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## Sex Differences in Cardiovascular Actions of the Renin-Angiotensin System

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### Abstract

Cardiovascular disease remains a worldwide public health concern, despite decades of research and the availability of numerous targeted therapies. While the intrinsic physiological mechanisms regulating cardiovascular function are similar between males and females, marked sex differences are established in terms of cardiovascular disease onset, pathophysiology, manifestation, susceptibility, prevalence, treatment responses and outcomes in animal models and clinical populations. Premenopausal females are generally protected from cardiovascular disease when compared to men of similar age, and tend to develop cardiovascular complications later in life following menopause. Emerging evidence suggests this cardioprotection in females is, in part, attributed to sex differences in hormonal regulators such as the renin-angiotensin system. To date, research has largely focused on canonical renin-angiotensin system pathways, and shown that premenopausal females are protected from cardiovascular derangements produced by activation of angiotensin II pathways. More recently, a vasodilatory arm of the renin-angiotensin system has emerged that is characterized by angiotensin-(1–7), angiotensin-converting enzyme 2, and Mas receptors. Emerging studies provide evidence for a shift towards these cardioprotective angiotensin-(1–7) pathways in females, with effects modulated by interactions with estrogen. Despite well-established sex differences, female comparison studies on cardiovascular outcomes are lacking at both the preclinical and clinical levels. Furthermore, there are no specific guidelines in place for treatment of cardiovascular disease in men versus women, including for therapies targeting the renin-angiotensin system. This review summarizes current knowledge of sex differences in cardiovascular actions of the renin-angiotensin system, focusing on interactions with gonadal hormones, emerging data for protective angiotensin-(1–7) pathways, and potential clinical implications for established and novel therapies.

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Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

## Keywords

angiotensin; blood pressure; hypertension; sex; estrogen; clinical; animal models

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## Introduction

Cardiovascular disease (CVD) remains the leading cause of death for men and women worldwide, despite the availability of numerous targeted treatment approaches [1]. Hypertension is a leading independent risk factor for developing cardiovascular-related diseases including atherosclerosis, myocardial infarction, stroke, heart failure, end-stage renal disease, and peripheral arterial disease [2]. Importantly, there are recognized sex differences in blood pressure regulation as well as CVD susceptibility, onset, prevalence, clinical presentation, pathophysiology, treatment responses and outcomes in animal models and clinical populations [3]. For example, premenopausal women generally have a lower incidence and severity of hypertension compared with age-matched men [3]. Despite this, following a cardiovascular event, women do not respond as well to treatment and have higher risk of death [4]. This cardioprotection in premenopausal women is generally lost with obesity and type II diabetes and during menopause, with a greater incidence of CVD and more vascular and myocardial stiffness in hypertensive older women [1]. The manifestation of ischemic heart disease also exhibits sex-specific features, with men typically having obstructive dysfunction, whereas women suffer from non-obstructive or microvascular phenotypes [3].

The mechanisms underlying sex differences in CVD remain poorly understood, with females being underrepresented in preclinical and clinical research. Several mechanisms have been proposed including lifestyle and behavioral factors, functioning of cardiovascular end organs, sex hormones, and physiological regulatory systems including sympathetic and immune activation and vasoactive hormones such as nitric oxide (NO) and endothelin [4]. Emerging evidence suggests that differential activation of the renin-angiotensin system (RAS) also contributes to sex differences in CVD [5,4]. While the RAS intrinsically regulates blood pressure and cardiovascular end organ function in both sexes, premenopausal females exhibit a shift in this hormone system towards cardioprotective counter-regulatory pathways mediated by the hormone angiotensin (Ang)-(1–7) [4]. This protective role of Ang-(1–7) pathways in females appears to be modulated, in part, by interactions with estrogen. This review will highlight current knowledge of sex differences in cardiovascular actions of the RAS, including interactions with gonadal hormones, emerging data for protective Ang-(1–7) pathways, and therapeutic implications for established and novel therapies in clinical populations. In addition to hypertension, this review will describe evidence for sex differences in RAS actions in heart failure as well as diseases associated with vessel wall remodeling such as atherosclerosis and abdominal aortic aneurysm. While not a focus of this review, sex differences in the RAS have also been described in animal models and clinical populations for other CVD-related conditions including obesity, aging, and developmental programming [6–8].

## RAS Signaling Pathways in Cardiovascular Regulation

The RAS has been established as a critical contributor to blood pressure regulation, and the pathogenesis of CVD, for over a century. This system consists of a series of enzymes and bioactive peptides regulating cardiovascular function, with a simplified overview provided in Figure 1. In the canonical RAS, the enzyme renin (an aspartyl protease) is secreted from renal juxtaglomerular cells in response to various stimuli such as decreased perfusion in the afferent renal arterioles, locally acting prostanoids and NO, increased sympathetic activity, or low sodium chloride concentration in the macula densa [9]. Renin cleaves angiotensinogen to produce Ang I, which is hydrolyzed by Ang-converting enzyme (ACE) to form Ang II, the main effector of the canonical pathway [10]. Ang II acts at cell surface type I G protein-coupled receptors (AT<sub>1</sub>R) to elevate blood pressure via multiple mechanisms including vasoconstriction, sodium reabsorption, sympathetic and immune activation, impairment of arterial baroreceptor reflex sensitivity, and increases in aldosterone, oxidative stress, fibrosis, and inflammation [10]. Uncontrolled Ang II activation is a contributing factor to hypertension as well as cardiac and vascular dysfunction and fibrosis, to promote development of CVD [10]. To counteract AT<sub>1</sub>R actions, Ang II binds cell surface type II receptors (AT<sub>2</sub>R) to increase arterial baroreflex sensitivity and promote vasodilation, natriuresis, and NO production [11]. The precise mechanisms responsible for AT<sub>2</sub>R actions are incompletely understood, however, with activity of this receptor limited in adults due to low affinity and tissue expression [11].

More recently, a noncanonical counter-regulatory arm of the RAS has been discovered, which is characterized by the biologically active heptapeptide Ang-(1–7) [12]. As shown in Figure 1, Ang-(1–7) results from degradation of Ang I by endopeptidases (e.g., neprilysin, prolyl oligopeptidase, thimet oligopeptidase), or degradation of Ang II by carboxypeptidases such as ACE2. Ang I can also be converted to Ang-(1–9) by ACE2, which is then subsequently cleaved by ACE or neprilysin to form Ang-(1–7); however, this formation pathway has lower catalytic efficiency [13]. Ang-(1–7) elicits physiological effects by binding Mas G protein-coupled receptors (MasR), which are found in tissues pivotal to cardiovascular control including the heart, vasculature, kidneys, and brain [12]. The actions of Ang-(1–7) at cell surface MasR promotes vasodilation, NO release, and improved arterial baroreflex sensitivity [12]. Ang-(1–7) also has antihypertensive, sympathoinhibitory, antihypertrophic, antifibrotic, antiarrhythmogenic, and antithrombotic properties in animal models of CVD [13,12]. While most evidence suggests that *in vivo* actions of Ang-(1–7) require MasR, a few recent studies have shown potential heterodimerization and functional interactions between MasR and AT<sub>1</sub>R or AT<sub>2</sub>R [13]. Overall, Ang-(1–7)-ACE2-MasR pathways generally opposes the deleterious cardiovascular actions elicited by activation of the Ang II-ACE-AT<sub>1</sub>R axis.

As recently described [6], in addition to these Ang II and Ang-(1–7) pathways, research continues to reveal further layers of complexity in the RAS with more recent discoveries including: the renin-independent precursor angiotensin-(1–12); prorenin, and the prorenin receptor; localization of RAS components in tissues; intracellular RAS components; ACE-independent enzymatic pathways for Ang II formation; and binding of the endogenous heptapeptide alamandine to mas-related G protein-coupled receptor D to elicit

cardiovascular actions similar to Ang-(1-7). These alternate pathways will not be a focus of this review, as there is currently limited or non-existent data related to sex differences in cardiovascular outcomes.

## Effects of Sex Hormones on RAS Pathways

Accumulating evidence exists for profound effects of gonadal hormones such as estrogen and testosterone (as well as hormone replacement therapies) on expression and activity of RAS components, to contribute to sex differences in cardiovascular outcomes and pathophysiology. An overview of interactions of the RAS with gonadal hormones for cardiovascular control is described below, with findings for estrogen and testosterone summarized in Figure 2.

### RAS-Estrogen Interactions:

Estrogen is primarily produced by theca and granulosa cells in the ovaries, but can also be synthesized in extragonadal tissues such as adipose, brain, heart and vasculature [14]. The majority of circulating estrogen is in the form of  $17\beta$ -estradiol, but other naturally occurring and less potent estrogens include estrone and estriol. Levels of circulating angiotensinogen are generally higher in premenopausal women compared to men. This trend has a biological age feature with postmenopausal women having lower angiotensinogen levels compared with premenopausal women [5,15]. Oral estrogen replacement increases angiotensinogen secretion in postmenopausal women, irrespective of hypertensive status [16,5]. These overall findings suggest that estrogen increases synthesis of angiotensinogen, which is likely due to the presence of an estrogen response element in its gene promoter [5,15].

While increasing angiotensinogen production, most studies support that estrogen reduces plasma renin concentrations. This reflects, in part, estrogen-mediated reductions in sympathetic activity, to suppress renin secretion from renal juxtaglomerular cells [5]. For example, plasma renin concentration is lower in premenopausal women and postmenopausal women on estrogen replacement therapy when compared to either postmenopausal women without hormonal therapy or men [17,18]. Estrogen replacement can increase plasma renin activity in postmenopausal women, however, perhaps reflecting more angiotensinogen availability [15]. In addition, oral versus transdermal estrogen replacement can produce similar or differential effects on renin levels in postmenopausal women [15,5]. Despite this, estrogen consistently reduces serum and tissue ACE expression and activity,  $AT_1R$  expression and signaling in tissues (e.g. kidney, adrenal cortex, vasculature), and aldosterone production in animal models [19,5,15]. The ability of estrogen to downregulate  $AT_1R$  may result from direct interference with gene transcription via enhanced NO production as well as with translation by blocking ribosomal scanning [20,21].

There are varying effects of estrogen replacement on Ang II levels with one study showing a decrease in young female hypertensive rats, and another showing an increase in ovariectomized female rats [22,23]. A decrease in Ang II levels with estrogen is thought to result from reduced formation due to suppression of renin and ACE activity [5]. Despite controversy for modifying hormone levels, pressor responses to Ang II are attenuated following estrogen replacement in young female hypertensive rodents [15]. Furthermore,

treatment with  $17\beta$ -estradiol inhibits Ang II-induced cellular proliferation and oxidative stress in cardiac fibroblasts obtained from neonatal rats [24]. Clinically, oral estrogen replacement increases plasma renin activity, decreases circulating ACE, and increases Ang II in postmenopausal women, with no impact of transdermal estrogen replacement on these RAS components [15,5,25]. These divergent effects likely reflect that transdermal administration avoids hepatic metabolism (first pass effect), which can produce non-physiological elevations in estrone as well as renin substrate to increase Ang II formation [25]. Overall, while some conflicting evidence exists depending on route of administration, estrogen is generally accepted to reduce activation of Ang II-ACE-AT<sub>1</sub>R pathways [15,5].

In addition to inhibiting Ang II pathways, evidence from preclinical models indicates that estrogen upregulates protective Ang-(1-7)-ACE2-MasR-AT<sub>2</sub>R pathways (Figure 2). Circulating Ang-(1-7) levels are higher in obese young female mice and healthy premenopausal women when compared with their male counterparts [26,27]. In human endothelial cells, estradiol increases local Ang-(1-7) production through an estrogen receptor  $\alpha$  (ER $\alpha$ ) dependent mechanism [28]. Similarly, estrogen increases ACE2 gene expression in cultured 3T3-L1 adipocytes [26], and increases ACE2 and MasR expression in human atrial tissue from elderly men [29]. Administration of  $17\beta$ -estradiol to young female ovariectomized rats lowers blood pressure and attenuates Ang II-induced pressor responses, effects associated with reduced plasma ACE and Ang II and increased Ang-(1-7) levels [22]. In young obese female mice, loss of estrogen via ovariectomy increases resting blood pressure and decreases adipose ACE2 and plasma Ang-(1-7) levels [26]. Furthermore, young female rats are protected from renovascular hypertension, due to an estrogen-regulated increase in intratubular Ang-(1-7)-ACE2-MasR pathways [30]. Estrogen also upregulates AT<sub>2</sub>R expression and binding in cardiovascular tissues from young male and female rats [31,32], and reciprocally AT<sub>2</sub>R agonism increases estrogen production in ovaries from young female rabbits [33]. These protective estrogen-RAS interactions appear diminished during aging. A recent study showed a decline in circulating Ang-(1-7) levels during aging in clinical populations, which was particularly evident in overweight and obese individuals [34]. Furthermore, loss of vasodilatory responses to Ang-(1-7) is observed in older female mice, with vascular responsiveness and NO levels restored following estradiol replacement [35].

Overall, estrogen provides cardioprotection in premenopausal females by shifting the balance of the RAS towards Ang-(1-7)-ACE2-MasR-AT<sub>2</sub>R pathways. The increased production of Ang-(1-7) with estrogen is mediated by ER $\alpha$  to increase ACE2 activity and expression in a concentration-dependent manner [36]. In addition, estrogen promotes NO-dependent vasodilation through a mechanism involving crosstalk with MasR [36]. In premenopausal women, loss of estrogen to modulate these protective RAS pathways may contribute to increased cardiovascular risk in this population [4]. The precise mechanisms by which estrogen interacts with the RAS to induce cardioprotective properties are still incompletely understood, but appear dependent on the type of estrogen administered, the experimental context, and use of preclinical versus clinical models.

## RAS-Testosterone Interactions

In young male rats, renal and hepatic angiotensinogen gene expression are increased following testosterone administration, and conversely decreased following castration [5,37]. Testosterone administration to either castrated male or ovariectomized female rodents increases plasma renin activity suggesting a positive regulatory relationship [5,15]. Conversely, anti-androgen therapies decrease plasma renin in young male and female hypertensive rats [38,39]. Testosterone exposure also increases ACE activity and tissue AT<sub>1</sub>R expression and plays an essential role in the development and maintenance of Ang-II induced vascular dysfunction, hypertension, and cardiac hypertrophy in pregnant rats and in young male mice and rats [40–42]. Ang-II induced hypertensive and vasoconstrictor responses are attenuated in young castrated male rats and associated with a reduced vascular AT<sub>1</sub>R/AT<sub>2</sub>R ratio, with effects reversed by testosterone replacement [43]. Thus, in contrast to estrogen, sex-specific differences in CVD pathophysiology are amplified by androgens such as testosterone through stimulation of the Ang II-ACE-AT<sub>1</sub>R axis (Figure 2).

While increasing Ang II pathways, much less is known about the effects of testosterone on counter-regulatory pathways of the RAS. Testosterone downregulates AT<sub>2</sub>R expression in aorta from young male and female rats [44], with no effect on ACE2 expression in cultured 3T3-L1 adipocytes or testicular ACE2 activity in young male rats [26,45]. Conversely, AT<sub>2</sub>R expression is increased in young male castrated rats with heart failure, with effects prevented by testosterone replacement [46]. Taken together, testosterone shifts the balance of the RAS towards Ang II-ACE-AT<sub>1</sub>R pathways to induce vasoconstriction, vascular dysfunction, and cardiac hypertrophy and fibrosis. Further research is needed to better understand how testosterone, and anti-androgen therapies, interacts with protective Ang-(1–7)-ACE2-MasR-AT<sub>2</sub>R pathways to influence cardiovascular control.

## RAS Interactions with Other Sex-Related Hormones

In addition to estrogen and testosterone, there is some evidence the RAS interacts with other sex-related hormones for cardiovascular control. Progesterone is a hormone primarily produced by the ovaries, which plays a critical role in reproductive functions including the menstrual cycle, pregnancy, and embryonic development. Progesterone receptors are also widely expressed in the vasculature and can promote either vasodilation or vasoconstriction in humans depending on the vascular bed studied, dose administered, and if co-administered with estrogen [47]. Premenopausal women taking oral contraceptives containing both estrogen and progesterone have elevated circulating Ang II levels when compared with premenopausal women not taking oral contraceptives and men [48,49]. Progesterone increases AT<sub>1</sub>R gene and protein expression in vascular smooth muscle cells from thoracic aorta of young female rats [50], and increases AT<sub>1</sub>R binding in the arcuate nucleus of the hypothalamus in young ovariectomized female rats [51,52]. Despite potentially elevating Ang II and AT<sub>1</sub>R levels, progesterone does not appear to functionally affect pressor responses to Ang II in women [53]. Ang II can conversely produce dose- and time-dependent stimulatory effects on progesterone secretion in human granulosa and rat zona glomerulosa cells [54,55].

Progesterone is also a competitive inhibitor of the mineralocorticoid receptor, resulting in increased compensatory aldosterone production to maintain sodium homeostasis [56]. In support of this concept, aldosterone is higher in the luteal phase in cycling women in high-sodium balance, and progesterone increases aldosterone production in isolated rat zona glomerulosa cells [57]. This ability of progesterone to increase adrenal aldosterone production appears independent of changes in plasma renin activity or Ang II levels suggesting RAS-independent mechanisms [57,58]. More recently, it has been shown that progesterone upregulates endothelial mineralocorticoid receptor expression to contribute to leptin-induced endothelial dysfunction in young obese female mice [59]. Overall, progesterone appears to increase Ang II and aldosterone pathways, although there are limited data on the functional importance of these interactions for cardiovascular outcomes.

Much less is known about interactions of progesterone with Ang-(1–7) pathways. Ang-(1–7) attenuates the ability of Ang II to increase progesterone secretion in rat zona glomerulosa cells [55]. In addition, female rats lacking either estradiol or progesterone have decreased Ang-(1–7) levels and MasR gene expression in the cerebral cortex and hippocampus [60]. While less studied, Ang II interactions with relaxin and oxytocin have also been reported in rat models of cardiac fibrosis and hypertension [61,62]. Given this limited research, studies are needed to determine how these additional sex hormones interact with RAS components to contribute to cardiovascular control, with a major gap in knowledge existing related to their influence on cardioprotective Ang-(1–7) pathways.

## Sex Differences in Cardiovascular Actions of the RAS

The development and progression of CVD are enhanced by uncontrolled RAS activation and display sex-specific features. While blood pressure and cardiovascular end organ function are controlled by the same intrinsic mechanisms in males and females, differential activation of the vasoconstrictor versus vasodilatory arms of the RAS contributes to sex differences in cardiovascular control [4]. In males, activation of Ang II-ACE-AT<sub>1</sub>R pathways is well recognized to contribute to the etiology of CVD. In contrast, premenopausal women and animal models are generally protected from cardiovascular complications due to upregulation of counter-regulatory Ang-(1–7)-ACE2-MasR-AT<sub>2</sub>R pathways. This protection is diminished in postmenopausal women and ovariectomized animal models following loss of estrogen, with evidence for decreased circulating Ang-(1–7) levels as well as reduced responsiveness to the vasodilatory effects of exogenous Ang-(1–7) administration with aging to increase cardiovascular risk (Figure 3) [35,34]. Importantly, this differential RAS activation is multifaceted and affects cardiovascular control at multiple levels resulting in sex differences in CVD presentation, pathophysiology, and outcomes. This section will describe known sex differences in cardiovascular actions of RAS components, with a focus on hypertension, atherosclerosis, aneurysm formation, and heart failure.

## Blood Pressure Regulation and Hypertension

Hypertension affects over 85 million adults in the United States alone, and is a leading predictor for cardiovascular morbidity and mortality [1]. Sex differences in hypertension are well recognized, with premenopausal women generally having lower incidence and severity

of hypertension compared with age-matched men [4]. This protection declines after menopause with percentage of hypertensive women outweighing the number of men in elderly populations [4,3]. Hypertensive women are also more likely to develop vascular and myocardial stiffness, as well as isolated systolic hypertension, during aging when compared with men [3]. These sex differences in hypertension have been, in part, attributed to differential levels and activity of RAS components in males versus females.

The main precursor of the RAS, angiotensinogen, is primarily produced in liver but is also expressed in cardiovascular-regulatory tissues such as heart, kidney, vasculature, and adipose [5]. A handful of recent studies have focused on adipose tissue, as adipose-derived angiotensinogen is reported to contribute to up to 30% of total levels in mice. In young male mice, adipose over-expression of angiotensinogen elicits hypertension, whereas adipose-specific deletion lowers resting blood pressure and attenuates development of obesity hypertension [63,64]. Similar to males, adipose-specific deletion of angiotensinogen lowers resting blood pressure in both younger and older female mice [65]. As recently described [6], sex differences have been reported in stimuli influencing renin release, with females having reduced sympathetic activation, increased renal nitric oxide synthesis, and a differential pattern of renal transporters. Despite this, young male and female rats with global deletion of the renin gene exhibit similar reductions in resting blood pressure [66]. In addition, direct renin inhibition with aliskiren similarly lowers blood pressure in men and women with mild-to-moderate hypertension [67]. Adipose deletion of the prorenin receptor also exacerbates obesity hypertension in young female mice via an intra-renal Ang II-dependent mechanism [68]. Therefore, there does not appear to be major sex differences in the blood pressure regulatory actions of angiotensinogen and renin, particularly when targeting these components in adipose tissue.

In terms of Ang II pathways, elevated ACE activity is observed in the circulation, heart, and kidney of young male hypertensive rodents [5]. In humans, serum ACE activity is generally higher in men versus women, but declines during healthy aging in men [7]. In young mice, global ACE knockout lowers blood pressure in males but not females, despite similar reductions in serum ACE activity between sexes [69]. While plasma and tissue Ang II is higher in young male rodents compared with females, consistent sex differences for circulating levels of this hormone have not been observed clinically [5]. Despite this, males exhibit greater sensitivity to Ang II-induced cardiovascular effects in both animal models and clinical populations. One study showed that Ang II produces greater pressor and renal vasoconstrictor responses in healthy young men versus women [70]. Chronic Ang II infusion results in a greater pressor response in young male compared with female rodents, and appears androgen-dependent and associated with increased sympathetic activation as well as higher AT<sub>1</sub>R and lower AT<sub>2</sub>R gene expression in the vasculature and kidneys [71–74]. This protection from hypertension in female rodents appears mediated by AT<sub>2</sub>R as well as estrogen actions at centrally expressed ER $\alpha$  to inhibit Ang II-induced sympathetic activation and oxidative stress [75–77]. Similarly, young female rats are protected from Ang II-induced sensitization of hypertension via central estrogenic mechanisms [78]. Furthermore, obesity hypertension in young male mice is associated with increased circulating Ang II levels and is mediated by AT<sub>1</sub>R activation [26]. In contrast, young female mice do not exhibit elevated Ang II and are protected from obesity hypertension [26]. Similarly, the elevated blood



pressure in young male fructose fed rats is associated with increased cardiac ACE and AT<sub>1</sub>R expression, with females protected from this cardiovascular phenotype [79]. As a potential mechanism underlying these sex differences, recent studies have shown that chronic Ang II infusion increases renal pro-inflammatory T cells in young male rats, while increasing anti-inflammatory T regulatory cells in females [80]. These data suggest females are protected from hypertension resulting from activation of Ang II-ACE-AT<sub>1</sub>R pathways of the RAS.

In clinical populations, a few studies have shown that plasma and urinary Ang-(1-7) levels are higher in young healthy women versus men, and positively correlate with blood pressure only in women [27,81]. Another study, however, showed higher plasma Ang-(1-7) in young healthy men [82]. These disparate findings could reflect heterogeneity in blood sample collection and patient demographics. In addition, no differences in Ang-(1-7)-forming enzyme activities (e.g. ACE2, neprilysin) are observed between men and women during healthy aging [7]. In young hypertensive rats, circulating and renal Ang-(1-7) are higher in females versus males [83]. Similarly, young obese female mice have higher circulating Ang-(1-7) and adipose ACE2 and are protected from hypertension [26]. This protection from obesity hypertension in female mice is mediated by endogenous Ang-(1-7) actions at MasR and interactions with estrogen [26]. In addition, the lower blood pressure and increased Ang-(1-7) levels in young female hypertensive rats is associated with more anti-inflammatory T regulatory cells and cytokines compared with males [84].

In addition to elevated Ang-(1-7) levels, endogenous activation of ACE2, MasR and AT<sub>2</sub>R induces cardioprotective effects, particularly in females. Endogenous brain Ang-(1-7) pathways are upregulated via estrogen -dependent mechanisms to protect against hypertension development in young female rats [85]. Adipocyte ACE2 deficiency increases resting blood pressure as well as pressor responses to Ang II in young obese female but not male mice [86]. In Chinese individuals with untreated essential hypertension, the ACE2 alleles rs2074192 and rs2106809 are associated with reduced circulating Ang-(1-7) levels, which correlates with increased blood pressure only in women [87]. Furthermore, global MasR deletion in young mice increases sympathetic tone in both sexes, but only reduces heart rate variability in females [88]. In young obese female mice, global MasR deletion increases blood pressure compared to lean within genotype controls, suggesting endogenous MasR protect females from obesity-related hypertension [89]. In contrast to females, young obese male MasR knockout mice have reduced blood pressure, which is attributed to chronically elevated Ang II levels to impair cardiac function. The depressor and natriuretic effects elicited by activation of AT<sub>2</sub>R are also greater in females, with levels of this receptor modulated by estrogen [90]. These overall findings suggest that in females, activation of Ang-(1-7)-ACE2-MasR-AT<sub>2</sub>R pathways promotes blood pressure lowering to protect against development and progression of hypertension.

### **Vessel wall remodeling**

Changes in vessel wall structure are implicated in numerous CVD including atherosclerosis, abdominal aortic aneurysm (AAA), myocardial infarction, and peripheral arterial disease. Sex differences in these diseases are established, with men dying from atherosclerosis and myocardial infarction at a younger age compared with women [3]. The estrogenic effect in

the vasculature is claimed as one of the main reasons for this beneficial cardiovascular profile in premenopausal women, with more women developing atherosclerosis following menopause [91]. In addition, there is a 4- to 5-fold higher incidence of AAA in men compared to age-matched women, as well as higher incidence in males in the Ang II-infused mouse model of AAA [92]. Interactions of the RAS with testosterone are implicated in these sex differences in aneurysm formation. Testosterone upregulates ACE activity, with older healthy men having lower ACE activity compared with younger healthy men [7]. Furthermore, the higher prevalence of AAA in Ang II-infused male mice is associated with androgen-mediated upregulation of AT<sub>1</sub>R in the abdominal aorta [93]. Despite occurring less frequently, postmenopausal women exhibit rapid growth rates of AAA and rupture at smaller sizes [94]. This has been attributed to sex hormones, with exogenous estrogen administration reducing progression and severity of Ang II-induced AAA in ovariectomized female mice [95]. Similar to males, AT<sub>1</sub>R blockade attenuates Ang II-induced AAA formation in young hypercholesterolemic female mice [96].

Sex differences in protective RAS pathways in diseases associated with vessel wall remodeling are less understood. In severe coronary atherosclerosis, there is a high Ang II to Ang-(1-7) ratio in both sexes, which may increase vascular proliferation and inflammation to promote plaque development [97]. In young and middle aged male hypercholesterolemic mice, global knockout of either ACE2 or MasR exacerbates Ang II-induced atherosclerosis and aortic aneurysm ruptures, and conversely pharmacologic ACE2 activation reduces incidence of AAA [98,99], with no information in females. In human brain vascular smooth muscle cells and young mice, Ang-(1-7) counteracts Ang-II induced vascular remodeling by attenuating proinflammatory pathways [100]. In young female hypercholesterolemic mice, AT<sub>2</sub>R antagonism increases incidence and severity of AAA and atherosclerosis, providing evidence for a protective role of AT<sub>2</sub>R against Ang II-mediated vascular pathophysiology [96]. Thus, while some evidence exists for a protective role of Ang-(1-7)-ACE2-MasR-AT<sub>2</sub>R pathways against atherosclerosis and AAA, sex differences in these mechanisms have yet to be defined. Further, while emerging literature suggests a potential role for sex chromosomes to regulate atherosclerosis and AAA development [92], whether this involves interactions with the RAS is unclear. Given these known sex disparities, guidelines were recently established for the design and reporting of sex as a biological variable in preclinical studies on atherosclerosis, aneurysms, and peripheral arterial disease [92].

### Heart failure

Emerging evidence suggests the epidemiology and clinical characteristics of heart failure, as well as response to heart failure therapies including those inhibiting the RAS, are sex dependent. The RAS is a major contributor to heart failure with chronic Ang II exposure promoting cardiac hypertrophy via activation of growth factors and increased mitochondrial oxidative stress [101]. In addition, Ang II-mediated activation of AT<sub>1</sub>R induces structural remodeling of the ventricular wall including proliferation of cardiac fibroblasts, deposition of extracellular matrix proteins such as collagen, and hypertrophic growth of cardiomyocytes [101]. Indeed, cardiac Ang II levels increase with age and are linked to hypertrophy, fibrosis, and diastolic dysfunction [102]. Sex differences in RAS mechanisms involved in cardiac dysfunction have been reported, particularly related to aging and heart

failure. In a mouse model of chronically elevated cardiac Ang II, age-related deterioration of cardiomyocyte function is sex-dependent with females more susceptible to contractile deficits but having low spontaneous activity, and males maintaining contractile function but having desensitization of myofilaments to calcium and heightened vulnerability to arrhythmias [102]. Over-expression of AT<sub>1</sub>R in cardiomyocytes results in heart failure in both male and female mice, but with females having increased susceptibility to dilated cardiomyopathy and higher mortality following cardiac Ang II overstimulation [103]. In addition, young female rats at the onset of the decompensated phase of congestive heart failure exhibit higher mortality, associated with increased renal vascular responsiveness to norepinephrine and Ang II [104]. These findings suggest female rodents are more sensitive to Ang II in terms of susceptibility to heart failure, although the precise mechanisms underlying this finding are not fully understood. This is consistent with clinical findings showing that women are more susceptible to developing heart failure in response to hypertension compared with men [105].

Clinically, pressure overload-induced hypertrophy is associated with a smaller left ventricular chamber and larger wall thickness in women than in men [106,107]. These findings are in agreement with animal studies showing that female mice develop concentric myocardial hypertrophy whereas males develop eccentric myocardial hypertrophy [3]. This may be explained by a reduced number of ventricular myocytes with age via apoptosis in men but not women [108]. These findings could help explain why older men are more likely than age-matched women to experience heart failure with reduced ejection fraction [102]. Interestingly, hypertensive women do not respond as well to long-term RAS inhibition with the angiotensin receptor blocker (ARB) losartan in terms of left ventricular structure and function when compared to men [109]. Despite this, ARBs appear more effective to improve survival in women with congestive heart failure compared with ACE inhibitors irrespective of hypertensive status [110]. These collective data suggest that prolonged activation of Ang II pathways plays a pathophysiological role in cardiac remodeling, and that sex differences exist in terms of susceptibility for developing myocardial hypertrophy and heart failure as well as responsiveness to therapies targeting Ang II for treatment of hypertensive cardiac abnormalities.

In contrast to Ang II, emerging evidence suggests that activation of non-canonical RAS pathways involving Ang-(1-7) provides protection against heart failure [111,112]. In support of this, in rodent models: Ang-(1-7) prevents Ang II-induced cardiac remodeling in young male rats; the MasR agonist AVE-0991 attenuates myocardial infarction-induced heart failure in young male rats; and genetic deletion of ACE2 accelerates hypertension-induced cardiac dysfunction in young male mice [111-113]. Unfortunately, these preclinical studies showing protective effects of Ang-(1-7) pathways in heart failure have been limited to males. In heart failure patients, ACE2 is upregulated in the myocardium and associated with the degree of left ventricular ejection fraction in both men and women [114]. This ACE2 upregulation is suggested as an adaptive compensatory mechanism to prevent myocardial remodeling in both sexes. While similar cardiac ACE2 has been observed, a recent study suggests plasma ACE2 concentrations are higher in men versus women with heart failure, and not associated with ACE inhibitor or ARB treatment [115]. Further research is needed to determine the importance of these sex differences in ACE2 levels to outcomes, as well as the

effectiveness of targeting Ang II versus Ang-(1-7) pathways in men versus women, in heart failure.

## Sex Differences in Cardiovascular Therapeutic Formulations Targeting the RAS

Despite well-established sex differences in CVD, preclinical and clinical research efforts have largely focused on males. Even with recent efforts to include females equally in CVD research, women still only represent ~37% of study participants in controlled NIH-funded trials, with only 35% of published cardiovascular clinical studies analyzing outcomes according to sex [116]. As a result, sex-specific guidelines have not been developed for hypertension or CVD treatment, including for therapies targeting the RAS. The current knowledge of sex differences in cardiovascular outcomes for RAS therapies is shown in Table 1.

RAS inhibition has been one of the most common treatment approaches for CVD for decades. Pharmacological agents such as direct renin inhibitors, ACE inhibitors, ARBs, and mineralocorticoid antagonists have helped to convert what was once a lethal condition into a chronic one. These therapies all elicit antihypertensive, antifibrotic, and anti-remodeling effects and are generally safe and efficacious across diverse clinical populations. For direct renin inhibition, a retrospective meta-analysis of eight clinical trials showed no differences in blood pressure lowering efficacy of aliskiren in hypertensive men versus women [67]. In contrast, evidence suggests potential dimorphic responses to therapeutics blocking Ang II activity based on sex. In 2008, a systematic review was published on sex-based analysis of controlled clinical trials using ACE inhibitors or ARBs for hypertension treatment [117]. Sex-specific results were only published in 43% of the trials included, with data suggesting both of these therapies are slightly more effective in men versus women. Additional literature, however, suggests women respond more favorably to ARBs in terms of cardiovascular outcomes, whereas ACE inhibitors appear more effective in men. Women treated with ACE inhibitors are more likely to develop adverse reactions such as cough and angioedema, with effectiveness potentially decreasing over time and offering less benefit for total mortality [118–120]. A lower dose of the ARB irbesartan is required for young healthy women to inhibit Ang-II mediated reduction in renal blood flow compared to men [121]. Women also have a greater antihypertensive response to treatment with the ARB valsartan combined with amlodipine, a calcium channel blocker, when compared with men treated with the same agents [122]. Another study described better outcomes in women with congestive heart failure treated with an ARB versus an ACE inhibitor, while no differences in outcomes were observed in men between these therapeutics [110]. Additionally, women appear more responsive to blockade of aldosterone actions with mineralocorticoid receptor antagonists. This is particularly evident in obese women where increased aldosterone correlates with visceral adiposity, body mass index, and blood pressure [123,124]. Women also appear to have greater salt sensitivity of blood pressure compared with men, irrespective of age or hypertensive status, due to increased adrenal aldosterone production [125]. These findings highlight emerging data for sex differences in therapeutic effectiveness to

established RAS therapies, with women potentially responding more favorably to ARBs and mineralocorticoid receptor antagonists.

In addition to blockade of Ang II pathways, direct targeting of Ang-(1–7) has been explored for treatment hypertension and CVD. Unfortunately, Ang-(1–7) has unfavorable pharmacokinetic properties including a short half-life [13]. Therefore, novel therapeutics are in development to increase Ang-(1–7) levels or actions such as oral formulations, stable analogs, MasR agonists, ACE2 activators, and recombinant human ACE2 [12]. These therapies reduce blood pressure and attenuate cardiovascular damage in animal models, making them attractive for clinical development [12]. There are limited published or ongoing trials targeting Ang-(1–7) pathways in humans, however, with none designed to identify sex-specific differences in cardiovascular outcomes. Importantly, targeting Ang-(1–7) pathways may provide advantage over use of ACE inhibitors and ARBs. ACE inhibitors are limited in up to 11% of patients by cough due to bradykinin production [126]. Furthermore, both ACE inhibitors and ARBs increase endogenous Ang-(1–7) generation, which contributes to the beneficial cardiovascular and metabolic effects of these therapies in young male rodent models of hypertension and obesity [127–129]. Direct targeting of Ang-(1–7) pathways may therefore provide cardiovascular benefit while avoiding potentially limiting side effects of therapies blocking Ang II activity. Overall, these limited data illustrate the critical need for additional preclinical and clinical studies to determine the impact of sex on cardiovascular effects of RAS inhibition, for both established therapies targeting Ang II pathways and more novel therapies targeting Ang-(1–7) pathways.

## Conclusions

The identification of sex-specific mechanisms by which the RAS influences cardiovascular control, including potential interactions with gonadal hormones, remains an active area of research. There are known sex differences in expression and activity of several RAS components, with estrogen upregulating protective Ang-(1–7)-ACE2-MasR-AT<sub>2</sub>R pathways [4]. On the other hand, testosterone favors activation of Ang-II-ACE-AT<sub>1</sub>R pathways, thus enhancing the vasoconstrictive arm of the RAS [15,5]. These variations in RAS activity can contribute to differential CVD clinical presentation, pathophysiology, and treatment responses and outcomes between men and women. The role of the RAS in regulating cardiovascular function also exhibits a biological age pattern, offering cardioprotection to premenopausal women with effects decreasing in menopause following loss of estrogen [35]. There remain several gaps in our knowledge of sex differences in RAS mechanisms regulating cardiovascular function that require future research, as summarized below and in Table 2. While interactions with estrogen and testosterone are well described, there are limited data on how other sex-related hormones (e.g. progesterone, relaxin, oxytocin) or newer forms of contraception influence RAS components and activity. Importantly, we still do not fully understand sex differences in therapies targeting the RAS, due to underrepresentation of females in clinical trials. Furthermore, therapeutic management of CVD is similar between men and women, despite initial evidence for sex-specific outcomes in therapies blocking Ang II activity. In particular, women may respond better to ARBs and tend to experience increased side effects with ACE inhibitors [120,118]. While targeting of Ang-(1–7) and other components of the vasodilatory arm of the RAS seems to be a

promising therapeutic target, there is currently a paucity of clinical data. Preclinical studies have shown that novel therapeutic formulations targeting Ang-(1–7) pathways are protective in CVD, but the cellular mechanisms underlying these effects are not fully understood [12]. Additionally, further studies are needed to determine the effects of Ang-(1–7) on cardiovascular outcomes in clinical populations with hypertension and CVD. Overall, there is crucial need for additional research to clarify the impact of sex-specific RAS differences on the cardiovascular system, to aid in the development of novel cardioprotective agents.

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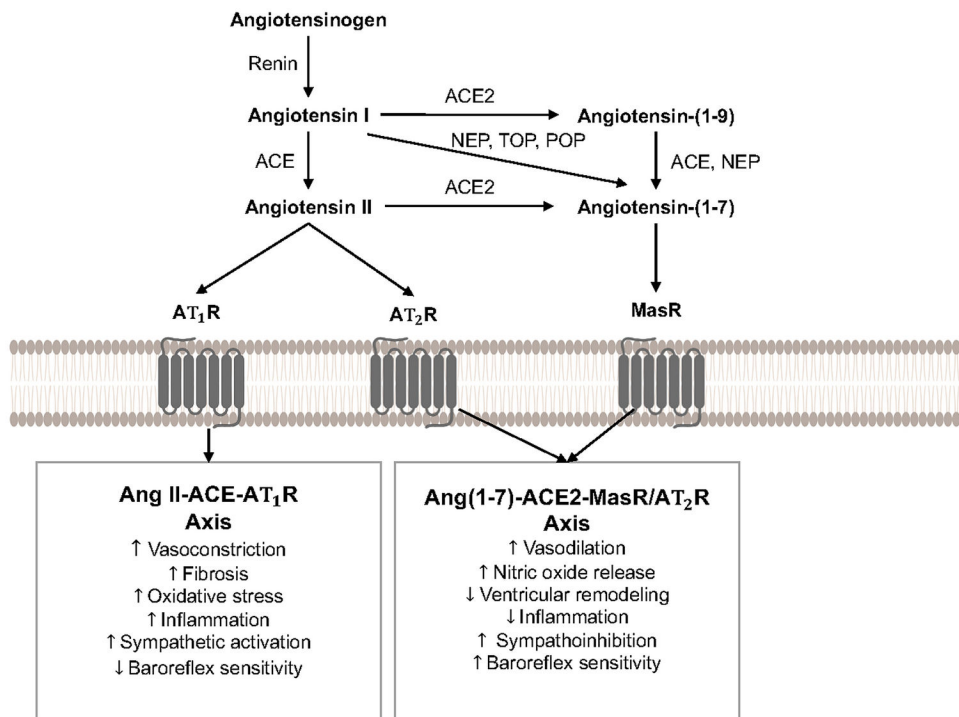
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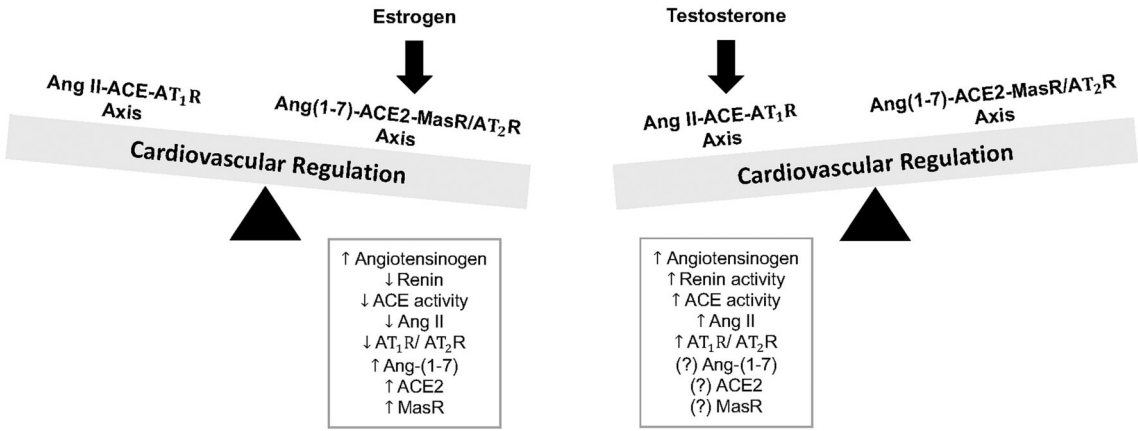
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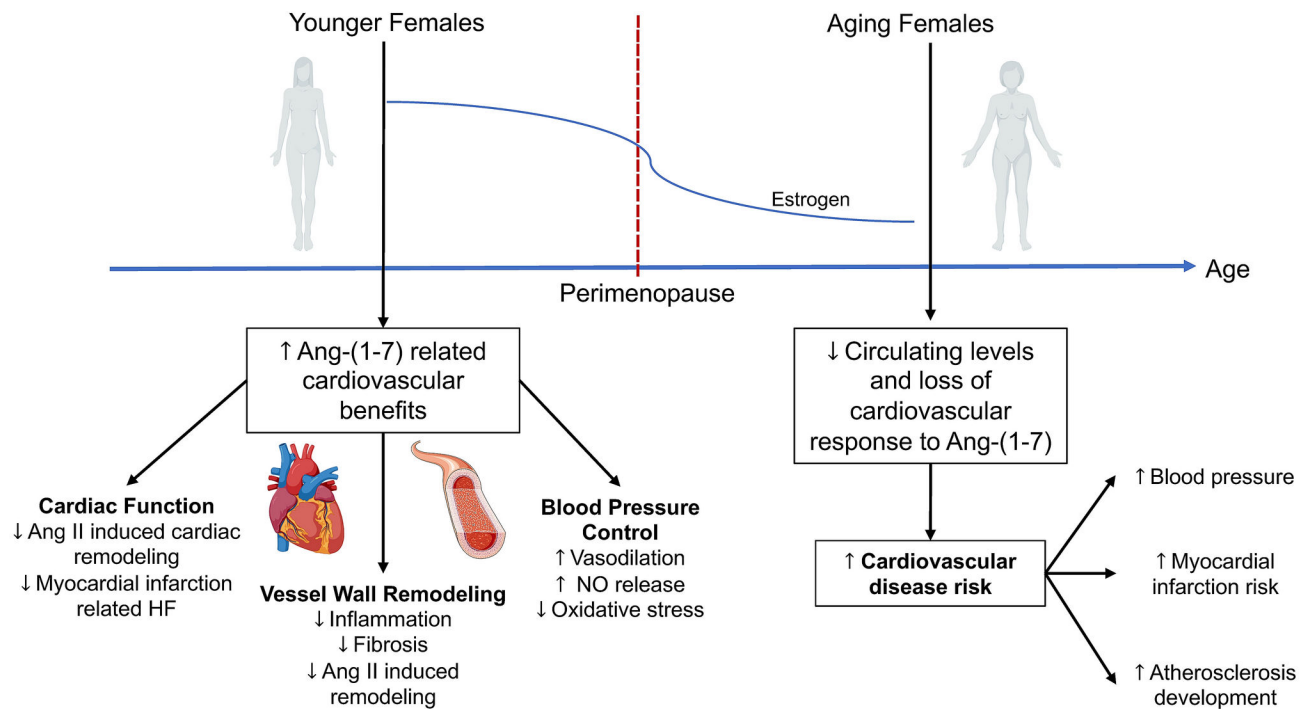


**Fig. 1.** Simplified overview of the renin-angiotensin system, including primary cardiovascular effects elicited by activation of angiotensin II and angiotensin-(1–7) signaling pathways. ACE, angiotensin converting enzyme; ACE2, angiotensin converting enzyme 2; Ang, angiotensin; AT<sub>1</sub>R, angiotensin II type 1 receptor; AT<sub>2</sub>R, angiotensin II type 2 receptor; MasR, angiotensin-(1–7) mas receptor; NEP, neprilysin; POP, prolyl oligopeptidase; TOP, thimet oligopeptidase.



**Fig. 2.** Interactions between sex hormones and components of the renin-angiotensin system for cardiovascular regulation. Estrogen shifts the balance of the renin-angiotensin system towards angiotensin-(1-7)-ACE2-mas receptor-AT<sub>2</sub> receptor pathways to elicit cardiovascular protection. In contrast, testosterone shifts the balance of the renin-angiotensin system towards angiotensin II-ACE-AT<sub>1</sub> receptor pathways to elicit deleterious cardiovascular actions. ACE, angiotensin converting enzyme; ACE2, angiotensin converting enzyme 2; Ang, angiotensin; AT<sub>1</sub>R, angiotensin II type 1 receptor; AT<sub>2</sub>R, angiotensin II type 2 receptor; MasR, mas receptor.





**Fig. 3.** Biological aging feature of angiotensin-(1-7) pathways and effects on the cardiovascular system. In premenopausal females, angiotensin-(1-7) elicits protective effects on cardiac function, vessel wall remodeling, and blood pressure control. These protective responses to angiotensin-(1-7) appear reduced in postmenopausal women following loss of estrogen, to potentially increase risk for developing cardiovascular diseases such as hypertension, myocardial infarction, and atherosclerosis. Ang, angiotensin; HF, heart failure; NO, nitric oxide.

**Table 1**

Sex-specific responses to established and novel pharmacological therapeutic formulations targeting the renin-angiotensin system in clinical populations.

Therapeutic Agent	Mechanism of Action	Sex-Specific Responses
ACE inhibitors	Decrease Ang II formation	Men demonstrate better blood pressure control [117], with women having increased susceptibility to side effects [119,120].
Angiotensin receptor blockers (ARBs)	Block Ang II effects at AT <sub>1</sub> R	Women exhibit greater antihypertensive effect and require lower drug dosage [121,122,110]. Improved survival in women with CHF when compared to ACE inhibitors [110].
Mineralocorticoid antagonists	Block aldosterone effects at mineralocorticoid receptors	More effective to lower blood pressure in women and in obesity-related hypertension [123,124].
Direct Renin Inhibitors	Antagonize renin to prevent downstream activation of the RAS	Similar blood pressure lowering efficacy in hypertensive men and women treated with aliskiren [67].
MasR Agonists	Increase activity of Ang-(1-7) mas receptors	Unknown
ACE2 Activators	Decrease Ang II and increase Ang-(1-7) levels	Unknown
Recombinant human ACE2	Decrease Ang II and increase Ang-(1-7) levels	Unknown

ACE, angiotensin converting enzyme; ACE2, angiotensin converting enzyme 2; Ang, angiotensin; AT<sub>1</sub>R, angiotensin II type 1 receptor; CHF, congestive heart failure; MasR, angiotensin-(1-7) mas receptor; RAS, renin-angiotensin system.

**Table 2**

Potential Questions for Future Research on Sex Differences in Cardiovascular Actions of the Renin-Angiotensin System (RAS)

<b>Effects of Sex Hormones on RAS Pathways</b>	
•	What are the precise cellular mechanisms by which estrogen and testosterone interact with the RAS to influence cardiovascular control?
•	How do other sex hormones (such as progesterone, relaxin, and oxytocin) interact with the RAS, and in particular with cardioprotective Ang-(1-7) pathways?
•	How do more modern contraceptive methods influence circulating RAS components?
<b>Sex Differences in Cardiovascular Actions of the RAS</b>	
•	Are there sex differences in the role of emerging RAS components in cardiovascular control, such as prorenin, alamandine, and tissue and intracellular components?
•	What are the cellular mechanisms underlying the cardioprotective effects of Ang-(1-7) in females?
•	What is the role of sex chromosomes and genetics?
•	Do sex differences in ACE2 levels correlate with outcomes in heart failure?
<b>Sex Differences in RAS Therapies</b>	
•	How do we improve underrepresentation of women in clinical trials for RAS inhibitors and other cardiovascular-related therapies?
•	Are current RAS therapies more effective in men versus women in terms of blood pressure control and end organ damage, and if so, what are the underlying mechanisms?
•	Should we have sex-specific guidelines for treatment with RAS inhibitors?
•	Are novel therapies targeting Ang-(1-7) pathways effective in clinical populations, and are there sex differences in responsiveness?