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Chemical evidence for the tradeoff-in-the-nephron hypothesis to explain secondary hyperparathyroidism

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Abbreviations: [*x*], concentration of *x*; $[x]_p$, concentration of *x* in plasma; $[x]_s$, concentration of *x* in serum; $[x]_u$, concentration of *x* in urine; Ca, calcium; Ca^{++}, ionized calcium; $Ca_3(PO_4)_2$ (am., s.), calcium phosphate (amorphous, solid); $Cacit^-$,

Abstract

Background

Secondary hyperparathyroidism (SHPT) complicates advanced chronic kidney disease (CKD) and causes skeletal and other morbidity. In animal models of CKD, SHPT was prevented and reversed by reduction of dietary phosphate in proportion to GFR, but the phenomena underlying these observations are not understood. The tradeoff-in-the-nephron hypothesis states that as GFR falls, the phosphate concentration in the distal convoluted tubule ($[P]_{DCT}$) rises, reduces the ionized calcium concentration in that segment ($[Ca^{++}]_{DCT}$), and thereby induces increased secretion of parathyroid hormone (PTH) to maintain normal calcium reabsorption. In patients with CKD, we previously documented correlations between [PTH] and phosphate excreted per volume of filtrate (E_P/C_{cr}), a surrogate for $[P]_{DCT}$. In the present investigation, we estimated $[P]_{DCT}$ from physiologic considerations and measurements of phosphaturia, and sought evidence for a specific chemical phenomenon by which increased $[P]_{DCT}$ could lower $[Ca^{++}]_{DCT}$ and raise [PTH].

Methods and findings

We studied 28 patients ("CKD") with eGFR of 14–49 mL/min/1.73m^2 (mean 29.9 ± 9.5) and 27 controls ("CTRL") with eGFR > 60 mL/min/1.73m^2 (mean 86.2 ± 10.2). In each subject, total $[Ca]_{DCT}$ and $[P]_{DCT}$ were deduced from relevant laboratory data. The Joint Expert Speciation System (JESS) was used to calculate $[Ca^{++}]_{DCT}$ and concentrations of related chemical species under the assumption that a solid phase of amorphous calcium phosphate ($Ca_3(PO_4)_2$ (am., s.)) could precipitate. Regressions of [PTH] on eGFR, $[P]_{DCT}$, and $[Ca^{++}]_{DCT}$ were then examined. At filtrate pH of 6.8 and 7.0, $[P]_{DCT}$ was found to be the sole determinant of $[Ca^{++}]_{DCT}$, and precipitation of $Ca_3(PO_4)_2$ (am., s.) appeared to mediate this result. At pH 6.6, total $[Ca]_{DCT}$ was the principal determinant of $[Ca^{++}]_{DCT}$, $[P]_{DCT}$ was a minor determinant, and precipitation of $Ca_3(PO_4)_2$ (am., s.) was predicted in no CKD and five CTRL. In CKD, at all three pH values, [PTH] varied directly with $[P]_{DCT}$ and inversely with $[Ca^{++}]_{DCT}$, and a reduced $[Ca^{++}]_{DCT}$ was identified at which [PTH] rose unequivocally. Relationships of [PTH] to $[Ca^{++}]_{DCT}$ and to eGFR resembled each other closely.

calcium citrate, transitory form; CaHCO3+, calcium bicarbonate, transitory form; CaHPO4.2H_2O, calcium monohydrogen phosphate dihydrate; brushite in solid form; CaHPO40, calcium monohydrogen phosphate in solution; $CaSO_4$, calcium sulfate; Ca_{uf}, ultrafilterable calcium; C_{cr}, creatinine clearance, units of volume/time; CKD, chronic kidney disease; CTRL, control; DCT, distal convoluted tubule; eGFR, estimated GFR, units of mL/min/1.73m^2; E_x, excretion rate of *x*, units of mass/time; E_x/C_{cr}, amount of *x* excreted per volume of filtrate (assuming C_{cr} ~ GFR); FD_{Ca}, fractional delivery of Ca (herein, to the DCT); FD_f, fractional delivery of filtrate (herein, to the DCT); GFR, glomerular filtration rate, units of volume/time; IAP, ion activity product; JESS, Joint Expert Speciation System; K_{sp}, solubility product constant; MDRD, modification of diet in renal disease; P, phosphorus or phosphate; PHPT, primary hyperparathyroidism; SHPT, secondary hyperparathyroidism; SI, solubility index; Stage G_3, stage of CKD in which eGFR is 30–60 mL/min/1.73m^2; Stage G_4, stage of CKD in which eGFR is 15–30 mL/min/1.73m^2.

Conclusions

As $[P]_{DCT}$ increases, chemical speciation calculations predict reduction of $[Ca^{++}]_{DCT}$ through precipitation of $Ca_3(PO_4)_2$ (am., s.). [PTH] appears to rise unequivocally if $[Ca^{++}]_{DCT}$ falls sufficiently. These results support the tradeoff-in-the-nephron hypothesis, and they explain why proportional phosphate restriction prevented and reversed SHPT in experimental CKD. Whether equally stringent treatment can be as efficacious in humans warrants investigation.

Introduction

The parathyroid hormone concentration ([PTH]) rises as the glomerular filtration rate (GFR) falls in patients with chronic kidney disease (CKD) [1]. This phenomenon, secondary hyperparathyroidism (SHPT), causes skeletal morbidity and may contribute to other uremic manifestations [2–4]. PTH also raises concentrations of fibroblast growth factor 23, which may exert its own toxic effects [5–7].

During the past 50 years, seven theories have been advanced to explain the pathogenesis of SHPT [8]. The most recent of these, the tradeoff-in-the-nephron hypothesis, attributes SHPT to an increased phosphate concentration in the distal convoluted tubule ($[P]_{DCT}$), where PTH regulates reabsorption of ionized Ca (Ca^{++}) [8–11]. According to this hypothesis, high $[P]_{DCT}$ reduces $[Ca^{++}]_{DCT}$, and [PTH] rises to maintain Ca^{++} reabsorption at a rate compatible with normocalcemia [8,12,13]. The hypothesis integrates the micropuncture observation that $[P]_{DCT}$ rose in animals with CKD fed a standard diet [9], and explains why dietary restriction or intestinal binding of phosphate prevented, mitigated, or reversed SHPT in animals and humans [14–22]. The term "tradeoff-in-the-nephron" invites a comparison to the original tradeoff hypothesis, which attributed SHPT to an interaction between phosphate and Ca^{++} in plasma [23].

If creatinine clearance (C_{cr}) is accepted as a surrogate for GFR, the ratio of the phosphorus excretion rate (E_P) to C_{cr} quantifies the amount of P excreted per volume of filtrate. Moreover, E_P/C_{cr} is proportional to $[P]_{DCT}$ if fractional delivery of filtrate to the DCT is assigned a constant value [8,12,13,24,25]. In a cohort of 30 patients with stages G_3 or G_4 CKD, significant correlations between [PTH] and E_P/C_{cr} were demonstrated under multiple conditions, but a chemical mechanism to explain these correlations was not investigated [24].

The Joint Expert Speciation System (JESS) employs a compilation of thousands of equilibria to predict concentrations of ions and other chemical entities under defined conditions [26,27]. In the present study, we deduced concentrations of Ca and P in the DCT ($[Ca]_{DCT}$ and $[P]_{DCT}$) from laboratory measurements and physiologic considerations, and posited evidence-based assumptions concerning other constituents in that segment. With this information, JESS was used to calculate $[Ca^{++}]_{DCT}$ under various modeling scenarios, of which the most germane proved to be ones that excluded or included possible precipitation of amorphous calcium phosphate ($Ca_3(PO_4)_2$ (am., s.)). Regressions of [PTH] on $[Ca^{++}]_{DCT}$ were then performed at pH 6.6, 6.8, and 7.0, which are representative values over the documented pH range in the DCT [28]. If precipitation of amorphous calcium phosphate ($Ca_3(PO_4)_2$ (am., s.)) occurred as filtrate reached saturation with this solid, total $[P]_{DCT}$ reduced $[Ca^{++}]_{DCT}$ in advanced CKD to values associated with increased [PTH]. Relationships of [PTH] to $[Ca^{++}]_{DCT}$ and to eGFR were virtually identical.

Methods

Subjects and laboratory determinations

Between 2010 and 2013, data were collected from 28 control subjects with estimated GFR (eGFR) > 60 mL/min/1.73m^2 and 30 patients with eGFR 14–49 mL/min/1.73m^2. GFR was estimated with the 4-variable MDRD formula. All participants were normocalcemic (8.5–10.2 mg/dL in our hospital laboratory). Two patients with CKD were excluded from the present study, one because the serum ultrafilterable calcium concentration ($[Ca_{uf}]_s$) was reported to be lower than the ionized calcium concentration ($[Ca^{++}]_s$)–a physiologic impossibility–and one because the association of [PTH] of 169 pg/mL with $[Ca^{++}]_s$ of 1.35 mM implied autonomy of PTH secretion. A control subject who failed to collect a 24-hour urine specimen was also excluded. The study sample in the present report therefore includes 28 patients with CKD (denoted as "CKD") and 27 control subjects (denoted as "CTRL"). CKD and CTRL were not matched for age, race, or gender.

The data considered herein were obtained before experimental interventions were initiated [12,13,24,25]. Aliquots of urine, serum, and plasma were obtained at a clinic visit occurring between 8:00 and 10:00 a.m. Subjects performed a urine collection during the 24 hours preceding the visit and were instructed to take no medicines or food after midnight of their appointment day. Serum (s) and urine (u) concentrations of creatinine, calcium, and phosphorus were measured by autoanalyzer. $[Ca^{++}]_s$, $[Ca_{uf}]_s$, and plasma [PTH]1–84 ([PTH]) were measured as previously described [12].

Estimation of total phosphate and calcium concentrations in the DCT

Phosphate and calcium exist in filtrate of the DCT as either free ions (PO_4^{3-} and Ca^{++}) or chemical species derived from the ions by formation reactions. Most derived species, such as $H_2PO_4^-$, $HPO_4^=$, and $CaHPO_4^0$, exist in solution (*i.e.*, are dissolved), but solid phases such as brushite and amorphous calcium phosphate may precipitate if their solubility product constants are exceeded. The extent of formation of any chemical species, including precipitates, can be calculated from the total concentrations $[P]_{DCT}$ and $[Ca]_{DCT}$ using thermodynamic relationships and equilibrium constants described in the chemical literature. However, it is necessary in such calculations to stipulate *a priori* which if any precipitates may be formed. Such stipulations are based on certain empirical rules of thumb, particularly Ostwald's Rule of Stages, as described below.

Total $[P]_{DCT}$ and $[Ca]_{DCT}$ were estimated in this work under the following assumptions: the rate of phosphate delivery to the DCT equals the excretion rate of phosphorus (E_P) [29]; the rate of calcium delivery to this segment is 10% of the filtration rate of calcium, or 0.1 (eGFR)$[Ca_{uf}]_s$ [30]; and fractional delivery of filtrate (FD_f) to the DCT is 0.2 in controls and 0.35 in subjects with CKD [31–33]. Accordingly, total $[Ca]_{DCT}$ was computed as 0.1(eGFR) $[Ca_{uf}]_s$/(0.35)eGFR in CKD and as 0.1[(eGFR)$[Ca_{uf}]_s$/(0.2)eGFR in CTRL. Similarly, total $[P]_{DCT}$ was calculated as (24h E_P)/0.35(eGFR) in CKD and as (24h E_P)/0.2(eGFR) in CTRL. Equations for total $[Ca]_{DCT}$ and $[P]_{DCT}$ thus simplified to 0.1$[Ca_{uf}]_s$/FD_f and (24h E_P)/ {FD_f(eGFR)}, respectively.

Estimation of other total concentrations in the DCT

Filtrate pH and total concentrations in the DCT of sodium, potassium, magnesium, chloride, urate, sulfate, citrate, oxalate, bicarbonate, creatinine, and urea were estimated by appropriate combinations of the following: ultrafilterable concentrations in serum; estimated GFR (eGFR); published micropuncture data [28,30–38]; studies of urine dilution with water loading [39];

excretion rates of anions not reabsorbed or secreted in the distal nephron [40–45]; and assumptions concerning FD_f at normal and reduced GFR [31–33]. The concentration of ammonium in the DCT was assumed to be negligible. Details concerning assignment of concentrations, including pH, are provided in Supporting Information.

Determination of $[Ca^{++}]_{DCT}$ using JESS

From estimated total concentrations and relevant equilibria, routine chemical speciation models were constructed to solve mass balance equations for each applicable DCT component. The calculated ionic strength of DCT filtrate allowed the ionic activity quotients of all chemical reactions to be inferred. For each possible solid phase, the saturation index (SI) was calculated as IAP/K_{sp}, where IAP is the ion activity product and K_{sp} is the solubility product constant [27]. If logSI is < 0, it follows that SI is < 1 and the compound in question is fully dissolved at equilibrium. If logSI is ≥ 0, it follows that SI is ≥ 1 and filtrate is saturated or supersaturated with the solid.

In the present work, possible solid phases in the DCT included brushite ($CaHPO_4{\cdot}2H_2O$) and $Ca_3(PO_4)_2$, (am., s.), but the former was dismissed because logSIbrushite was uniformly negative in both groups and all scenarios. In contrast, at pH 6.8, logSI$Ca_3(PO_4)_2$ (am., s.) exceeded zero in approximately half of CKD and a majority of CTRL when precipitation of this solid was not assumed. Ostwald's rule of stages suggests that in a fluid supersaturated with multiple solids, the one with logSI closest to 0 is likely to precipitate first [27,46]. In the present study, JESS analyses indicated that this solid phase was $Ca_3(PO_4)_2$ (am., s.) in the DCT of both CKD and CTRL. Two additional modeling scenarios, one with and one without precipitation of $Ca_3(PO_4)_2$ (am., s.), were therefore investigated. When precipitation was assumed in states of saturation or supersaturation, consequences were examined at pH 6.6, 6.8, and 7.0. In each scenario, $[Ca^{++}]_{DCT}$, $[CaHPO_4^0]_{DCT}$, $[Cacit^-]_{DCT}$, $[Caox^0]_{DCT}$, $[CaHCO_3^+]_{DCT}$, and $[CaSO_4^0]_{DCT}$ were calculated as concentrations of the most likely pertinent species of dissolved calcium.

Statistical analysis

The ultimate goals of the present study were to identify a chemical phenomenon by which increased $[P]_{DCT}$ could reduce $[Ca^{++}]_{DCT}$ in CKD; to ascertain whether [PTH] would vary significantly with $[Ca^{++}]_{DCT}$ if that reduction occurred; and to consider the possibility that [PTH] was related to eGFR because it was related to $[Ca^{++}]_{DCT}$. Table 1 summarizes the sequence of questions addressed and the examinations conducted to pursue these goals.

In each subset of subjects, mean values were determined for parameters not affected by pH or precipitation of $Ca_3(PO_4)_2$ (am., s.), including eGFR, [PTH], 24h E_P, 24h E_{Ca}, $[P]_s$, $[Ca^{++}]_s$, $[Ca_{uf}]_s$, $[P]_{DCT}$, and $[Ca]_{DCT}$. For each of these parameters, normality of distribution was examined with plots and with the Shapiro-Wilk test. When distributions were judged to be normal, differences between means were assessed with t-tests for unpaired values (unequal variances assumed). When distributions were skewed, differences between medians were assessed with the Mann Whitney U test. Results were considered to be statistically significant at $p < 0.05$.

The hypothesis investigated in the present study was that $[Ca^{++}]_{DCT}$ induced by $[P]_{DCT}$ determines [PTH] in stages G_3 and G_4 CKD. For comparative purposes, we examined least-squares regressions of total $[P]_{DCT}$ and total $[Ca]_{DCT}$ on eGFR, and regressions of [PTH] on total $[P]_{DCT}$, total $[Ca]_{DCT}$, and eGFR (Fig 1). None of these regressions was affected by pH or the state of precipitation of $Ca_3(PO_4)_2$ (am., s.).

Since JESS identified $Ca_3(PO_4)_2$ (am., s.) as the Ca species most likely to precipitate in the DCT, we examined the maximum, minimum, median, mean, and 25^{th} to 75^{th} percentiles of

Table 1. Rationale for inquiries into the tradeoff-in-the-nephron hypothesis.

QUESTIONS	RELEVANT EXAMINATIONS
Were [PTH], total $[P]_{DCT}$, and total $[Ca]_{DCT}$ related to eGFR?	Regressions of $[P]_{DCT}$ and $[Ca]_{DCT}$ on eGFR, and of [PTH] on 100/eGFR (Fig 1)
Was [PTH] related to total $[P]_{DCT}$ and total $[Ca]_{DCT}$?	Regressions of [PTH] on $[P]_{DCT}$ and $[Ca]_{DCT}$ (Fig 1)
Did pH affect precipitation of $Ca_3(PO_4)_2$ (am., s.) in the DCT?	Box-and-whisker plots of $logSICa_3(PO_4)_2$ at pH 6.6, 6.8, and 7.0 (Fig 2); plot of $logSICa_3(PO_4)_2$ against pH at fixed $[P]_{DCT}$ and $[Ca]_{DCT}$ (S1 Fig)
Was $[Ca^{++}]_{DCT}$ related to total $[P]_{DCT}$? To total $[Ca]_{DCT}$?	Regressions of $[Ca^{++}]_{DCT}$ on $[P]_{DCT}$ and $[Ca]_{DCT}$ at pH 6.6, 6.8, and 7.0 (Figs 3, 4, S2 and S3)
If $[Ca^{++}]_{DCT}$ was related to $[P]_{DCT}$, did precipitation of $Ca_3(PO_4)_2$ (am., s.) mediate that relationship?	Linkage of precipitation of $Ca_3(PO_4)_2$ (am., s.) (Fig 2) to regressions of $[Ca^{++}]_{DCT}$ on $[P]_{DCT}$ (Figs 3, 4, S2 and S3)
Was $[Ca^{++}]_{DCT}$ related to $[CaHPO_4^0)_{DCT}$?	Plots of $[Ca^{++}]_{DCT}$ against $[CaHPO_4^0]_{DCT}$ at pH 6.6, 6.8, and 7.0 (S4 and S5 Figs)
Did anions other than phosphate affect $[Ca^{++}]_{DCT}$?	Plots of $[Ca^{++}]_{DCT}$ against $[Cacit^+]_{DCT}$, $[Caox^0]_{DCT}$, $[CaHCO_3^+]_{DCT}$ and $[CaSO_4^0]_{DCT}$ at pH 6.8 (S5 Fig)
Was [PTH] related to $[Ca^{++}]_{DCT}$?	Regressions of [PTH] on $[Ca^{++}]_{DCT}$ (Figs 3, 4, S2 and S3)
Did the relationship of [PTH] to $[Ca^{++}]_{DCT}$ explain the relationship of [PTH] to eGFR?	Comparison of regressions of log[PTH] on $log[Ca^{++}]_{DCT}$ and log[PTH] on log(eGFR) after standardization of logarithmic values (Fig 5)

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$logSICa_3(PO_4)_2$ (am., s.) at pH 6.8 with precipitation excluded, and at pH 6.6, 6.8, or 7.0 with precipitation assumed if $logSICa_3(PO_4)_2$ (am., s.) equaled or exceeded zero (Fig 2).

Factors determining $[Ca^{++}]_{DCT}$ ("determinants") were sought with least-squares regressions of $[Ca^{++}]_{DCT}$ on total $[P]_{DCT}$ and total $[Ca]_{DCT}$. The tradeoff-in-the-nephron hypothesis was tested with regressions of [PTH] on $[Ca^{++}]_{DCT}$ at pH 6.6, 6.8, and 7.0, assuming precipitation of $Ca_3(PO_4)_2$ (am., s.) if logSI was ≥ 0 (Figs 3,4, S2 and S3). In combined CKD and CTRL, inverse curvilinear relationships between [PTH] and either eGFR or $[Ca^{++}]_{DCT}$ were assessed as power functions in the form $y = kx^{-1} + c$, where y = [PTH] and x = eGFR (Fig 1) or $[Ca^{++}]_{DCT}$ (Figs 3 and 4). Relationships between [PTH] and both eGFR and $[Ca^{++}]_{DCT}$ were then investigated by log-log transformation of these variables and subsequent standardization of logarithmic values (Fig 5).

To investigate the possibility that $CaHPO_4^0$ was a mediator of the effect of total $[P]_{DCT}$ on $[Ca^{++}]_{DCT}$, we produced scatterplots of $[CaHPO_4^0]_{DCT}$ against total $[P]_{DCT}$, and of $[Ca^{++}]_{DCT}$ against $[CaHPO_4^0]_{DCT}$, at pH 6.6, 6.8, and 7.0 (S4 Fig). To determine whether other Ca species had affected $[Ca^{++}]_{DCT}$, we examined regressions of $[Ca^{++}]_{DCT}$ on $[Cacit^-]_{DCT}$, $[Caox^0]_{DCT}$, $[CaHCO_3^+]_{DCT}$, and $[CaSO_4^0]_{DCT}$, assuming pH 6.8 and precipitation of $Ca_3(PO_4)_2$ (am., s.) if logSI was ≥ 0 (S5 Fig).

Although we assumed in the present work that fractional delivery of calcium to the DCT (FD_{Ca}) was 0.1 in both CKD and CTRL, we also considered the possibility that in CKD, a higher FD_{Ca} might increase the effect of total $[Ca]_{DCT}$ on $[Ca^{++}]_{DCT}$. To investigate that possibility, we performed regressions of $[Ca^{++}]_{DCT}$ on total $[Ca]_{DCT}$, $[Ca^{++}]_{DCT}$ on $[P]_{DCT}$, and [PTH] on $[Ca^{++}]_{DCT}$ at FD_{Ca} 0.15 and 0.2 and pH 6.6, 6.8, and 7.0 (S6 and S7 Figs).

Statistical analyses were carried out with Microsoft Excel and R version 4.0.3 (R Core Team, 2020) [47].

Clinical research policies

The research project that provided the data reported herein [12,13] was approved and periodically reviewed by the Institutional Review Board (IRB) of the Stratton Veterans' Affairs Medical Center, Albany, NY, USA. The project was conducted with adherence to the Declaration of Helsinki, and written informed consent was obtained from all participants. Data employed in

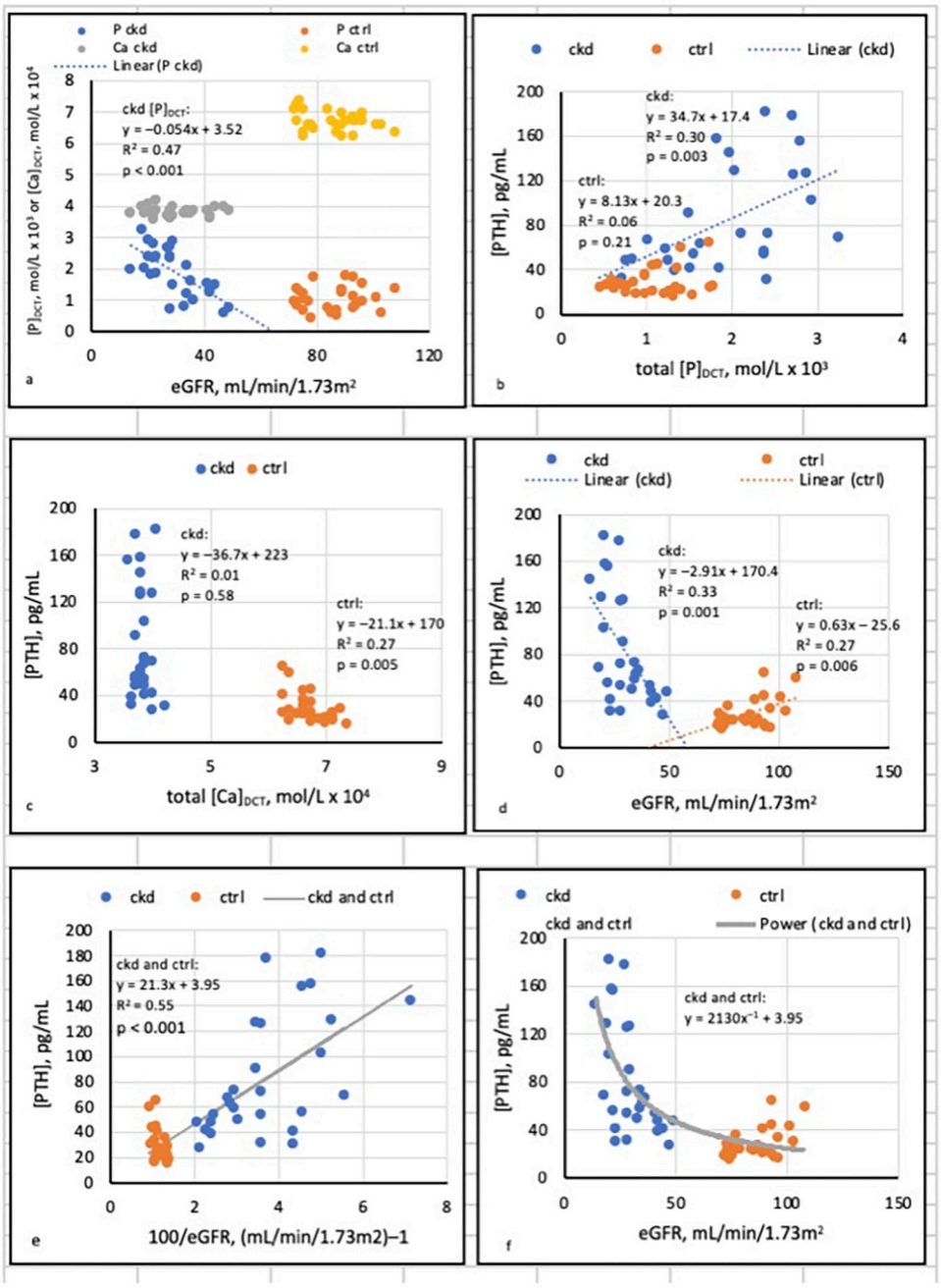

Fig 1. Linear regressions unaffected by pH or precipitation of $Ca_3(PO_4)_2$ (am., s.).

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the present study were obtained from tests on urine, serum and plasma obtained at an IRB-approved research clinic visit. IRB oversight over access to all data was in place.

Results

We present our results in accordance with the order of questions posed in Table 1. Table 2 compares means of variables that were not affected by filtrate pH or precipitation of $Ca_3(PO_4)_2$ (am., s.) in the DCT.

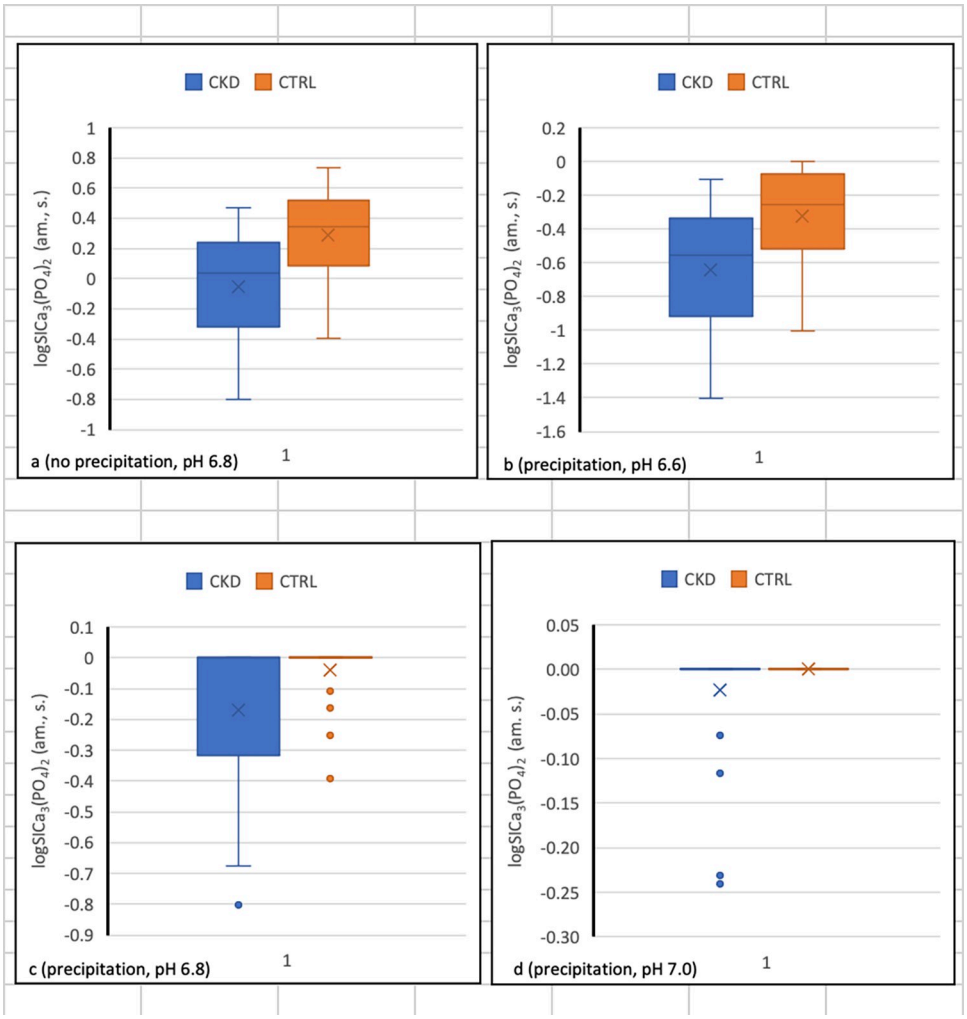

Fig 2. Dependence of logSI$Ca_3(PO_4)_2$ (am., s.) on pH in the DCT.

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Fig 1 depicts regressions that were not affected by filtrate pH or precipitation of $Ca_3(PO_4)_2$ (am., s.).

Fig 1A shows that total $[P]_{DCT}$ varied inversely with eGFR in CKD but not CTRL, and total $[Ca]_{DCT}$ was unrelated to eGFR in each group. [PTH] rose with $[P]_{DCT}$ in CKD but not CTRL (Fig 1B); in contrast, [PTH] was inversely related to $[Ca]_{DCT}$ in CTRL but not CKD (Fig 1C).

Fig 1D shows that [PTH] varied inversely with eGFR in CKD and directly with eGFR in CTRL. However, a scatterplot of [PTH] (y) against eGFR (x) in the combined groups appeared to depict a hyperbola described in part by the formula $xy = k$ (Fig 1D). Accordingly, a least-squares regression of [PTH] (y) on 100/eGFR ($100/x$) displayed a linear relationship (Fig 1E). The associated equation was then modified to express [PTH] (y) as a power function of eGFR (x) in the form $y = kx^{-1} + c$. The hyperbola described by the function is included in Fig 1F.

Fig 2 depicts the median, mean, 25^{th}-75^{th} percentile range, and upper and lower limits of logSI-$Ca_3(PO_4)_2$ (am., s.) in CKD and CTRL under four combinations of conditions. LogSI$Ca_3(PO_4)_2$ (am., s.) was 0 at saturation and > 0 at supersaturation. In Fig 2A, pH was 6.8 and precipitation was considered not to occur; in Fig 2B–2D, at pH 6.6, 6.8, and 7.0, precipitation was assumed to occur when DCT filtrate was saturated or supersaturated with $Ca_3(PO_4)_2$ (am., s.).

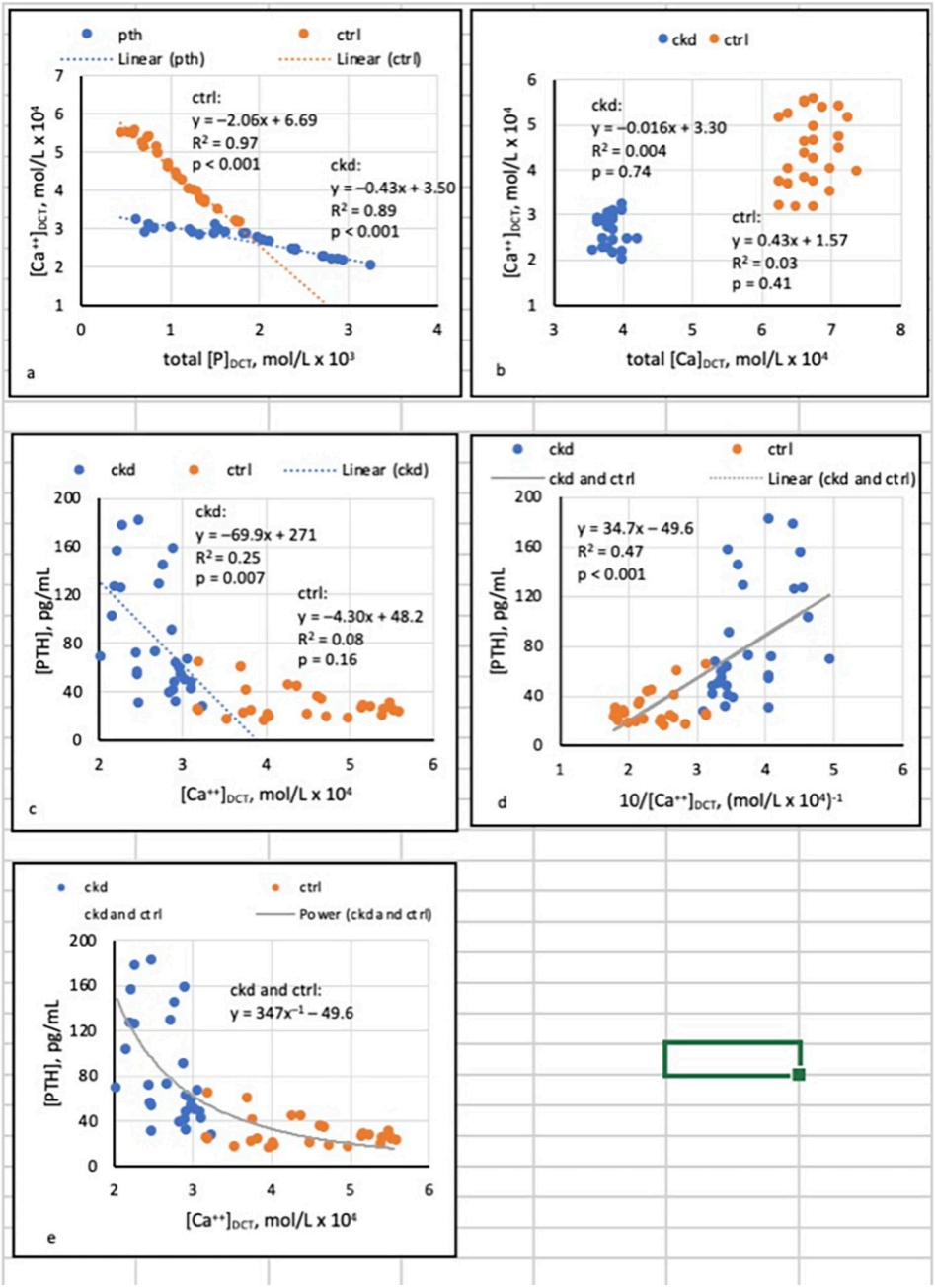

Fig 3. Regressions assuming pH 6.8 and precipitation of $Ca_3(PO_4)_2$ (am., s.).

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JESS predicted that with no precipitation, the DCT would be saturated or supersaturated with this compound at pH 6.8 in half of CKD and a majority of CTRL (Fig 2A). In contrast, at pH 6.6, saturation with $Ca_3(PO_4)_2$ (am., s.) was predicted in no subjects with CKD and in five CTRL (Fig 2B). At pH 6.8, saturation with and consequent precipitation of $Ca_3(PO_4)_2$ (am., s.) were predicted in the top two quartiles of CKD and in all but four CTRL (Fig 2C). At pH 7.0, precipitation was predicted in all but four CKD and in all CTRL (Fig 2D). If $[Ca]_{DCT}$ and $[P]_{DCT}$ were fixed hypothetically at 0.5 mM and 1.5 mM respectively, saturation with $Ca_3(PO_4)_2$ (am., s.) occurred at pH 6.73 (see S1 Fig and related discussion in SI).

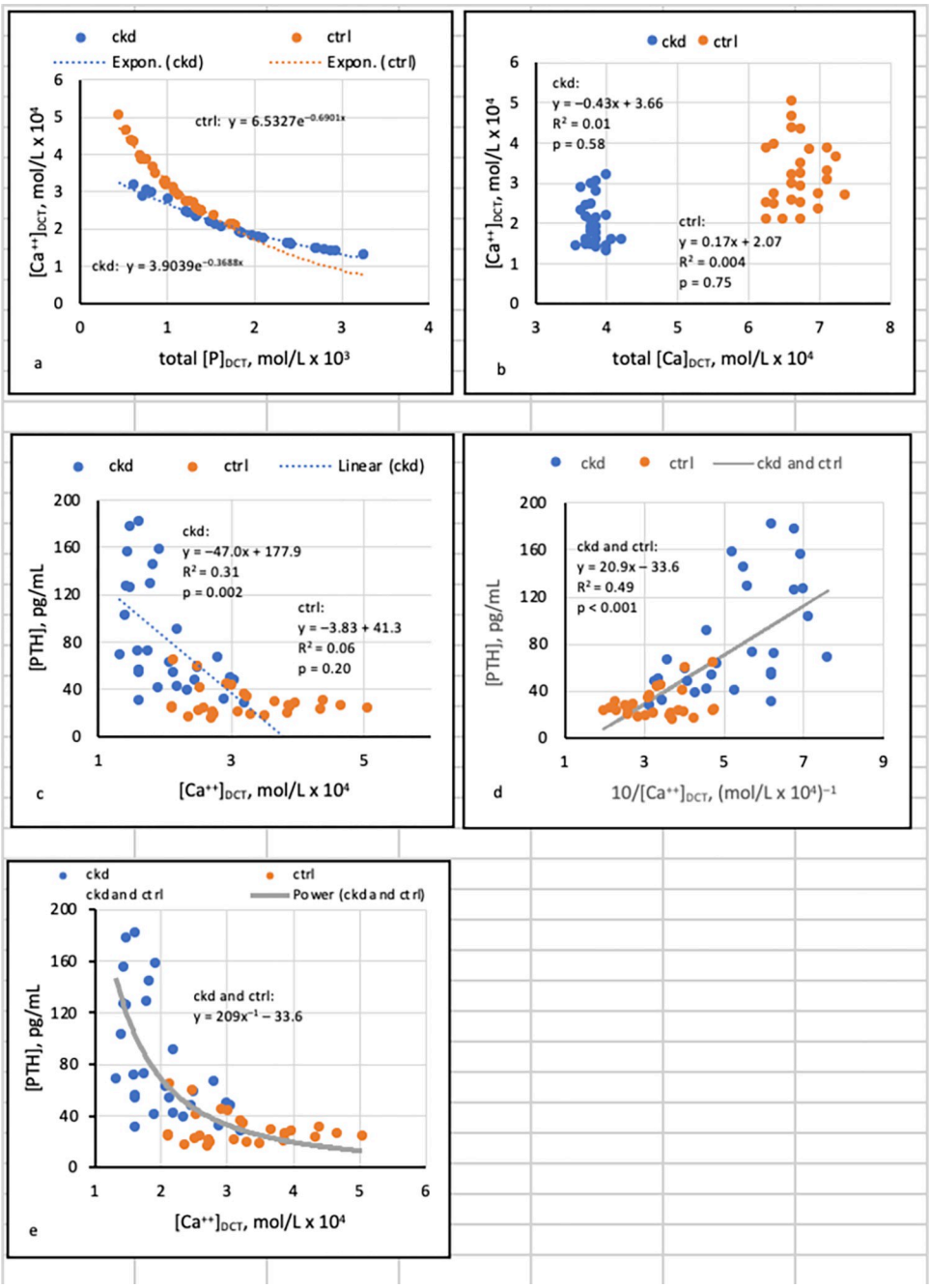

Fig 4. Regressions assuming pH 7.0 and precipitation of $Ca_3(PO_4)_2$ (am., s.).

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Under conditions in which precipitation of $Ca_3(PO_4)_2$ (am., s.) did not occur (Fig 2A and 2B), linear regressions showed that total $[Ca]_{DCT}$ was a major and $[P]_{DCT}$ was a minor determinant of $[Ca^{++}]_{DCT}$ (see S2 and S3 Figs and related discussion in SI). In contrast, when precipitation was assumed to occur in states of supersaturation (Fig 2C and 2D), regressions showed that $[P]_{DCT}$ was the sole determinant of $[Ca^{++}]_{DCT}$ (Figs 3A, 3B, 4A and 4B).

At pH 6.8, [PTH] varied inversely with $[Ca^{++}]_{DCT}$ in CKD but not CTRL, and began to rise unequivocally in CKD at $[Ca^{++}]_{DCT}$ of approximately 2.8 x 10^{-4} mol/L (Fig 3C). In the

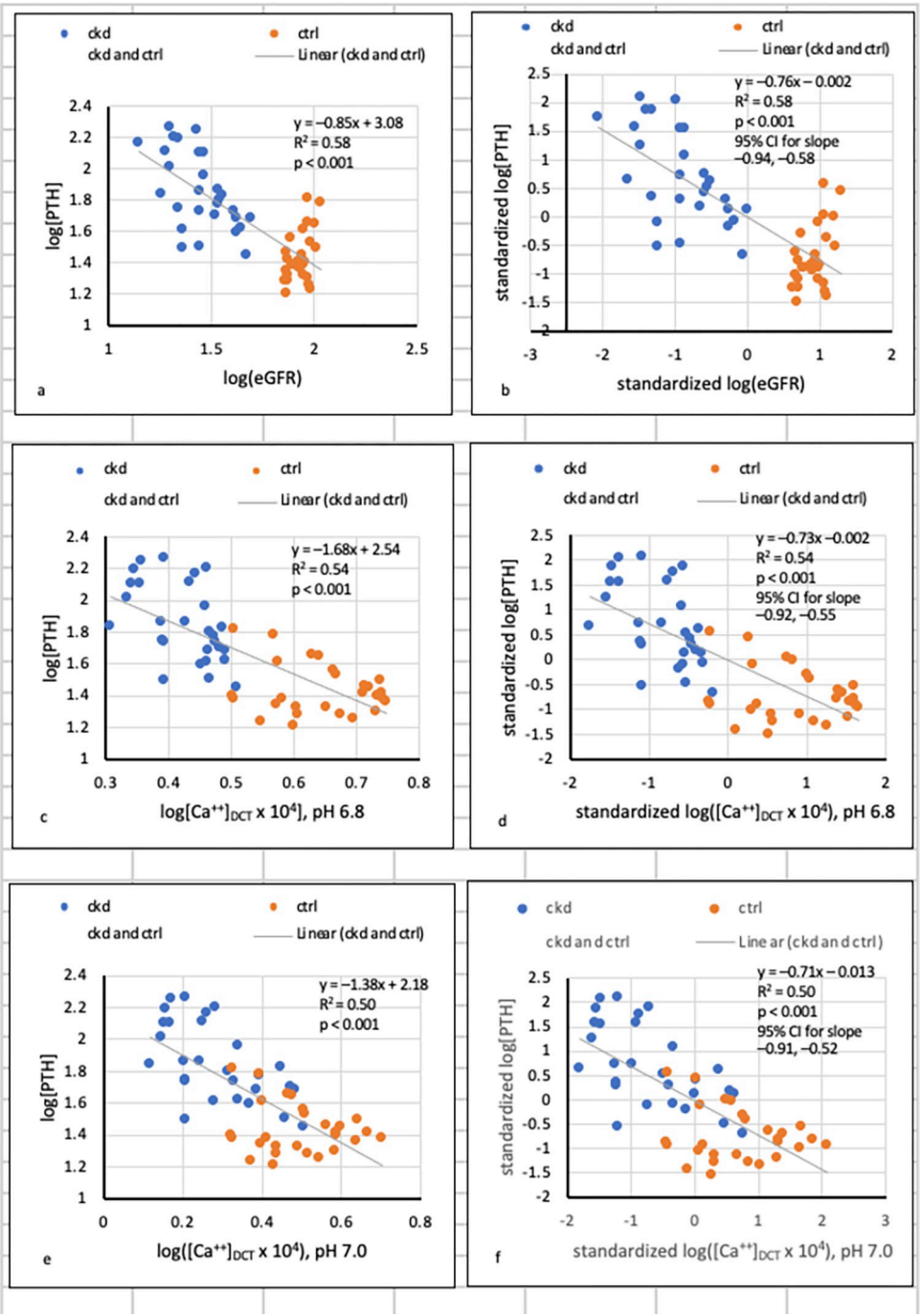

Fig 5. Regressions of [PTH] on eGFR and $[Ca^{++}]_{DCT}$ after log-transformation of variables and standardization of logarithmic values.

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combined groups, a scatterplot of [PTH] (y) against $[Ca^{++}]_{DCT}$ (x) appeared to depict a hyperbola described in part by the formula $xy = k$ (Fig 3C). A significant least-squares regression of [PTH] (y) on $10/[Ca^{++}]_{DCT}$ ($10/x$) was accordingly documented (Fig 3D), and the associated linear equation was modified to express [PTH] (y) as a power function of $[Ca^{++}]_{DCT}$ (x) in the form $y = kx^{-1} + c$. The hyperbola described by the function is included in Fig 3E.

Table 2. Parameters unaffected by pH or precipitation of $Ca_3(PO_4)_2$ (am., s.) in the DCT[a].

Parameter	Subjects with CKD (n = 28)	Control subjects (n = 27)	p
eGFR, mL/min/1.73m^2	29.9 (9.5)	86.2 (10.2)	< 0.001
[PTH], pg/mL	82.9 (47.6)	29.0 (12.4)	< 0.001
$[Ca^{++}]_s$, mol/L x 10^3	1.24 (0.05)	1.26 (0.03)	0.1
$[Ca_{uf}]_s$, mol/L x 10^3	1.34 (0.05)	1.34 (0.06)	0.9
$[P]_s$, mol/L x 10^3	1.15 (0.24)	1.11 (0.20)	0.7
E_P, mol/24h x 10^2	2.60 (0.83)	2.66 (1.03)	0.8
E_{Ca}, mol/24h x 10^3	1.05 (0.74)	3.31 (1.84)	< 0.001
Total $[Ca]_{DCT}$, mol/L x 10^4	3.82 (0.14)	6.71 (0.31)	< 0.001
Total $[P]_{DCT}$, mol/L x 10^3	1.89 (0.75)	1.07 (0.38)	< 0.001

[a]Values are mean (SD).

$[Ca^{++}]_s$, $[Ca_{uf}]_s$, $[P]_s$, and 24-hour E_P were not different in CKD and CTRL. In CKD, eGFR was lower, [PTH] higher, 24h E_{Ca} lower, total $[Ca]_{DCT}$ lower, and total $[P]_{DCT}$ higher than in CTRL.

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At pH 7.0, results resembled and accentuated those obtained at pH 6.8. $[Ca^{++}]_{DCT}$ was again entirely dependent on $[P]_{DCT}$ in both groups, but relationships were curvilinear rather than linear (Fig 4A). $[Ca^{++}]_{DCT}$ remained independent of total $[Ca]_{DCT}$ (Fig 4B). [PTH] rose as $[Ca^{++}]_{DCT}$ fell in CKD but not CTRL, and the trajectory of [PTH] became more positive at $[Ca^{++}]_{DCT}$ of approximately 2.0 mol/L x 10^{-4} (Fig 4C). Again, a scatterplot of [PTH] against $[Ca^{++}]_{DCT}$ in both groups appeared to depict a hyperbola described in part by the equation $xy = k$ (Fig 4C). A significant linear regression of [PTH] (y) on $10/[Ca^{++}]_{DCT}$ (x) was accordingly demonstrated (Fig 4D), and the associated equation was modified to express [PTH] (y) as a power function of $[Ca^{++}]_{DCT}$ (x) in the form $y = kx^{-1} + c$. The hyperbola described by the function is included in Fig 4E.

Because relationships of [PTH] to eGFR and to $[Ca^{++}]_{DCT}$ were visually similar (Figs 1F, 3E, and 4E), we performed an additional test of the hypothesis that the first relationship resulted from the second. Whereas ($kx^{-1} + c$) is not amenable to log transformation, the general formula $y = kx^n$–a simpler power function of x–transforms to the linear equation $\log y = \log k + n(\log x)$.

Fig 5 presents results of this modification for y = [PTH] and x = eGFR or $[Ca^{++}]_{DCT}$ at pH 6.8 or 7.0.

Fig 5A, 5C, and 5E show that at either pH value, log transformations yielded significant linear regressions of log[PTH] on both log(eGFR) and $\log([Ca^{++}]_{DCT} \times 10^4)$. These results indicate that in addition to the hyperbolic formulas in Figs 1F, 3E, and 4E, power functions of the form $y = kx^n$ related [PTH] (y) to both eGFR and $[Ca^{++}]_{DCT}$ (x) in combined CKD and CTRL. In Fig 5B, 5D, and 5F, each value of log[PTH], log(eGFR) and $\log([Ca^{++}]_{DCT} \times 10^4)$ was assigned a z-score. After this standardization procedure, slopes of lines relating log[PTH] to either log(eGFR) or $\log([Ca^{++}]_{DCT} \times 10^4)$ were virtually identical, as is indicated by the extreme overlap in confidence intervals.

Other issues are examined in Supporting Information. Although precipitation of $Ca_3(PO_4)_2$ (am., s.) was assumed in most scenarios, we also considered the possibility that formation of $CaHPO_4^0$ might cause a reduction of $[Ca^{++}]_{DCT}$ and an elevation of [PTH] as $[P]_{DCT}$ rose (S4 Fig). In CKD and CTRL, $[CaHPO_4^0]_{DCT}$ increased with total $[P]_{DCT}$ at pH 6.6, 6.8, and 7.0 (S4A and S4B Fig). $[Ca^{++}]_{DCT}$ fell as $[CaHPO_4^0]_{DCT}$ rose, but at a given $[CaHPO_4^0]_{DCT}$, $[Ca^{++}]_{DCT}$ varied markedly with pH and therefore did not appear to be controlled by $[CaHPO_4^0]_{DCT}$ *per se* (S4C and S4D Fig). The small calculated formation of $CaHPO_4^0$ as a

percentage of total calcium argues strongly against a role for this species in determination of $[Ca^{++}]_{DCT}$.

Assuming pH 6.8 and precipitation of $Ca_3(PO_4)_2$ (am., s.), we examined regressions of $[Ca^{++}]_{DCT}$ on concentrations of $Cacit^{-}$ and other Ca complexes to ascertain whether these compounds could reduce $[Ca^{++}]_{DCT}$ and raise [PTH] secondarily. $[Ca^{++}]_{DCT}$ varied inversely with $[CaHPO_4^{0}]_{DCT}$, presumably because of the relationship of $[CaHPO_4]_{DCT}$ to $[P]_{DCT}$ (S4 Fig), and directly with concentrations of all other complexes (S5 Fig). Citrate, oxalate, bicarbonate, and sulfate did not reduce $[Ca^{++}]_{DCT}$ significantly.

In principle, fractional delivery of filtered calcium to the DCT (FD_{Ca}) could have been higher in CKD than the assigned value, 0.1, with the result that total $[Ca]_{DCT}$ exerted an effect on $[Ca^{++}]_{DCT}$ that was not evident at FD_{Ca} 0.1. We therefore examined regressions of $[Ca^{++}]_{DCT}$ on total $[Ca]_{DCT}$, $[Ca^{++}]_{DCT}$ on total $[P]_{DCT}$, and [PTH] on $[Ca^{++}]_{DCT}$ at FD_{Ca} 0.15 or 0.2. At pH 6.6, 6.8, and 7.0, $logSICa_3(PO_4)_2$ (am., s.) was essentially zero in all subjects at both FD_{Ca} values (data not shown), and therefore indicated universal precipitation of $Ca_3(PO_4)_2$ (am., s.) at the increased values of FD_{Ca}. At pH 6.6, a previously significant relationship of $[Ca^{++}]_{DCT}$ to total $[Ca]_{DCT}$ disappeared (S3B Fig), and a strong relationship of $[Ca^{++}]_{DCT}$ to total $[P]_{DCT}$ emerged (S6 and S7 Figs). At pH 6.8 and 7.0, $[Ca^{++}]_{DCT}$ remained independent of $[Ca]_{DCT}$ and exclusively dependent on $[P]_{DCT}$ (S6 and S7 Figs). Regressions of [PTH] on $[Ca^{++}]_{DCT}$ were significant at both values of FD_{Ca} and all three pH values; R^2 for these regressions was similar to values obtained at FD_{Ca} 0.1 (Figs 3 and 4).

Discussion

Background and summary of principal findings

In stages G_3 and G_4 CKD, one of the cardinal features of SHPT is persistence of normal $[Ca^{++}]_s$ and $[Ca_{uf}]_s$ until CKD is far advanced [12]. If C_{cr} is assumed to approximate GFR, $[Ca_{uf}]_s$ equals $E_{Ca}/C_{cr} + TR_{Ca}/C_{cr}$, *i.e.*, the summed amounts of Ca excreted and reabsorbed per volume of filtrate [12,48]. Since flux of Ca into plasma determines and equals E_{Ca}, the ratio E_{Ca}/C_{cr}, calculated as $[Ca]_u[cr]_p/[cr]_u$, quantifies the contribution of net influx from all sources to $[Ca_{uf}]_s$ [48]. TR_{Ca}/C_{cr}, the difference between $[Ca_{uf}]_s$ and E_{Ca}/C_{cr}, describes the simultaneous contribution of tubular Ca reabsorption. Ordinarily, influx provides 1–2% and reabsorption 98–99% of the flux maintaining normal $[Ca_{uf}]_s$ [12].

PTH regulates reabsorption of the 10% of filtered Ca that reaches the DCT by controlling expression of the apical calcium channel transient receptor potential vanilloid 5 (TRPV5), the intracellular transporter calbindin-D_{28K}, and the basolateral extrusion proteins sodium-calcium exchanger 1 (NCX1) and plasma membrane calcium ATPase 1b (PMCA1b) [10,11,30]. In primary hyperparathyroidism (PHPT), elevated [PTH] causes hypercalcemia by increasing TR_{Ca}/C_{cr} [12,49]; in SHPT, comparable or higher [PTH] is associated with and presumably required to achieve normal TR_{Ca}/C_{cr} and normocalcemia [12]. This presumption is consistent with the observation that cinacalcet, a calcimimetic that suppresses synthesis and secretion of PTH, reduced tubular Ca reabsorption and caused hypocalcemia as it lowered [PTH] in CKD stages G_3 and G_4 [50].

The tradeoff-in-the-nephron hypothesis states that as GFR falls, $[Ca^{++}]_{DCT}$ also falls in response to increased total $[P]_{DCT}$; if $[P]_{DCT}$ reduces $[Ca^{++}]_{DCT}$ sufficiently, [PTH] rises to preserve Ca reabsorption and maintain normocalcemia [8]. Whereas the hypothesis was previously supported by significant relationships of [PTH] to E_P/C_{cr}, a surrogate for $[P]_{DCT}$ [12,13,24,25], the present study showed additionally that $[Ca^{++}]_{DCT}$ is related to total $[P]_{DCT}$ in CKD and CTRL (Figs 3A and 4A). If precipitation of amorphous $Ca_3(PO_4)_2$ is posited in states of supersaturation, the results imply that in CKD, [PTH] increases as $[P]_{DCT}$ rises and

$[Ca^{++}]_{DCT}$ falls. At FD_{Ca} 0.1 and pH 6.8 or 7.0, the upward trajectory of [PTH] is accentuated at a sufficiently reduced $[Ca^{++}]_{DCT}$ (Figs 3E and 4E). A critical observation is that in combined CKD and CTRL, curvilinear relationships of [PTH] to $[Ca^{++}]_{DCT}$ and to eGFR are essentially identical after log-transformation of variables and standardization of logarithmic values (Fig 5).

Estimation of total $[Ca]_{DCT}$ and $[P]_{DCT}$

Our estimations of total $[Ca]_{DCT}$ and $[P]_{DCT}$ are based on published physiologic observations and measurements of $[Ca_{uf}]_s$, eGFR, and 24-hour E_P. Given that micropuncture studies of normal rodents showed fractional Ca delivery to the DCT of 10% [30], we calculated total $[Ca]_{DCT}$ as the rate of Ca delivery divided by the rate of filtrate delivery to the DCT, or (0.1)eGFR $[Ca_{uf}]_s/\{FD_f(eGFR)\}$, where FD_f = fractional delivery of filtrate to that segment. This formula simplified to total $[Ca]_{DCT} = (0.1)[Ca_{uf}]_p/FD_f$. We assumed FD_f of 0.2 in CTRL and 0.35 in CKD [31–33]; since $[Ca_{uf}]_s$ was not different in CKD and CTRL, FD_f was solely responsible for differences in total $[Ca]_{DCT}$ in the two groups. In CKD, higher FD_f lowered total $[Ca]_{DCT}$; this consequence could possibly have obscured an effect of higher FD_{Ca} on $[Ca^{++}]_{DCT}$, but when FD_{Ca} was increased to 0.15 or 0.2, $[Ca^{++}]_{DCT}$ remained independent of total $[Ca]_{DCT}$, and total $[P]_{DCT}$ continued to be the sole determinant of $[Ca^{++}]_{DCT}$ (S6 and S7 Figs).

Diurnal variation in $[P]_s$ necessitated a different line of reasoning to estimate total $[P]_{DCT}$ [51]. Because E_P approximates the rate at which phosphate is delivered to the DCT [29], we inferred, in accordance with classic micropuncture observations [9], that normal phosphate influx from the gut raises $[P]_{DCT}$ as GFR falls. We also recognized that a correlation between [PTH] and $[P]_{DCT}$ would explain why limiting intestinal phosphate influx has consistently prevented, mitigated, or reversed SHPT in animal and human studies [8,14–22].

Determinants of $[Ca^{++}]_{DCT}$

According to JESS calculations, the principal determinant of $[Ca^{++}]_{DCT}$ depended on whether precipitation of $Ca_3(PO_4)_2$ (am., s.) occurred in the DCT. When no precipitation, FD_{Ca} 0.1, and pH 6.8 were assumed (S2 Fig), total $[Ca]_{DCT}$ was found to be the major factor and total $[P]_{DCT}$ a minor factor determining $[Ca^{++}]_{DCT}$ in CKD and CTRL. At pH 6.6, mean logSI-$Ca_3(PO_4)_2$ (am., s.) was substantially negative, the DCT was not saturated with this solid (with five exceptions in CTRL), and relationships of $[Ca^{++}]_{DCT}$ to $[Ca]_{DCT}$ and $[P]_{DCT}$ resembled those predicted in the absence of precipitation at pH 6.8 (S2 and S3 Figs). When precipitation, FD_{Ca} 0.1, and pH 6.8 or 7.0 were assumed, supersaturation of the DCT with $Ca_3(PO_4)_2$ (am., s.) was more prevalent, and $[P]_{DCT}$ emerged as the sole determinant of $[Ca^{++}]_{DCT}$ (Figs 3 and 4). When precipitation and FD_{Ca} 0.15 or 0.20 were assumed, supersaturation with $Ca_3(PO_4)_2$ (am., s.) was universal at pH 6.6, 6.8, and 7.0, and $[Ca^{++}]_{DCT}$ was determined exclusively by $[P]_{DCT}$ (S6 and S7 Figs). We therefore conclude that the effect of $[P]_{DCT}$ on $[Ca^{++}]_{DCT}$ is dominant when precipitation of $Ca_3(PO_4)_2$ (am., s.) occurs and negligible when precipitation does not occur. By inference, tradeoff-in-the-nephron is not applicable when FD_{Ca} is $\leq$ 0.1 and pH is $\leq$ 6.6 simultaneously, but it is applicable under the more likely conditions that either pH is $\geq$ 6.7 (S1 Fig), or FD_{Ca} is $\geq$ 0.1 (S6 and S7 Figs), or both are true. In all scenarios, we presume that the solid phase of $Ca_3(PO_4)_2$ (am., s.) may dissolve downstream in a more acidic milieu [52].

If precipitation of $Ca_3(PO_4)_2$ (am., s.) was not assumed, $[CaHPO_4{}^0]_{DCT}$ became the only plausible phosphate-containing determinant of $[Ca^{++}]_{DCT}$, but JESS calculations rendered this scenario unlikely; $[Ca^{++}]_{DCT}$ was consistently predicted to be an order of magnitude higher

than $[CaHPO_4^0]_{DCT}$, and $[Ca^{++}]_{DCT}$ varied substantially with pH at a given $[CaHPO_4^0]_{DCT}$ (S4 Fig). We conclude that $CaHPO_4^0$ did not mediate the effect of $[P]_{DCT}$ on $[Ca^{++}]_{DCT}$.

We also investigated the effect of anions other than phosphate on $[Ca^{++}]_{DCT}$. Regressions of $[Ca^{++}]_{DCT}$ on $[Cacit^-]_{DCT}$, $[Caox^0]_{DCT}$, $[CaHCO_3^+]_{DCT}$, and $[CaSO_4^0]_{DCT}$ were performed to ascertain whether these compounds had reduced $[Ca^{++}]_{DCT}$, but $[Ca^{++}]_{DCT}$ and concentrations of each complex varied in the same direction (S5 Fig). These results support the inference that precipitation of amorphous $Ca_3(PO_4)_2$ determined $[Ca^{++}]_{DCT}$, and $[Ca^{++}]_{DCT}$ determined complex concentrations other than $[CaHPO_4^0]_{DCT}$. Low relative concentrations of these complexes preclude the possibility that $[Ca^{++}]_{DCT}$ was suppressed by substances such as citrate or oxalate.

Regressions of [PTH] on total $[P]_{DCT}$, $[Ca^{++}]_{DCT}$, and eGFR

[PTH] rose with total $[P]_{DCT}$ in CKD (Fig 1B). Although $[Ca^{++}]_{DCT}$ was inversely related to $[P]_{DCT}$ in CKD and CTRL, reductions in $[Ca^{++}]_{DCT}$ were apparently sufficient to raise [PTH] in CKD only. When precipitation of $Ca_3(PO_4)_2$ (am., s.) was excluded from consideration or appeared not to have occurred, total $[Ca]_{DCT}$ was the primary determinant of $[Ca^{++}]_{DCT}$, and substantial elevations of [PTH] were predicted over a narrow range of $[Ca^{++}]_{DCT}$ (S2 and S3 Figs). When precipitation of $Ca_3(PO_4)_2$ (am., s.) occurred at pH 6.8 or 7.0, $[P]_{DCT}$ was the sole determinant of $[Ca^{++}]_{DCT}$; [PTH] increased to abnormal levels over a wider and more plausible range of $[Ca^{++}]_{DCT}$, and a continuous hyperbolic relationship between [PTH] and $[Ca^{++}]_{DCT}$ emerged if CKD and CTRL were considered together (Figs 3 and 4). After log-log transformation of variables and standardization of logarithmic values, relationships of [PTH] to eGFR and to $[Ca^{++}]_{DCT}$ were found to be virtually identical (Fig 5).

At pH values typical of the DCT [28], we infer that increased $[P]_{DCT}$ mediates a decline in $[Ca^{++}]_{DCT}$ through precipitation of $Ca_3(PO_4)_2$ (am., s.). If $[Ca^{++}]_{DCT}$ is sufficiently reduced, [PTH] rises to maintain normal Ca^{++} reabsorption. The similarity of relationships of [PTH] to $[Ca^{++}]_{DCT}$ and [PTH] to eGFR suggests that the first relationship causes the second (Figs 1F, 3E, 4E, and 5).

Strengths and limitations of the present study

In the present study, we employed laboratory data and evidence-based physiologic assumptions to estimate total $[P]_{DCT}$ and $[Ca]_{DCT}$ [29, 30], and we drew on published information to assign concentrations to other constituents of DCT filtrate [31–45]. JESS calculations using representative values of $[P]_{DCT}$ and $[Ca]_{DCT}$ predicted precipitation of $Ca_3(PO_4)_2$ (am., s.) at pH $\geq$ 6.73 (S1 Fig); similarly, calculations based on measured values showed that at pH $\geq$ 6.8, $[P]_{DCT}$ determined $[Ca^{++}]_{DCT}$ by inducing precipitation of $Ca_3(PO_4)_2$ (am., s.). [PTH] rose in curvilinear fashion as eGFR and $[Ca^{++}]_{DCT}$ fell (Figs 1F, 3E, and 4E); moreover, after log-transformation of variables and standardization of logarithmic values, lines relating [PTH] to eGFR and [PTH] to $[Ca^{++}]_{DCT}$ had virtually identical slopes. A strength of our study is the coincidence of robust methodology with chemical evidence that in CKD, phosphate raises [PTH] by reducing $[Ca^{++}]_{DCT}$. The effect of $[P]_{DCT}$ on $[Ca^{++}]_{DCT}$ is consistent at FD_{Ca} 0.1, 0.15, and 0.2, and it explains why cinacalcet reduced Ca reabsorption and caused hypocalcemia in CKD stages G_3 and G_4 [50].

Although our observations support the tradeoff-in-the-nephron hypothesis, it is reasonable to question why R^2 values for some pertinent regressions are not higher. Several explanations present themselves. First, precipitation that reduces $[Ca^{++}]_{DCT}$ occurs only when the DCT is supersaturated with $Ca_3(PO_4)_2$ (am., s.). Since supersaturation was not universal in CKD at FD_{Ca} of 0.1 (Fig 2C and 2D), $[P]_{DCT}$ affected $[Ca^{++}]_{DCT}$ differently in individual subjects. A

related consideration is that uromodulin, a protein secreted by the thick ascending limb of Henle's loop, prevents aggregation of calcium phosphate crystals and may therefore have interfered with precipitation of $Ca_3(PO_4)_2$ in the DCT (am., s.) [53].

A second potential limitation is that loop diuretic therapy could have contributed to SHPT in CKD [54]. However, patients were instructed to abstain from food or medicines for at least eight hours before plasma was obtained to measure [PTH], and 24-hour E_{Ca} was reduced in proportion to eGFR in CKD (Table 1). We suspect that the contribution of loop diuresis to SHPT was minimal.

A third limitation is that we associated [PTH] at a single moment with calculations of $[P]_{DCT}$ and $[Ca^{++}]_{DCT}$ from 24-hour data. Since $[P]_{DCT}$ varies through the day with consumption of food and changes in phosphate reabsorption [51], it is unlikely that our calculated concentrations were identical to those present in the fasting state when blood was sampled for PTH assays. Given that the half-life of PTH is a few minutes [55], we presume that [PTH] was more closely related to a contemporaneous than to an average $[P]_{DCT}$.

Additional limitations arise from assumptions that FD_f to the DCT was fixed at 0.2 in CTRL and 0.35 in CKD, and filtrate pH was uniformly 6.6, 6.8, or 7.0. Errors were also inherent in estimations of GFR and determinations of 24-hour E_{Ca} and E_P. Single-nephron GFR varies in animal models of CKD [9], and it is unclear how this variability affected our data. Although equilibrium constants, especially solubility products of solid phases, are somewhat uncertain, we doubt that these uncertainties undermine our results.

Summary and conclusions

We describe an examination of the tradeoff-in-the-nephron hypothesis with the Joint Expert Speciation System. At pH values typical of the DCT, we present evidence that $[P]_{DCT}$ determines $[Ca^{++}]_{DCT}$ by inducing precipitation of $Ca_3(PO_4)_2$ (am., s.). Although this phenomenon occurs in both CKD and CTRL, $[Ca^{++}]_{DCT}$ appears to fall sufficiently to raise [PTH] in CKD only. Whether they are defined by the equation $y = kx^{-1} + c$ or the simpler power function $y = kx^n$, relationships of [PTH] to $[Ca^{++}]_{DCT}$ and [PTH] to eGFR are so similar that the former seems likely to cause the latter. Our observations strongly suggest that the tradeoff-in-the-nephron hypothesis explains SHPT in stages G_3 and G_4 CKD. They also suggest that humans with SHPT may be treated successfully by reducing phosphate influx in proportion to the reduction in GFR.

Supporting information

S1 Fig. Plot of logSI(Ca_3PO4_2) (am.,s.) *vs.* pH.
(TIFF)

S2 Fig. Regressions assuming pH 6.8 and no precipitation of $Ca_3(PO_4)_2$ (am., s.).
(TIFF)

S3 Fig. Regressions assuming pH 6.6 and precipitation of $Ca_3(PO_4)_2$ (am., s.).
(TIFF)

S4 Fig. Relationship of $[Ca^{++}]_{DCT}$ to $[CaHPO40]_{DCT}$ at pH 6.6, 6.8, and 7.0.
(TIFF)

S5 Fig. Effect of Ca-anion complexes on $[Ca^{++}]_{DCT}$.
(TIFF)

S6 Fig. Regressions at FD_{Ca} to DCT of 0.15 and pH of 6.6 (a-c), 6.8 (d-f), and 7.0 (g-i).
(TIFF)

S7 Fig. Regressions at FD_{Ca} of 0.2 and pH of 6.6 (a-c), 6.8 (d-f), and 7.0 (g-i).
(TIFF)

S1 Table. Assumed anion excretion rates at normal and reduced GFR.
(TIFF)

S2 Table. Estiimated total concentrations in the DCT.
(TIFF)

S1 File. Supplemental information.
(PDF)

S2 File. Raw data.
(PDF)

S3 File.
(PDF)

S4 File.
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S5 File.
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S10 File.
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References

1. Levin A, Bakris GL, Molitch M, Smulders M, Tian J, Williams LA, et al. Prevalence of abnormal serum vitamin D, PTH, calcium and phosphorus in patients with chronic kidney disease: results of the study to evaluate early kidney disease. Kidney Int. 2007; 71: 31–38. https://doi.org/10.1038/sj.ki.5002009 PMID: 17091124
2. Malluche HH, Ritz E, Lange HP, Kutschera J, Hodgson M, Seiffert U, et al. Bone histology in incipient and advanced renal failure. Kidney Int. 1976; 9: 355–362. https://doi.org/10.1038/ki.1976.42 PMID: 940274
3. Kim SM, Long J, Montez-Rath M, Leonard M, Chertow GM. Hip fracture in patients with non-dialysis-requiring chronic kidney disease. J Bone Miner Res. 2016; 31: 1803–1809. https://doi.org/10.1002/jbmr.2862 PMID: 27145189
4. Ritz E, Stefanski A, Rambausek M. The role of the parathyroid glands in the uremic syndrome. Am J Kidney Dis. 1995; 26: 808–813. https://doi.org/10.1016/0272-6386(95)90448-4 PMID: 7485137
5. Lavi-Moshayoff V, Wasserman G, Meir T, Silver J, Naveh-Many T. PTH increases FGF23 gene expression and mediates the high FGF23 levels of experimental kidney failure: a bone parathyroid hormone feedback loop. Am J Physiol Renal Physiol. 2010; 299: F882–F889. https://doi.org/10.1152/ajprenal.00360.2010 PMID: 20685823
6. Mace ML, Gravesen E, Nordholm A, Hofman-Bang J, Secher T, Olgaard K, et al. Kidney fibroblast growth factor 23 does not contribute to elevation of its circulating levels in uremia. Kidney Int. 2017; 92: 165–178. https://doi.org/10.1016/j.kint.2017.01.015 PMID: 28341272
7. Kendrick J, Cheung AK, Kaufman JS, Greene T, Roberts WL, Smits G, et al. FGF-23 associates with death, cardiovascular events, and initiation of chronic dialysis. J Am Soc Nephrol. 2011; 22: 1913–1922. https://doi.org/10.1681/ASN.2010121224 PMID: 21903574
8. Phelps KR. Tradeoff-in-the-nephron: a theory to explain the primacy of phosphate in the pathogenesis of secondary hyperparathyroidism. Nutrients 2017 Apr 26. 9: E427. https://doi.org/10.3390/nu9050427 PMID: 28445401
9. Bank N, Su WS, Aynedjian HS. A micropuncture study of renal phosphate transport in rats with chronic renal failure and secondary hyperparathyroidism. J Clin Invest. 1978; 61: 884–894. https://doi.org/10.1172/JCI109014 PMID: 659581
10. DeGroot T, Bindels RJM, Hoenderop JGJ: TRPV5: an ingeniously controlled calcium channel. Kidney Int. 2008; 74:1241–1246. https://doi.org/10.1038/ki.2008.320 PMID: 18596722
11. DeGroot T, Lee K, Langeslag M, Xi Q, Jalink K, Bindels RJM, et al. Parathyroid hormone activates TRPV5 via PKA-dependent phosphorylation. J Am Soc Nephrol. 2009; 20: 1693–1704. https://doi.org/10.1681/ASN.2008080873 PMID: 19423690
12. Phelps KR, Stote KS, Mason D. Tubular calcium reabsorption and other aspects of calcium homeostasis in primary and secondary hyperparathyroidism. Clin Nephrol. 2014; 82: 83–91. https://doi.org/10.5414/CN108223 PMID: 24985952
13. Phelps KR, Stote KS, Mason D. Use of sevelamer to examine the role of intraluminal phosphate in the pathogenesis of secondary hyperparathyroidism. Clin Nephrol. 2014; 82: 191–201. https://doi.org/10.5414/cn108227 PMID: 25079864
14. Slatopolsky E, Caglar S, Gradowska L, Canterbury J, Reiss E, Bricker NS. On the prevention of secondary hyperparathyroidism in experimental chronic renal disease using "proportional reduction" of dietary phosphorus intake. Kidney Int. 1972; 2: 147–151. https://doi.org/10.1038/ki.1972.84 PMID: 4669450
15. Kaplan MA, Canterbury JM, Bourgoignie JJ, Veliz G, Gavellas G, Reiss E, et al. Reversal of hyperparathyroidism in response to dietary phosphorus restriction in the uremic dog. Kidney Int. 1979; 15: 43–48. https://doi.org/10.1038/ki.1979.6 PMID: 491396
16. Lopez-Hilker S, Dusso AS, Rapp NS, Martin KJ, Slatopolsky E. Phosphorus restriction reverses hyperparathyroidism in uremia independent of changes in calcium and calcitriol. Am J Physiol. 1990; 259: F423–F437. https://doi.org/10.1152/ajprenal.1990.259.3.F432 PMID: 2396669

17. Ritter CS, Martin DR, Lu Y, Slatopolsky E, Brown AJ. Reversal of secondary hyperparathyroidism by phosphate restriction restores parathyroid calcium-sensing receptor expression and function. J Bone Miner Res. 2002; 17: 2206–2213. https://doi.org/10.1359/jbmr.2002.17.12.2206 PMID: 12469914

18. Nagano N, Miyata S, Abe M, Kobayashi N, Wakita S, Yamashita T, et al. Effect of manipulating serum phosphorus with phosphate binder on circulating PTH and FGF23 in renal failure rats. Kidney Int. 2006; 69: 531–537. https://doi.org/10.1038/sj.ki.5000020 PMID: 16395276

19. Combe C, Aparicio M: Phosphorus and protein restriction and parathyroid function in chronic renal failure. Kidney Int. 1994; 46: 1381–1386. https://doi.org/10.1038/ki.1994.408 PMID: 7853797

20. Combe C, Morel D, de Precigout V, Blanchetier V, Bouchet JL, Potaux L, et al. Long-term control of hyperparathyroidism in advanced renal failure by low-phosphorus low-protein diet supplemented with calcium (without changes in plasma calcitriol). Nephron 1995; 70: 287–295. https://doi.org/10.1159/000188606 PMID: 7477615

21. Oliveira RB, Cancela ALE, Graciolli FG, Dos Reis LM, Draibe SA, Cuppari L, et al. Early control of PTH and FGF23 in normophosphatemic CKD patients: a new target in CKD-MBD patients? Clin J Am Soc Nephrol. 2010; 5:286–291. https://doi.org/10.2215/CJN.05420709 PMID: 19965540

22. Sprague SM, Abboud H, Qui P, Dauphin M, Zhang P, Finn W. Lanthanum carbonate reduces phosphorus burden in patients with CKD stages 3 and 4: a randomized trial. Clin J Am Soc Nephrol. 2009; 4: 178–189. https://doi.org/10.2215/CJN.02830608 PMID: 19056618

23. Bricker NS. On the pathogenesis of the uremic state: an exposition of the "trade-off hypothesis." N Engl J Med. 1972; 286: 1093–1099. https://doi.org/10.1056/NEJM197205182862009 PMID: 4553202

24. Phelps KR, Mason DL, Stote KS. Phosphate homeostasis, parathyroid hormone, and fibroblast growth factor 23 in stages 3 and 4 chronic kidney disease. Clin Nephrol. 2016; 85: 251–261. https://doi.org/10.5414/CN108686 PMID: 26951967

25. Phelps KR, Mason DL. Parathyroid hormone, fibroblast growth factor 23, and parameters of phosphate reabsorption. Am J Nephrol. 2018; 47: 343–351. https://doi.org/10.1159/000489270 PMID: 29779023

26. May PM, Rowland D. JESS, a Joint Expert Speciation System–VI: thermodynamically-consistent standard Gibbs energies of reaction for aqueous solutions. New J Chem. 2018; 42: 7617–7629.

27. Hill MG, Konigsberger E, May PM. Mineral precipitation and dissolution in the kidney. Am Min. 2017; 102: 701–710.

28. Rector FC Jr, Carter NW, Seldin DW: The mechanism of bicarbonate reabsorption in the proximal and distal tubules of the kidney. J Clin Invest. 1965; 44: 278–290. https://doi.org/10.1172/JCI105142 PMID: 14260168

29. Biber J, Murer H, Mohebbi N, Wagner CA. Renal handling of phosphate and sulfate. Compr Physiol. 2014; 4: 771–792. https://doi.org/10.1002/cphy.c120031 PMID: 24715567

30. Costanzo LS, Windhager EE. Calcium and sodium transport by the distal convoluted tubule of the rat. Am J Physiol. 1978; 235: F492–F506. https://doi.org/10.1152/ajprenal.1978.235.5.F492 PMID: 727266

31. Wen S-F, Wong NLM, Evanson RL, Lockhart EA, Dirks JH. Micropuncture studies of sodium transport in the remnant kidney of the dog. J Clin Invest. 52: 386–397. https://doi.org/10.1172/JCI107195 PMID: 4683878

32. Wong NLM, Quamme GA, Dirks JH. Tubular handling of bicarbonate in dogs with experimental renal failure. Kidney Int. 1984; 25: 912–918, 1984. https://doi.org/10.1038/ki.1984.109 PMID: 6471673

33. Bank N, Aynedjian HS. A micropuncture study of potassium excretion by the remnant kidney. J Clin Invest. 1973; 52: 1480–1490. https://doi.org/10.1172/JCI107322 PMID: 4703232

34. Kunau RT, Whinnery MA. Potassium transfer in distal tubule of normal and remnant kidneys. Am J Physiol. 1975; 235: F186–F191.

35. Simon EE, Martin D, Trigg D, Buerkert J. Contribution of the distal tubule to potassium excretion in experimental glomerulonephritis. Kidney Int. 1988; 34: 53–59. https://doi.org/10.1038/ki.1988.144 PMID: 3172637

36. Clapp JR, Robinson RR. Osmolality of distal tubular fluid in the dog. J Clin Invest 1966; 45: 1847–1853. https://doi.org/10.1172/JCI105488 PMID: 5926630

37. Quamme GA, Wong NLM, Dirks JH, Roinel N, De Rouffignac C, Morel F. Magnesium handling in the dog kidney: a micropuncture study. Pflugers Arch. 1978; 377: 95–99. https://doi.org/10.1007/BF00584380 PMID: 569286

38. Curry J, Yu A. Magnesium handling in the kidney. Adv Chronic Kidney Dis. 2018; 25: 236–243. https://doi.org/10.1053/j.ackd.2018.01.003 PMID: 29793662

39. Allon M, Harrow A, Pasque CBV, Rodriguez M. Renal sodium and water handling in hypothyroid patients: the role of renal insufficiency. J Am Soc Nephrol. 1990; 1: 205–210. https://doi.org/10.1681/ASN.V12205 PMID: 2104264

40. Uribarri J, Douyon H, Oh MS. A re-evaluation of the urinary parameters of acid production and excretion in patients with chronic renal acidosis. Kidney Int. 1995; 47: 624–627. https://doi.org/10.1038/ki.1995.79 PMID: 7723250

41. Marangella M, Vitale C, Manganaro M, Cosseddu D, Martini C, Petrarulo M, et al. Renal handling of citrate in chronic renal insufficiency. Nephron 1991; 57: 439–443. https://doi.org/10.1159/000186347 PMID: 2046827

42. Taylor EN, Curhan GC. Determinants of 24-hour urinary oxalate excretion. Clin J Am Soc Nephrol. 2008; 3: 1453–1460. https://doi.org/10.2215/CJN.01410308 PMID: 18650406

43. Waikar SS, Srivastava A, Palsson R, Shafi T, Hsu C-Y, Sharma K, et al. Association of urinary oxalate excretion with the risk of chronic kidney disease progression. JAMA Intern Med. 2019; 179: 542–551. https://doi.org/10.1001/jamainternmed.2018.7980 PMID: 30830167

44. Rieselbach RE, Steele TH. Intrinsic renal disease leading to abnormal urate excretion. Nephron. 1975; 14: 81–87. https://doi.org/10.1159/000180437 PMID: 1124138

45. Sui W, Calvert JK, Kavoussi NL, Gould ER, Miller NL, Bejan CA, et al. Association of chronic kidney disease stage with 24-hour urine values among patients with nephrolithiasis. J Endourol. 2020; 34: 1263–1271. https://doi.org/10.1089/end.2020.0403 PMID: 32578450

46. Chung S-Y, Kim Y-M, Kim J-G, Kim Y-J. Multiphase transformation and Ostwald's rule of stages during crystallization of a metal phosphate. Nat Phys. 2009; 5: 68–73.

47. R Core Team. R. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org, 2020.

48. Phelps KR, Gosmanova EO. A generic method for analysis of plasma concentrations. Clin Nephrol. 2020; 94: 43–49. https://doi.org/10.5414/CN110056 PMID: 32383639

49. Kristiansen JH, Brochner-Mortensen J, Pedersen KO, Jensen S, Glud T. Renal tubular reabsorption of calcium and sodium in primary hyperparathyroidism. Acta Endocrinol. (Copenh) 1990; 123: 194–202. https://doi.org/10.1530/acta.0.1230194 PMID: 2220260

50. Chonchol M, Locatelli F, Abboud HE, Charytan C, de Francisco ALM, Jolly S, et al. A randomized, double-blind, placebo-controlled study to assess the efficacy and safety of cinacalcet HCl in participants with CKD not receiving dialysis. Am J Kidney Dis. 2009; 53:197–207. https://doi.org/10.1053/j.ajkd.2008.09.021 PMID: 19110359

51. Portale AA, Halloran BP, Morris RC Jr. Dietary intake of phosphorus modulates the circadian rhythm in serum concentration of phosphorus. J Clin Invest. 1987; 80: 1147–1154. https://doi.org/10.1172/JCI113172 PMID: 3654974

52. Tiselius H-G, Lindback B, Fornander A-M, Nilsson M-A. Studies on the role of calcium phosphate in the process of calcium oxalate crystal formation. Urol Res. 2009; 37: 181–182. https://doi.org/10.1007/s00240-009-0191-7 PMID: 19444436

53. Wolf MTF, Zhang J, Nie M. Uromodulin in mineral metabolism. Curr Opin Nephrol Hypertens. 2019; 28: 481–489. https://doi.org/10.1097/MNH.0000000000000522 PMID: 31205055

54. Isakova T, Anderson CAM, Leonard MB, Xie D, Gutierrez OM, Rosen LK, et al: Diuretics, calciuria and secondary hyperparathyroidism in the chronic renal insufficiency cohort (CRIC). Nephrol Dial Transplant 2011; 26: 1258–1265. https://doi.org/10.1093/ndt/gfr026 PMID: 21382989

55. Schmitt CP, Huber D, Mehls O, Maiwald J, Stein G, Veldhuis JD, et al. Altered instantaneous and calcium-modulated oscillatory PTH secretion patterns in patients with secondary hyperparathyroidism. J Am Soc Nephrol. 1998; 9: 1832–1844. https://doi.org/10.1681/ASN.V9101832 PMID: 9773784