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Publication Date

2023-09-08

DOI

10.1038/s41581-023-00757-2

nature reviews nephrology

https://doi.org/10.1038/s41581-023-00757-2

Review article

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Sex differences in renal transporters: assessment and functional consequences

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Abstract

Mammalian kidneys are specialized to maintain fluid and electrolyte homeostasis. The epithelial transport processes along the renal tubule that match output to input have long been the subject of experimental and theoretical study. However, emerging data have identified a new dimension of investigation: sex. Like most tissues, the structure and function of the kidney is regulated by sex hormones and chromosomes. Available data demonstrate sex differences in the abundance of kidney solute and electrolyte transporters, establishing that renal tubular organization and operation are distinctly different in females and males. Newer studies have provided insights into the physiological consequences of these sex differences. Computational simulations predict that sex differences in transporter abundance are likely driven to optimize reproduction, enabling adaptive responses to the nutritional requirements of serial pregnancies and lactation — normal life-cycle changes that challenge the ability of renal transporters to maintain fluid and electrolyte homeostasis. Later in life, females may also undergo menopause, which is associated with changes in disease risk. Although numerous knowledge gaps remain, ongoing studies will provide further insights into the sex-specific mechanisms of sodium, potassium, acid–base and volume physiology throughout the life cycle, which may lead to therapeutic opportunities.

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Key points

- Renal tubule organization differs in female compared with male rodents, notably in the abundance and expression of transporters in individual segments; generally, androgens regulate transporter abundance along the proximal tubule and oestrogens regulate transporter abundance along the distal tubule.
- Compared with males, female rats exhibit lower fractional reabsorption of sodium along the proximal nephron (associated with a lower activity of NHE3 and lower abundance of claudin2 and AQP1) and higher fractional reabsorption of sodium along distal segments (associated with a higher abundance of NKCC2, NCC, ENaC and phosphorylated AQP2).
- Female rats excrete a saline load more rapidly than males and achieve sodium homeostasis with a high salt diet more rapidly than males; moreover, female, but not male, diabetic mice maintain normotension when administered a high-salt diet.
- Angiotensin infusion provokes similar changes in blood pressure, ENaC activation and K^+ loss in both sexes, along with a rise in the expression of distal renal tubule transporters and a lowering of proximal transporters in females.
- Male and female kidneys differ in the mechanisms used to maintain acid–base homeostasis; for example, they demonstrate differences in baseline ammoniagenesis and their acid–base transporters, and prioritize different adaptations to acid load; key differences are androgen receptor dependent.
- Computer simulations of pregnant rat kidney function indicate that known sex differences in renal transporters can serve to prepare females to meet the fluid and electrolyte demands of the offspring.

Introduction

Renal sodium, glucose and water transporters are effective targets for the treatment of hypertension, diabetes and cardiovascular diseases. Although a young field, the study of sex differences in renal transporters holds great promise to improve understanding of how sex differences impact kidney disease epidemiology, manifestations and outcomes. The prevalence of chronic kidney disease (CKD), though rising in both sexes, has been consistently higher in women than in men (15% versus 13%, respectively) over the past 80 years[1]. By contrast, the lifetime risk of kidney failure is nearly 50% higher in men than in women across racial and ethnic groups[2–6]. These sex differences remain significant, even after adjusting for clinical and sociodemographic factors, supporting the existence of sexual dimorphisms in the risk of kidney failure. Theories postulate that sex disparities in CKD progression can be attributed to a variety of factors, including sex hormones, differences in renal haemodynamics and differences in kidney mass between men and women[5,7–10]. The aetiology of CKD also differs between sexes: diabetes and hypertension are more prevalent causes of CKD in male patients, whereas autoimmune diseases are more common causes in women. Independent of CKD, hypertension is one of the most common causes of death worldwide[11]. As reviewed previously, premenopausal women have a lower incidence and severity of hypertension than men, but a higher risk after menopause[12–14]. Key systems that affect both the development of hypertension and the activity of renal transporters include the sympathetic nervous system, the renin–angiotensin–aldosterone system and the immune system — all of which exhibit differential activation in females compared with males based on animal studies[14–18]. Diabetic kidney disease (DKD) is the leading cause of kidney failure[19]. Similar to hypertension, the prevalence of type 2 diabetes is higher among men than among women[20], along with a greater risk of DKD; however, the risk profile of women changes after menopause such that postmenopausal women exhibit a higher risk than men of DKD, glomerular hyperfiltration and diabetes-associated kidney failure[19,21]. The changes in disease risk that follow menopause can be attributed, at least in part, to differences in gene expression and sex hormone signalling within the kidney that contribute to the regulation of blood pressure and glomerular filtration rate (GFR)[22]. These statistics provide a very strong rationale for uncovering mechanistic explanations for sexual dimorphisms in the risk of disease onset and progression, which may lead to the identification of additional disease mechanisms and therapies.

Recent comprehensive studies in rodents have detailed sexually dimorphic patterns in both the expression and abundance of electrolyte, acid–base, water and organic solute 'transporters', which we consider to include renal solute and electrolyte co-transporters, pumps, channels, claudins, and their regulators[17,23–35]. These studies have established that renal tubule organization and operation are distinctly different in female and male rodents. Such sexual dimorphism likely evolved to optimize reproduction. Unlike males, females can undergo profound changes in kidney function as a consequence of pregnancy and lactation to divert large volumes of circulating fluids and electrolytes to the offspring and mammary glands, respectively (Fig. 1). Later in life, females also undergo menopause, which potentially blunts the differences between females and males and alters their disease risk.

This Review summarizes our current understanding of sex differences in renal fluid and electrolyte transporters between female and male kidneys, primarily based on studies in rodents. We focus on Na^+, K^+, acid–base, sugar and organic acid transporters, in which major sex differences have been documented. We describe the physiological consequences of differences in transporter profiles, based on information from experimental studies and from model predictions, and discuss the differential impact of sex on transporter regulation by hormones, diet and acid–base status. A number of knowledge gaps exist and further work is needed to better understand the extent to which these sexual dimorphisms exist in humans; however, the available data provide insights into sex-specific mechanisms that regulate salt and volume physiology throughout the life cycle, and may also have potential therapeutic implications.

Transporter patterns along the renal tubule

Renal tubular transport has an essential role in maintaining homeostasis. As the filtrate flows along the renal tubule, the tubular epithelia secrete or reabsorb water and solutes. Under normal physiological conditions in which GFR is sufficient, nearly 99% of filtered Na^+ is reabsorbed and the remaining 1% of the filtered Na^+ is excreted in the urine to match dietary intake minus extrarenal excretion. The precise adaptation of urinary Na^+ excretion to dietary Na^+ intake is a consequence of the tight coordination between the filtered load of Na^+ and renal transport systems. Transcellular reabsorption and secretion processes are fuelled by the transmembrane Na^+ gradients created by the basolateral Na,K-ATPase. Sodium pump activity drives transcellular

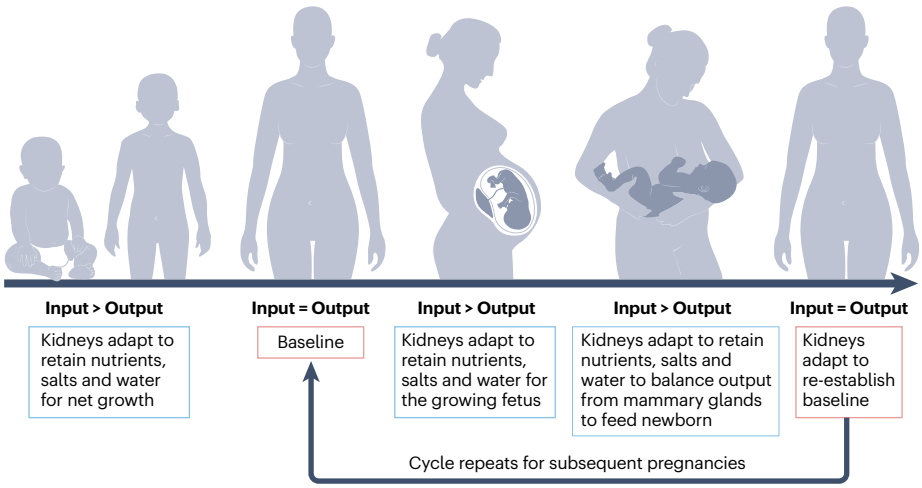

Fig. 1 | Female-specific changes in kidney function that occur through life stages. As in males, growth from childhood to adulthood in females is accompanied by adaptations to retain nutrients, salts and water that are necessary to sustain growth. However, pregnancy and lactation are both characterized by substantial increases in plasma volume and electrolyte retention. Kidneys must adapt quickly to shift nutrients, fluid and electrolyte demands from the kidney to the growing placenta and fetus, and then shift nutrients, fluid and electrolytes to the mammary glands. At weaning, the maternal kidneys adapt to re-establish baseline fluid and electrolyte homeostasis until the next pregnancy occurs. Throughout these stages, the kidneys are responsible for maintaining homeostasis, normal plasma electrolyte levels and acid–base balance in the mother. Sex differences in kidney function likely prime females for the adaptations in fluid and electrolyte retention that are required of pregnancy and lactation.

transport systems, which comprise a collection of cotransporters, antiporters, channels, and other pumps on the apical and basolateral membranes. The paracellular transport system is mediated primarily by claudins, which are tight-junction proteins that determine the permeability and charge selectivity of the paracellular pathway to small ions. The kidney adapts to variations in blood volume and composition by modulating the expression, abundance, and/or activity of its epithelial transporters. These changes are mediated by a variety of hormonal and neuronal signals and often involve complex signalling cascades. The renal tubule is categorized into 14 segments, each with distinct cell types[36,37]; the heterogeneity of the different renal tubule segments is crucial for this adaptive capacity. Whereas the proximal tubules reabsorb large amounts of solutes and water iso-osmotically owing to the abundance of ion and water transporters and channels, downstream tubular segments exhibit more specialized functions. For example, the loops of Henle exhibit high Na^+ transporter activity and low water channel activity to create gradients that are important for concentrating and diluting urine. Along the distal renal tubule, the distal convoluted tubules and principal cells fine-tune the transport of Na^+ and K^+, whereas intercalated cells fine-tune the acid–base balance. Water permeability is variably regulated by water channels to maintain body fluid osmolality. The 14 renal tubule segments continuously work in concert to adjust fluid and electrolyte handling to match output to intake, recover filtered nutrients and respond to pathophysiological stimuli. Available and emerging evidence suggests that sex differences in transport expression may contribute to this malleability.

Considerations in assessing sex differences

Most of our understanding of sex differences in renal transporters comes from studies in young adult, virgin rodents (rats and mice). Studies of the impact of age, genotype versus phenotype, and lactation are just beginning to enter the literature and are not considered here; however, information on the effects of sex differences in response to altered diets and hormones is burgeoning. Determining how sex affects epithelial transport along the renal tubule requires consideration of factors that determine the pool size and activity of 'transporters', which are defined to include co-transporters, channels, pumps and claudins (Tables 1 and 2). Transporter activity is defined by solute delivery to the segment and the abundance of active transporters in the cell membrane. The abundance of transporters in the membrane is a function of their respective rates of synthesis and degradation as well as their trafficking to and from the membrane[38–40]. Transporter activity in the membrane is, in turn, influenced by covalent modifications including phosphorylation and dephosphorylation[41], cleavage[42], protein–protein associations (for example, complexes of NHE3-DPPIV[43], ClC-K-barttin[44] and Na,K-ATPase subunits[45]), and the delivery of transporter activators or inhibitors via the tubule fluid[46,47]. At the organ level, transporter activity is also influenced by kidney size, the relative length of tubule segments, haemodynamics and age; generally, sex differences are dependent on sex chromosomal and/or hormonal complements[48]. Of note, females have smaller kidneys and shorter proximal tubules than males.

The heterogeneity of epithelial and non-epithelial cell types in the kidney complicates analyses of individual cell types in vivo. However, in the past few years, atlases of renal transporter mRNA[24,36] and protein[17,37,49,50] have provided a treasure-trove of new information. Analyses of microdissected tubules[37,49,51] and single cells[24,52,53] have revealed previously unidentified kidney cell types[52,54], and cell-specific responses to disease onset and progression[53,55].

Studies that have compared transcriptomic and proteomic data are especially useful as they provide unbiased information about the nature of regulatory responses to rapid and chronic stimuli to maintain fluid and electrolyte homeostasis[37,49,56,57]. However, most studies provide atlases of RNA or protein data (rather than both), which justifies

Table 1 | Sex differences in transporter abundance — proximal tubule through medullary thick limb

Transporter: protein (gene names)	Renal tubule region	Species	Female-to-male abundance ratio	Refs.
Na/H exchanger 3 (*Nhe3, Slc9a3*)	PT apical	Rat	1.20	17
		Mouse	0.59	
NHE3pS552 (*Nhe3, Slc9a3*)	PT apical	Rat	1.30	17
		Mouse	0.64	
Na-phosphate cotransporter-2A (*NaPi-2, Slc34a1*)	PT apical	Rat	0.75	17
		Mouse	0.54	
Electrogenic Na-bicarbonate cotransporter (*Nbce1, Slc4a4*)	PT basolateral	Rat	1.5	17
		Mouse	0.78	
Claudin 2 (*Cldn2*)	PT tight junction	Rat	0.38	17
		Mouse	0.7	
Na-glucose cotransporter- 2 (*Sglt2, Slc5a2*)	PT apical S1, S2	Rat	1.2 (cortex)	A.A.M., unpublished work
		Rat	3.0 (BBM)	28
		Mouse	1.11	A.A.M., unpublished work
Na-glucose cotransporter- 1 (*Sglt1, Slc5a1*)	PT apical S3, MD	Rat	1.31	67
		Mouse	0.5	156
Organic anion transporter 1 (*Oat1, Slc22a6*)	PT basolateral S2,S3	Rat	0.2	27
		Mouse	0.25	29
Organic anion transporter 2 (*Oat2, Slc22a7*)	PT apical S3	Rat	F » M	68
		Mouse	F » M	
Organic anion transporter 3 (*Oat3, Slc22a8*)	PT basolateral S1, S2 PC	Rat	0.61	27
		Mouse	2	29
K-Cl cotransporter 3 (*Kcc3, Slc12a6*)	PT	Rat	1	Mercado and A.A.M., unpublished work
		Mouse	No assay	
K-Cl cotransporter 4 (*Kcc4, Slc12a7*)	PT, DCT, CD	Rat	1	Mercado and A.A.M., unpublished work
		Mouse	No assay	
Aquaporin 1 (*Aqp1*)	PT; DTL	Rat	0.44	17
		Mouse	1.45	65
mNa/H exchanger 3 (*Nhe3, Slc9a3*)	mTAL	Rat	0.9	17
		Mouse	0.77	
mNHE3pS552 (*Nhe3, Slc9a3*)	mTAL	Rat	1	17
		Mouse	0.65	
mNa-K-2Cl cotransporter (*mNkcc2, Slc12a1*)	mTAL	Rat	1.2	17
		Mouse	1.3	128
			1	31
mNKCC2pT96T101 mNKCC2pS87	mTAL	Rat	1.4	17
		Mouse	0.44	31
mNaK-ATPase alpha (m*Nka α, Atp1a1*)	mTAL small amount in mCD	Rat	1.3	17
		Mouse	1.0	31
mNaK-ATPase beta (*mNka β, Atp1b1*)	mTAL small amount in mCD	Rat	1.3	17
		Mouse	0.75	31

Sex differences in transporter abundance are illustrated as the ratio of female-to-male abundance for transporters expressed from the proximal tubule through to the medullary thick ascending limb. The values provided here come with the following caveats: the tabulation is likely incomplete as additional examples are continually being published, and findings are presented without analysis of statistical significance. BBM, brush border membranes; CD, collecting duct; CNT, connecting tubule; DCT, distal convoluted tubule; DTL, descending thin limb; mCD, medullary collecting duct; MD, macula densa; mTAL, medullary thick ascending limb; PC, principal cells; PT, proximal tubule; S1, S2, S3, segments of the PT.

a brief consideration of each output and the relationship between these distinct assessments. mRNA levels can be considered a surrogate measure of protein synthesis rates, but not of the protein pool size, which requires knowledge of other factors such as the protein half-life and translational efficiency. The protein pool size is determined by its rate of synthesis and half-life[37,56,58]. Consider two proteins, A and B, with equivalent cellular protein pool sizes: if the mRNA expression of protein A is 100-fold greater than that of protein B, coupled to an 100-fold shorter protein half-life[58], then Protein A mRNA will be readily detectable by mRNA analysis whereas Protein B mRNA will be 100-fold less readily detectable. The synthesis and assembly of the sodium pump alpha (catalytic) and beta (glycoprotein) subunits provide an informative case in point: pre-translational up-regulation of the rate-limiting beta mRNA alone increases the rate of assembly of the beta subunit with existing cellular alpha subunits to form αβ heteromers, which exhibit a much longer half-life than unassembled alpha or beta subunits, without any change in alpha mRNA. Thus, the Na,K-ATPase pool size is increased by increasing expression of the beta

subunit and by reducing degradation rates of the alpha and beta subunits. Moreover, αβ heteromerization permits trafficking of Na,K-ATPase from the Golgi to the plasma membrane, which increases cellular Na,K-ATPase activity[45,59]. In addition to the impact of turnover rates, steady-state mRNA and protein levels can be affected by differential mRNA stability[60]; a number of studies from the past few years point to a role for sex-dependent transcriptional and epigenetic regulation in this process[57,61–63].

Table 2 | Sex differences in transporter abundance — cortical thick limb through collecting ducts in the distal renal tubule

Transporter: protein (gene name)	Renal tubule region	Species	Female-to-male abundance ratio	Refs.
Na-K-2Cl cotransporter (*Nkcc2, Slc12a1*)	cTAL	Rat	1.2	17
		Mouse	2.2	128
			1.3	17
NKCC2pS91	cTAL	Rat	1.04	A.A.M., unpublished work
NKCC2pS87		Mouse	0.59	31
NaK-ATPase alpha[a] (*Nka α, Atp1a1*)	Cortex	Rat	1.2	17
		Mouse	1.7	31
NaK-ATPase beta[a] (Nkaβ, *Atp1b1*)	Cortex	Rat	1.2	17
		Mouse	0.8	31
Na-Cl cotransporter (*Ncc, Slc12a3*)	DCT	Rat	1.61	17
		Mouse	1.0	127
			1.6	17
			2	31
NCCpT53 (*Ncc, Slc12a3*)	DCT	Rat	2	17
		Mouse	4	127
			2.7	25
			1.6	31
Claudin7 (*Cldn7*)	DCT-CD	Rat	1.4–1.7	17
		Mouse	1.5–1.7	31
Claudin 8 (*Cldn8*)	DCT-CD	Rat	1.75	A.A.M., unpublished work
		Mouse	2	
Claudin 10 (*Cldn10*)	All along the nephron	Rat	1	A.A.M., unpublished work
		Mouse	No assay	
Epithelial Na channel α subunit — full length (*αEnac, Scnn1a*)	CNT-CD	Rat	1.5	17
		Mouse	1.2	
Epithelial Na channel α subunit — cleaved (*αEnac, Scnn1a*)	CNT-CD	Rat	1.2	17
		Mouse	1.0	
Epithelial Na channel β subunit (*βEnac, Scnn1b*)	CNT-CD	Rat	1.2	17
		Mouse	1.3	31
			1	17
Epithelial Na channel γ subunit — full length (*γEnac, Scnn1g*)	CNT-CD	Rat	1.2	17
		Mouse	1.3	31
Epithelial Na channel γ subunit — cleaved (*γEnac, Scnn1g*)	CNT-CD	Rat	1.2	17
		Mouse	1	31
Renal outer medulla K channel — two bands (*Romk, Kcnj1*)	TAL, OMCD	Rat	1	17
		Mouse	1	A.A.M., unpublished work
Big K channel α subunit (*BKα, Kcnma1*)	CD	Rat	1	157
		Mouse	F > M	158
Rhesus glycoprotein b (*Rhbg, Slc42a2*)	CNT-CD	Rat	No assay	
		Mouse	1.6	74
Rhesus glycoprotein c (*Rhcg, Slc42a3*)	CNT-CD	Rat	No assay	
		Mouse	F > M	74
Aquaporin 2 (*Aqp2*)	CNT-CD	Rat	1.1, cortex	17
		Mouse	1.2–1.45	31
AQP2pS256 (*Aqp2*)	CNT-CD	Rat	2.5	A.A.M., unpublished work
		Mouse	1.0–2.0	31

Sex differences in transporter abundance are illustrated as the ratio of female-to-male abundance for transporters expressed from the cortical thick ascending limb (cTAL) through the collecting duct. The values provided here come with the following caveats: the tabulation is likely incomplete as additional examples are published continually, and findings are presented without analysis of statistical significance. [a]The ubiquitously expressed cortical Na,K-ATPase subunits are arbitrarily placed in the distal cortex. CD, collecting duct; CNT, connecting tubule; DCT, distal convoluted tubule; MD, macula densa; OMCD outer medullary collecting duct; PC, principal cells; TAL, thick ascending limb.

Most transporters within the kidney tubule exhibit segment- and/or cell-specific expression, which simplifies analyses of transporter sex differences. Studies over the past decade have revealed sex differences in both the expression and the abundance of transporters along the proximal tubule at both mRNA[24,64] and protein levels[17,23,28,29,65]. For example, semi-quantitative immunoblotting has been used to compare the pool sizes of major renal transporters in the cortex and medulla of adult male and female rats and mice[17,66]. Other studies have reported sex differences in the expression level of specific transporters, including aquaporin 1 (AQP1), organic ion transporters (OATs), organic cation transporters (OCTs), sodium–glucose cotransporters (SGLTs)[28,29,67–69] and acid–base transporters[23,70]. Moreover, single-cell RNA sequencing (RNAseq) of adult male and female mouse kidney revealed that the transcriptomes of proximal tubule cells cluster according to sex[24] — these sex differences can be visualized and further explored using the Kidney Cell Explorer database. Further analysis of the sexual dimorphisms specific to solute carrier (SLC) transporters[49] demonstrated that most differences in the proximal tubule are evident in the S2–S3 segment, in agreement with previous reports[30,64]. The SLCs that were more strongly expressed in males than in females were predominantly of the OAT family, (for example, SLC22), whereas the SLCs that were expressed more frequently in the females than in males were predominantly amino acid transporters. Although this transcriptome comparison is novel and informative, caution must be exercised in its interpretation because it does not take into account sex differences in tubule length[70,71]. Moreover, only 10% of the expected S3 cells were represented in the RNAseq profile from the male mice, suggesting that these cells succumbed to stress-induced cell death during the cell dissociation and isolation process[24,72].

As described above, the relationship between mRNA and protein dynamics is not expected to be linear; however, sex differences can provide insights into the relationship between the synthesis rate and the pool size of a protein. Compared with male mice, young adult female virgin mice have smaller protein pools of NHE3, NaPi2, claudin 2, villin[17], OAT1 (ref. 29), and SGLT2 (ref. 28) by ~0.5-fold (in part, because of the shorter proximal tubules of females)[71,73,74], but 1.5-2-fold greater protein pool sizes of AQP1 (ref. 17) and OAT3 (ref. 29). At the mRNA level in mice, transcripts encoding NHE3, NaPi2, villin and OAT1 are also detected at lower levels in females than in males; SGLT2 and AQP1 at equivalent levels across sexes; and claudin 2 and OAT3 at higher levels in females than in males[24,51,69]. OAT1 has both higher expression and greater pool size in males whereas OAT3 has both higher expression and greater pool size in females, which is attributed to sex hormone expression along the proximal tubule[29,69]. The pattern of lower protein expression of claudin 2, combined with higher mRNA in females than in males may reflect higher rates of protein degradation or lower levels of mRNA translation. Interestingly, a single-cell RNAseq analysis[24] reported very low levels of transcripts that encode a set of proteins that are abundantly expressed in the proximal tubule brush border, including NHE3 (ref. 39), NaPi2 (ref. 40), di-peptidyl peptidase 4 (DPP-4)[75] and AQP1 (ref. 65). One interpretation of this finding is that these proteins, which are key to reabsorbing two-thirds of the glomerular ultrafiltrate, have very long half-lives and thus, low levels of mRNA. Another possibility is that these mRNAs — which are located in the metabolically active proximal tubule cells — are adversely affected by the single cell isolation protocol itself, which may reduce oxygen availability and signals from neighbouring cells for a period of time[76]. However, this latter interpretation is undermined by the fact that transcripts of other major proximal tubule transporters, including *Sglt2*, *Cldn2*, *Nbce1* (also known as *Slc4a4*), *Oat1* and *Oat3*, are detected at high levels in single-cell RNAseq studies[24]. Nevertheless, these comparisons illustrate that mRNA levels are not a surrogate measure of protein levels in a tissue; rather, they are one measure of the synthesis rate of a specific protein. Likewise, the absence of detection of an mRNA cannot be interpreted as the surrogate of its encoded protein, as evidenced by proximal tubule NHE3 and its regulator DPP4.

Comparing relative transporter abundance in female versus male homogenates by semi-quantitative immunoblotting requires clear demonstration of the linearity of the detection system; assuming a general protein loading amount for the detection of all proteins is not sufficiently rigorous for protein quantification. This last point is key because of the highly variable abundance of transporters in the homogenates and the very wide range of antibody affinities[66]. Typically, we assay male and female samples at two (pre-verified) amounts on the same blot, which also provides two measurements, and validate with a form of densitometry whereby doubling the sample amount doubles the immunoblot signal on every blot; some transporter–antibody combinations are within the linear range at 0.5–1 µg, and others at 40–80 µg (as outlined elsewhere[17]). Equivalent protein loading across samples is validated by quantifying a parallel Coomassie stained gel or blot for total protein[66]. As apical and paracellular transporters exhibit specific expression patterns along the renal tubule (as indicated in Tables 1,2 and Fig. 2), mapping their regional abundance from homogenate immunoblots is feasible. By contrast, mapping the regional abundance of a ubiquitous transporter such as the Na,K-ATPase (sodium pump) from crude homogenates is not possible by homogenate immunoblots. With more confidence, we can assign medullary Na^+-K^+-ATPase subunits to the medullary thick ascending limb — a region that is highly enriched in Na,K-ATPase compared with other medullary tubule segments.

As studies on sex differences in renal transporter expression progress, it will be important to recognize that impactful discoveries will come from complementing transcriptomic analyses with protein and functional measurements. It will also be important to consider other factors that influence sex differences in renal transporter expression. Studies of transporter protein levels have demonstrated that sex differences vary among species[17,25,28,29,50,69] (Tables 1,2, Fig. 2). Moreover, it will be important to consider the biologic variables that can influence sex differences in protein levels, including relative tubule lengths[77], age[78], hormonal status[79], as well as methodological variables such as isolation methods and the strategies used to normalize relative expression or abundance levels. Moreover, as transporter activity is a function of multiple physiological variables, including trafficking, covalent modifications, and protein–protein associations, it will be important to consider that changes in transporter activity often occur independent of changes in protein or RNA abundance[17,25,80], especially in response to acute stimuli or inhibition[81].

Sex differences in renal transporters

Tables 1 and 2 summarize the ratios of female to male transporter protein abundance reported in rats and mice, organized according to location along the nephron from the proximal tubule to the collecting duct. These ratios are, for the most part, extracted from studies that assessed protein abundance in homogenates of dissected renal cortex (which contain proximal tubules, cortical thick ascending limbs, distal convoluted tubules, connecting tubules and cortical collecting ducts) and renal medulla (which contain some proximal tubule S3 segments, medullary thick ascending limbs and medullary collecting ducts) — an approach that minimizes the effects of differential recovery when

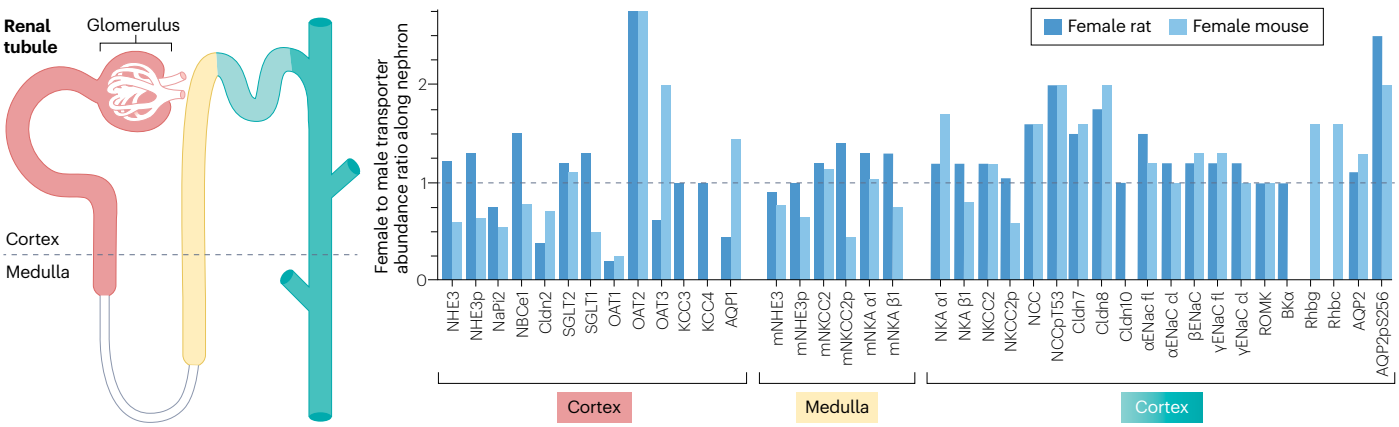

Fig. 2 | Sex- and species-specific differences in the abundance of renal transporters along the renal tubule. The female-to-male transporter abundance ratios, assessed in rat and mouse cortical and medullary homogenates, are plotted relative to the mean abundance of each transporter in males defined as 1 (dashed line). Data are obtained from the female-to-male ratios summarized in Tables 1 and 2. Specific citations for individual transporter ratios are provided in Tables 1 and 2. The location of each transporter is indicated by the coloured bars on the left, which correspond to the segment with the same colour in the renal tubule cartoon. Cortical Na,K-ATPase subunits, which are ubiquitously expressed along the renal tubule, are arbitrarily illustrated here in the distal cortex, adjacent to the medullary Na,K-ATPase subunits. Transporters that localize to both the cortical and medullary collecting duct are not duplicated.

purifying cell membranes. Overall, in rats and mice, the female to male transporter ratios are ≤1 along the proximal tubule to the medullary thick ascending limb and ≥1 from the cortical thick ascending limb through to the collecting duct (with exceptions). This pattern is partially attributed[71,82] to the presence of shorter proximal tubules in females than in males in both mice[74] and rats[8,83,84].

Sodium and water handling in the proximal tubule

Male rats reabsorb approximately two-thirds of the filtered Na^+ and volume along the proximal tubule. Female rats excrete a saline load twice as fast as males, exhibit one-third lower proximal tubule bicarbonate reabsorption versus males, and twice the lithium clearance rate (a marker of volume flow from the proximal nephron). These measures indicate reduced fractional sodium and volume reabsorption along the proximal tubule of females; that is to say that female rats reabsorb only about half of the filtered load of Na^+ and volume along the proximal tubule[17]. As a result, a higher fraction of the filtered Na^+ is delivered and reabsorbed downstream of the proximal tubule, which raises questions about the mechanisms responsible for the reduced fractional Na^+ reabsorption along the proximal tubule in rats, as well as the consequences of shifting sodium reabsorption downstream. A number of observations provide insights into these questions. First, although the Na/H exchanger, NHE3 — which is responsible for a considerable fraction of transcellular Na,K-ATPase-driven Na^+ reabsorption — was not less abundant in female than in male rats[17] (Table 1), immunohistochemistry localizes more NHE3 to the base of the proximal tubule brush border microvilli in female than in male rats and mice[17,31]. At the base, NHE3 transport activity is low[85,86], and NHE3 phosphorylation (NHE3pS552), which provides a marker of NHE3 in this less active zone, is more abundant in female than in male rats (not mice). Second, the abundance of claudin 2 is lower in females than in males by 60% and 30% in rats and mice, respectively[17] (Table 1). This finding is key, because claudin 2 promotes the passive paracellular reabsorption of about 50% of the Na^+ reabsorbed with Cl^- along the late proximal tubule[87]. The low Cl^- permeability of the early proximal tubule means that the tubular concentration of Cl^- increases along the proximal tubule as other salts and water are reabsorbed transcellularly driven by the Na,K-ATPase. Along the late proximal tubule, the permeability of Cl^- is greater than that of HCO_3^-, which drives the paracellular reabsorption of Cl^-, generating a lumen-positive potential, which then drives the paracellular reabsorption of Na^+ via claudin 2. This claudin 2-mediated transport is passive, independent of Na,K-ATPase and lowers the overall metabolic cost of proximal tubule sodium reabsorption compared with that of downstream segments. The lower abundance of claudin 2 in females than in males not only lowers proximal tubule NaCl reabsorption, but also raises the overall metabolic cost of renal sodium transport, which correlates with a higher abundance of Na,K-ATPase subunits in female rats and mice[17] (Table 1). Third, compared with males, female rats exhibit a 50% lower abundance of the water channel, AQP1, along the proximal tubule, which affects transepithelial water permeability; the Na–phosphate co-transporter NaPi2a is also 25% lower in female than in male rats. In contrast to the lower abundance of Na^+ and water transporters in female than in male rats, expression of the sodium–glucose cotransporters SGLT1 and SGLT2 is 20–30% higher in females than in males — an adaptive difference that may enable the complete reabsorption of filtered glucose along the shorter female proximal tubule[71].

Interestingly, the female-to-male ratios for proximal tubule transporters are quite different between rats and mice. Like rats, NHE3 in female mice is localized to the base of the proximal tubule brush border[17,31], where transport activity is lower[85,86]. Unlike rats, NHE3 abundance is 40% lower and AQP1 abundance is 45% greater in female than in male mice. Moreover, the diuretic and natriuretic responses to a saline challenge are indistinguishable in female and male mice, suggesting that the lower levels of AQP1 in female rats contribute to their enhanced response to a saline challenge[17].

Organic anion transporters

OATs, which are expressed in many tissues, are found along the proximal tubule basolateral and apical membranes. These transporters bind organic anions — including toxins, many drugs including diuretics, and metabolites — with a very broad specificity. OATs capture organic anions that diffuse from the peritubular capillaries and facilitate their

secretion into the tubular fluid[88], driven by basolateral Na,K-ATPase activity[89]. Male and female sex hormones strongly affect the expression and abundance of OATs: in rats, the female-to-male ratios of OAT1 and OAT3 are 0.2 and 0.61, respectively, whereas OAT2 is far more abundant in female rats than in males[27,68] (Table 1). Of interest, the abundance of cortical OAT1 in male rats was strongly reduced by castration; levels were restored to normal with testosterone treatment and further depressed by oestradiol. Similarly, levels of OAT3 in the cortex of male rats were stimulated by androgens and inhibited by oestrogens[27]. Conversely, OAT2, which is expressed on the apical surface of proximal tubule S3 segment cells in rats, was upregulated by castration, downregulated by ovariectomy, strongly downregulated by testosterone and weakly upregulated by oestradiol and progesterone treatment[68]. Extensive discussion of the physiological relevance of function and the relevance of these sex differences can be found elsewhere[90].

Sodium transporters along the ascending loop of Henle

The thick ascending limb of the loop of Henle, also known as the diluting segment, reabsorbs Na^+ without water against a steep electrochemical gradient, thereby diluting the tubular fluid and concentrating the medullary interstitium — processes that are key to concentrating and diluting the urine. The lower proximal tubule reabsorption of female rats than of males results in the increased delivery of fluids and electrolytes to the thick ascending limb. A compensatory 'downstream shift' in Na^+ and volume reabsorption must therefore occur post-proximal tubule to maintain homeostasis. This shift, in female rats, moves Na^+ transport from a region with low metabolic cost (discussed above) to regions with higher metabolic costs, such as the thick ascending limb and later segments. The impact of this sex difference is evident along the medullary loop of Henle where the abundance of the apical Na^+-K^+-$2Cl^-$ cotransporter (NKCC2) as well as its activated phosphorylated form (NKCC2p) are 20% and 40% greater in female rats than in male rats, and the basolateral Na,K-ATPase subunits are 30% more abundant in females than in males, evidence that the transporter profile in female rats is accompanied by a higher metabolic cost[17]. This pattern of higher metabolic cost along with lower proximal Na^+ reabsorption was also observed in male, claudin 2-knockout mice that have blunted paracellular Na^+ reabsorption[87].

In contrast to rats, female versus male mice have greater proximal AQP1 abundance and a similar excretory response to saline challenge. Moreover, the downstream medullary thick ascending limb of female mice does not exhibit greater abundance of NHE3, NKCC2 or Na,K-ATPase than that of male mice[17], indicating species differences in renal fluid and electrolyte handling and raising further questions about the mechanisms and consequences of sex differences in fluid and electrolyte handling along the proximal and medullary tubules.

Sodium transporters along the distal renal tubule and collecting duct

Beyond the thick ascending limb, the distal renal tubule exerts fine control over Na^+, K^+, acid–base and water homeostasis. Under physiological conditions, the ratio of female-to-male transporters in this region in rats is elevated, reflecting the downstream shift in fluid and electrolyte reabsorption in females compared with males. The sodium–chloride transporter (NCC) and its phosphorylated form (NCCp), which is specifically localized to the apical surface[38], are 2-fold more abundant in the distal convoluted tubule of female rats and mice compared with males (Table 2, Fig. 2). NCC meters Na^+ delivery downstream to the epithelial sodium channel (ENaC), which has a female-to-male ratio of 1.2 in rats. Na^+ reabsorption by ENaC generates a favourable transmembrane gradient for apical K^+ secretion mediated by the renal outer medulla K^+ channel (ROMK, which is expressed at equivalent levels in females and males) and Big K^+ channel (BK α, which is also expressed at equivalent levels in females and males). In addition, the abundance of cortical collecting duct claudin 7 and claudin 8, which facilitate net NaCl reabsorption by ENaC[91,92], are both 1.7-fold greater in female than in male rats and mice, which may amplify ENaC transport activity in females compared with males. Female rats that fast overnight present with lower plasma K^+ concentrations (not evident without fasting owing to the rapid impact of food intake on plasma K+)[17,93] and similar plasma aldosterone concentrations to males, which likely reflects higher ENaC activity in females than in males. If this assumption is correct, the elevated NCCp in females may represent a mechanism to defend plasma K^+ concentrations in response to greater ENaC activation[94], and may potentially have a role in preventing hyperkalaemia in states of high dietary K^+ intake, such as during pregnancy and lactation[95] (Fig. 1). The highly regulated AQP2 in its phosphorylated, activated state (AQP2p) is ~2-fold more abundant in female than in male rats, which predicts higher water permeability and water reabsorption along the collecting duct in the presence of the AQP2-activating antidiuretic hormone[96]. Overall, the pattern of transporters along the distal renal tubule is quite similar in mice and rats (Table 2, Fig. 2). Nonetheless, plasma K^+ levels are not lower in female mice; rather, Na^+ and K^+ concentrations overlap in the two sexes in mice[17].

Contribution of morphological differences

How sex differences in tubule length and/or volume contribute to sexual dimorphisms in transporter abundance along the renal tubule is a topic for investigation. Sex differences in renal structure have been well-documented, as reviewed elsewhere[97]. Segment-specific comparisons of morphometric differences between male and female mouse nephrons[74,98] have not identified a simple correlation between tubule length or volume and transporter abundance. The female-to-male ratio of proximal tubule volume density is 0.67 (ref. 74); however, the female-to-male ratios of proximal tubule proteins range from 0.54 for NaPi2 to 3.0 for OAT2 (Table 1). By contrast, distal convoluted tubules are of similar length in female and male mice, whereas the abundance of NCC is two-fold greater in females[98]. The female-to-male volume density of cortical collecting ducts is ~1.5 (ref. 74), which does roughly correspond to the female-to-male ratios of full-length ENaC subunits (1.2–1.3), AQP2 (~1.3) and Rhbg (1.6) (Table 2).

Electrolyte and hormonal challenges

High salt diet consumption or Angiotensin II (ANGII) infusion challenge fluid and electrolyte homeostasis. Emerging findings provide new insights into the effects of sex dimorphisms on the homeostatic responses.

High salt diet

Kidneys match salt excretion to salt intake to maintain circulatory volume. Male rodents respond to a 4% NaCl 'high salt' diet through a number of adaptations, including translocating NHE3 to the base of the microvilli, decreasing NCC abundance, phosphorylation and surface expression, and reducing ENaC cleavage[38,99,100]. A 2022 study by Gohar et al.[101] reported that female rats fed a high salt diet for 5 days exhibited a much more rapid natriuretic response, and maintained serum Na^+ and osmolality better than males, consistent with the previously reported enhanced natriuresis of female rats subjected to an acute saline challenge[17]. When female and male C15BL/6 J mice were fed a high salt (4% NaCl) versus a normal salt (0.26% NaCl) diet for 15 days, NHE3

trafficked to the base of the microvilli in males, as reported before[99], and remained at the base of the microvilli without further retraction in females[31]. The changes in sodium transporter abundance with high versus normal salt diets (Fig. 3a) were similar along the nephron between sexes, including reduced NCC and ENaC, whereas the sex differences in normal salt transporter abundance, including lower NHE3 in females, persisted. The pattern is consistent with, and provides a mechanism for, the findings of Gohar et al.[101] that females adapt to excreting a high salt diet more rapidly and thus maintain serum volume and electrolytes more closely than male mice. Of relevance to this 'female advantage', another study showed that a 4-week, high salt diet raised blood pressure in male but not female diabetic (db/db) mice[26]. Moreover, although the high salt diet decreased levels of NHE3, NKCC2 and NCC in both sexes, ENaC subunit abundance and cleavage were reduced in control and db/db female mice, but not in db/db males — a finding that the researchers attributed to the effects of inflammation[26].

Angiotensin II

In contrast to the actions of a high salt diet, which increases Na^+ excretion, ANGII is a sodium-retaining hormone. Infusion of 400 ng/kg/min of ANGII via an osmotic minipump is a widely used experimental model of hypertension. In male rodents, 3-day ANGII infusion activates sodium transporters all along the renal tubule, leading to a positive sodium balance[102]. With continued ANGII infusion, cortical NKCC2, NKCC2p, NCC, NCCp and cleaved ENaC subunits remain activated, whereas proximal and medullary NHE3 and medullary NKCC2 pool sizes decrease, secondary to the positive sodium balance and hypertension responses, which reinstate fluid and electrolyte homeostasis at the cost of persistent hypertension[25,103–105]. Interestingly, the transporter profile of male mice during ANGII-induced hypertension resembles that of female mice under physiological conditions; that is, they exhibit a lower abundance of proximal and medullary transporters and a higher abundance of Na^+ transporters along the distal tubule and collecting duct — a pattern that facilitates pressure natriuresis in the face of persistent ANGII-induced ENaC activation[105]. ANGII infusion into female C57BL/6 J mice amplifies the baseline profile of transporter expression, by decreasing the pool size of NHE3 and increasing the pool sizes of NCC and ENaC[25] (Fig. 3b). Related to this point, another study reported that wild type female mice excreted twice the amount of Na^+ as males in response to an NCC inhibiting thiazide diuretic, and that this difference was eliminated in mice with ANGII receptor deletion (AT1R KO) owing to a doubling of the diuretic response in AT1R KO males coupled with a lack of response in AT1R KO females[33]. At the transporter level in AT1R KO mice, NHE3 abundance was reduced by 50% in both sexes compared with that of wild type (confirming a previously reported role for AT1R in regulating NHE3 abundance)[106]. By contrast, levels of NCC and NCCp abundance were doubled in AT1R KO compared with wild type males (not females), implicating the downstream shift in NaCl from the proximal to the distal nephron in driving the increased NCC abundance in AT1RKO mice[33].

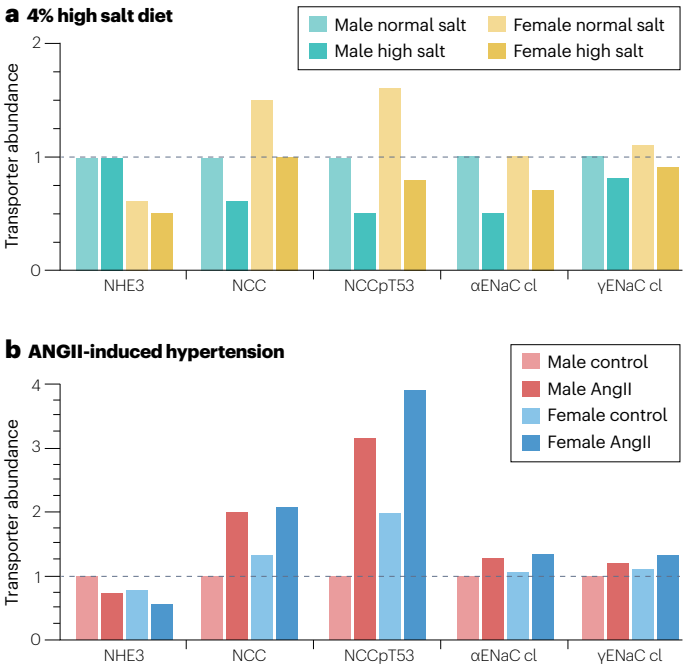

Fig. 3 | Regulation of selected sodium transporters in female versus male mice. a, Regulation of sodium transporter abundance in response to 15-day, 4% NaCl (high salt) versus a 0.26% NaCl (normal salt), diet. Transporter abundance, from Torres-Pinzon et al.[31], are normalized to transporter levels in males on a normal salt diet, defined as 1. **b**, Regulation of sodium transporter abundance in response to 14-day infusion of Angiotensin II (ANGII) (400 ng/kg/min by osmotic minipump) versus sham surgery controls. Transporter abundance, from Veiras et al.[25], are normalized to control uninfused males on a normal salt diet.

Acid–base handling

Kidneys maintain tight acid–base homeostasis by eliminating 50–100 mEq H^+ per day (50–100 mmol/l per day), mainly derived from the diet, as ammonium ion (NH_4^+) and titratable acid. This process is driven by glutamine uptake from the circulation into the proximal tubule via a basolateral sodium–glutamine transporter (SNAT3; expressed at equivalent abundance in female and male mice)[70]. In mitochondria of the proximal tubule, glutamine is metabolized to ammonia and generates 'new' bicarbonate, in a process that involves PEPCK and glutamine synthetase[107,108]. Even at baseline, acid is excreted every day to replenish lost bicarbonate, a process mediated by secretion of ammonia into tubular fluid via apical NHE3, which is coupled to the production and absorption of new bicarbonate into the bloodstream via basolateral NBCe1 (refs. 80,81,107,109–113). Further downstream, in the thick ascending limb, tubular ammonium is absorbed into the interstitium by NKCC2 (refs. 107,114,115), and further down the renal tubule, ammonia and ammonium are secreted into the tubular fluid of the collecting duct, primarily by the Rhesus (Rh) glycoproteins Rhbg and Rhcg[107,114,115], where it can be trapped by secreted H^+. In the end, the amount of ammonia and ammonium trapped and excreted is a measure of the new bicarbonate that has been absorbed. Mice demonstrate significant sex-dependent variations in several proteins involved in ammonia transport. Under basal conditions, females excrete twice as much urinary ammonium as males[74]. This sexual dimorphism is associated with greater expression of PEPCK, glutamine synthetase, NKCC2, Rhbg and Rhcg in females, and greater NHE3 expression in males[70] (Tables 1,2 and Fig. 2).

Metabolic acidosis, one of the earliest recognized complications of decreased kidney function, may affect as many as 40% of patients with kidney disease[116]. Acid loading induces adaptive increases in the production of ammonia and the abundance of NHE3, Rhbg and Rhcg, which facilitate acid excretion along with the absorption of new bicarbonate[115]. Interventional clinical trials have shown that correcting

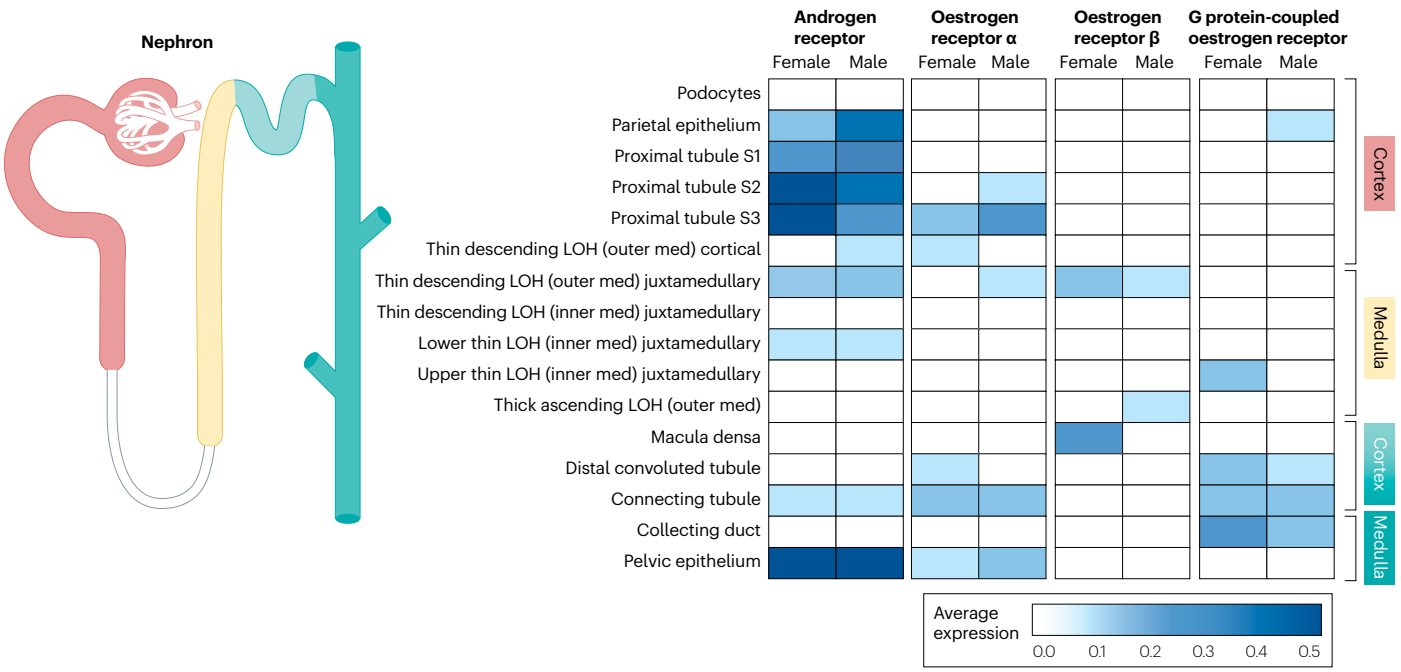

Fig. 4 | Expression of sex hormone genes along the renal tubule. The average expression of genes encoding sex hormone receptors is shown for adult male and female C57BL/6 J mice, based on single-cell RNA sequencing[24] and are available for segment-wise viewing in the Kidney Cell Explorer. LOH, loop of Henle.

metabolic acidosis decreases CKD progression and the need for kidney replacement therapy[117,118]. To our knowledge, sex-specific differences in the rate of CKD progression have not been conclusively determined. However, acid loading in mice induced a significantly greater relative increase in ammonium excretion in males than in females, despite similar acid intake and blood values, culminating in similar absolute responses[70]. Interestingly, the greater increase in ammonium excretion observed in acid-loaded male mice was associated with increases in PEPCK, Rhbg and NBCe1 expression, and enriched apical localization of Rhcg[70], whereas female mice exhibited greater increases in the glutamine transporter (SNAT3) than males. Thus, sex differences in acid–base homeostasis not only involve differences in ammonia production but also contrasting mechanisms of transporter regulation along the renal tubule. Understanding the impact of sex on ammonia metabolism is important for improving patient-centred medical care and optimizing acid–base balance in patients with kidney disease.

Sex hormones and their receptors

Gonadal hormones mediate sex differences in the structure and function of the kidney[17,74,119]. In particular, androgens enhance salt reabsorption[120] and water retention[121] in the proximal tubule, and increase total kidney volume in males[120]. Testosterone also regulates urinary calcium clearance[122], as well as ammonia metabolism and excretion[23,123]. Gonad removal and hormone injection studies indicate that testosterone has an important role in regulating sex dimorphism in both the mouse and the rat kidney[23,28,29,61,68,119,123–126]. In line with these findings, studies from the past few years have revealed that androgen receptor mRNA and protein are expressed exclusively in the proximal tubule[24,123] (Fig. 4). By contrast, oestrogens are reported to regulate NCC[127] and NKCC2 (ref. 128) abundance and/or activity in more distal segments. Ovariectomy and renal tubule-specific knockout of oestrogen receptor α had little impact on gene expression in general in the mouse kidney;[61] and motif analysis revealed a lack of direct involvement of oestrogen receptors in the proximal tubule[57,61], although transcripts for oestrogen receptor α and β were detected in proximal and distal tubules[24] (Fig. 4). In rats, treatment of ovariectomized females with oestradiol strongly decreased the expression of OAT1 (ref. 27). G protein-coupled oestrogen receptor (GPER) is expressed in distal tubules[24] (Fig. 4), and studies from the past couple of years have demonstrated the role of GPER in regulating the activities of medullary Na,K-ATPase in female rats[129] and NCC in distal convoluted tubules[130]. However, the role of GPER in regulating sex differences remains to be evaluated comprehensively.

Sex differences in hormone receptor abundance, as well as potential variability conferred by oestrus cycling in females, are often provided as a rationale for restricting experimental studies to males, raising the question of whether transporter abundance does indeed vary with stage in the oestrus cycle. An evaluation of transporter expression by oestrus stage[131] did not identify any correlation between stage of oestrus cycle (proestrus, oestrus, metoestrus, dioestrus) and the abundance or activity (phosphorylation) of renal cortical NHE3, NBCe1, NCCpS71 or medullary NKCC2p[17]. A comprehensive analysis of the effect of oestrus stage on phenotypic variability in 50 inbred strains of rats identified sex differences in variance in 74 of the 142 phenotypes examined. However, 59% of those differences were greater within males (which do not undergo cycling) than in females, prompting the study investigators to conclude that the "differential treatment of males and females for the purposes of experimental design is unnecessary until proven otherwise, rather than the other way around[132]."

X-inactivation

During fetal development, one copy of the X chromosome in females will undergo random inactivation, thereby achieving a gene dosage

equivalence to males, which have only one copy of the X chromosome. This inactivation is random; it is expected that cells have a 50:50 chance of expressing genes from the maternal copy or the paternal copy. However, this inactivation can be skewed in as many as 80% of cells, which subsequently display preferential inactivation of maternal or paternal chromosomes. This skewing of X chromosome inactivation is an epigenetic risk factor for various kidney disease outcomes[133,134]. Renal transporters are expressed across the chromosomes, and of those discussed in this Review, only claudin 2 is X-linked (Supplementary Table 1). Using data from whole kidney bulk RNA-seq, expression levels of *Cldn2* do not differ between male and female newborn C57BL/6 mice or throughout their lifetime, suggesting that the gene dosage of *Cldn2* is balanced between the sexes[61].

Physiological implications

As noted above, marked differences exist in the expression pattern of membrane transporters between male and female rodents; however, the functional implications of these differences are only beginning to emerge. Given the many types of renal epithelial cells and their complex cellular composition, analyses of the impact of sexual dimorphisms can be facilitated by computational models[135]. In particular, computational models that simulate epithelial transport along the renal tubule have been developed to provide insights into the effects of reported sex differences in transporter abundance. Given a set of model parameters, models of renal epithelial transport can predict tubular fluid and solute flow, water and solute fluxes through individual transporters or channels, and urine flow and solute excretion rates. Until recently, such models were built exclusively for male rats[136–138]. To simulate kidney function in females, model parameters should be adjusted to incorporate known or hypothesized sex differences, including but not limited to membrane transporters (Table 1, Fig. 2). In a series of studies, Layton, McDonough and co-workers developed sex-specific computational epithelial transport models, which account for sex differences in the abundance of apical and basolateral transporters (as reported in rats and extrapolated to humans), in single-nephron GFR, and in tubular dimensions[71,73,139,140].

These model simulations predict a shift in the Na^+ transport load to the distal tubules in females compared with males, consistent with their transporter abundance profiles (Figs. 2,5) and physiological measurements[17]. As previously noted, the lower fractional Na^+ reabsorption in the proximal tubules of the female rat kidneys is attributed to their smaller transport area, lower NHE3 activity and lower abundance of claudin 2 and AQP1, culminating in significantly larger fractional delivery of water and Na^+ to the downstream renal tubule segments[71]. Conversely, the female distal renal tubule exhibits a higher abundance of key Na^+ transporters, including NKCC2, NCC, ENaC, claudin 7 and claudin 8 as well as AQP2. The higher abundance of these transporters accounts for enhanced Na^+ and water reabsorption along the distal renal tubule in females compared with males, resulting in similar urine excretion in the two sexes. Consequently, in response to a saline load, the Na^+ load delivered distally is greater in female rats than in male rats, overwhelming the transport capacity of the distal renal tubule and collecting duct and resulting in higher natriuresis in female rats, as reported in both experimental and theoretical assessments[17,71]. Male and female rodents also exhibit differential responses to diuretics and to mutations in Na^+ transporters, with a stronger resulting diuresis, natriuresis and kaliuresis response in females when some Na^+ transporters (for example, NHE3, NKCC2 and NCC) are inhibited and a stronger response in males when others (for example, ENaC) are inhibited[73], consistent with the report of greater thiazide diuresis in female mice, discussed above[33,34]. It is noteworthy that, unlike most Na^+ transporters, which are less abundant in the proximal tubule of females than in that of males, SGLT2 is more abundant in females. Model simulations suggest that the higher SGLT2 expression in females is necessary to compensate for their smaller tubular transport area in order to achieve a hyperglycaemic tolerance comparable with that of males[71].

During pregnancy and lactation, females face unique physiological challenges, which include the extra fluid and electrolyte demands of the offspring; hormonal changes, volume overload and vasodilation, leading to cardiac hypertrophy[141]; and major changes in renal and systemic haemodynamics. Indeed, all aspects of renal physiology are impacted by pregnancy, and those effects evolve throughout pregnancy and differ among species. In pregnant females, GFR increases by ~50% and renal plasma flow increases up to 80% above non-pregnant levels[142]. Renal tubular function, together with electrolytes and water handling, are altered, leading to mild proteinuria and glucosuria, and to lower serum osmolality and sodium levels. In part due to water retention, hydronephrosis is common in pregnancy.

The first computational models of renal tubule transport in the kidney of pregnant rats[143] incorporate known renal adaptations during

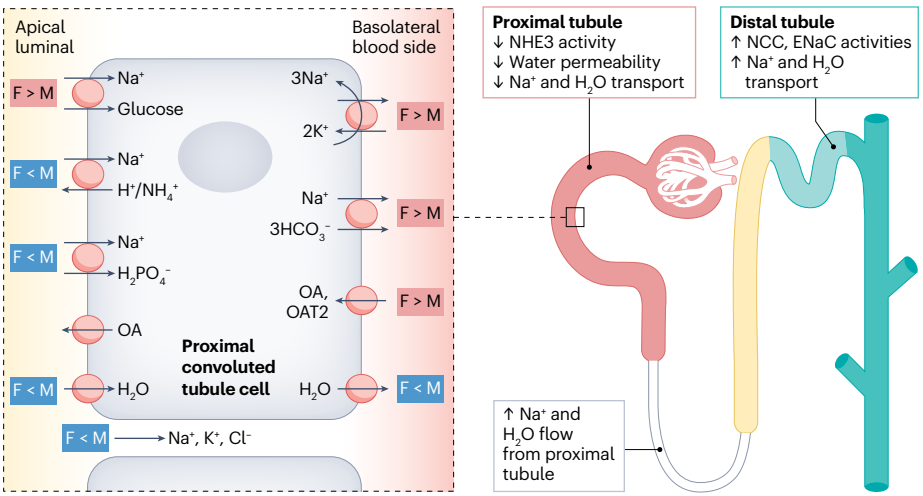

Fig. 5 | Impact of transporter sex differences on fluid and electrolyte handling along the renal tubule. A number of sex-specific differences exist in the abundance of membrane transporters along the renal tubule, as illustrated for rat proximal convoluted tubule cells. Such differences impact fluid and electrolyte handling, with lower Na^+ and water reabsorption in females than in males along the proximal tubule, and higher transport activities along the distal renal tubule in females than in males.

Box 1

Sex differences in renal transporters along the renal tubule

Pending issues:

- To what extent do sex differences in renal transporter patterns observed in rodents translate to humans?
- What are the functional implications of the sex differences in renal transporters, in normal physiology, pathophysiology and pharmacological therapy?
- The non-linear relationship between mRNA and protein dynamics, and between renal transporter protein abundance and activity, would benefit from a more comprehensive understanding and wider appreciation that includes estimates of turnover rates.
- There are major knowledge gaps to be filled in our understanding of the life cycle of the female kidney, including development, pregnancy, lactation, menopause and aging.
- Animal studies and computational models are often based on young virgin rats and mice, even though studies of chronic diseases such as hypertension, chronic kidney disease and diabetic kidney disease would be more clinically relevant if older rodents equivalent to middle-aged humans were studied.
- To what extent can sex differences in renal transporters explain the differences in the prevalence and progression of kidney-related chronic diseases? A better understanding of the 'female advantage' in cardiovascular disease may guide therapeutic targets in both sexes across the life cycle.
- A better understanding of the consequences of sexual dimorphisms on drugs that target transporters or their regulation may improve drug effectiveness.

Opportunities moving forward:

- Further use of optical clearing of kidneys to quantify morphometrics in females versus males in rodents and humans.
- Use of human kidney biopsy banks to study and quantify sexual dimorphisms, e.g. the Kidney Precision Medicine Project.
- Further exploration of human urinary exosomes to assess human renal transporters by applying ratiometric approaches, e.g. the ratio of proximal versus loop versus distal transporters.
- Exploit Four Core Genotype mice and rats to understand whether sexual dimorphisms are due to chromosomal or hormonal differences.
- Explore the impact of gender reassignment on renal transporter function.

pregnancy, including the marked elevation in GFR, renal hypertrophy and alterations in transporter expression[144,145]. Exactly how renal transporters are regulated in pregnancy remains to be fully characterized, but pregnancy-induced changes have been reported in Na^+ transporters (including Na,K-ATPase, NaPi2, NKCC2, NCC and ENaC)[146], K^+ transporters (ROMK and BK channels, H,K-ATPase)[147] and AQP2[148]. Model simulations and analyses have been used to identify the renal adaptations that have the largest effect on Na^+, K^+ and volume retention during pregnancy. Models of pregnant rats predicted that morphological adaptations and increased NHE3 and ENaC activity are essential for the enhanced Na^+ reabsorption that is observed during pregnancy[143]. Model simulations showed that for sufficient K^+ reabsorption to occur in pregnancy, increased activity of H,K-ATPase and decreased K^+ secretion along the distal segments are required. The model also suggested that certain known sex differences in renal transporter pattern (for example, the higher NHE3 protein abundance but lower activity in the proximal tubules of virgin female rats compared with male rats) may serve to better prepare females for the increased transport demand in pregnancy and the need to divert large volumes of circulating electrolytes and fluids to the fetuses.

Application to the human kidney

The laboratory rat is arguably one of the most well-studied animals. In particular, many of the morphological and molecular properties of its kidney have been experimentally measured. As such, computational models of the kidney have traditionally been based on the rat. These virtual rat kidney models have been used to simulate kidney function under altered Na^+ and K^+ loads[149,150], to better understand glucose handling via the SGLTs[137,151,152], and to shed light on the role of the renin–angiotensin system in hypertension[153,154]. However, the clinical value of these models is limited, given the major differences in anatomy and haemodynamics between the rat kidney and the human kidney. In other words, although results obtained using a rat kidney model may provide insights into kidney function, those results will not always or entirely translate to the human.

The extent to which tubular transport and function differs between the kidneys of men and women remains an open question. Limitations in variable sample quality and variation amongst individuals sampled are confounding factors in human studies and thus, the full scope of human renal sex differences awaits further investigation. Of note, a comparative study published in preprint form based on single-nuclear multiomic data demonstrated conserved sexually dimorphic gene expression in the human and mouse kidney[61].

Given the essential role of the kidney in blood pressure control, sex differences in the incidence of hypertension and CKD may be attributable, in part, to differences in kidney structure and kidney transporter regulation over the life cycle. To obtain a better understanding of the female advantage in cardiovascular disease, one may develop sex-specific computational models for the human kidneys. This would require data that characterize human renal haemodynamics, morphology and transporter activities. Human GFR and gross kidney size have been measured, but the patterns of transport activity are mostly uncharacterized in the human kidney, let alone sex differences. Despite the relative sparsity of data for the human kidneys, computational models have been developed by extrapolating our knowledge from rodents to humans[82,140,155]. Those models assume that the human kidneys exhibit sex differences similar to those characterized for the rat. That extrapolation may be justified by the similar challenges that female rats and women face in circulating volume adaptation during pregnancy and lactation (Fig. 1). Nonetheless, although human kidney

models offer a valuable platform for in silico biomedical research, the substantial uncertainty in model parameters must be acknowledged.

Conclusions

Emerging studies in rodents have yielded exciting findings that highlight the fact that the kidneys of a female rodent are not simply smaller versions of male kidneys; rather, rodent kidneys exhibit dimorphic patterns in transporter expression and salt handling, with major implications for kidney function (Box 1). A first step in assessing how the structure and function of the kidney is regulated by sex hormones and chromosomes involves determining the abundance and activity of transporters. Given the many factors that affect transporter abundance and activity, such assessments would benefit from combining transcriptomics with protein and functional measurements to properly analyse sex differences in renal transporters. Available data demonstrate that renal transporter protein abundance is higher in male rats and mice relative to females along the proximal tubule to medullary thick ascending limb; by contrast, from the cortical thick ascending limb through to the collecting duct, transporter protein abundance is higher in females, reflecting a compensatory downstream shift in Na^+ and volume reabsorption that is likely necessary to maintain homeostasis (Tables 1,2, Figs. 2,5). Sex differences have also been reported in acid–base homeostasis as a result of sex-specific transporter regulation along the renal tubule, as well as differences in ammonia production. The physiological consequences of the sex differences in kidney structure have been revealed in computational modelling studies. In particular, we hypothesize that the larger transporter reserve in the proximal tubules of virgin female rats than in those of their male counterparts may serve to better prepare females for the increased transport demands and stress of pregnancy, regardless of whether or not they become pregnant. The field of sex differences in renal transporters is growing; major knowledge gaps remain to be filled concerning the life cycle of the female kidney, including changes occurring during development, pregnancy, lactation, menopause and aging. A better understanding of the physiological challenges that are unique to females may yield new explanations for the sex differences in renal transporters beyond pregnancy and lactation. Furthermore, given the observed similarities in how sex impacts the prevalence and progression of kidney disease in humans and rodents, an understanding of transporter sex differences in rodent kidneys, especially in aged animals, may provide insights into novel disease mechanisms and potential therapies for humans, despite the major differences in the anatomy and haemodynamics between humans and rodents.

Published online: 08 September 2023

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Acknowledgements

A.A.M. discloses support for the research of this work from the National Institutes of Health (DK083875). A.N.H. discloses support for the research of this work from the National Institutes of Health (K08DK120873). L.(I.)X. discloses support for the research of this work from the National Institutes of Health (R01 DK126925-01). A.T.L. discloses support for the research of this work from: the Canada 150 Research Chairs program, the Natural Sciences and Engineering Research Council (NSERC Discovery award: RGPIN-2019-03916) and the Canadian Institutes of Health Research of Canada, and the Canadian Institutes of Health Research (CIHR) [TNC-174963].

Author contributions

All authors researched the data for the article, discussed the content, wrote the text, reviewed and edited before submission.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41581-023-00757-2.

Peer review information *Nature Reviews Nephrology* thanks Eman Gohar, Michael Butterworth and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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