Role of Female Sex Hormones and Immune Response in Salt-Sensitive Hypertension Development: Evidence from Experimental Models

Sandra G. Vlachovsky[1,2] · Luis A. Di Ciano[1] · Elisabet M. Oddo[1,2] · Pablo J. Azurmendi[1,2] · Claudia Silberstein[3] · Fernando R. Ibarra[1,2,3]

Abstract

Purpose of Review Female sex hormones have systemic effects unrelated to their reproductive function. We describe experiences of different research groups and our own, on aspects related to the importance of female sex hormones on blood pressure (BP) regulation and salt-sensitivity-mediated BP response and salt sensitivity without alterations in BP, as well as renal sodium handling and interactions with the immune system.

Recent Findings Changes in sodium intake in normotensive premenopausal women cause more BP variations than in men. After menopause, women often develop arterial hypertension (HT) with a profile of sodium sensitivity. Besides, experimental results have shown that in adult rat models resembling the postmenopausal hormonal state induced by ovariectomy, controlling BP is not enough to avoid renal and other tissue infiltration with immune cells, which does not occur when sodium intake is low or normal. Therefore, excess sodium promotes an inflammatory state with the involvement of immune cells. The evidence of activation of adaptive immunity, besides changes in T cell subpopulations, includes changes in sodium transporters and receptors.

Summary More studies are needed to evaluate the particular sodium sensitivity of women and its meaning. Changes in lifestyle and sodium intake reduction are the main therapeutic steps. However, to face the actual burden of salt-sensitive HT in postmenopausal women and its associated inflammatory/immune changes, it seems reasonable to work on immune cell activity by considering the peripheral blood mononuclear cell phenotypes of molecules and transport proteins related to sodium handle, both to screen for and treat cell activation.

Keywords Salt-sensitive hypertension · Immune cells · Female sex hormones · High sodium diet · Ovariectomy-menopause

Abbreviations

Ang II	Angiotensin II
BP	Blood pressure
CV	Cardiovascular
CYP4A	Cytochrome P-4504A
D1DR	D1-like dopamine receptors
DOCA	Deoxycorticosterone acetate
ECFV	Extracellular fluid volume
ENaC	Epithelial sodium channel
ER	Estrogen receptors
GAGs	Glycosaminoglycans
GPER-1	Membrane receptor G protein-coupled estrogen receptor 1

Claudia Silberstein and Fernando R. Ibarra contributed equally to this manuscript.

✉ Claudia Silberstein
csilber@fmed.uba.ar

✉ Fernando R. Ibarra
ibarraf@hotmail.com; fibarra@fmed.uba.ar

1 Universidad de Buenos Aires, Instituto de Investigaciones Medicas A. Lanari, Laboratorio de Nefrología Experimental y Bioquímica Molecular, Combatientes de Malvinas 3150, Buenos Aires 1427, Argentina

2 Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Instituto de Investigaciones Médicas A. Lanari, Buenos Aires, Argentina

3 Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Facultad de Ciencias Médicas, Departamento de Ciencias Fisiológicas. Instituto de Fisiología y Biofísica B. Houssay (IFIBIO-Houssay), Laboratorio de Fisiología Renal, Paraguay 2155, piso 4, Buenos Aires 1121, Argentina

HDZ	Hydralazine
20-HETE	20-hydroxyeicosatetraenoic acid
HS	High sodium
HT	Arterial hypertension
IF rats	Intact female rats
NCC	Neutral sodium chloride cotransporter
NK	Natural killer
NKA	Na^+, K^+-ATPase pump
NKCC1	Type 1 $Na^+–K^+–2Cl^-$ cotransporter
NKCC2	Type 2 $Na^+–K^+–2Cl^-$ cotransporter
NS	Normal salt or normal sodium
oVx	Ovariectomized
oVx HS	Ovariectomized hypersodic
oVx NS	Ovariectomized normosodic
PKC	Protein kinase C
PBMC	Peripheral blood mononuclear cells
sgkl gene	Serum- and glucocorticoid-induced kinase 1 gene
SGK1	Serum- and glucocorticoid-induced kinase 1
SHR	Spontaneously hypertensive rats
SSHT	Salt-sensitive hypertension
Th	T helper lymphocyte
Tregs	Regulatory T lymphocytes
VEGF-C	Vascular endothelial growth factor C
WNK 1	Lysine-deficient protein kinase 1 [with no lysine (K)]

Introduction

Female sex hormones have multiple systemic and organ-specific effects that are not related to their reproductive function. In this article, we describe the experiences of different research groups and our own, concerning aspects of blood pressure (BP) regulation, sodium intake/balance, salt-sensitivity-mediated BP responsiveness, and salt-sensitive activity other than BP, as well as renal sodium handling and interaction with the immune system. Female sex hormones regulate immune system activity, and immune response can intermediate between sex hormones and specific organ effects. In both reproductive-age women and young female animals, female sex hormones play a key role in the regulation of blood pressure, both by their renal protective effects and by their modulation of the immune system. The response of blood pressure to changes in salt intake may vary according to the presence or absence of female sex hormones, through the regulation of renal sodium handling, and to the activation of immune system cells (Fig. 1).

Regarding BP, the development of arterial hypertension (HT) in postmenopausal women often displays a pattern of salt sensitivity [1•, 2, 3•] and over the last years, an immune component linked to innate and adaptive immunity has been observed [4]. Although in this article we highlight the
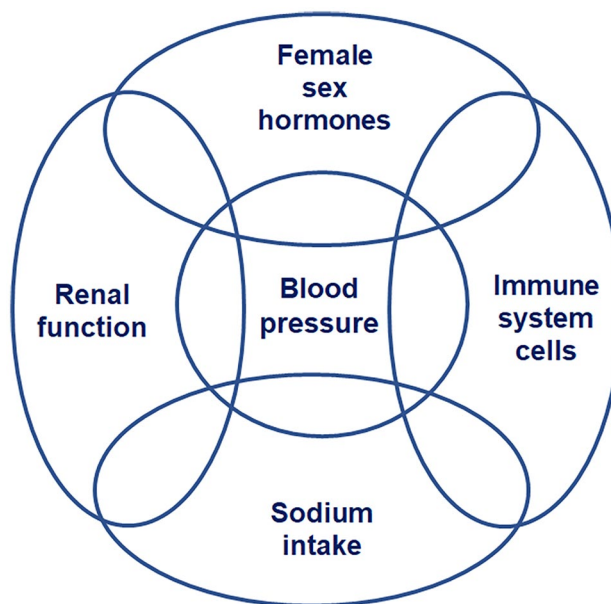
Fig. 1 The diagram represents the interrelation among the different factors that may be involved in the regulation of blood pressure

importance of excessive sodium intake, we must also take into account that this behavior is frequently accompanied by other eating habits and inappropriate physical activity such as excessive consumption of carbohydrates and a sedentary lifestyle, respectively. The combination of these factors leads to the development of metabolic syndrome [5•].

Sex Hormones and Salt-Sensitive Hypertension

Clinical and experimental studies have shown that HT is associated with an increase in sodium intake [6•]. Excess sodium can exacerbate ongoing HT or promote HT development in susceptible individuals or animals, so-called salt sensitive hypertension (SSHT) [7]. In fact, HT and high sodium (HS) consumption are nowadays worldwide health problems, especially in Western countries [5••]. These reports, however, do not distinguish salt-sensitive from saltresistant HT. Salt sensitivity, independent of BP, is a risk factor for cardiovascular (CV) morbidity and mortality, as well as other diseases [8••, 9, 10••]

The profile of sensitivity to salt or SSHT is observed when increasing the intake of NaCl or table salt increases BP. This is the predominant concept, but it is also defined as a decrease in BP through reduced NaCl intake. The BP response in SSHT is more marked in some individuals that are afterward named salt-sensitive patients. However, there is not yet a clear definition of SSHT [11•]. The people most affected by it are the elderly since 80% of hypertensive patients over 60 years of age suffer from SSHT, while only 50% of patients under 40 years

of age present it [8•]. Sensitivity to salt is associated with increased CV disease morbidity and mortality including renal disease [5••], such as that occurring in other HT conditions.

Once the CV response pattern is identified, it could be avoided by decreasing NaCl intake. In clinical practice, this occurs both in patients with SSHT and those with HT with other etiologies who improve when NaCl intake is decreased. In addition, it is interesting to note that a yet undetermined number of people classified within the normotensive ranges also exhibit the phenomenon of salt sensitivity: their BP changes more than in other normotensive individuals when salt intake changes, without reaching the HT levels. This response pattern is also associated with a higher prevalence of CV disease and other diseases such as neoplasms [9].

When comparable groups of women and men, as well as female and male experimental animals from hypertensive strains, are studied [12, 13], consistently lower BP in fertile women/females than in men/males is observed [14•, 15]. In normotensive rat strains, males and females have similar levels of BP [16•]. Studies in experimental animals show that females can excrete sodium load faster and more efficiently than males [17•, 18]. Along these lines, CV events are also lower in women than in men, unless other factors such as smoking, obesity, or drug use concur. In addition, it is observed that if kidney disease or injury occurs, women of reproductive age have a better and slower evolution than men [19••]. Similarly, experimental studies of acute kidney injury also indicate a pattern of recovery or deterioration of the renal function according to age and sex, with young female rats resisting acute injury better and recovering much faster [20]. This profile changes in postmenopausal women. This change in the CV response profile and sodium intake is associated with a decrease in ovarian hormone levels, mainly estrogens [19••], a feature also described in several experimental animal models [13]. A decade after menopause, the pattern of CV and renal protection decreases and approaches the biological or systemic response of the men counterpart [21, 22•]. It is common for postmenopausal women to develop HT with a response pattern of salt sensitivity [22•]. However, a careful review of recent information shows that women have more frequency of CV and renal disease during their fertile age than previously thought. Therefore, the protection that female hormones give should not be taken as a paradigm, although the general trend of protection, as stated above, and the decline after menopause are still sustained [23••].

Female Sex Hormones and Renal Regulation of Sodium and Blood Pressure

Gonadal hormones and, in particular, female hormones play an important role in the maintenance of body fluid homeostasis and BP regulation [19••]. In young adult normotensive humans, the renin-angiotensin-aldosterone system,

extracellular fluid volume (ECFV), and BP differ according to sex. Men have more systolic BP, plasma aldosterone, and ECFV than women. This is particularly observed under conditions of moderate-high sodium intake versus low sodium intake [24•]. Thus, variables related to ECFV, as stated here and below, are under the control of sex hormones.

Another particularly interesting study due to the number of participants and a design with a clear division of results into women and men under a dietary intervention is the GenSalt study [25••]. The authors showed that sex, age, and previous levels of BP are important to determine the intensity of BP response or variation when individuals change from low sodium to moderate-high sodium intake and to moderate-high sodium intake plus potassium. The importance of sex lies in the fact that women have lower basal BP but greater variation in BP levels with both types of sodium intake than men.

Researchers have traditionally shown that the presence or alterations of ovary hormones seem to play a relevant role in the regulation of vascular relaxation, and hence BP, even in aging female spontaneously hypertensive rats (SHR) [26]. Other BP regulatory mediators such as the renal kallikrein-kinin system also play an important role in SHR and show sexual and gonadal dimorphism [12]. Premenopausal women and adult female rats are protected against hemodynamic stress such as sodium loading, but this condition changes in the postmenopausal condition. The lack of estrogens may lead to a maladaptive response to sodium load [22•, 27•]. In addition, the role of progesterone seems to be more complex. Progesterone could interact with aldosterone depending on the sodium status [28]. Progesterone regulation of ECFV is more evident in circumstances such as pregnancy where a marked fluid retention is observed without an increase in BP [29]. Thus, progesterone could stimulate mineralocorticoid receptors during low aldosterone conditions and phosphorylate and activate the neutral sodium chloride cotransporter (NCC). Both effects could result in BP increments or sodium retention, as observed in some postmenopausal women receiving hormone replacement [29].

Classical experimental studies were performed on male or female animals with normal renal function and receiving mineralocorticoids and deoxycorticosterone acetate salt (DOCA-salt) to evaluate the systemic CV effect [30••] or with the chronic infusion of angiotensin II (Ang II) [31, 32•]. These traditional studies combine SSHT with an excess of salt-retaining hormones. Thus, HT ensues as a consequence of factors that retain sodium or increase the arterial vascular tone, such as Ang II and norepinephrine.

This early hypothesis of work has been expanded over the years because other studies have extended the knowledge on this topic, showing that the low availability of natriuretic

and vasodilator factors, usually described as modulators, may predispose to HT. Several lines of evidence suggest that renal dopamine [33•, 34] and renal kallikrein in intact and gonadectomized SHR [12] play an important role in regulating sodium excretion, BP, and vascular tone. Other vasodilator and natriuretic factors, such as atrial natriuretic factor and intrarenal dopamine, interact to regulate sodium metabolism [35], while nitric oxide and prostaglandins also play a leading role in the pathophysiology of HT.

In most studies, men/males have been taken as the standard sex to be studied. However, as previously stated, it is increasingly evident that women/females must be included in this type of research, since findings and behavior regarding BP regulation and related issues like sodium handling or interaction with immune cells differ between the sexes.

In this conceptual framework, our group has developed an experimental model of SSHT in adult ovariectomized (oVx) Wistar rats. The studies were performed in female Wistar rats, a normotensive rat strain. Rats were bilaterally oVx at 60 days of life. Later, 90 days after oVx, rats received normal salt (NS, 0.24% NaCl) or HS intake (1% NaCl in drinking water) for 5–7 days. After this period, we conducted several studies to evaluate the profile of BP response and transporters or mediators linked to sodium balance in the kidney and its behavior under NS or HS intake.

The first investigations were conducted in oVx rats under NS intake. It was found that the activity of the renal kallikrein-kinin system, urinary kallikrein, and kallikrein gene expression in kidney tissue were increased in oVx rats. In this NS model, BP remained normal or even decreased [36]. However, upon treatment with the kallikrein-kinin system blocker, aprotinin, BP only increased in oVx rats. Therefore, even under normal conditions of sodium intake and BP, a vasodilator renal system was active to keep BP normal.

In subsequent studies, we analyzed the BP response and sodium transporters in the kidney, by comparing a baseline situation with NS intake and another one with HS intake [37•]. Importantly, oVx NS rats were normotensive, but HS intake uncovered the propensity to develop HT. Upon sodium challenge, oVx rats excreted less sodium than intact female rats and developed salt-sensitive HT. In this study, we found that oVx rats had an overexpressed renal Na^+, K^+-ATPase (NKA) pump. Even in normotensive rats under NS intake, NKA in renal tubular epithelial cells in the cortex and medulla was overexpressed. Another important hemodynamic finding was that under NS intake, oVx rats had decreased renal vascular resistance. Instead, under HS, while intact female (IF) rats increased renal plasma flow, oVx did not, resembling the characteristics of nonresponding hypertensive humans [38]. The finding of an overexpressed renal NKA, even under NS intake, prompted us to investigate the possible cause. NKA expression/activity in renal tubular epithelial cells is regulated, among other mechanisms, by posttranslational phosphorylation/dephosphorylation reactions at Ser 23 residue. Phosphorylation of NKA reduces its activity while dephosphorylation of NKA increases it [39•, 40]. Therefore, we studied whether a defect in NKA phosphorylation could be responsible for decreased natriuresis and sodium retention, together with HT. For this purpose, we examined the regulation of NKA by renal dopamine [41•]. Although renal dopamine synthesis was equal in IF and oVx rats, several steps of the dopamine signaling pathway in renal tubular epithelial cells were altered. There was a lower expression of D1-like dopamine receptors (D1DR) in oVx rats. Besides, upon sodium load, oVx rats were unable to increase the expression of cytochrome P-4504A (CYP4A), which is the enzyme that increases 20-hydroxyeicosatetraenoic acid (20-HETE) production. The 20-HETE is a natriuretic factor that stimulates protein kinase C (PKC) alpha. PKC alpha phosphorylates NKA alpha-1 subunit at Ser 23 and diminishes NKA activity, which promotes sodium excretion. PKC alpha was also reduced. Therefore, in oVx rats, an altered renal dopaminergic pathway causes a failure in handling sodium load.

Three observations about this SSHT model are noteworthy. First, although the decrease in female sex hormones was abrupt (surgically induced), it was performed in adult rats, thus resembling the hormonal conditions of postmenopausal women or decline of ovary hormones for causes other than menopause [42]; second, the amount of sodium used as sodium loading was similar to that observed in office consulting patients and was administered for a short period of time (5–7 days); finally, the model was not Ang II or aldosterone-dependent. Aldosterone levels were reduced after sodium loading. Therefore, experimental evidence corroborates that ovarian hormones, through presence or absence, play a key role in integrating homeostatic aspects related to kidney function, ECFV, and BP.

Estrogens, Sodium, Immune Cells, and Sodium Sensitivity with or Without Hypertension

Estrogens and Immune Cells

Female sex hormones interact with innate and adaptive immune mechanisms. Given that sex hormones can regulate immune system physiology, that sodium intake influences immune cell activity, and that sex hormones and immune cells can induce and release, respectively, different types of cytokines, female hormones, and immune cells should be expected to have several points at which they can interact. Therefore, the interaction of estrogens with the immune system is both rich and complex [43••]. First,

estrogens act by activating different estrogen receptors (ER) such as ER alpha, ER beta, and membrane receptor G protein-coupled estrogen receptor 1 (GPER-1). According to the presence or absence of these receptors, estrogen can promote different and, sometimes, opposite responses in tissues. All mononuclear immune cells in peripheral blood, like monocytes and B and T cells, express ER, but CD4$^+$ T cells express more ER alpha than the other cell types while B cells express more ER beta [44].

As an overall estrogenic activity, exogenous administration of estrogen avoids T cell maturation and decreases the abundance of the thymus T cell subset. Estrogen also causes thymus atrophy [45]. Through the stimulation of receptors, estrogens can regulate T cell maturation, proliferation, and polarization, affecting different subsets depending on the interaction with specific immune cells, tissue physiology, pathological conditions, or cytokines in the environment. Thus, this is a complex network of possible interactions [46•]. For example, estradiol affects the cytotoxicity of natural killer (NK) cells, activation and differentiation of monocytes, macrophages, and dendritic cells, in a dose-dependent manner [47–51]. However, otherwise stated, we will focus on adaptive immunity. Specifically, estradiol decreases the differentiation of T lymphocytes towards proinflammatory subpopulations like T helper 17 (Th17) and increases regulatory lymphocytes or anti-inflammatory regulatory (Tregs) T lymphocytes [52•, 53••]. Low concentrations of estradiol promote a skew to a Th1 immune response, while at high concentrations (pregnancy as an extreme condition) trigger a Th2 immune response [54]. In addition, other physiological conditions, besides pregnancy, with normal-high levels of estrogens increase regulatory Treg lymphocytes [55]. Thus, in general terms, it can be stated that ER stimulation by estradiol in T cells favors an anti-inflammatory immune response by decreasing Th17 polarization, and importantly, by expanding the regulatory

T cell compartment. On the other hand, if female sex hormones induce this kind of immune response, the absence or decrease of estrogens could be expected to promote an abnormal immune response with a bias of immune response towards inflammation in pathological conditions like HT (Fig. 2) [27, 42, 56••].

Although progesterone is not usually regarded as an important player in pathophysiological conditions such as immune-mediated hypertension, progesterone also acts on immunity by inducing an anti-inflammatory response. Progesterone, like estradiol, also has receptors expressed in different types of immune cells [57]. Progesterone shifts the immune response from Th1 to Th2 or Treg cells [58] and suppresses the differentiation to Th17 cells.

Female Hormones, Sodium, BP, and Adaptive Immunity

It has been well established in experimental settings that innate and adaptive immunity participate in the pathogenesis of arterial HT [59••, 60]. Guzik et al. [59••] were the first to show in male rats that T lymphocytes participate in the hypertensive response to chronic infusion of Ang II. Subsequently, it was observed that in female rats the model did not behave in the same way as it did in males [61••]. In other words, the response of T cells is specific to the sex in which it is studied, highlighting the importance of female hormones. However, regardless of sex, it is still unclear whether the immune cells found in renal tissue, blood vessels, or skin [62–64••] may induce HT development or whether the presence of immune cells is secondary to the increase in BP [61••]. Investigations into the role of the immune system in HT have analyzed the presence of CD4$^+$ T helper cells or CD8$^+$ T cytotoxic cells. In general, CD4$^+$ T helper cells and their different subsets have been described as the predominant cells in the pathophysiology of HT in the kidney and other organs [43••, 61••, 65••].

Fig. 2 The scheme shows that at high sodium intake, women of reproductive age, and adult female rats may not develop salt-sensitive hypertension due to the protective effect of female sex hormones. However, in postmenopausal women and ovariectomized rats, the protective effect is lost. Therefore, in the absence of female sex hormones, an impaired renal sodium handling and activation of the immune system may occur, leading to the development of salt-sensitive hypertension
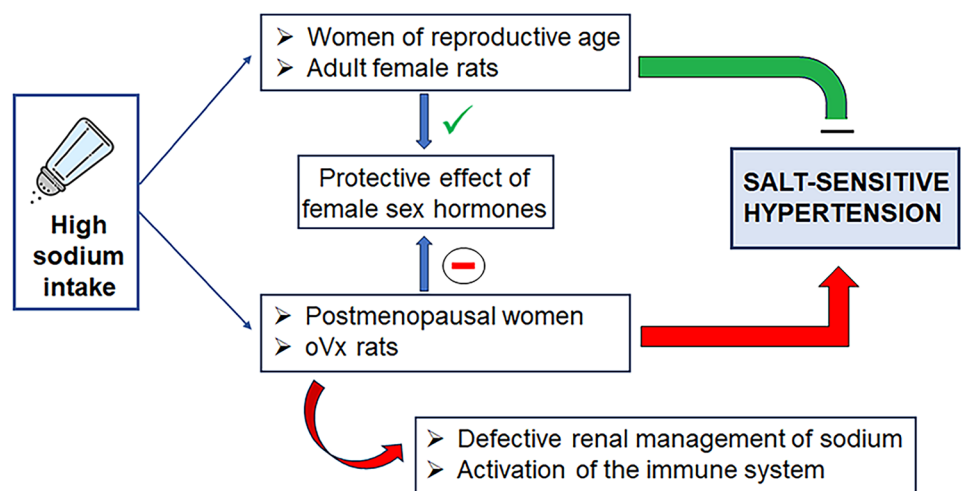
Female sex hormones and sodium intake are also important players that can modulate the lymphocyte physiology and thus the direction the adaptive immune response may take. As stated above, the interplay between the immune system and female hormones is complex [43••, 61••, 66] since female sex hormones have a regulatory role in cells from both the innate and adaptive immune systems [53••]. Estrogen-mediated immunomodulation causes a reduced activation of effector T cells [52•] and potentiation of Treg cells [3•, 53••, 67]. On the other hand, the absence of female sex hormones may be expected to cause a different immune response in comparison with intact females [27•]. In addition, HS intake may induce changes in immune T cell subsets, like activation and polarization of CD4$^+$ Th cells towards the proinflammatory Th17 subset [68, 69•]. To be activated and proliferating and also during migration, lymphocytes require several transporters and intracellular signals or receptors that mainly participate in the ion flux increase upon T cell activation [70, 71••, 72]. The increase in ion fluxes is the motor that drives lymphocyte activation, expansion, and migration. To perform this task, lymphocytes are endowed with sodium and chloride transporters, as well as sodium channels, specific receptors, and intracellular mediators also present in other cells. The sodium-dependent serum- and glucocorticoid-induced kinase 1 (SGK1) is expressed in lymphocytes. In the kidney, SGK1 increases the activity of ion transporters such as electroneutral NCC, type 2 Na$^+$–K$^+$–2Cl$^-$ cotransporter (NKCC2), epithelial sodium channel (ENaC), and NKA pump [73].

Thus, T lymphocytes CD4$^+$ and CD8$^+$ subsets express NKA, type 1 Na$^+$–K$^+$–2Cl$^-$ cotransporter (NKCC1), SGK1, dopamine receptors, mainly D1DR, lysine-deficient protein kinase 1 (WNK1), and other sodium and chloride transporters or channels as well [27•, 69••, 72, 74]. Then, T cells are good candidates to study the response of sodium transporters when facing changes in the surrounding environment, since, as mentioned above, immune cells could be part of the regulatory mechanisms of BP, compromised with HT and under the individual or interrelated influence of female sex hormones, and sodium intake. In this way, a study on T lymphocytes showed the importance of SGK1 and NKCC1 during the development of HT by the chronic infusion of Ang II or by DOCA-salt treatment, in which *sgkl* gene deletion or NKCC1 inhibition blunted BP increase [69••].

As previously described, the model of SSHT in adult oVx Wistar rats, besides deranged sodium handling by renal tubules with sodium retention and high BP, is associated with an overexpressed and stimulated NKA. To explore whether this is a specific alteration in kidney function or whether other systems/tissues that express sodium transport proteins could also be involved in this pathophysiological scenario, we extended studies to the immune response by exploring the behavior of sodium transporters in peripheral blood mononuclear cells (PBMC) from IF and oVx rats under normal or high salt intake. The results of this study demonstrate that the response of PBMC to HS differs according to the hormonal background. PBMC in oVx rats sensed sodium intake in a completely different way, compared with IF rats. Sodium transporters NKA and NKCC1 increased their expression in PBMC from oVx rats under HS intake. Besides, CD4$^+$-specific protein and D1DR also increased in oVx HS, in comparison with oVx NS rats. oVx under NS intake did not develop HT. Therefore, high sodium was required to increase BP. In contrast, IF rats upon HS did not become hypertensive, and CD4 + protein and NKA were decreased by HS, as compared with IF NS rats and oVx HS rats.

We then explored whether findings suggesting immune cell activation in oVx HS rats were only present in PBMC or in renal tissues as well. In fact, inflammatory/immune CD45$^+$ cells were found at the basolateral peritubular space. One of the main findings of these studies was that PBMC and their ion transport proteins are not neutral to variations in sodium intake and react very differently to HS intake in oVx in comparison with IF rats. Thus, in the absence of female sex hormones, HS intake and HT are variables that PBMC must face in their environment, showing patterns of different sensitivities to these stimuli.

Findings in renal tissue by immunohistochemistry show that in oVx HS rats the kidney cortex begins to be infiltrated by inflammatory/immune cells as part of this complex scenario. Activated immune cells release local Ang II, inflammatory cytokines, and oxidative stress intermediaries, and aggravate ongoing HT and cause kidney injury [56••] Hypothetically, a similar scenario probably develops in the basolateral space of intestinal cells and in the interstitial lymphatic compartment. In the absence of female sex hormones and with increased BP, HS intake at these interfaces could be processed as a neoantigen to which inflammatory/ immune cells react. Therefore, neoantigens [75, 76] or direct stimulation by Na$^+$ [68, 69••] can be behind immune cell stimulation in this model of SSHT.

However, if immune cells are responsible for HT development or if once high BP ensues, somehow immune cells could be recruited, remains to be elucidated. Another question is whether this alteration is caused by HS intake, BP, or both, in SSHT models with infiltration of renal tissue by immune cells. In this regard, several interesting articles have described different findings. Rudemiller et al. [77] reported that CD247 T lymphocytes participate in the hypertension mechanism in Dahl SS rats, since genetically modified CD247 -/- rats do not show the magnitude of renal infiltration and HT that CD247 + / + rats develop. A particular finding of this study was that peripheral blood T lymphocytes do not develop the level of inflammatory cytokine mediators that renal lymphocytes have. The authors interpreted

it as an indication that renal immune cells are active while PBMC are not. In addition, while Evans et al. [78] found that a reduction in renal perfusion pressure by a servo control mechanism in the left kidney of Dahl salt-sensitive rats decreases renal T cell infiltration, Pai et al. [79] observed that the pharmacological reduction of BP in Envigo rats is not as effective in reducing renal infiltration with immune cells. Thus, it seems clear that, at least in these attractive experimental works, the involvement of immune cells in SSHT is evident but the final pathophysiological mechanism is still under debate.

Our results have shown that a BP increase in oVx rats under HS intake is accompanied by activation of PBMC and renal infiltration with inflammatory/immune cells CD45$^+$ leukocytes. These findings are all associated with the absence of female hormones. Recently, we also explored in oVx HS rats whether the changes observed in overexpressed NKA in PBMC, renal epithelial tubular cells, and kidney infiltration with CD45$^+$ leukocytes were secondary to HS intake, high BP, or both (Vlachovsky et al., Communication at the Joint Meeting SAIC SAI&FAIC SAFIS 2022. Abstract published in: Medicina, Buenos Aires) [80]. All experimental animals under NS or HS intake, even IF rats, received hydralazine (HDZ) to reduce BP. HDZ decreased BP in all groups including IF NS rats. Despite effective BP reduction, HDZ did not cause any of the observed changes in oVx and IF rats. Therefore, the main changes are ascribed to high sodium, rather than high BP.

Two findings are relevant to this review. One is that NKA is already overexpressed in the kidney of oVx NS rats that are normotensive and become hypertensive upon HS intake, and the other one is that the location of CD45$^+$ renal infiltrates is basolateral, like NKA overexpression, which opens the possibility of high sodium fluxes in the peritubular basolateral space of renal epithelial tubular cells. Potentially, this fact could create micro-domains of high sodium where immune cells can be activated. Therefore, expressed in terms of homeostasis or altered regulation, overexpressed NKA is the primary event and HS is the perturbation to the system.

Effects of Estrogens at the Arterial and Cardiovascular Levels

As stated above, sodium salt sensitivity, independent of BP, causes several organs and systems of the body to suffer pathological consequences either because of an excess in sodium intake or an abnormal response to sodium. This kind of damage depends on the environment in which sodium interacts with other mediators, including sex hormones. For instance, the endothelial function is impaired in rodents receiving a HS diet with no alterations in BP [81, 82]. However, it should be taken into account that in those studies, BP was measured in anesthetized rats [81], which may alter

BP values, or by tail cuff [82], which may be inappropriate for detecting small changes in BP, in evaluating pathologic changes in high salt intake.

Humans' arterial function is also affected during HS intake [83]. Oxidative stress seems to play an important role in this response. Regarding arterial dysfunction, women seem to have relative protection, since female sex hormones, particularly estrogens, have a positive action on nitric oxide generation, in comparison with men [84].

Various groups of researchers have focused on the vascular aspects of HT that occur in postmenopausal women. In some hypertensive postmenopausal women and in old female mice, 17-β estradiol treatment favors the synthesis of vascular nitric oxide, which reduces the salt sensitivity of BP and restores vasodilatory response, while its absence causes an increase in the response to Ang II followed by vasoconstriction and HT [85–87].

However, the consideration of the role of female hormones in protecting against CV, metabolic, and kidney diseases has changed drastically over the last years. Some time ago, the protective role of female sex hormones was taken for granted although it was considered to depend on age and the onset of menopause [3•, 19••]. Nevertheless, current studies and observational descriptions have shown that women are not so protected against CV diseases and renal dysfunction [23••], at least not as protected as they were thought to be before. Perhaps, women require a new definition of adequate systolic BP to avoid CV events, like myocardial infarction or heart failure [3•, 15]. Despite these observations, women are still considered to be more protected against CV injuries than men, this effect being mainly due to the protection offered by estrogens [88], or in the case of other injuries, like renal injuries, the progression to end-stage renal disease at a given reduced glomerular filtration rate is slower in females [23••]. In clinical studies, supplement or treatment with estrogens reduces risk factors for CV disease [89] or stress-related hypertension [90]. However, in a recent vascular ex vivo study using human samples, other researchers do not agree, at least partially, that estrogen supplementation is so effective in improving vascular dysfunction [91]. Therefore, here we have new issues for the ongoing discussion about estrogens as entirely protective, and perhaps may contribute to finding a new optimal threshold for women's BP at different stages of life [3, 92].

Sex Hormones, Skin, Sodium, and Lymphatic Response

In the context of SSHT or HT, it was observed that sodium can induce the activation of immune cells in the kidney and skin [56, 93]. The sodium complexed with anionic molecules in the skin and the relationship with innate immunity and the lymphatic system has gained importance over the last years. Although still under debate [94••], these findings

have changed the model of two compartments for sodium distribution and have possibly added part of the skin structures, the hypodermis, as a third compartment that regulates systemic sodium handling under the form of osmotically non-compromised sodium. This means that Na^+ binds to glycosaminoglycans (GAGs), especially with carboxyl and sulfated anionic GAGs, which can facilitate the non-osmotic storage of sodium.

Skin approximately represents 6% of body weight and contributes to a great portion of the interstitium [95•]. Previous investigations have shown a positive correlation between the amount of sodium, GAGs, and NKA density in the extracellular matrix of bovine cartilage [96], and in rats under sodium loading, storage in the dermis correlated with an increment in GAGs [97••]. This reasoning comes from observations that both salt-sensitive and salt-resistant individuals without HT showed similar degrees of body sodium retention after salt loadings, and that sodium retention occurred without an increase in BP in salt-resistant individuals [98, 99]. Indeed, sodium retention was associated with weight gain in salt-sensitive individuals, while no difference in water balance or weight was observed in salt resistant participants. Then, another compartment could play an important role in sodium balance in the salt-resistant response.

Titze et al. [100, 101] proposed that osmotically inactive sodium is deposited in the skin and that sodium-resistant rats have more sodium in the skin than salt-sensitive animals. In these experiments, oVx Sprague–Dawley had a smaller capacity to store sodium in the skin than intact female rats [102•]. Later, these observations were extended to humans participating in a space flight simulation experiment [103]. In addition, other researchers working on hemodialysis patients have shown that patients with end-stage renal disease had more sodium in the skin than usual [104].

Wiig et al. [105] and others have reported the importance of innate immunity in this kind of sodium balance by describing the involvement of macrophages, TonEBP (NFAT5) tonicity protein, and vascular endothelial growth factor C (VEGF-C) and its receptor. According to this description, there is a linkage between high sodium, these osmosensitive intermediary proteins, and growth factors, which finally, under a sodium load, would induce an increase in GAGs storing sodium. At the same time, lymphangiogenesis through a signal generated in macrophages and mediated by TonEBP induced an increase in VEGF-C. According to this proposal, these events induce a higher amount of lymphatic vessels, which, after sodium is buffered by GAGs, could release the accumulated amount of the ion into the general circulation without harming uncontrolled volume expansion.

Nevertheless, Thowsen et al. [106] worked on male rats and found that under very high sodium load or sodium plus DOCA, sulfated and unsulfated GAGs were not increased.

They also observed that sodium excess was redistributed to the intracellular compartment, mainly to skeletal and cardiac muscle cells. However, it is still under debate whether this mechanism plays a role in sodium balance and whether skin accumulation is hypertonic as proposed in initial studies [101]. Even if sodium is stored in the interstitium of the skin or other tissues, it is still unclear whether it is isotonic instead of hypertonic, as proposed by Rossitto et al. [107••].

The other discussion is whether this kind of storage prevents HT or, on the contrary, whether it promotes HT by vascular inflammation and endothelial/glycocalyx damage [95•]. This also creates the opportunity to establish the concept of skin sodium as a potential "double-edged sword" [95•]. Is it good or bad to accumulate sodium in the skin? This concept, in which female hormones could play an important role, is yet to be resolved. Lymphatic endothelial cells express estrogen receptors and females have higher serum levels of VEGF-C and VEGF-D [108].

High salt intake expands and increases the number of lymphatic vessels in the skin, as well as lymph flow in the skin and skeletal muscle [109]. These results are from studies in male rats with a high amount of sodium in the diet. In fact, rats developed HT only with high salt in the order of 8% intake. Then, when macrophages were inhibited, there was a reduction in lymphatic vessels and in lymph flow in skin and muscle. This demonstrates the importance of lymph flow regulated by macrophages to handle a HS intake.

However, it has also been shown that high sodium can induce an inflammatory pattern response in adaptive immune system cells [65•, 68, 69••], vascular inflammation, and endothelial/glycocalyx damage [95•], which finally cause arterial HT. However, in another report [110••] working with a much lower amount of sodium (1%), which did not induce hypertension in male rats, hypertension ensued when macrophages were inhibited.

Therefore, by regulating lymph flow probably via NFAT 5/TonBEP and VEGF-C, macrophages may work as buffers of sodium load mainly under conditions of moderate sodium intake. When sodium intake is massive, the operation of the system seems to be out of range to keep BP normal, probably by generating an inflammatory response. A sexual dimorphic pattern appears when plasma levels of VEGF-C are measured. Women have more angiogenic circulating factors than men [111]. As stated above, VEGF-C participates in lymphatic growth. In gonadectomized animals, lymph flow is decreased in comparison with intact females [112]. In a scenario in which lymph flow is decreased, female rats have a lower capacity of sodium storage in the skin, and the absence of estradiol reduces the arterial ability to increase nitric oxide synthesis upon sodium load creating a favorable scenario for HT development.

Similarly, Selvarajah et al. [113••] described a response to sodium with certain sex specificity since skin in men can buffer

sodium intake in normotensive participants, maintaining stable BP, while women do not accumulate salt in the skin and their BP in a normotensive range, as stated above, is more unstable. Besides, researchers have reported that no change occurred in plasma VEGF-C between the placebo and sodium-loaded groups, which did not show a clear involvement of TonEBP or VEGF-C activation. Compromise of sex hormones, by presence or absence, agrees with and extends the concept that the immunomodulation exerted by sodium depends on the local microenvironment [93].

In research on humans, protocols often differ in the stimulus to find salt sensitivity. Therefore, some studies use an intravenous sodium load while others use different amounts of oral sodium intake for days. Another important fact is that until very recently, the sex of the involved participants was not taken into account. Experimental individuals were either all men or women and men pooled together. In the study by Barnett et al. [114•], a crossover design involving young people, normotensives, with oral placebo vs. sodium, found an increase in GFR and NGAL in urine, no change in BP, but an increase in body weight and plasma volume. Toering et al. [24•] reported on women's and men's responsiveness to sodium at intakes of 50 to 200 mmol/day. The basal BP and renin-angiotensin-aldosterone system responses differed greatly between the sexes. Men had a significantly higher plasma aldosterone, extracellular volume, and systolic BP than women during HS intake. Other studies, which also differentiated between women and men and included a good amount of participants, reported that BP behavior and body weight differed according to sex. BP, even within the normal range, is more unstable in women than in men [25••] and women tend to gain body weight during changes from low to HS intake [113••]. However, the fractional excretion of sodium seems to be sharper in women.

More standardized studies are probably needed to elucidate physiological and pathological characteristics and their importance in this field. Therefore, more studies to directly assess the relationship between BP and HT development with skin sodium and lymphatic response in females and males are required. So far, it has been difficult to determine what is abnormal or not when no precision exists about the "normal" amount of sodium that humans should consume. This is especially so because SSHT is not easy to study and it is currently restricted to research work and only clinically available by observation [4]. With no intent to extrapolate experimental findings to humans, if the model of SSHT in Wistar rats resembles that of menopausal women, a word of caution should be given to this population regarding sodium intake. Some reports state that patient groups with CV risk and a good response to HT treatment are not entirely free of potential CV events [115]. Therefore, it seems that factors other than HT, like inflammation and immune activation, are active.

Many postmenopausal hypertensive women receive BP-reducing therapy, which often fails to properly control high BP, as shown in the excellent study by Mill et al. [116••]. Besides, this population has an increased risk of developing chronic renal disease and chronic renal failure [23••]. Information and control in terms of why and how to reduce sodium intake should be reinforced in postmenopausal women, since HS intake may well be behind treatment failure and progression of CV and renal disease.

Conclusions and Perspectives

In this review, we describe the relationship among female sex hormones, sodium handling, salt-sensitive hypertension, and characteristics of sodium sensitiveness other than increased arterial pressure, the potential of a new putative compartment in the skin to buffer sodium body balance, and the possible role of immune mechanisms compromised with BP regulation. Although they look like a sum of different items, they are parts of a continuum that is described according to which piece is analyzed. Altogether, when deranged, it is a systemic pathophysiological alteration triggered on the homeostasis of the biological system (Fig. 3). This occurs in the context of the presence, absence, or low levels of female sex hormones.

Taking into account that, in middle- or high-income societies, menopause starts at age 47–52 years and that a great number of women with this condition lead an active life, with a life expectancy of roughly 30 more years, it is evident that health issues in this group are meaningful [118]. According to experimental and clinical studies, HS intake may be behind the failure in achieving appropriate results with anti-hypertensive therapies. Even reaching a good level of BP with therapy, inflammatory/immune cells can still be present in different tissues, especially in the kidneys and vessels. Therefore, tissue damage may continue regardless of BP control if HS intake is not controlled. In addition, adequate control of BP does not completely prevent CV events. The first step in treating this disorder is to control sodium intake. It requires public policies related to community education and commitments to the food industry, since 75% of ingested sodium comes in ultra-processed foods. It is also possible to administer synthetic female hormones, but sometimes it could be dangerous due to adverse side effects. Another possible treatment to solve this problem is the use of immunosuppressive or immunomodulation medications. However, this option of using current drugs is not viable because the consequences could be even worse. However, a point to work on concerning immune cell activity is to consider PBMC phenotypes of molecules and transporters as a new therapeutic target [117••]. In fact, transporters or receptors expressed in PBMC, such as NKA, NKCC1, SGK1, and
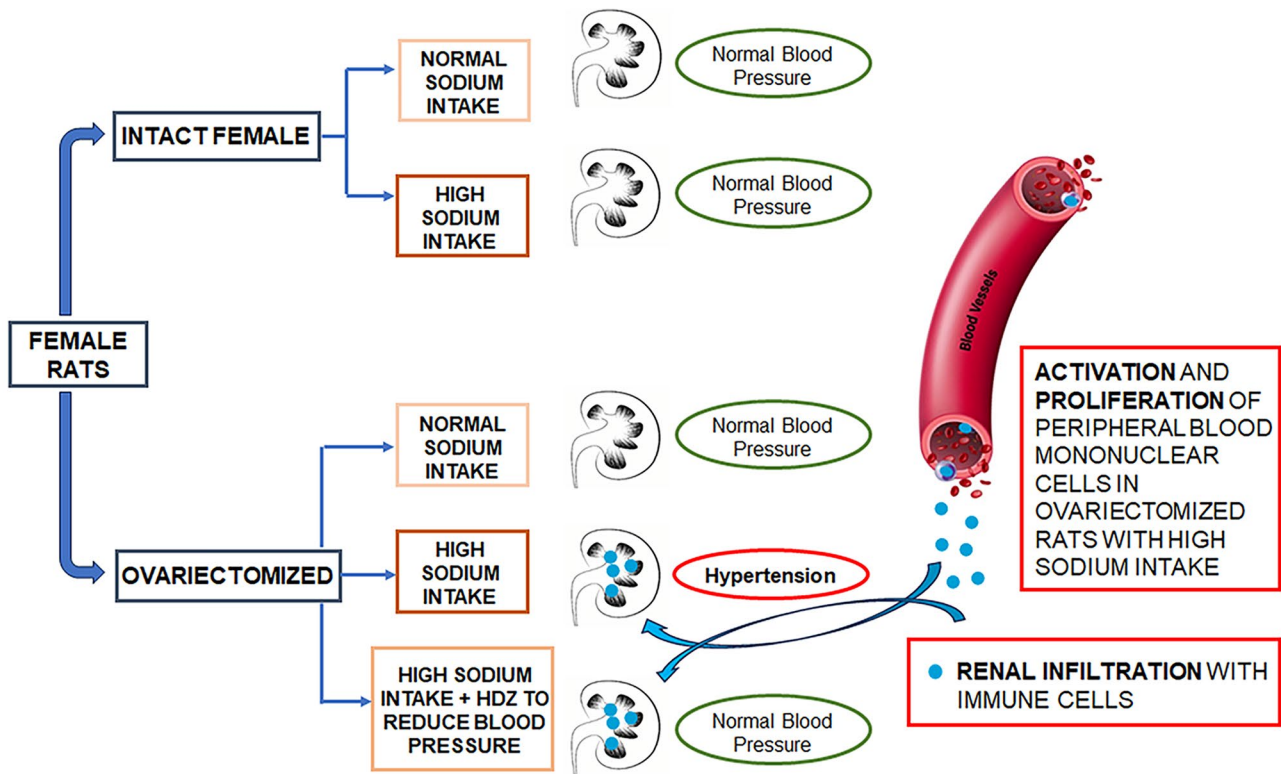
Fig. 3 Animal model of salt-sensitive hypertension. The scheme shows how blood pressure varies in intact and ovariectomized rats with normal or high sodium intake. Under high sodium intake, intact rats maintain the normal blood pressure, while ovariectomized rats increase their blood pressure, activate peripheral blood mononuclear cells, and show immune cell infiltration in their renal tissues. With the administration of hydralazine (HDZ), a vasodilator drug that reduces blood pressure, ovariectomized rats with high sodium intake show normal blood pressure values but maintain the leukocyte infiltration

D1DR, may play a dual role as screening molecules [27•, 69••, 71••] for sodium sensitivity and as targets to reduce immune cell activation and could thus be added to current therapies aimed at lowering BP. This is a new field in which sex differences are considered. The study of female physiology and pathophysiology will help to uncover current failures in our understanding of such specific issues.

Compliance with Ethical Standards

Ethics Approval Ethical approval was not required as our study did not involve humans or animals.

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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