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Review

Sex steroids and the kidney: role in renal calcium and phosphate handling

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ABSTRACT

Calcium and phosphate are vital for the organism and constitute essential components of the skeleton. Serum levels are tightly hormonally regulated and maintained by exchange with three major sources: the intestines, the kidney and the bone. The effects of sex steroids on the bone have been extensively studied and it is well known that sex steroid deficiency induces bone loss, indirectly influencing renal calcium and phosphate homeostasis. However, it is unknown whether sex steroids also directly regulate renal calcium and phosphate handling, hereby potentially indirectly impacting on bone. The presence of androgen receptors (AR) and estrogen receptors (ER) in both human and rodent kidney, although their exact localization within the kidney remains debated, supports direct effects. Estrogens stimulate renal calcium reabsorption as well as phosphate excretion, while the effects of androgens are less clear. Many of the studies performed with regard to renal calcium and/or phosphate homeostasis do not correct for the calcium and phosphate fluxes from the bone and intestines, which complicates the differentiation between the direct effects of sex steroids on renal calcium and phosphate handling and the indirect effects via the bone and intestines.

The objective of this study is to review the literature and current insight of the role of sex steroids in calcium and phosphate handling in the kidney.

Contents

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1. Introduction

Calcium is involved in many cellular processes, such as muscle contraction, enzyme activation, cell differentiation, immune response, programmed cell death, and neuronal activity (Pu et al., 2016). Phosphate is essential for several cellular functions as well, including cell signaling, energy exchange, and the formation of lipid bilayers (Tatsumi et al., 2016). In addition, calcium and phosphate are essential components of the skeleton. Together, they form hydroxyapatite, the main component of bone, responsible for its rigidity. Thus, an optimal calcium and phosphate balance is indispensable for bone mass and strength.

In the serum, calcium and phosphate levels are maintained between narrow ranges through several exchange routes: dietary intake via intestinal absorption, renal reabsorption/excretion, and mobilization from bone (Fig. 1). Several 'calciotropic' hormones tightly regulate the in- and effluxes in order to stabilize serum levels of calcium and phosphate: parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), klotho, and 1,25$(OH)_2$-vitamin D. The endocrine actions will be further discussed in the sections below. Chronic imbalances in calcium and phosphate homeostasis can not only lead to bone abnormalities (kidney-bone axis), but also affect other tissues and organs, and lead for example to ectopic calcifications in blood vessels and the kidney, resulting in chronic kidney disease and cardiovascular complications (Pu et al., 2016; Tatsumi et al., 2016).

Besides their well-known effects on the male and female reproductive systems, sex steroid hormones play a role in many other systems and processes, such as the cardiovascular system, the immune system, erythropoiesis, lipid and protein metabolism, and psychosexual and cognitive behavior (De Leon-Nava et al., 2009; Ikeda et al., 2005; Luine, 2008). Furthermore, sex steroids have an impact on the musculoskeletal system (Dubois et al., 2014, 2012; Vanderschueren et al., 2014). It is well established that sex steroid deficiency induces bone loss, leading to osteoporosis, and increased risk for osteoporotic fractures (Almeida et al., 2017; Vanderschueren et al., 2014). Not only female postmenopausal osteoporosis, but also male osteoporosis – for example in men with prostate cancer receiving androgen deprivation therapy - represents a major burden for public health (Vanderschueren et al., 2014).

It was previously demonstrated that the severe osteoporotic phenotype observed in global AR knock out mice could not be fully reproduced in bone cell-specific knock out mice, suggesting that sex steroids regulate processes in other organs which in turn have an impact on bone (Sinnesael et al., 2015, 2012). It can therefore be hypothesized that sex steroids have direct renal effects, hereby influencing the kidney-bone axis. There are several other arguments for the kidney as an important target of sex steroid action. First of all, the kidney expresses both AR and ER, as will be reviewed in the sections below. Furthermore, the kidney mass is sexually dimorphic, and several renal functions display gender differences, including the glomerular filtration rate, inulin clearance, activity of multiple enzymes, pharmacokinetics and pharmacodynamics of various drugs and substances, and the transport of organic compounds and ions (reviewed in (Sabolić et al., 2007)). Several kidney diseases show gender differences in occurrence and/or development as well; sex steroids have been suggested to play a role in the susceptibility to acute kidney injury (Crawford and Moul, 2015; Hodeify et al., 2013; Lapi et al., 2013; Soljancic et al., 2013), the risk and outcome of chronic kidney disease (Khurana et al., 2014; Kummer et al., 2012), the morbidity and mortality of kidney transplant recipients (Antus et al., 2001; Müller et al., 1999), compensatory renal growth after unilateral nephrectomy (Mulroney et al., 1999), kidney stone formation (Lieske et al., 2014; Naghii et al., 2014), as well as effects on the renin-angiotensin

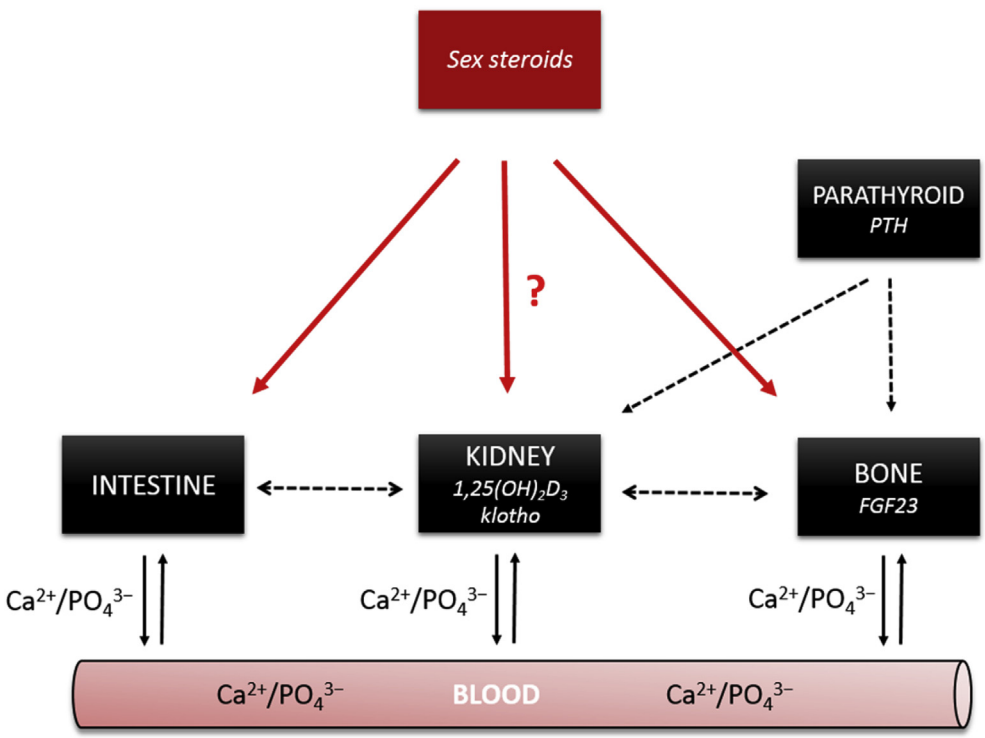

Fig. 1. Regulation of calcium and phosphate homeostasis and the potential contribution of sex steroids. Serum calcium and phosphate levels are kept stable through exchange via the intestines, the kidney and bone. These processes are controlled by calciotropic hormones: 1,25$(OH)_2$-vitamin D and klotho formed by the kidney, PTH secreted by the parathyroid glands, and the bone-derived FGF23.

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Table 1
Overview of AR expression in the male and female kidney.

Species	Male	Female	Localization	Method	Reference
Human Mouse	+	+	NS	In vivo luciferase activity + IHC	(Dart et al., 2013)
Rat Mouse	+	NI	NS	qPCR + WB + IHC	(Hsu et al., 2014)
Human	+	−	NS PT	qPCR + WB	(Quinkler et al., 2005)
Rat	+	NI	Glomeruli: endothelial, mesangial and visceral epithelial cells	IHC	(Shortliffe et al., 2014)
Mouse	+	+	Glomeruli	qPCR + WB	(Elliot et al., 2007)
Mouse	NI	+	Glomeruli: podocytes	WB	(Doublier et al., 2011)
Mouse	+	NI	DT-CT	IHC	(Hsu et al., 2010)
Rat	+	NI	PT, CCD	qPCR	(Boulkroun et al., 2005)
Mouse	+	+	PT	qPCR + WB	(Grimont et al., 2009)
Mouse	+	NI	PT	qPCR	(Krid et al., 2012)

NI = not investigated, NS = not specified, PT = proximal tubule, DT = distal tubule, CT = connecting tubule, CCD = cortical collecting duct, IHC = immunohistochemistry, WB = Western Blot.

system (Reckelhoff et al., 2000).

In this review we will focus on the current knowledge of the effects of sex steroids on renal calcium and phosphate handling.

2. Expression of sex steroid receptors in the kidney

There are several gender differences in renal function and occurrence and development of kidney diseases. In order to better understand these differences, it is important to know where sex steroid receptors are located. Uncovering the tissue-specific expression of the AR and ER could aid in distinguishing the functional roles of each receptor in normal renal physiology as well as pathophysiology.

2.1. Androgen receptor

It has been established that the AR is expressed in mouse, rat and human kidney, in males as well as females (Dart et al., 2013; Hsu et al., 2014), although one study revealed low mRNA levels and no apparent protein expression in females (Quinkler et al., 2005) (Table 1). However, the exact localization of the receptor within the kidney remains debated (Fig. 2). While some showed expression in mouse and rats both *in vitro* and *ex vivo* in the glomeruli (Doublier et al., 2011; Elliot et al., 2007; Shortliffe et al., 2014) and in distal nephron segments (Boulkroun et al., 2005; Hsu et al., 2010), others showed that the AR is predominantly expressed in the proximal tubule (PT) by analyzing expression in

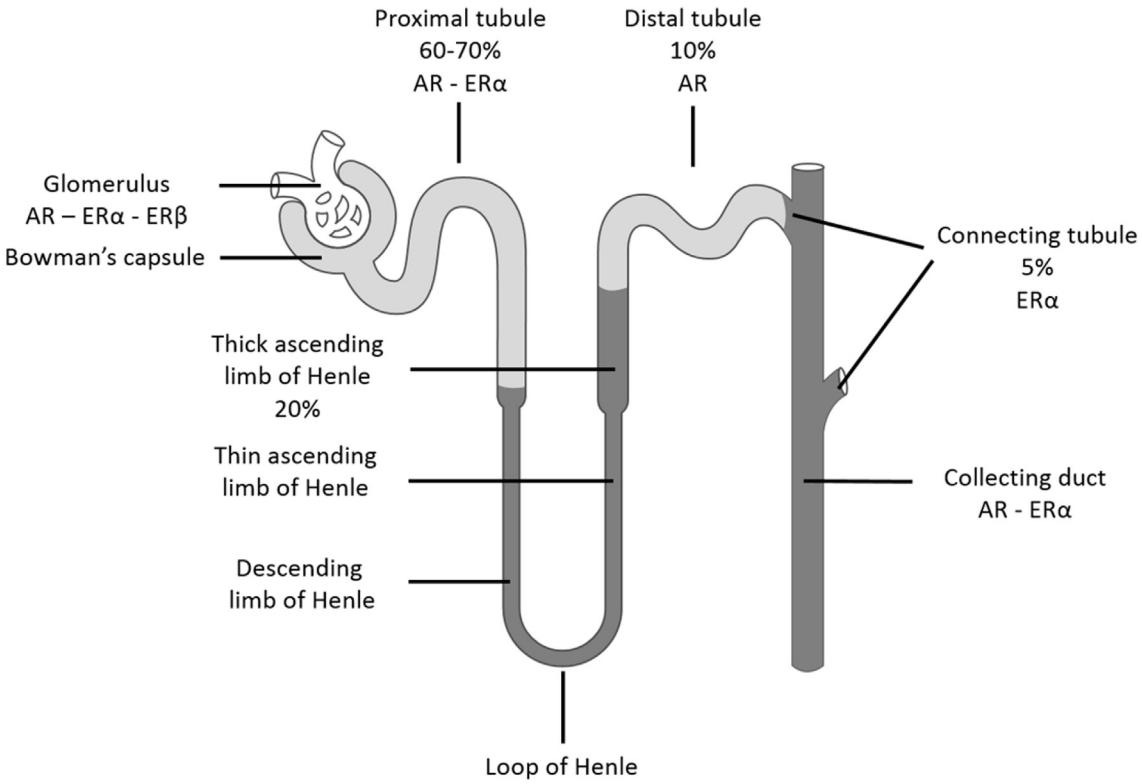

Fig. 2. Schematic representation of the nephron, with expression of androgen (AR) and estrogen receptors (ERα and ERβ) and the relative contribution (expressed as %) of each tubular segment to calcium reabsorption. The kidney, consisting of the cortex and the medulla, contains approximately 1 million nephrons. Each nephron consists of a renal corpuscle, comprising the glomerular capillaries and Bowman's capsule, and the renal tubules. The components of the renal tubule are the proximal tubule (PT), the descending limb of Henle, the loop of Henle, the thin and thick ascending limb of Henle (TAL), the distal tubule (DT) and the connecting tubule (CT). The CT connects the DT to the collecting duct (CD). AR and ER localization is still debated, with variable expression shown in glomeruli, PT, DT, CT and CD.
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Table 2
Overview of ER expression in male kidney.

Species	ERα	ERβ	Localization	Method	Reference
Mouse	NS	NS	NS	Radioligand binding	(Hagenfeldt and Eriksson, 1988)
Mouse	+	−	NS	RPA	(Couse et al., 1997)
Mouse	+	−	NS	SB + WB	(Sharma and Thakur, 2004)
Mouse	+	−	Nuclear ER: PT, low expression in distal segments (CT, CCD, OMCD) Membrane bound ER: distal part of nephron	qPCR + WB	(Grimont et al., 2009)
Mouse	+	−	PT	qPCR	(Krid et al., 2012)
Mouse	+	+	ERα66: Vasculature, glomerulus ERα46: NS ERα36: mesangial and tubular epithelial cells ERβ: mesangial cells	WB + IHC	(Irsik et al., 2013)
Mouse	+	−	NS	PCR	(Kuiper et al., 1997)

NS = not specified, PT = proximal tubule, CT = connecting tubule, CCD = cortical collecting duct, OMCD = outer medullary collecting duct, SB = Southern Blot, WB = Western Blot, IHC = immunohistochemistry.

isolated rat or mouse PT, immortalized PT cell lines as well as primary human PT cells (Boulkroun et al., 2005; Grimont et al., 2009; Krid et al., 2012; Quinkler et al., 2005). The apparent discrepancy in the literature on AR expression could be due to species-specific differences and to the possible artifacts known to occur in immunohistochemistry for AR.

2.2. Estrogen receptor

Estrogens are usually considered to bind soluble intracellular receptors, ERα and ERβ. However, recently the existence of membrane bound ERs has been demonstrated as well. While it is generally accepted that the ERα is expressed in both male (Table 2) and female kidneys (Table 3), it is still uncertain whether the ERβ is present as well and where both receptors are located precisely. ERα expression was shown in both male and female mice, while ERβ was absent (Couse et al., 1997; Kuiper et al., 1997; Lim et al., 1999; Sharma and Thakur, 2004). In contrast, both isoforms were located in the glomerulus and more specifically in podocytes in humans and mice (Doublier et al., 2011; Gross et al., 2004; Kummer et al., 2011). More recent analyses revealed expression of ERα, but not ERβ, in mouse PT with little to no expression in distal segments of the nephron (Krid et al., 2012). More specifically, Grimont et al. described that ERα in the PT was found in the nuclei, while in the distal parts a membrane-bound ER was found, also referred to as GPR30 (Krid et al., 2012). GPR30, a 7-transmembrane G protein −coupled receptor, has been recently shown to be localized both in the plasma membrane, as well as on intracellular structures, including cytokeratin intermediate filaments in Madin-Darby canine kidney cells expressing the native receptor and in HEK239 cells ectopically expressing the receptor (Broselid et al., 2014; Sanden et al., 2011).

With the identification of different splice variants of ERα and ERβ (Maruyama et al., 1998; Petersen et al., 1998), localization studies have become even more challenging. Irsik et al. studied the distribution of ERα and its different splice variants in detail in both males and female mice. IHC showed low expression of the full length ERα (ERα66) in the kidney of both genders, where it was detectable in the glomerulus although it was not present in podocytes. In addition, its expression was also evident in the brush border membrane of PT cells and cortical CD (CCD) in female mice. Expression of the splice variant ERα36 was much higher in females than males. It was detectable in mesangial cells and in tubular epithelial cells including the PT in both genders, and in podocytes in females. ERα46 was also found in the kidney, but localization of this splice variant was not possible due to lack of a specific antibody. ERβ was detected in both genders, but with a higher expression level in females as well. It was present in mesangial cells in both genders and in podocytes of female mice as well. These variable expression patterns of the different splice variants highlight the importance of identifying the different ERs when studying estrogen effects on the kidney (Irsik et al., 2013). The localization of the different ERβ splice variants in the kidney has not been investigated yet.

Considering the identification of these multiple splice variants, some of the discrepancies might be accounted for by the use of different antibodies, or by the variations in the detection methods used.

In conclusion, in order to gain further insight in renal AR/ER expression, there is a need for immunohistochemical localization

Table 3
Overview of ER expression in female kidney.

Species	ERα	ERβ	Localization	Method	Reference
Mouse	NS	NS	NS	Radioligand binding	(Hagenfeldt and Eriksson, 1988)
Mouse	+	−	NS	RPA	(Couse et al., 1997)
Mouse	+	−	NS	SB + WB	(Sharma and Thakur, 2004)
Mouse	+	−	Cortex	qPCR	(Lim et al., 1999)
Mouse	+	+	Glomeruli	IHC	(Gross et al., 2004)
Mouse	+	+	Glomeruli: podocytes	WB	(Doublier et al., 2011)
Human Mouse	+	NI	Glomeruli: podocytes	IHC + WB	(Kummer et al., 2011)
Mouse	+	−	Nuclear ER: PT, low expression in distal segments (CNT, CCD, OMCD) Membrane bound ER: distal part of nephron	qPCR + WB	(Grimont et al., 2009)
Mouse	+	+	ERα66: Vasculature, glomerulus, Brush border of PT and CCD ERα46: NS ERα36: mesangial and tubular epithelial cells, podocytes ERβ: mesangial cells, podocytes	WB + IHC	(Irsik et al., 2013)

NI = not investigated, NS = not specified, PT = proximal tubule, CNT = connecting tubule, CCD = cortical collecting duct, OMCD = outer medullary collecting duct, SB = Southern Blot, WB = Western Blot, IHC = immunohistochemistry.

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along with co-localizations using established markers for the different renal cell types both in rodents and humans, using highly qualitative antibodies and appropriate positive and negative controls. By gaining more insight into the expression and the exact localization of the different receptors and their isoforms, the effects of sex steroids on kidney structure and function and the related gender differences could be better understood.

3. Renal calcium and phosphate handling

3.1. Renal calcium handling

In the kidney, about 98–99% of the filtered calcium is reabsorbed. Most of the calcium is taken up by the PT (60–70%), while the thick ascending limb of the loop of Henle (TAL), the DT and the CT reabsorb 20%, 10% and 5% respectively (Blaine et al., 2015; Friedman, 2000) (Fig. 1). This calcium re-absorption occurs via paracellular and transcellular transport. Paracellular, passive transport is dependent on electrochemical gradients. In contrast, transcellular transport is an active process, consisting of three steps: entry of calcium through the apical membrane, followed by transport to the basolateral membrane, and efflux into the blood circulation (Friedman, 2000; Moor and Bonny, 2016).

The epithelium of the PT has a high permeability for calcium, where it is reabsorbed mainly by paracellular transport and influenced by several elements, including sodium. The PT expresses claudins, epithelial tight junctions proteins involved in regulating the permeability to water, solutes and small ions, including calcium (Hou et al., 2013). Numerous claudins are present throughout the nephron, with expression of claudin-2, 10a, 12, and 17 in the PT (Hou et al., 2013). Claudin-2 knockout mice show hypercalciuria, suggesting that claudin-2 is involved in calcium reabsorption (Muto et al., 2010). Furthermore, PT cells play a role in calcium homeostasis through the expression of the 1α-hydroxylase enzyme, which is responsible for the conversion of 25(OH)-vitamin D into the active $1,25(OH)_2$-vitamin D3. (Hou et al., 2013; Moor and Bonny, 2016). $1,25(OH)_2$-vitamin D3 increases transcellular calcium transport and hence dietary calcium absorption from the intestines and calcium reabsorption in the distal part of the nephron (Moor and Bonny, 2016).

Transport in the TAL is also believed to be paracellular and is dependent on sodium reabsorption. Many claudins are localized in the TAL, with claudin-16 and claudin-19 likely to play a role in calcium reabsorption, as supported by the observed hypercalciuria in claudin-16 and claudin-19 knockout mice (Hou et al., 2009, 2007). Claudin-14 is believed to be a negative regulator of calcium reabsorption, by blocking the channel formed by claudin-16 and 19 (Gong et al., 2012).

As the DCT-CT epithelial cells are more tightly connected, calcium is predominantly actively transported through the transcellular pathway, mediated by several calcium transport proteins (Fig. 3). Apical entry by the transient receptor potential cation subfamily V member 5 (TRPV5) is followed by intracellular binding by calbindin-D_{9K} and calbindin-D_{28K} and transport to the basolateral membrane, after which calcium exits the cells and enters the blood circulation through the calcium-ATPase PMCA and the sodium-calcium exchanger NCX1 (Blaine et al., 2015; Friedman, 2000; Jeon, 2008; Moor and Bonny, 2016). Even though the majority of filtered calcium is reabsorbed proximally, the fine tuning occurs distally. Both the hormones $1,25(OH)_2$-vitamin D3 and PTH regulate this fine tuning process. PTH, secreted by the parathyroid glands in case of low serum calcium, acts on the kidney by enhancing active calcium reabsorption in the distal part of the nephron including the stimulation of TRPV5 expression, and stimulating 1α-hydroxylase activity in the PT. At the same time, PTH affects bone osteoblasts and even more osteoclasts, resulting in increased bone resorption and compensated serum calcium levels (Hoenderop et al., 2005). Klotho, present in soluble and membrane-bound form, is formed mainly by the kidney and exhibits opposite effects on calcium homeostasis. It inhibits $1,25(OH)_2$-vitamin D3 synthesis, resulting in reduced intestinal calcium absorption, and acts directly on the kidney by upregulating TRPV5 channels, hereby increasing calcium reabsorption (Huang and Moe, 2011).

3.2. Renal phosphate handling

About 75%–85% of the filtered phosphate is reabsorbed via

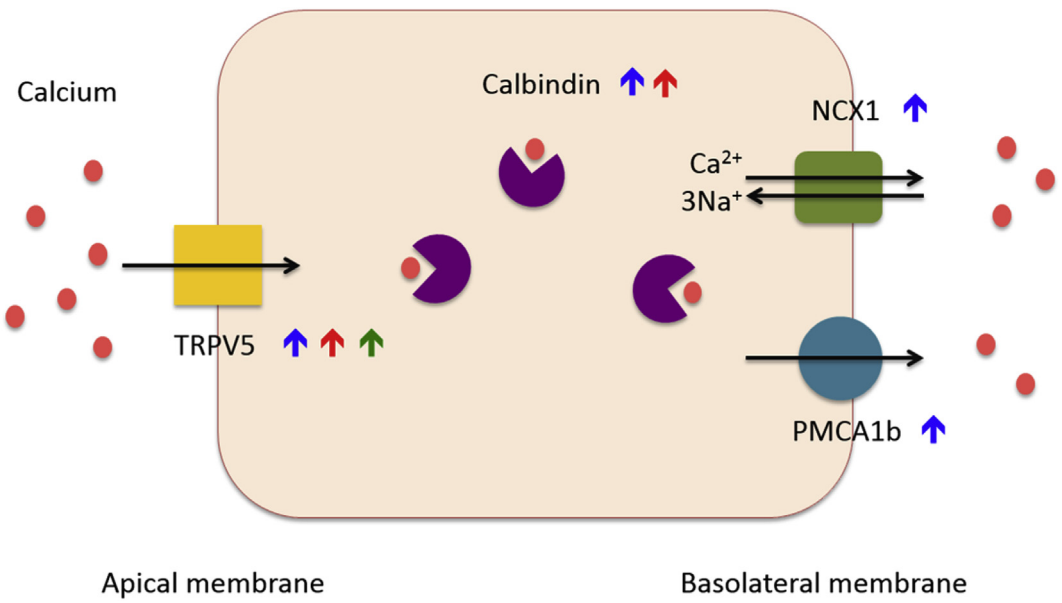

Fig. 3. Hormonal regulation of calcium reabsorption in the distal tubule. Calcium is taken up on the apical side by TRPV5, followed by binding to calbindin in the cytoplasm and exit of the cell through NCX1 and PMCA1b. In case of low serum calcium levels, calcium reabsorption in the distal tubule is increased by PTH (blue arrows), $1,25(OH)_2$-vitamin D3 (red) and klotho (green).

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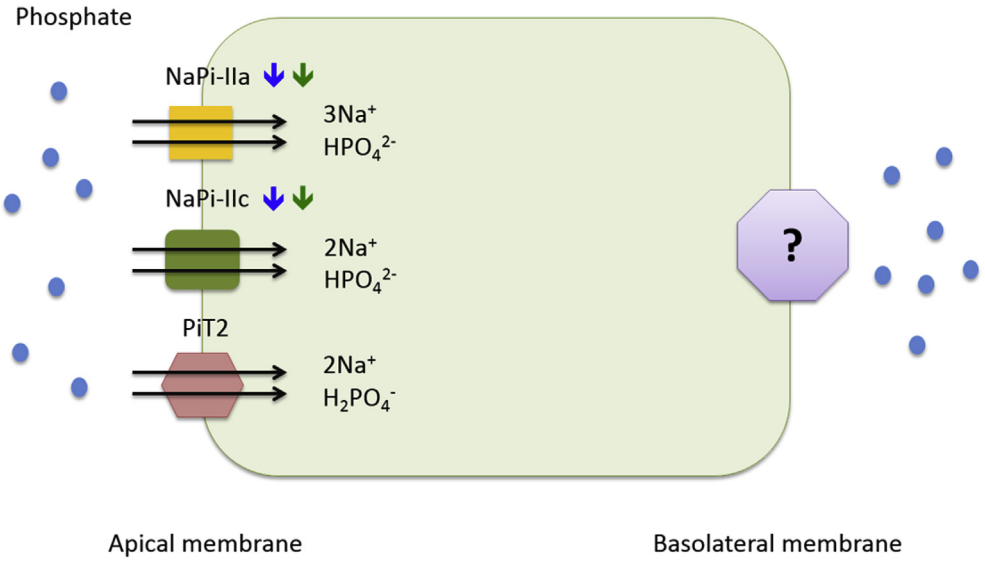

Fig. 4. Hormonal regulation of phosphate reabsorption in the proximal tubule. Phosphate is taken up on the apical side by three sodium/phosphate cotransporters: type II (NaPi-IIa and NaPi-IIc) and type III (PiT2) transporters. In case of high serum phosphate, phosphate reabsorption is inhibited by the action of PTH (blue arrows) and FGF23 and klotho (green).

sodium-dependent active cotransport (Blaine et al., 2015). The majority of reabsorption takes place in the PT, where phosphate is taken up from the tubular lumen at the apical membrane, followed by efflux at the basolateral membrane (Fig. 4). Several types of sodium/phosphate cotransporters have been identified in the apical membrane of proximal tubuli: type I (NaPi-I or Npt1), although these are believed to transport mainly organic phosphate and multiple other anions as well, type II (NaPi-IIa and NaPi-IIc) and type III (PiT2) transporters (Tatsumi et al., 2016). Patients with a homozygous duplication in the gene encoding NaPi-IIa suffer from autosomal recessive Fanconi's syndrome, hypophosphatemic rickets and renal failure, illustrating the critical role of the transporter in renal phosphate handling and control of phosphate homeostasis (Magen et al., 2010). This phenotype is mimicked partially in rodent knockout models (Beck et al., 1998). In contrast, NaPi-IIc appears to be important in humans (Bergwitz et al., 2006), while in mice this transporter is not essential for phosphate transport (Segawa et al., 2009). The mechanism of basolateral efflux of phosphate to the blood remains to be identified (Tatsumi et al., 2016).

Serum phosphate levels are hormonally regulated by PTH, 1,25$(OH)_2$-vitamin D3 and bone-derived FGF23.1,25$(OH)_2$-vitamin D3 stimulates the intestinal absorption of dietary phosphate and mobilization from bone through bone resorption (Tatsumi et al., 2016). In case of high serum phosphate on the contrary, action of PTH and FGF23-klotho is important. PTH decreases phosphate reabsorption by downregulating the expression of NaPi-IIa and NaPi-IIc. FGF23 is synthesized by the bone osteoblasts and requires its cofactor klotho, produced in the kidney, to exert phosphaturic effects. It acts by diminishing NaPi-IIa and NaPi-IIc levels, and by lowering 1,25$(OH)_2$-vitamin D3 levels via inhibition of 1α hydroxylase and stimulation of its catabolizing enzyme 24,25 hydroxylase (Blaine et al., 2015; Tatsumi et al., 2016).

4. The effects of sex steroids on renal calcium handling

4.1. Androgens

It has been shown in both humans (Davis et al., 1970; Morgan and Robertson, 1974) and mice (Hsu et al., 2010) that males exhibit a higher renal calcium excretion than females, suggesting a role for sex steroids in the regulation of calcium handling. Androgen replacement therapy in young adult and older hypogonadal men has been shown to both decrease serum calcium levels (Katznelson et al., 1996), while other studies showed no significant changes (Morley et al., 1993; Tenover, 1992). Treatment of prostate cancer patients with a GnRH analog to induce sex steroid deficiency did not alter serum calcium levels (Maillefert et al., 1999). On the contrary, treatment of healthy adult men with a GnRH analog increased serum calcium levels after 12 weeks of treatment (Burnett-Bowie et al., 2007). However, these effects could be mediated through effects on bone, as serum bone resorption markers were increased as well (Burnett-Bowie et al., 2007). Furthermore, in male mice calcium excretion has been shown to increase with age (Lin et al., 2016; van Abel et al., 2006).

In mice, it was suggested that androgens have stimulatory effects on urinary calcium excretion and that these effects are independent of estrogen or calciotropic hormones. Two weeks after orchidectomy of adult male mice a decreased calcium excretion was observed, along with an upregulation of renal TRPV5, calbindin-D_{28K}, PMCA and NCX1 mRNA and increased TRPV5 and calbindin-D_{28K} protein levels. T supplementation restored urinary calcium excretion and decreased the expression of renal calcium transporters. Serum levels of 1,25$(OH)_2$-vitamin D3, PTH and estrogens two weeks post-orchidectomy were similar to sham-operated mice, which led the authors to hypothesize that androgens may affect the expression of renal calcium transporters directly. In addition, an inhibition of apical to basolateral calcium transport was observed after treatment of rabbit DCT-CT cells with dihydrotestosterone (DHT) (Hsu et al., 2010). Similar results were obtained after incubating PT cells with T, leading to an inhibition of calcium uptake (Han et al., 2000). In contrast, others demonstrated that orchidectomy of male rats induced hypercalciuria at 2 (Lin et al., 2016) and 8 weeks (Gaumet-Meunier et al., 2000) after orchidectomy, which was prevented by either T or DHT supplementation respectively. However, these results should be interpreted with caution, as serum calcium levels as well as calciuria are also influenced by exchanges via the intestines and the bone. As acute sex steroid deficiency is also known to induce fast and severe

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bone resorption, it cannot be excluded that the observed renal effects could also be explained by the effects of androgens on bone.

In vitro experiments performed on rabbit kidney tubules also revealed contradictory results. While T had no effect on calcium reabsorption in PT, incubation of DT with T increased calcium reabsorption in a dose-dependent manner. The increase was already maximal after 5 min of incubation, suggesting non-genomic actions (Couchourel et al., 2004). In contrast, 24 h of incubation with T and DHT was necessary to increase PMCA activity, but not PMCA protein expression, in immortalized DT cells (Dick et al., 2003).

In conclusion, there are conflicting *in vivo* and *in vitro* data on the role of androgens in direct renal control of calcium homeostasis, which could possibly be accounted for by variations in species or strains as well as in methodology and experimental design. As an example, renal calcium handling could be differentially affected in case of acute versus chronic androgen deprivation. Furthermore, the observed effects could be mediated by the effects of androgens on bone and/or intestine. Moreover, serum sex steroid concentrations may not always be a good reflection of intra-organ sex steroids, as for example metabolizing enzymes might be differentially expressed. Although little is known about sex steroid metabolisation in the kidney, it appears that multiple enzymes involved are expressed in the kidney, including aromatase (Prabhu et al., 2010) and 5α-reductase (Panter et al., 2005; Quinkler et al., 2003). In addition, renal calcium reabsorption is dependent on several other factors, such as sodium reabsorption. It has been demonstrated that sex steroids can also have an influence on sodium reabsorption (Brunette and Leclerc, 2001; Quinkler et al., 2005), which in turn could impact calcium handling. Thus, it is clear that the investigation of androgen effects on renal calcium handling is complex and challenging and that more studies are necessary to further clarify the role of androgens in this process.

4.2. Estrogens

Estrogen deficiency in postmenopausal women is associated with increased serum calcium levels and/or increased urinary loss of calcium, and this hypercalcemia and hypercalciuria can be restored with estrogen replacement therapy (Bansal et al., 2013; Castelo-Branco et al., 1992; Falch and Gautvik, 1988; Kotowicz et al., 1990; Prince et al., 1991). In a cross-sectional study, Dick et al. studied the association of endogenous serum E2 and urinary calcium excretion in elderly women and showed that high E2 concentrations were associated with reduced renal calcium excretion (Dick et al., 2005). E2 was measured using an immunoassay however, which has been shown to overestimate serum E2 concentrations, with low reproducibility and precision in postmenopausal women in comparison to mass spectrometry (Lee et al., 2006). Nevertheless, in agreement with these data, postmenopausal women using estrogen therapy have lower serum calcium levels and a lower fractional calcium excretion (Bansal et al., 2013). These effects could however again be related to effects of estrogens on bone and/or intestines, rather than to direct renal effects of estrogen.

Opposed to the human data, estrogen deficiency in rats, induced via ovariectomy, reduced calcium excretion compared to sham operation, which was increased with estrogen administration. Calcium perfusion of these ovariectomized rats revealed an increased ability to reabsorb calcium at a given filtered load (Dick and Prince, 1997). In contrast, estrogen deficient female rats fed a low calcium diet displayed a negative calcium balance, including a downregulation of the renal transporters TRPV5, calbindin$_{28K}$ and PMCA1b (Dong et al., 2014).

There is discussion on whether estrogen acts directly on the kidney, or whether it exerts its effects on calcium metabolism by influencing other factors such as calciotropic hormones. When the calcium-perfused ovariectomized rats receiving estrogen replacement were also parathyroidectomized, no changes in calcium excretion were observed, suggesting that PTH is an important mediator of the estrogen effects on calcium metabolism (Dick and Prince, 1997). In contrast, other studies suggest that estrogens acts directly on the kidney. Ovariectomy of mature rats had no effect on serum calcium levels or renal transporter expression, but supplementation with different doses of estradiol (E2) decreased serum calcium and upregulated the renal mRNA and/or protein levels of TRPV5, calbindin$_{28K}$, NCX1 and PMCA1b. To address the question whether these estrogen effects were dependent on local 1,25(OH)$_2$-vitamin D3 production, the experiments were repeated in 1α hydroxylase-deficient mice. Similar results were obtained, indicating that E2 influences calcium metabolism via upregulation of TRPV5 in a 1,25(OH)$_2$-vitamin D3 independent manner (Van Abel et al., 2002). More supporting evidence came from ovariectomized mice, which showed decreased expression of calbindin-D$_{28K}$. Treatment with E2 increased the expression levels, while having no influence on 1,25(OH)$_2$-vitamin D3 (Criddle et al., 1997). Estrogen deficient mice (caused by aromatase deficiency) also show hypercalciuria and decreased mRNA and protein expression levels of several renal calcium transporters, including TRPV5, PMCA1b, NCX1 and calbindin-D$_{28K}$. E2 treatment of aromatase knockout females normalized calcium excretion, restored the expression of the calcium transporters and increased calbindin$_{28K}$ activity. These effects were independent of PTH and 1,25(OH)$_2$-vitamin D3 (Oz et al., 2007).

Similar to humans however, the above observed renal effects in rodents could be mediated by estrogen effects on bone and/or intestines.

The direct effects of estrogens on renal calcium handling were also studied *ex vivo* in rabbit PT cells, incubated with E2 linked to BSA, to explore non-genomic effects of estradiol on calcium reabsorption. E2-BSA enhanced calcium uptake (Han et al., 2000). In contrast, incubation of rabbit PTs and DTs with E2 decreased calcium reabsorption in the DTs, while no changes were observed in the PTs. This effect was dose-dependent and already present several minutes after stimulation, suggesting a non-genomic action as well (Brunette and Leclerc, 2001). In immortalized DT cells however, E2 increased PMCA activity without affecting protein levels via an ER-mediated mechanism, suggesting that estrogens regulate calcium transport by increasing the transporter's activity rather than its abundance (Dick et al., 2003). In contrast to the results of the group of Brunette et al., 24 h of incubation was needed. In CCD, E2 also increased calcium uptake, however here it occurred through an activation of the TRPV5 channel (Irnaten et al., 2009).

In conclusion, estrogens appear to stimulate renal calcium reabsorption directly by increasing probably both the activity as well as the abundance of calcium transporters. Nevertheless, it should be noted that estrogens might also give rise to altered calcium handling in an indirect manner, through effects on bone or intestines.

5. Effects of sex steroids on renal phosphate handling

5.1. Androgens

The Osteoporotic Fractures in Men study revealed a significant inverse association between T and serum phosphate levels, independent of FGF23 levels (Meng et al., 2010). A weaker non-linear association was found in men participating in the National Health and Nutrition Examination Survey III (NHANES III) (Wulaningsih

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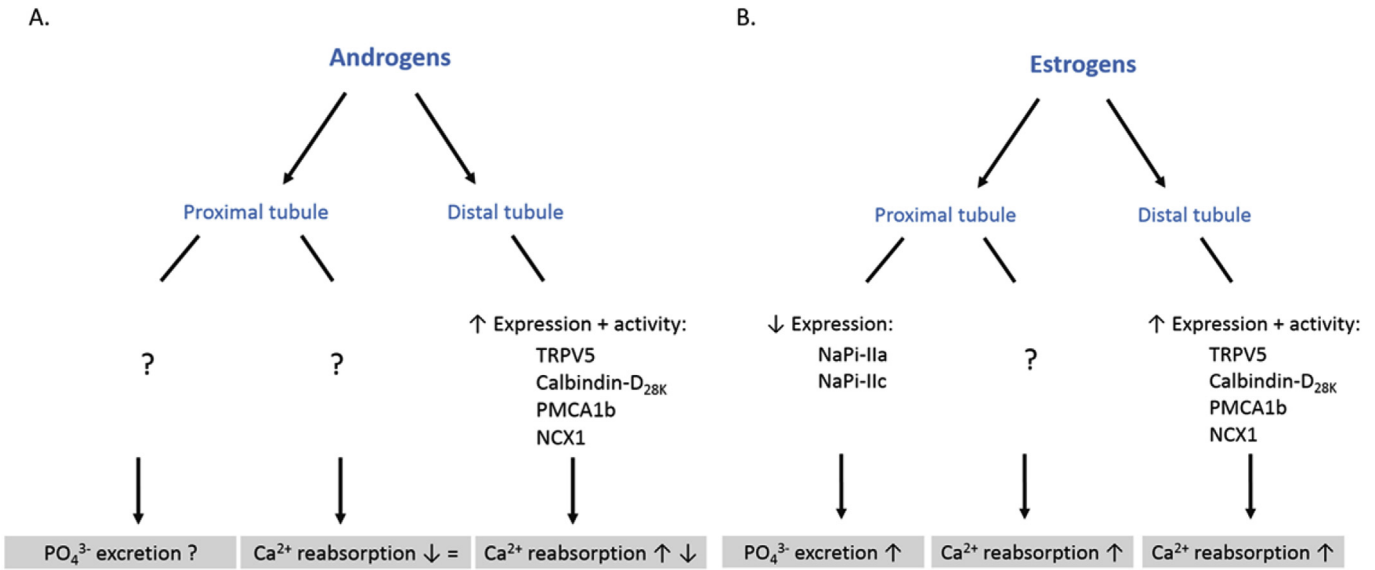

Fig. 5. Androgen (A) and estrogen (B) effects on calcium and phosphate handling in the kidney. Current studies on the effects of androgens on renal calcium handling show conflicting results, while very little is known about the effects on phosphate handling. Estrogens act on the proximal tubule, inducing phosphaturia by downregulating the expression of type II sodium/phosphate cotransporters, and increasing calcium reabsorption via a yet unknown mechanism. In the distal tubule, estrogens upregulate the expression of several calcium transporters, resulting in increased calcium reabsorption.

et al., 2014). The lower mean age of these men could be a possible explanation for the weaker association. In certain subgroups, a stronger association was observed, suggesting that race/ethnicity might play a role in phosphate homeostasis.

Consistent with these findings, men receiving GnRH analogs to induce androgen deprivation had increased serum phosphate levels (Burnett-Bowie et al., 2007; Maillefert et al., 1999) and increased renal phosphate reabsorption, while FGF23 levels remained unaffected (Burnett-Bowie et al., 2007). Similarly, hypogonadal men exhibit a decrease in serum phosphate levels after treatment with T (Wang et al., 2001). T treatment in aging men reduced plasma phosphate as well, while it did not alter levels of FGF23 or soluble klotho (Pedersen et al., 2017). Several human studies have described lower serum phosphate levels in elderly men compared to postmenopausal women of the same age (Cirillo et al., 2008; de Boer et al., 2009; Dhingra et al., 2007; Ix et al., 2009; Onufrak et al., 2009; Zhang et al., 2014).

In preclinical studies where hypogonadism was mimicked in young male rats by orchidectomy, no effect on serum phosphate levels or urinary phosphate excretion was detected, while an increase in PTH was observed. DHT administration lowered PTH levels, although concentrations were still higher compared to sham-operated mice (Gaumet-Meunier et al., 2000). In mice, T supplementation upregulated mRNA and protein levels of renal klotho expression, which could possibly have an effect on phosphate handling (Hsu et al., 2014).

Taken together, clinical data indicate that T reduces serum phosphate levels and renal reabsorption in humans, while in rats it does not seem to have an effect. Limited data are available however, with very few experiments performed in animal models, highlighting the need for more (pre)-clinical investigations on the direct role of androgens in renal phosphate handling, independent of effects on bone or intestines.

5.2. Estrogens

In both men (Meng et al., 2010) and women (Uemura et al., 2000), E2 levels as measured by mass spectrometry and immunoassays respectively, are correlated with reduced serum phosphate levels. In women, E2 has been associated with lower tubular phosphate reabsorption (Uemura et al., 2000). Accordingly, women treated with estrogens have reduced serum phosphate levels and/or a decrease in renal phosphate reabsorption (Castelo-Branco et al., 1992; Uemura et al., 2000; Zhang et al., 2014) or increased fractional phosphate excretion (Bansal et al., 2013). On the contrary, estrogen-depleted women have increased serum phosphate levels and renal phosphate reabsorption (Falch and Gautvik, 1988). This estrogen effect seems to be similar in males, as hypophosphatemia was also observed in prostate cancer patients treated with estrogens (Citrin et al., 1984). Together, clinical data suggest that estrogens might suppress active phosphate transport in the kidney, although possible effects on bone or intestines can not be excluded.

These human data are in agreement with animal experiments, suggesting that E2 induces phosphaturia. Preclinical experiments in rats indeed demonstrated decreased phosphate excretion following ovariectomy, while estrogen replacement stimulated excretion (Dick and Prince, 2001). These findings are in agreement with the hypophosphatemia and hyperphosphaturia observed in estrogen-treated ovariectomized rats (Faroqui et al., 2008). Moreover, reduced mRNA and protein levels of NaPi–IIa cotransporter in the kidneys were observed. This was independent of changes in food intake and PTH, and likely not mediated by the ERα as simultaneous treatment of ovariectomized rats with estrogen and an ERα inhibitor did not prevent the downregulation of NaPi–IIa. The increased urinary phosphate excretion, together with the decrease in serum phosphate and NaPi-IIa expression were later confirmed by the same research group. Remarkably, they also found that co-activation of both ERα and ERβ was necessary for the observed effects, suggesting that these receptors form a heterodimer complex in the rat kidney (Burris et al., 2015). A too low dose of the ERα inhibitor in their previous study could possibly account for the discrepancy in results. Moreover, they discovered that estrogens influenced phosphate reabsorption by specifically downregulating the NaPi–IIa cotransporter without acting on the NaPi–IIc cotransporter or the PiT2 transporter (Burris et al., 2015).

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Interestingly, the same group discovered that in mice estrogen treatment induced hypophosphatemia and phosphaturia due to downregulation of both NaPi-IIa and NaPi-IIc. The reduced levels of NaPi-IIa protein were likely caused by direct effects, as evidenced by *in vitro* studies demonstrating NaPi-IIa downregulation after estrogen treatment of U20S cells expressing the ERs, and were independent of PTH, FGF23 and 1,25$(OH)_2$vitamin D_3. In contrast, the estrogen effects on NaPi-IIc were likely mediated by PTH and FGF23 (Webster et al., 2016). These experiments emphasize that sex steroids might not act through the same mechanism across different species.

Estrogens might have comparable effects in male kidneys as well, as a decrease in serum phosphate was observed following E2 treatment in orchidectomized rats (Gaumet-Meunier et al., 2000).

These findings were consistent with *ex vivo* data. Incubation of renal brush border membranes from ovariectomized and parathyroidectomized rats with E2 resulted in an inhibition of Na^+-P_i cotransport, suggesting that E2 may have phosphaturic effects independent of PTH (Beers et al., 1996).

Together, these data suggest that estrogens exhibit phosphaturic effects, by acting both directly and indirectly on multiple renal phosphate transporters. The physiological significance of this phosphaturia, however, remains to be determined. Nevertheless, indirect effects through bone and intestines cannot be excluded. Fig. 5 shows an overview of the current insight in estrogen effects on renal calcium and phosphate handling.

6. Conclusion

Both the bone and the kidney play a crucial and interrelated role in calcium and phosphate homeostasis, referred to as the bone-kidney axis. Even though it is well recognized that calcium and phosphate represent crucial components of the bone matrix and the effects of sex steroids on skeletal development and homeostasis have been extensively demonstrated, little is known about the mechanisms of sex steroid action in the regulation of renal calcium and phosphate handling and the role of sex steroids on the kidney-bone axis. The presence, abundance, and exact localization of sex steroid receptors within the kidney is still debated. Moreover, in order to study the effects of sex steroids on renal calcium and phosphate handling, indirect effects via the bone and intestine should be accounted for, which was not the case in previous studies and represents a drawback. By gaining more insight in the direct effects of sex steroids on renal calcium and phosphate handling, new strategies could be developed for the treatment of several disorders related to calcium and phosphate imbalances, such as vascular calcification, chronic kidney disease, or osteoporosis. However, a lot of research is still necessary in this field. The development of kidney-specific AR and ER knockout mice represents a first and feasible step to improve our insight into the direct versus indirect effects of sex steroids on calcium and phosphate handling and the bone-kidney axis.

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