Polycystic ovary syndrome: Insights from pre-clinical research

Jane Reckelhoff, Noha Shawky, Damian Romero, and Licy Yanes Cardozo

Abstract:
Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive-aged women, affecting approximately 10%. PCOS is diagnosed by the presence of at least two of these three criteria: hyperandrogenemia, oligo- or anovulation and polycystic ovaries. The most common type (80%) of PCOS includes hyperandrogenemia. PCOS is also characterized by obesity or overweight (in 80% of US PCOS women), insulin resistance with elevated plasma insulin but not necessarily hyperglycemia, dyslipidemia, proteinuria, and elevated blood pressure. Although elevated compared to age-matched controls, the blood pressure may not reach levels considered treatable according to the current clinical hypertension guidelines. However, it is well known that elevated blood pressure, even modestly so, increases the risk of cardiovascular disease. We have developed a model of hyperandrogenemia in rodents that mimics the characteristics of PCOS in women, with increases in body weight, insulin resistance, dyslipidemia, proteinuria and elevated blood pressure. This review discusses potential mechanisms responsible for the elevated blood pressure in the adult and aging PCOS rat model that may be extrapolated to PCOS women.

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Polycystic ovary syndrome: Insights from pre-clinical research

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Abstract

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive-aged women, affecting approximately 10%. PCOS is diagnosed by the presence of at least two of these three criteria: hyperandrogenemia, oligo- or anovulation and polycystic ovaries. The most common type (80%) of PCOS includes hyperandrogenemia. PCOS is also characterized by obesity or overweight (in 80% of US PCOS women), insulin resistance with elevated plasma insulin but not necessarily hyperglycemia, dyslipidemia, proteinuria, and elevated blood pressure. Although elevated compared to age-matched controls, the blood pressure may not reach levels considered treatable according to the current clinical hypertension guidelines. However, it is well known that elevated blood pressure, even modestly so, increases the risk of cardiovascular disease. We have developed a model of hyperandrogenemia in rodents that mimics the characteristics of PCOS in women, with increases in body weight, insulin resistance, dyslipidemia, proteinuria and elevated blood pressure. This review discusses potential mechanisms responsible for the elevated blood pressure in the adult and aging PCOS rat model that may be extrapolated to PCOS women.
Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive-age women, affecting approximately 10% [1]. Based on the “Rotterdam Criteria” (2003) [2] and the Androgen Excess Society (2006) criteria for PCOS [3], two of three characteristics must be present for a diagnosis of PCOS: hyperandrogenemia, oligo- or anovulation, and polycystic ovaries. PCOS occurs as early as menarche, but is rarely diagnosed until women are in their 20s. In fact, it often takes more than 2 years of consultation with physicians to receive the diagnosis of PCOS [4]. One of the major reasons for PCOS women to seek healthcare is due to issues with fertility, and assisted reproduction is common in PCOS women [4].

In addition to fertility difficulties and hyperandrogenemia, PCOS women also exhibit hirsutism, depending on the level of androgens, and metabolic characteristics that are associated with cardiovascular disease (CVD), such as obesity, insulin resistance (even in the absence of obesity), type 2 diabetes mellitus, dyslipidemia, endothelial dysfunction and elevated blood pressure [5, 6]. The mechanisms responsible for development of PCOS are not clear. While a genetic component has been suggested, studies in daughters of PCOS women are not consistent as to whether they develop PCOS themselves or not and whether similar metabolic and hypertensive phenotypes exist in daughters, or even sons, of PCOS women [7-13].

Approximately 50% of PCOS women exhibit increased microalbuminuria [14-17] that was found to be closely associated with increased diastolic blood pressure and area under the curve for an oral glucose tolerance test. Unfortunately, there are minimal studies on kidney function in PCOS women. Gozukara and colleagues reported that PCOS women
do not develop overt glomerular hyperfiltration, yet their glomerular filtration rate (GFR) was significantly higher than controls [18]. The increased GFR was also positively correlated with uric acid. The same study also found that PCOS women have increased proteinuria [18]. There are several caveats regarding this study, however. The PCOS women included were young (approximately 25 years of age). In addition, GFR was not measured using tracer techniques, but was estimated from serum creatinine levels. Finally, the PCOS cohort was not overweight or obese (BMI<25) [18], which is inconsistent with the majority of PCOS women especially in the US.

In young PCOS women, blood pressure is typically only mildly elevated and often does not reach the level required for antihypertensive treatment according to the clinical guidelines [19]. Interestingly, meta-analyses studies show that blood pressure is elevated in PCOS women compared to controls prior to menopause (RR=1.7 fold), but after menopause, there are no differences in prevalence of hypertension [20], suggesting that blood pressure increases in control postmenopausal women just as in PCOS women [20]. However, it is well known that increases in blood pressure are associated with increased risk of CVD in the future [21], but whether PCOS women have a higher prevalence of CVD after menopause is not clear.

**Animal model of PCOS**

Over the past several years we have studied an animal model of PCOS that was first described by Manneras and colleagues [22]. The model uses female Sprague Dawley (SD) rats that are implanted with dihydrotestosterone pellets (DHT, 7.5 mg/90 days, Innovative Research, Inc.) beginning at 4 weeks of age; pellets are replaced every 85
days throughout the life of the rat [23]. This dose results in approximately 3-fold increase in circulating DHT [23], similar to androgen levels in women with PCOS [24]. DHT is used for the animal model since it cannot be converted to estradiol. Furthermore, this level of DHT is not sufficient to reduce the endogenous synthesis of testosterone and estradiol, such that estradiol levels are similar to age-matched, control female SD rats [23]. In the original studies by Manneras and colleagues, they compared the DHT-female model and compared it with the letrozole model of PCOS [22]. They found that the DHT-model more closely resembled the phenotypes found in PCOS women. However, they did not measure blood pressure or proteinuria in the models since these investigators were mainly interested in whether the model would replicate the reproductive issues [22]. As in women with PCOS, the DHT-treated female rats develop an increase in body weight due to an increase in food intake [22,23]. Early in the development of obesity in the DHT-treated rats, CT scans of adipose tissue show that there is an increase in subcutaneous adipose tissue [25] that is accompanied by increases in plasma leptin, insulin, area under the curve of an oral glucose tolerance test, and increases in plasma cholesterol [23]. The DHT-treated females remain normoglycemic, however. Similar to PCOS women, DHT-treated rats have fertility difficulties with 6-day estrus cycles rather than 4-day cycles [23], and only 50-60% become pregnant when they are placed with males, compared to control SD females who become pregnant 99% of the time [26-28]. As adults, the DHT-treated females also exhibit a modest (10 mm Hg) increase in blood pressure similar to the small increases in blood pressure in PCOS women [23,25]. Also, just as in PCOS women, the DHT-treated female rats develop proteinuria [23].
As noted above, while the DHT-female PCOS model is one of the most common, there are other animal models of PCOS. The letrozole PCOS model is produced by giving letrozole, a non-steroidal aromatase inhibitor, (given by oral gavage for at least 12 weeks [22]. Vaginal smears in the letrozole model show they are anovulatory, and they have cysts in the ovary, increased body weight, elevated testosterone levels, abnormal glucose and lipid metabolism, and insulin resistance [22,29,30]. Because the letrozole-treated rats or mice are anovulatory, this model cannot be used for PCOS reproductive studies. The dehydroepiandrosterone (DHEA) rat model of PCOS is developed using daily subcutaneous injects of the testosterone precursor [30,31]. The DHEA model exhibits increases in androgens and ovarian cysts along with abnormal estrous cycling [30,31], and thus is used to study the reproductive consequences of PCOS. However, the DHEA model does not exhibit any metabolic phenotype, no adiposity, no insulin resistance [30,31]. Finally, there are other models of PCOS produced in monkey and sheep female offspring by androgens or estrogens given to the dam during pregnancy, as noted in this review (32,33). These models have variable levels of insulin sensitivity and do not completely recapitulate all the symptoms of PCOS in women. Thus the DHT-treated female rat and mouse models of PCOS most closely resemble the majority of the symptoms in PCOS women and is the best in which to study the cardiovascular-renal and metabolic consequences of androgens in adult and aging females and the consequences of pregnancy on the DHT-treated dams throughout their pregnancy and as they age and on their offspring.
Mechanisms responsible for elevated blood pressure in PCOS women and the rat model

Role of obesity in PCOS women

Obesity is a frequent clinical finding in women with PCOS [34], and exacerbates the increases in BP in PCOS women. However, one should keep in mind that BP is also elevated in PCOS women with a normal BMI [35,36]. Obesity plays a major role in the clinical manifestations of the syndrome, and PCOS women commented in a recent online survey that difficulty in losing weight was their most common clinical concern [4]. Weight loss, the first-line therapeutic recommendation for PCOS women, is associated with improvements in infertility, insulin resistance, and hyperandrogenism [37,38]. However, whether weight loss in PCOS women decreases their BP remains unclear.

Two major fat depots were characterized in PCOS women; the subcutaneous adipose tissue (SAT), localized beneath the skin, and the visceral adipose tissue (VAT), which lines the internal organs. Computed tomography (CT), which clearly distinguishes fat from other tissues, allows the identification and quantification of VAT and SAT. When the SAT storage capacity is exceeded, either due to an inability to generate sufficient new adipocytes (hyperplasia) or an inability to further expand existing adipocytes (hypertrophy), adipose tissue begins to accumulate in the VAT. PCOS women have an expansion of both SAT and VAT even after adjusting for BMI. Epidemiological studies demonstrated that VAT accumulation is associated with insulin resistance in white women [39]. A recent study showed that PCOS women, independent of their BMI, had elevated
VAT compared to controls [40]. In the same study, VAT correlated positively with adverse cardiometabolic complications and elevated testosterone levels in PCOS women [40]. Associations between adipose tissue accumulation in specific depots and blood pressure regulation have been not studied in PCOS. Furthermore, the molecular mechanism(s) by which obesity exacerbates the cardiometabolic complications in PCOS remains poorly understood. Since androgens are also increased in VAT with obesity in non-PCOS women (41,42), it is probable that the molecular mechanisms responsible for cardiometabolic complications in PCOS are in part mediated by adipose tissue and androgens, although the mechanisms are as yet unclear. Finally, to date, effective and safe therapeutic options to treat the obesity-associated cardiometabolic consequences in PCOS are limited to exercise, weight loss and metformin to treat insulin resistance [43].

**Obesity and sympathetic activation in PCOS rat model**

As mentioned above, the DHT-treated rat model exhibits increased food intake and body weight, and CT scans show that SAT, but not VAT, is increased by 14-16 weeks of age [23]. By 12 months of age, CT scans show that both SAT and VAT (retroperitoneal depots) are increased in DHT-treated rats [23, 44]. Along with the increase in adipose tissue, plasma leptin levels, produced by adipose tissue, are also increased in DHT-treated females [23].

Since obesity is associated with sympathetic activation, the contribution of the sympathetic nervous system to the elevated blood pressure in DHT-treated rats was tested. Rats were given terazosin and propranolol to block the $\alpha_1$, and $\beta_{1,2}$-adrenergic receptors, and blood pressure decreased to a greater extent in DHT-treated females than
in controls, suggesting that the model did have sympathetic activation [25]. Renal
denervation also decreased the blood pressure in DHT-treated females, suggesting that
the renal nerves were involved in the elevated blood pressure [25].

The MC4R in POMC neurons is thought to be upregulated in response to increased levels
of leptin [45]. The increased MC4R then activates the sympathetic nervous system which
is manifested by increases in blood pressure or thermogenesis [45]. Because plasma
leptin is elevated in the DHT-treated rat model, the hypothesis was tested that the
melanocortin-3/4 receptor (MC4R) in pro-opiomelanocortin (POMC) neurons may
mediate the increased sympathetic activity in the PCOS model. Previous studies in
female SHR that exhibited increased sympathetic activation and reduction in blood
pressure in response to adrenergic blockade and renal denervation did not exhibit a
reduction in blood pressure in response to the MC4R blocker, SHU-9119 [45]. In contrast,
SHU-9119 reduced the blood pressure in the DHT-treated female PCOS model, and
interestingly, the protein expression of the MC4R in the hypothalamus was significantly
increased [25]. Furthermore, in the absence of an active MC4R in MC4R−− rats, DHT was
not able to increase blood pressure [25]. Taken together, the data strongly suggest that
activation of the MC4R does increase sympathetic activity that is responsible at least in
part for the elevated blood pressure in the DHT-treated model. The mechanism
responsible for the upregulation of the MC4R in the model is unclear; however, it could
be due to either increased leptin or DHT, neither of which are upregulated in SHR females
[46]. Whether the MC4R plays a role in mediating the elevated blood pressure in PCOS
women has not been investigated to our knowledge.
In summary, we hypothesize that sympathetic activation in the DHT-treated females is due to adipose-induced increases in leptin or to DHT-mediated upregulation of the MC4R in the POMC neurons that then stimulates the SNS, activates the adrenergic receptors and the renal nerves to cause an increase in blood pressure. It is possible also that the adipose tissue and/or sympathetic activation could upregulate the RAS as discussed below.

Role of the Renin-Angiotensin System (RAS) in PCOS

The RAS plays a major role in blood pressure regulation, fluid homeostasis, and metabolism that was extensively reviewed by Ferrario and colleagues [47]. In the RAS system, the substrate angiotensinogen is hydrolyzed by renin to angiotensin (Ang) I which in turn is converted to Ang II by the Ang I converting enzyme (ACE). Ang II binds and activates the Ang II type 1 receptor (AT1R) to elicit its bioactivity, thus making up the vasoconstrictor arm of the RAS. Ang II can also bind the AT2R or the ACE2 enzyme to generate the vasodilator Ang(1-7) that binds and activates the MAS receptor, making up the vasodilator arm of the RAS.

Young women with PCOS have increased plasma renin levels and activity [48, 49] and increased plasma prorenin [50]. Similarly, PCOS women have higher levels of ACE and Ang II, compared to control subjects. ACE gene polymorphism is also associated with increased metabolic comorbidities in PCOS women [51,52, ]. Furthermore, there is evidence that AT1R antagonists (ARBs) and AT2R activation normalize androgen levels in PCOS women, although the studies have small numbers of participants [53,54] and the mechanisms responsible for how ARBs or AT2R activation could change androgen levels
are not clear. Quigley and colleagues reported several years ago that losartan blocks the androgen-mediated increases in proximal tubule sodium reabsorption [55]. As expected, a similar ARB to losartan, Telmisartan, significantly reduced elevated blood pressure in PCOS women [54].

Angiotensinogen is highly expressed in adipose tissue and is constitutively secreted by mature adipocytes from separate adipose depots in animal models and humans [56]. The concentration of androgens in adipose tissue from non-PCOS women is several-fold higher than in plasma [42]. Overexpression of angiotensinogen in adipose tissue increases adiposity and blood pressure in transgenic mice [57]; therefore, it is possible that an elevated level of androgens in adipose tissue in PCOS women activates the intra-adipose RAS in PCOS women. Recently ACE2 deficiency in adipose tissue was shown to increase blood pressure in females, fed a high-fat diet, but not in males [58]. Whether androgen-mediated increases in Ang II and decreases in Ang(1-7) in adipose tissue play a role in mediating the elevated blood pressure in obese PCOS women remains to be determined.

As mentioned, the RAS plays a role in the androgen-mediated increase in blood pressure, and we demonstrated that the vasoconstrictor arm of the intrarenal RAS is activated by androgens in the DHT-treated female rat [23] and in male models of hypertension and renal injury, such as Dahl Salt Sensitive rats and spontaneously hypertensive rats (SHR) [59]. Intrarenal mRNA expression of angiotensinogen and ACE are increased and AT1R expression is decreased in DHT-treated females [23]; enalapril (ACE inhibitor) reduces blood pressure in the model [60]. Interestingly, the body weight and blood pressure in
DHT-treated female rats remain elevated 6 months after DHT withdrawal (termed ex-DHT), and activation of the intrarenal RAS plays a major role in the remaining elevated BP in ex-DHT. These data are important because they suggest that chronic upregulation of the intrarenal and adipose tissue androgen receptor may explain the long-lasting effects of androgens [61], and that once androgen excess initiates obesity and upregulation of the RAS, the system remains upregulated.

**Role of 20-hydroxyeicosatetraenoic acid (20-HETE) in PCOS**

20-HETE is a lipid produced from arachidonic acid, whose presence in the kidney can be either pro- or anti-hypertensive [62,63], depending on its cellular location. If 20-HETE is increased in the intrarenal microvasculature, it causes renal vasoconstriction, thus reducing GFR and causing increases in blood pressure [63]. If 20-HETE is increased in the renal tubules, it acts as a diuretic reducing sodium reabsorption and is thus anti-hypertensive [63,64]. In collaboration with Dr. Richard Roman, we measured the levels of 20-HETE in renal microvessels of DHT-treated female rats and found that 20-HETE was increased, but not in renal tubules [64]. In addition, we found that the intrarenal cytochrome P450 4A2 mRNA expression was increased 15-fold in the PCOS model [64]. These data led us to studies in which a P450 4A2-/- rat was used to determine if DHT could increase the blood pressure as in control females. In fact, we found that DHT was unable to increase the blood pressure in 4A2-/- rats, unlike in DHT-treated control females [64]. These data suggest that DHT-mediated 20-HETE increase in the renal microvasculature is at least in part responsible for the increased blood pressure in the PCOS model.
As mentioned above, 20-HETE is a metabolite of arachidonic acid [62], and both Ang II and endothelin are able to increase the release of arachidonic acid leading to increases in 20-HETE [62]. However, whether Ang II or endothelin are elevated in this model and contribute to the elevated blood pressure is not clear and will need to be studied. Furthermore, whether 20-HETE is elevated in kidneys of women with PCOS has not been studied to our knowledge.

**PCOS, menopause and CVD**

Few studies have been done in postmenopausal women to determine the age-related cardiovascular-renal consequences of having PCOS throughout their lives. Interestingly, meta-analyses studies show that blood pressure is elevated in PCOS women compared to controls prior to menopause (RR=1.7 fold), but after menopause, there are no differences in prevalence of hypertension [20], suggesting that blood pressure increases in control postmenopausal women just as in PCOS women [20], and hypertension is a major risk factor for future CVD [21]. Some reports show that PCOS women may go through the menopause transition at a later age (53.3 years vs 51.2 years) than control women [65], suggesting that PCOS women may have longer exposure to estrogens than control women. A recent review by Helvaci and Yildiz evaluated all studies published in English between 1990 and 2020 that used MeSH terms “polycystic ovary syndrome”, “menopause”, and other terms associated with CVD [66]. Unfortunately, the diagnosis of PCOS was not specific for the Rotterdam or Androgen Excess-PCOS Society criteria in all of the studies included. The evaluation showed that menopause in the general population was associated with increased prevalence of obesity, glucose intolerance,
hypertension, dyslipidemia and metabolic syndrome [66]. Pre-menopausal PCOS women had a higher prevalence of these conditions than did pre-menopausal controls [67]. However, despite the earlier exposure to CVD-promoting conditions, postmenopausal women with PCOS did not exhibit an increased risk of CVD events or mortality compared to age-matched controls [66]. In another study PCOS women that were diagnosed according to the Rotterdam criteria and controls were studied at 70 years of age and compared to themselves at 50 years of age [68]. At 70 years of age, there were few differences between control women and PCOS women with regard to metabolic factors and blood pressure [68]. Again, as shown in other studies, at age 50 years, blood pressure was significantly higher in PCOS women than controls, but by 70 years of age, there were no differences between the groups [68]. Whether postmenopausal women have increased proteinuria compared to pre-menopausal women was not studied; nor are there any studies, to our knowledge, in which kidney function has been reported in postmenopausal PCOS women.

**Aging in the PCOS rat model**

Aging in the DHT-treated rat model of PCOS is associated with increases in blood pressure compared to young adults and aging controls [26, 44]. The mechanisms for the further elevations in blood pressure after the DHT-treated rats stop estrus cycling (at 10-12 months of age) are not clear. We recently reported that chronic administration of the Glucagon Like Peptide-1 Receptor Agonist (GLP1-RA), Liraglutide, in post estrus cycling DHT-treated rat model results in significant improvement in several cardiometabolic risk factors, such as insulin resistance, obesity (9% reduction in body weight), dyslipidemia,
and leptin levels [69]. Despite these improvements, the well-known blood pressure lowering effect of GLP-1 RA was not observed DHT-treated rats, only in control rats [69]. In contrast, there is a report that GLP-1RAs did decrease BP in reproductive-age DHT-treated rats [70], suggesting differences in mechanisms of hypertension with aging in PCOS rat models. Similarly, administration of sodium-glucose cotransporter 2 inhibitor in PCOS women causes a significant reduction in adipose tissue and leptin levels with only modest (approximately 2 mm Hg) decreases in blood pressure [71].

Aging control female SD rats are protected from hypertension [26]. However, DHT-treated female rats, aged 22-24 months, exhibit a 20 mm Hg increase in blood pressure compared to age-matched controls (controls: 110+/-5; DHT: 130 +/- 6 mm Hg; p,0.01) [72]. Along with the increase in blood pressure, the aging DHT-treated rats have renal hypertrophy, GFR is decreased by 60%, renal plasma flow is reduced by 50%, and proteinuria, factored for GFR, is increased by 20-fold [72]. The kidneys of aging DHT-treated females also exhibit focal segmental glomerulosclerosis and interstitial fibrosis [72]. Whether aging in PCOS women exhibit a higher incidence of chronic kidney disease than control aging women is unknown.

As mentioned above, the therapeutic options for the cardiovascular-renal and metabolic consequences of PCOS are few. The use of anti-androgens in PCOS women is not recommended by the FDA due to the high prevalence of hepatotoxicity found in European studies of PCOS women [73]. However, based on the data discussed above and as shown in our animal model, the increase in androgens certainly sets the cardiometabolic symptoms of the disease in motion. However, once established, normalization of
androgens and reduction in body weights may not be enough to decrease blood pressure in PCOS women, as we found in our ex-DHT model [61]. These data suggest then that the mechanisms responsible for the elevated blood pressure in PCOS women may be more complicated than just androgen excess and obesity. Future studies in PCOS women will be necessary to determine the contributions of other hypertensive mechanisms, such as renal dysfunction, the RAS, endothelin, or 20-HETE as we found in our rat model of PCOS, and whether these interactions change or synergize with aging.

**Cardiovascular-renal consequences of previous pregnancy in PCOS women and DHT-treated female rats**

To our knowledge there are no studies reported in which CVD and blood pressure in PCOS women have been separated based on previous pregnancy. As mentioned above, PCOS women often have difficulty becoming pregnant and require reproductive assistance [74,75]. PCOS women have a somewhat higher incidence of preeclampsia, gestational diabetes, and children born either small or large for gestational age [66-68], likely due to the extent of the gestational diabetes [76-79].

Just as in PCOS women, the DHT-treated female rat model of PCOS also has difficulty becoming pregnant. However, the number of pups per litter of DHT-treated rats is similar to control pregnancies, although the pups are born small for gestational age [26-28]. If left to age to 16-18 months, the DHT-treated dams have similar metabolic characteristics as virgin DHT-treated rats, including similar body weight, plasma insulin, leptin, and cholesterol, all of which are higher than age-matched control females [26]. Surprisingly, the aged previously-pregnant DHT-treated dams have significantly lower blood pressure.
than the virgin DHT-treated rats [26], and in fact, the blood pressure did not increase in the DHT-treated dams after 10 months of age, whereas blood pressure continued to increase in virgin DHT-treated rats [26].

The mechanism(s) by which previous pregnancy protected aging DHT-treated rats from hypertension is not clear. There are increases in intrarenal mRNA expression of vasodilator systems in the aging, previously-pregnant DHT-treated dams, such as endothelial nitric oxide (NO) synthase (eNOS) and endothelin B (ETB) receptor, and reductions in intrarenal vasoconstrictor systems, such as mRNA expression of renin, AT1R, and the endothelin ETA receptor, compared to DHT-treated virgins [26]. Taken together, these data suggest that the previously-pregnant DHT dams had an upregulation of intrarenal vasodilator systems, and a downregulation of intrarenal vasoconstrictor systems.

As mentioned, to our knowledge there are no studies on the extent of CVD in aging, postmenopausal cohorts of PCOS women that are separated based on whether they have been previously pregnant or not. Thus whether pregnancy protection against CVD and hypertension occurs in aging PCOS women is not clear. Taken with the data described above in which comparison was made between PCOS cohorts who were 50 years of age compared to 70 years of age [20], the fact that blood pressure was similar between PCOS women and controls at 70 years of age is surprising based on the early exposure to cardiometabolic dysfunction in PCOS women. In addition, PCOS women have a higher prevalence of gestational diabetes, preeclampsia, and superimposed preeclampsia than control women [79,80]. Other studies have reported that women who
have experienced preeclampsia during their pregnancies are at increased risk of CVD and hypertension later in life compared to control women [80]. Thus, it is surprising that DHT-treated dams are protected from age-related CVD and hypertension. One caveat is that we do not know if the DHT-treated dams maintain elevated blood pressure or even a further increase in blood pressure during pregnancy, since the DHT-treated rats have elevated blood pressure prior to pregnancy [23]. Pregnancy in SHR, a model of essential hypertension, results in a reduction in blood pressure in late pregnancy, just as in normotensive rats and humans [81,82]. Another factor that may contribute is that although the DHT-treated rats have higher body weight than controls prior to pregnancy, by the end of the pregnancy, the dams have lower levels of fat mass than do age-matched virgin DHT-treated rats [26]. It is surprising that the differences that occur at 4-5 months of age would have such profound effects when rats reach 16-18 months of age. In addition, it is surprising that these differences show up only after they stop estrous cycling, since there were no differences in blood pressure or metabolic factors between the previously pregnant and virgin DHT-treated rats at 10 months of age. It should be noted that adult male offspring of DHT-treated rats that were born with intrauterine growth restriction, exhibit exaggerated responses to Ang II infusion [26, 27], suggesting that there are perinatal factors occurring in the DHT-treated dams that impact the offspring later in life. Future studies in PCOS women will be necessary to determine whether those who have been previously pregnant are actually protected against further increases in blood pressure with aging.

Summary
PCOS is the most common reproductive disorder in women affecting approximately 10% of the population. While there are numerous studies on the pregnancy consequences of PCOS, there are significantly fewer studies on the cardiorenal consequences of PCOS, especially with aging. As shown in Figure 1, we hypothesize that an increase in androgens in PCOS via the androgen receptor increases food intake leading to obesity and insulin resistance, increases in subcutaneous and visceral fat depots that release leptin, and in combination with androgens and androgen receptor, upregulate the MC4R. Activation of the MC4R upregulates the SNS which increases renal sympathetic nerve activity that decreases GFR. In addition, increase in androgens and upregulation of the androgen receptor also increases synthesis of angiotensinogen to increase Ang II also reducing GFR. Androgens via the androgen receptor and Ang II-mediated release of arachidonic acid upregulates and activates the omega-hydroxylase, respectively, that produces 20-HETE in the intrarenal microvasculature which also reduces GFR. Taken together the reduction in GFR leads to increased renal injury that then causes increases in blood pressure, which in turn becomes a feed forward mechanism to cause further renal injury and further increases in blood pressure, especially with aging.

Based on this hypothesis, future studies are needed to determine the long term consequences of PCOS on renal function. Based on animal studies, long term exposure in PCOS women to the cardiometabolic consequences of PCOS, including elevated blood pressure, insulin resistance, hyperlipidemia, activation of the SNS, RAS and 20-HETE systems, likely will have an adverse impact on renal function, especially with aging. Furthermore, whether PCOS women who have been previously pregnant may be
protected from age-related renal injury and hypertension, and the mechanisms responsible also remain to be determined.

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Author Contributions

Jane Reckelhoff: Conceptualization; Formal analysis; Funding acquisition; Investigation; Project administration; Writing - original draft; Writing - review and editing. Noha Shawky: Data curation; Formal analysis; Investigation; Methodology; Writing - review and editing. Damian Romero: Formal analysis; Investigation; Methodology; Writing - review and editing. Licy Yanes Cardozo: Conceptualization; Formal analysis; Investigation; Methodology; Writing - original draft; Writing - review and editing.
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Figure 1: Hypotheses regarding the mechanisms responsible for the elevated blood pressure in PCOS women.
Polycystic ovary syndrome

↑ androgens

↑ androgen receptor

↑ food intake

obesity, insulin resistance

↑ SAT/VAT

↑ leptin

MC4R

upregulate SNS

↑ angiotensinogen

↑ Ang II

Arachidonic Acid

CYP4A2 omega-hydroxylase

↑ 20-HETE

↓ GFR

renal nerve activity

↑ renal injury

hypertension