio (profile similar to cis-men). The overall finding of this study was that changes in APOA1 significantly differ between young trans-men and trans-women.

APOA1 in plasma correlates with duration and concentration of plasma estrogen in trans-women but not with testosterone in trans-men. Overall, these data support testosterone modulating a connected network of atherogenic lipids in men, while estrogen favors an atheroprotective lipid profile in women. The effects on APOA1 remained statistically significantly even after adjusting for ethnicity [40].

Together, these data indicate that steroid hormones mediate sex differences in lipid profiles independent of sex chromosome dosage; however, this remains to be further investigated. Along this line, the Four Core Genotype (FCG) mouse model [65], which independently segregates the development of gonads from the sex chromosomes to generate four sex genotypes, i.e. XX and XY mice with ovaries and XX and XY mice with testes, was recently used to study the effects of gonadal sex and chromosomal sex on plasma lipid levels in a hypercholesterolemic state [66]. Mice with testes had higher cholesterol levels than those with ovaries, regardless of sex chromosome type [66]. In addition, free fatty acid levels in hypercholesterolemic mice were influenced by gonadal type, with higher levels in mice with testes than in mice with ovaries [66]. However, since the presence of two X chromosomes has been associated with increased adiposity and dyslipidemia in mouse models and in XXY men and the enhanced expression of genes that escape X chromosome inactivation has been suggested as a major contributor [67], more research is necessary to better understand the impact of sex chromosomes on lipid profiles.

Despite all this, further caution should be exercised, as the effects of gender-affirming hormone therapies on cardiovascular risk in transgender individuals are not fully understood [68]. This is mainly due to the frequent retrospective nature of the studies, subjective assessments, as well as incomplete datasets available for analysis [68]. In addition, the various forms of administration could lead to differences in cardiovascular risk profiles of transgender individuals. This includes the start of administration - before vs. after the onset of puberty, the mode of administration - oral vs. transdermal, the chemical nature of therapies - a single steroid hormone vs. a combination of steroid hormones [69, 70]. The changes in lipid profiles are evident. However, their effects on cardiovascular physiology remain unclear and require further investigation in standardized, prospective studies. In addition, long-term/lifelong analyses are necessary, since gender-affirming hormone therapies must be maintained for a lifetime. In current studies, however, the studied period is more or less limited, which does not allow conclusions to be made about the long-term/lifelong effects of exogenous hormone therapies on lipid metabolism and cardiovascular physiology.

Conclusions

APOA1 is a key player in metabolic and adipocyte physiology. It is evident that there are pronounced sex differences in the regulation and actions of APOA1. However, the underlying mechanisms are poorly understood. In this context, it is necessary that relevant studies in the field include sex as a biological variant and report data stratified by sex. In addition, it will be required that studies with experimental animals investigate these phenomena, including the role of sex and sex hormones. In fact, the role and actions of sex steroids are of particular interest for CVD-related risk and mortality not only in cis-gender individuals, but also in transgender individuals, who appear to have an increased risk of CVD [71]. Nevertheless, the contributing factors and processes are largely unknown. Interestingly, as summarized in the present article, exogenous administration of estrogen as part of gender-affirming therapy in transgender female individuals leads to an increase in APOA1 levels, a profile that is similar to cis-women and considered protective. However, both endogenous and exogenous estrogen might exert detrimental actions in male cardiovascular tissues and cells [72, 73], which, in turn, may contribute to increased mortality as shown in cis-gender men with systolic chronic heart failure [74]. Together, these findings show complex interactions and effects on physiology that lead to important sex-biased and sex steroid hormone-dependent effects on pathophysiology. A better understanding is necessary to improve clinical-decision making in an effort to decrease CVD-related risk and mortality. In this context, APOA1 levels could inform sex-based strategies for CVD risk management. Collectively, these measures could bring us closer to a more appropriate and personalized medical care.

Declarations of interest

None

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Figure legends

Figure. Schematic representation of sex differences in APOA1 and lipid profiles in youth, as well as the effects of sex steroid hormones in transgender individuals. APOA1, apolipoprotein A1; APOB, apolipoprotein B; CE, cholesterol ester; DHA, docosahexaenoic acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PL, phospholipids; PUFA, polyunsaturated fatty acid; TG, triglyceride; VLDL, very-LDL

Author, Year	APOA1 Sex Dependent Mechanisms	Major Findings
Sun X, <i>et al</i> , 2020 [56]	APOA1 levels and cardiovascular risk	Higher plasma APOA1 levels are associated with increased arterial stiffness in males but not females, despite the latter demonstrating overall higher concentrations.
Kouvari M, <i>et al</i> , 2020 [57]		APOA1 levels are inversely associated with 10-year cardiovascular risk in females but not males.
Yin RX, <i>et al</i> , 2011 [53]	Sex-biased effects of SNPs	Common gene variants of APOA1 (-75bpG>A) promote differences in triglycerides in males and HDL-C in females
Bai W, <i>et al</i> , 2019 [52]		Functional polymorphisms in APOA1/C3/A4/A5-ZPR1-BUD13 gene cluster are associated with sex-based dyslipidaemia. APOA1 rs5072 promotes hypertriglyceridemia in females only.
Duvillard L, <i>et al</i> , 2009 [60]	Exogenous sex hormones and APOA1	Oral contraceptive pill (estrogen and progestin) increases HDL-APOA1 production rate and pool size
Brinton EA, <i>et al</i> , 1996 [61]		Oral estrogen increases APOA1 in postmenopausal women
Robinson GA, <i>et al</i> , 2021 [40]		<p>Trans-women treated with puberty blockers and estrogen have an increase in APOA1 (profile similar to young cis-women).</p> <p>Trans-men treated with puberty blockers and testosterone have a decrease in APOA1 and an increase in APOB:APOA1 ratio (profile similar to cis-men)</p>

Table 1. Mechanisms of APOA1 Sex Differences

