Original Full Length Article

Acute and long term mineral metabolism adaptation in living kidney donors: A prospective study

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ABSTRACT

Background: Living kidney donors (LKDs) experience an abrupt decline in glomerular filtration rate (GFR). Mineral metabolism adaptations in early CKD are still debated and not well studied in LKDs. We prospectively studied acute and long term mineral metabolism adaptation of LKDs.
Methods: From May 2010 to December 2012, we included 27 adult LKDs. Their mineral parameters and renal function were repeatedly measured at days 0, 1, 2, 3, 180 and 360 after donation. We also measured in uninephrectomized rats' Klotho in the remnant kidney and FGF23 circulating levels.
Results: In the first days after nephrectomy, LKDs experience transient dilution hypocalcemia and secondary hyperparathyroidism. Urinary phosphate reabsorption decreases in spite of an abrupt decline in circulating FGF23 and Klotho. In a more chronic stage, at days 180 and 360 after donation, LKDs have lower GFR and $1,25(OH)_2D$ compared to pre-donation levels, with unchanged $25(OH)D$. PTH levels increase, resulting in decreased plasma phosphate levels and renal tubular reabsorption of phosphate. In comparison to pre-donation, FGF23 levels are not significantly changed whereas circulating Klotho levels are lower than pre-donation but higher than immediately post-donation. In uninephrectomized rats, Klotho kidney expression increases after three weeks, whereas circulating FGF23 levels are unchanged.
Conclusion: From six months after kidney donation, LKDs develop secondary hyperparathyroidism related to a decrease in $1,25(OH)_2D$, and decreased plasma phosphate levels. FGF23 levels do not rise in LKDs. Middle term mineral metabolism adaptations to decreased eGFR in donors include decrease in $1,25(OH)_2D$ and increase in PTH and fractional excretion of phosphate resulting in lowered plasma phosphate levels, independently of FGF23. These adaptations differ from those described in CKD patients.

Introduction

The exact mechanisms of mineral metabolism adaptation in response to loss of glomerular filtration rate (GFR) in early chronic kidney disease (CKD) are still debated. It has been hypothesized that increased phosphate retention is the primary event, leading to parathyroid hormone (PTH) elevation, mainly via complexation of calcium [1]. PTH secretion would, in turn, lead to a reduction in tubular maximum phosphate reabsorption per glomerular filtration rate ($TmPO^{4-}$/eGFR). In this hypothesis, the decrease in vitamin D renal hydroxylation is mainly related to reduced nephron mass and deemed unresponsive to phosphate intake [2]. According to another hypothesis, an increase of the intracellular inorganic phosphate (Pi) pool reduces the renal production of $1.25(OH)_2$ vitamin D and $TmPO^{4-}$/eGFR, which may then

be regulated by phosphate intake [3,4]. Experimental data suggests that FGF23 increase is an early event in CKD, decreasing 1.25 hydroxylation and enhancing phosphaturia [5]. Therefore, FGF23 is likely an important mediator of the tubular adaptation of phosphate reabsorption in CKD. On the other hand, its role in the presence of a reduced nephron number without kidney disease is not known.

Kidney donation allows us to determine the effect of a mild reduction in GFR on mineral metabolism. In this situation, as the patients are their own control, we can study the effect of the loss of renal function, without other confounding factors usually found in CKD, such as associated glomerular or tubular disease. The question of mineral bone metabolism alteration after living kidney donation is a matter of controversy [6]. A prospective controlled study including 201 donors has recently demonstrated 23% higher PTH and 5.4% lower phosphate serum levels 6 months after donation [7]. FGF23 has not been examined in this study and only few reports have previously described the evolution of FGF23 after kidney donation. In a study including 9 LKDs without controls, FGF23 levels remained unchanged 6 months after donation [8]. Conversely, Young et al., in a non-prospective study of 198 donors,

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found higher FGF23 and increased PTH levels five years after donation compared to control subjects [9]. Finally, whereas decreased expression of the transmembranous form of α-Klotho (hereafter named Klotho) is thought to be an early event in CKD [10,11], nothing is known about its adaptation in LKDs. Transmembranous Klotho expression may be assessed in the kidney in experimental models, but not in LKDs as it implies renal biopsies. Circulating soluble form of Klotho is measurable in patients [10], but it does not always correlate to kidney expression [12].

Hypothesizing that mechanisms of phosphate excretion might be different in LKDs than in patients with kidney disease, we have prospectively studied mineral parameters in LKDs and more specifically FGF23 and Klotho involvement in the tubular adaptation of phosphate reabsorption and modulation of the renal production of $1,25(OH)_2D$. We have further substantiated some of our results looking at the kidney expression of Klotho in a rat model of uninephrectomy.

Material, study population and methods

Patients

We conducted a prospective study in the Service of Nephrology at the University Hospital of Geneva (Switzerland) from 2010 to 2013. Patients were enrolled in 2010–12 and followed until 2013. All adult LKDs suitable for donation were offered to participate in the study after a written informed consent. Patients refusing to participate or unable to return for the late follow-up were not included. This study was approved by the ethical committee for human studies of Geneva and performed according to the Declaration of Helsinki.

Endpoints

Our primary endpoint was to analyze the changes in vitamin D ($25(OH)D$ and $1,25(OH)_2D$), PTH, FGF23, phosphate, calcium and Klotho levels between baseline and during the 6 and 12 months after donation (days 180 and 360, respectively).

Our secondary endpoint was to assess changes in renal function (creatinine and estimated GFR), and serum and urine bone markers from baseline and after donation.

Protocol and collected variables

Once they were eligible for donation, LKDs had their baseline 25(OH)D levels measured. Since vitamin D deficiency might alter PTH, FGF23 and other mineral metabolism parameters [13,14], we decided to supplement our patients to have a homogenous population and avoid this bias. A single dose of 300,000 units of native vitamin D (vitamin D3 Streuli®) was prescribed orally to patients with levels <70 nmol/l. A 24 hour ambulatory blood pressure measurement (ABPM) was performed at baseline and 1 year using an oscillometric and auscultatory devices validated by the British Hypertension Society (Diasys Integra II). Hypertension was defined as known treated hypertension or new office blood pressure ≥140/90 mm Hg confirmed by ABPM [15]. Estimation of phosphate intake was made before the intervention by a 3-day food diary where patients had to report their food and beverage intakes not only qualitatively but also quantitatively. The nutritional data were then entered in a validated computer program (PRODI 4.2 Plus: http://www.nutri-science.de/software/prodi.php) by a qualified dietician. Comorbidities were recorded.

The day before the intervention, blood was drawn and urine was collected for 24 h as well as spot urine after an overnight fast. This constituted the baseline values (day 0). At days 1, 2, 3, 180 and 360 after the nephrectomy, blood and spot urine were collected in fasting patients and constituted the follow-up values. The twenty-four hour urine collection was repeated at days 180 and 360.

Standard laboratory parameters were analyzed the same day on blood and urine samples. Samples were collected on plasma K + EDTA in fasting patients and always in the morning to avoid diurnal variations. These were immediately placed on ice and transported directly to the laboratory to be centrifuged 5 min at 3000 g. After centrifugation, samples were frozen down at −80° for storage. The maximum allowed delay between centrifugation and storage was 2 h. There was no freeze/thaw cycle.

Laboratory data

Clinical chemistry assays were performed by the University Hospital Clinical Laboratory on fresh blood samples. Plasma and urine creatinine were measured using the IDMS-traceable Jaffe kinetic compensated method (Roche Diagnostics, Switzerland, intra- and inter-batch CV: 2.9–0.7%). A quantitative immuno-nephelometry method was used to quantify albuminuria. Electrolytes were measured by indirect potentiometry (Unicel DxC 800 Synchron Clinical System). Corrected calcium was computed with the following formula: $CaCorr = total calcium \pm [0.02 * (40 - [albumin])]$. PTH was measured by immunodosage using Elecsys PTH (Roche Diagnostics, Switzerland) and Cobas 6000 (Roche Diagnostics, Switzerland), 25(OH)D was measured by immunodosage using Elecsys Vitamin D Total (Roche Diagnostics, Switzerland) and $1,25(OH)_2D$ was measured by ELISA using a 1.25-Dihydroxy Vitamin D EIA (ImmunoDiagnostic Systems Ltd., UK). FGF23 levels were measured as batched serum samples using the Kainos human intact FGF23 ELISA kit in humans and rats (maximum intra- and inter-CV: 2.–3.8%)[16]. Circulating Klotho levels were also batched and measured using an ELISA kit from IBL (Kyowa Hakko Kirin Co) (maximum intra- and inter-CV: 2.7–9.8%)[17]. Total P1NP and beta-crosslaps were measured using the Roche Elecsys P1NP and beta-crosslaps immuno-assays, respectively.

Calcium and phosphate tubular excretion

To assess calcium and phosphate tubular handling, we calculated for each time-point: fractional excretion of phosphate ($FePO^{4-}$), tubular reabsorption of phosphate ($TmPO^{4-}/eGFR$) and calcium ($TrCal/eGFR$), as well as calcium and phosphate over creatinine ratio (Ca/creat and PO^{4-}/creat). $FePO^{4-}$ was calculated as the product of urine phosphate and serum creatinine divided by the product of serum phosphate and urine creatinine. $TmPO_4^-/eGFR$ was computed using $FePO_4^-$ as previously described [18,19]: $TmPO^{4-}/eGFR = PO_4^- * (1 - FePO_4^-)$ if $FePO_4^- \geq 0.2$ or $TmPO^{4-}/eGFR = PO_4^- * e^{(10.318*(FePO4)2 - 5.18 * FePO4 + 0.4)}$ if $FePO_4^- < 0.2$. $TrCal/eGFR$ was computed as previously described [20,21]: $TrCal = Cacorr * (1.23 - 16. * FeCa + 426 * FeCa^2 - 6819 * FeCa^3 + 53,109 * FeCa^4 - 156,920 * FeCa^5)$ if $FeCa \leq 0.12$ or $TrCal = Cacorr * (0.99 - 1.921 * FeCa)$ if $FeCa > 0.12$. At days 0, 180 and 360, 24 hour phosphate and calcium urinary excretions were measured.

Renal function measurement

Renal function was measured using the 2 point chromium EDTA (3.7 MBq 51Cr-EDTA) clearance before donation and at day 360. The CKD-EPI equation was used to estimate renal function at baseline and during the long term follow-up only, as this formula has not been validated in the acute setting [22–24].

Nephrectomy

Nephrectomy was performed using a hand assisted retroperitoneoscopic technique. All patients received intravenous fluids during the procedure: ringer acetate, ringer lactate or isotonic saline 6 ml/kg/h. Further fluids were given during the procedure according to a goal-directed therapy. Postoperatively, patients were allowed to resume progressive fluid oral intake starting in the recovery room. Analgesics were prescribed as needed. Hydration with isotonic saline was maintained as

Table 1
Donors' clinical characteristics (n = 27).

Variables	Results
Age	54 ± 11
Male sex (%)	15 (56.6)
Smoking (%)	7 (25.9)
Hypertension (%)	5 (18.5)
Body Mass Index kg/m^2	24.9 ± 3.7
^{ct}EDTA ml/min/1.73 m^2	98.7 ± 17.4
ABPM mean SBP mm Hg	114.9 ± 13.1
ABPM mean DBP mm Hg	75.6 ± 7.4
25(OH)D at inclusion nmol/l (optimal range; ≥75 nmol/l)	54.4 ± 21.9
Daily dietary phosphate intake mg	1179 (883–1423)

^{ct}EDTA: renal clearance measured by EDTA radio-isotope. ABPM: 24 hour Ambulatory Blood Pressure Measurement. SBP: Systolic Blood Pressure. DBP: Diastolic Blood Pressure. 25(OH)D: 25 hydroxy-vitamin D.
Categorical variables are expressed as numbers and percentages (%) and continuous variables as mean ± standard deviation except phosphate expressed as median and 25th–75th percentiles.

long as the patient was unable to eat and drink sufficiently. Light eating was started the afternoon of post-operative day 1. Most patients left the hospital on post-operative day 4.

Statistics

Statistical analyses were conducted using STATA 12.0 (StataCorp, College Station, Texas, USA). Quantitative variables were expressed as mean ± standard deviation, or median and interquartile range as appropriate. Categorical variables are expressed as numbers and percentages. Mixed regression analyses taking into account random effect and the time were used to compare continuous variables between baseline and follow-up, analyzing separately short term (days 1, 2 and 3) and long term (days 180 and 360) follow-ups. We used Pearson's correlations to test associations between changes in FGF23, Klotho, PTH, and 1,25(OH)$_2$D and changes in serum or urine calcium and phosphate. Levels' changes are reported as median (interquartile range) and the correlations are expressed using correlation coefficient r. Mixed regression analyses, taking into account the day effect, were also conducted to look for longitudinal association between FGF, Klotho, and PTH; results are expressed using beta coefficient β and 95% confidence intervals (95%CI). A two-side p value <0.05 was considered significant.

Rat experiments

Uninephrectomy was performed as previously described [25]. Briefly, the ureter and vessels were exposed via a retroperitoneal incision. The renal artery and vein were ligated and then sectioned, the ureter was sectioned and the kidney was removed. The retroperitoneal wall and skin were closed, the skin was disinfected, and the animals were allowed to recover on a heating pad. In Sham rats, the retroperitoneal wall was opened by incision and the kidney was isolated. After 20 min, the retroperitoneal wall and skin were closed, the skin was disinfected, and the animals were allowed to recover on a heating pad. After 3 and 5, or 21 days, a total of 24 rats were sacrificed and their kidney was harvested (6, 6 and 12 respectively at the different time points).

Each kidney was dissected in three zones and protein samples from the cortex were prepared and processed for western blotting as previously described [26]. Anti-Human Klotho Monoclonal Antibody (Clone No. KM2076) from TransGenic Inc. was used for western blotting.

Results

From 38 eligible LKDs contacted between 2010 and 2012, 27 were included in the present analysis. One donor refused to participate, 9 were living abroad or donated in another city and were unable to come back for the later follow-up. Finally, 1 patient was secondarily excluded as she was diagnosed with and treated for primary hyperparathyroidism during the study. Three participants were lost to long term follow-up. Baseline clinical characteristics of the donors are presented in Table 1. Four of them had hypertension but none had diabetes. One was Hispanic and 26 were Caucasians. Baseline 25(OH)D levels before substitution and estimated phosphate intake are also shown in Table 1. The numbers of patients with 25(OH)D severe deficiency (<20 nmol/l), deficiency (<50 nmol/l) and insufficiency (50–75 nmol/l) were 2, 10 and 8 respectively at inclusion. Twenty patients were prescribed vitamin D supplementation. At the time of nephrectomy no patients had severe 25(OH)D deficiency, whereas 5 were still deficient and 9 were insufficient.

Intra-venous fluids were administered during all interventions but stopped in most patients after 24 h. The total amounts of i.v. fluid, blood pressure and body weight at baseline and after the procedure are described in Table 2. The evolution of the blood and urine parameters during the follow-up is detailed in Table 3.

Early follow-up after nephrectomy in LKDs (day 1 to day 3)

Uninephrectomy induced hypocalcemia, transient secondary hyperparathyroidism and decreased Klotho and FGF23 levels (Table 3 and Fig. 1A).

At day 1 after the nephrectomy, LKDs presented decreased total plasma calcium levels and hypoalbuminemia. We observed a median rise in PTH levels of 1.5 (0.4–2.5) pmol/l and a transient decrease of calcium excretion as assessed by the urinary calcium to creatinine ratio (Table 3). Corrected calcium decrease at day 1 correlated to calcium tubular reabsorption ($r = 0.49$; $p = 0.025$). Phosphatemia decreased significantly over the three days and relative phosphate excretion increased. The decrease in renal phosphate reabsorption and plasma phosphate persisted at day 3, when PTH had returned to baseline. As expected, phosphatemia changes were highly associated to tubular reabsorption (at day 3, $r = 0.82$; $p < 0.001$). PTH changes were negatively associated to calcemia ($r = -0.53$; $p = 0.009$) and calcium tubular reabsorption changes at day 1 ($r = -0.46$; $p = 0.026$).

After donation we observed a significant and immediate decrease in circulating Klotho and FGF23 levels that peaked at day 3. The median decreases from baseline values were 219.0 (110.4–274.2) pg/ml and 23.3 (4.0–37.7) pg/mol/l respectively (Fig. 1A). FGF23 decrease correlated to serum phosphate decrease at day 3 ($r = 0.67$; $p = 0.005$).

Using mixed regression analyses to adjust for the day effect, plasma phosphate level remained a determinant of FGF23 levels ($\beta = 21.2$

Table 2
Clinical parameters and hydration during the peri-operative period and following days.

Variables	Day 0	Day 1	Day 2	Day 3	P days 0 to 3
Hydration ml	3870 (2000–6000)	1000 (0–2000)	0 (0–2500)	0 (0–1000)	<0.001
Body weight kg	70.5 ± 11.8	NA	72.5 ± 11.9	71.0 ± 11.4	<0.001
Systolic blood pressure mm Hg	123.6 ± 16.4	115.6 ± 13.9	120.2 ± 14.8	118.2 ± 21.7	0.19
Diastolic blood pressure mm Hg	75.1 ± 9.8	69.4 ± 14.0	71.9 ± 10.0	74.4 ± 11.8	0.08
Heart rate per min	69.1 ± 8.9	70.5 ± 12.3	70.0 ± 8.6	69.4 ± 10.0	0.86

Variables are expressed as mean ± standard deviation or median (minimal–maximal range). NA: not available.

Table 3
Biological parameters before and after the nephrectomy in living kidney donors (n = 27).

Variables [Normal range]	Day 0	Day 1	Day 2	Day 3	P model [days 0 to 3]	Day 180	Day 360	P model [days 0 to 360]
Blood								
Ca mmol/l [2.3–2.52]	2.28 ± 0.08	2.07 ± 0.09***	2.11 ± 0.11***	2.15 ± 0.07***	<0.001	2.31 ± 0.06	2.31 ± 0.9	0.18
Cacorr mmol/l	2.34 ± 0.09	2.31 ± 0.08*	2.34 ± 0.11	2.39 ± 0.05**	<0.001	2.34 ± 0.06	2.35 ± 0.08	0.77
PO_4^- mmol/l [0.8–1.5]	1.27 ± 0.20	1.24 ± 0.20	0.91 ± 0.15***	0.93 ± 0.19***	<0.001	1.04 ± 0.16***	1.05 ± 0.18***	<0.001
PTH pmol/l [1.1–6.8]	4.3 (3.6–5.4)	5.9 (4.8–6.7)***	4.1 (3.7–4.8)	4.5 (3.5–5.5)	<0.001	6.1 (5.1–8.2)***	5.8 (4.9–7.5)***	<0.001
FGF23 pg/ml	48.1 (37.4–60.0)	36.3 (25.4–40.9)***	30.6 (22.2–37.5)***	26.9 (22.1–38.0)***	<0.001	49.8 (38.6–63.6)	45.2 (37.7–56.4)	0.86
Klotho pg/ml	526.2 (482.4–615.2)	418.1 (340.3–549.1)***	341.9 (293.4–432.9)***	304.4 (265.6–491.3)***	<0.001	478.4 (392.8–588.0)***	439.6 (398.3–613.0)**	<0.001
25(OH)D nmol/l	72 (55–93)	–	–	–		74 (66–101)	81 (52.5–108.5)	0.61
$1,25(OH)_2D$ pmol/l [49–140]	125 (105–163)	–	–	–		103 (76–121)	82 (67–96)***	<0.001
PALb µg/l [3.5–22.6]	9.7 (9.1–12.5)	–	–	–		10.8 (9.1–12.6)*	10.1 (8.3–11.2)	0.09
P1NP µg/l [14.3–76.3]	38.9 (32.1–54.9)	–	–	–		45.1 (32.1–58.3)	38.2 (29.6–53.6)	0.54
BxL ng/l [104–782]	494 (359–628)	–	–	–		420 (293–699)	347 (251–531)**	0.006
Alb g/l [35–48]	37.4 ± 2.7	28.7 ± 3.0***	28.5 ± 3.4***	28.6 ± 3.8***	<0.001	38.2 ± 2.5	38.2 ± 2.6	0.29
Creat µmol/l	69.2 ± 14.8	116.5 ± 26.7***	123.8 ± 31.3***	117.5 ± 25.3***	<0.001	102.5 ± 24.3***	103.2 ± 24.7***	<0.001
CKD-EPI ml/min/1.73 m^2	95.3 ± 11.2	–	–	–		62.6 ± 13.1***	62.9 ± 12.9***	<0.001
Urine								
$FePO_4^-$	0.12 ± 0.04	0.22 ± 0.07***	0.21 ± 0.10***	0.13 ± 0.08	<0.001	0.16 ± 0.06**	0.19 ± 0.08***	<0.001
TmPO$_4^-$/eGFR [0.8–1.35]	1.24 ± 0.31	0.96 ± 0.16***	0.79 ± 0.23***	0.91 ± 0.28***	<0.001	0.93 ± 0.20***	0.86 ± 0.27***	<0.001
PO_4^-/creat	2.23 ± 0.59	2.36 ± 0.77	1.72 ± 0.46**	1.02 ± 0.57***	<0.001	1.66 ± 0.52***	1.99 ± 0.67	<0.001
Ca/creat	0.38 ± 0.20	0.14 ± 0.10***	0.33 ± 0.24	0.41 ± 0.27	<0.001	0.16 ± 0.10***	0.12 ± 0.3***	<0.001
TrCal/eGFR [2.4–2.9]	2.62 ± 0.16	2.69 ± 0.18	2.57 ± 0.18	2.56 ± 0.16	0.02	2.69 ± 0.15	2.83 ± 0.58*	0.09
24 hPO$_4^-$U mmol [13–42]	26.4 ± 9.7	–	–	–		24.6 ± 9.8	26.9 ± 9.4	0.66
24 hCaU mmol [2.5–7.5]	5.3 ± 2.4	–	–	–		2.3 ± 1.0***	2.73 ± 1.40***	<0.001
24 h albuminuria mg	5 (0.001–8)	–	–	–	–	6 (0.01–17)	7.8 (4–15)	0.32

Ca: calcium. Ca corr: calcium corrected. PO_4^-: phosphate. 25(OH)D: 25 hydroxy-vitamin D. $1,25(OH)_2D$: 1,25 di-hydroxy-vitamin D. PALb: bone alkaline phosphatase. P1NP: propeptide type 1 N-terminal pro-collagen. BxL: betacrosslaps. Alb: Albumin. Creat: creatinine. $FePO_4^-$: phosphate excretion fraction. TmPO$_4$/GFR: tubular maximum phosphate reabsorption per glomerular filtration rate. PO_4^-/creat: urinary phosphate over creatinine ratio. Ca/creat: urinary calcium over creatinine ratio. TrCal: tubular reabsorption of calcium. 24 hPO$_4^-$U: 24 hour phosphaturia. 24 hCa^{2+}U: 24 hour calciuria. Variables are expressed as mean ± standard deviation or median (25th–75th percentile range).
*p < 0.04, **p < 0.01, ***p < 0.001 compared to day 0.

[95%CI: 7.7–34.6], p = 0.002), independently of PTH or renal function. Phosphate levels remained highly associated with tubular reabsorption even when adjusted for renal function, PTH or FGF23 (β = 0.51 [95%CI: 0.41–0.62]; p < 0.001]. Klotho and PTH were not found to be associated with plasma or urine phosphate and calcium.

Late follow-up (days 180 and 360): LKDs had reduced 1,25(OH)$_2$D levels, an elevation of PTH and decreased 24 hour urinary calcium excretion without significant changes in FGF23 levels (Table 3 and Fig. 1B)

At days 180 and 360, all patients were healthy. No patient reported a fracture or cardiovascular disease and dietary habits were anamnestically unchanged. None developed hypertension and the mean systolic and diastolic 24 h ABPMs measured at 1 year were similar to baseline: 113.5 ± 11.9 mm Hg (p = 0.8) and 75.1 ± 6.3 mm Hg (p = 0.7), respectively. EDTA measured GFR decreased to 67.1.0 ± 7.0 ml/min/1.73 m^2 (p < 0.001).

Biologically, plasma calcium and albumin levels had normalized by days 180 and 360 (Table 3). Although there was no significant change in 25(OH)D levels, most patients had a significant decline in 1,25(OH)$_2$D levels with a median decrease from baseline to 1 year of 52 (22–68) pmol/l. PTH secretion was clearly stimulated with an increase of 1.4 (0.8–2.5) pmol/l at 1 year. Furthermore, there was a persistent marked decrease in 24 hour urinary calcium excretion when compared with baseline. Plasma phosphate levels were decreased in line with enhanced urinary excretion, as demonstrated by the positive correlation with tubular reabsorption (at 6 months: r = 0.64; p = 0.001). We found no significant correlations between changes in PTH, vitamin D and calcium or phosphate levels.

Circulating Klotho remained lower than before donation at 180 and 360 days although levels were clearly higher than immediately after donation. There was no significant change in FGF23 levels when compared to baseline even though the median increased by 3.2

(−10.5–10.2) pg/ml at 1 year. FGF23 changes correlated positively with changes in plasma phosphate (r = 0.51, p = 0.014) and in 25(OH)D (r = 0.49; p = 0.028) at 1 year only. FGF23 changes did not correlate to changes in mGFR by EDTA clearance.

Using mixed regression analyses, FGF23 was no longer associated with phosphate levels when adjusted for renal function in the long term follow-up. However 25 (OH)D levels were still determinants of FGF23 levels (β = 0.07 [95%CI:0.01–0.12]; p = 0.032). Phosphate levels remained highly associated with tubular reabsorption even when adjusted for renal function, PTH or FGF23 (β = 0.45 [95%CI: 0.34–0.56]; p < 0.001).

At 1 year, markers of bone formation (PALB and P1NP) did not show significant changes compared with baseline, although there was a trend to increased PALB at 6 months. On the contrary betacrosslaps (BxL), a marker of bone resorption, had significantly decreased.

Experimental uninephrectomy in rats: uninephrectomy in rats increases Klotho expression in the remnant kidney after three weeks (Fig. 2)

Kidney transmembranous Klotho expression may be more physiologically relevant than circulating levels for FGF23 activity. This parameter can however not be easily assessed after kidney donation in LKDs. We therefore investigated kidney Klotho expression over time in a rat model of uninephrectomy. Indeed, since circulating Klotho decreased abruptly post-donation and tended to increase thereafter in LKDs, we hypothesized that kidney tissue α-Klotho expression was modified by nephrectomy over time in the remnant kidney. In rats, we observed that Klotho expression in the remnant kidney was unchanged at days 3 and 5 following uninephrectomy compared to Sham controls (n = 3 Sham and 3 uninephrectomized respectively at day 3 and at day 5) (Fig. 2A). Furthermore, at three weeks after nephrectomy Klotho expression increased locally in the remnant kidney (n = 6 sham and 6 uninephrectomized) (Fig. 2B). Finally, at three weeks, circulating
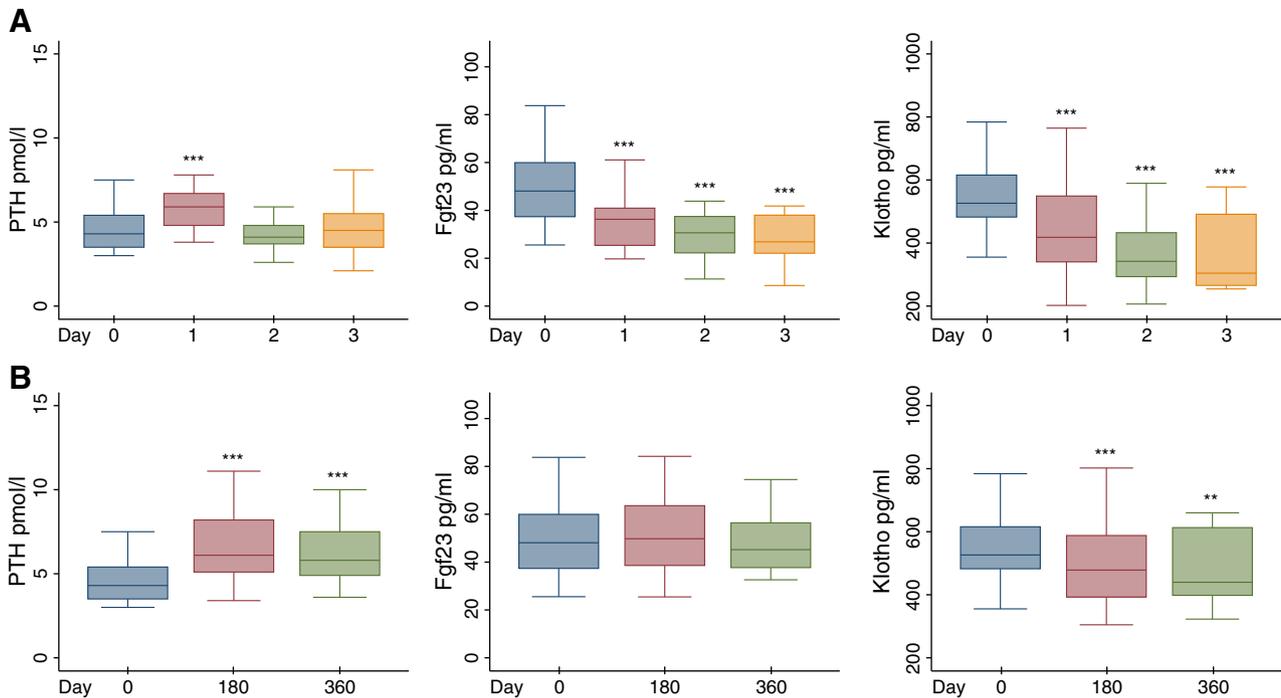
Fig. 1. Box plots showing the evolution of serum levels of PTH, FGF23 and Klotho circulating levels (A.) Short term (days 0 to 3) (B.) Long term (days 0 to 360). *** Compared to day 0: $p < 0.001$, **$p = 0.002$.

FGF23 levels were unchanged in uninephrectomized compare with sham rats (385 ± 35 versus 418 ± 34 pg/ml, n = 6 and 6 p = 0.56).

Discussion

We analyze the mineral metabolism of 27 LKDs during 3 consecutive days after the intervention and at 6 and 12 months. During postoperative days, we observe an abrupt decrease in serum calcium levels associated with a transient increase in PTH levels. Urinary phosphate reabsorption is lowered despite a decrease in FGF23 and circulating Klotho levels. Six and twelve months after nephrectomy 1,25(OH)$_2$D decreases, likely inducing an elevation of PTH levels. PTH probably participates in enhancing phosphate excretion, inducing a decrease in plasma phosphate levels although we did not find significant correlations between PTH and phosphate changes in our sample. Surprisingly, FGF23 levels decrease significantly in the acute phase but are unchanged relative to baseline at six and twelve months. Circulating Klotho levels also decrease in the acute phase and remain lower than

baseline later, although levels increase compared with acutely after nephrectomy. In the experimental uninephrectomy model, FGF23 circulating levels do not change whereas kidney Klotho expression increases in the remnant kidney after three weeks.

In our study, some of the changes in mineral metabolism observed acutely after kidney donation are likely related to acute loss of renal function and mass, and to factors related to the operation such as hydration and fasting. Post-operative hypocalcaemia (both total and ionized) and secondary increase in PTH are well described transient phenomenons in abdominal surgeries, and are not specific to uninephrectomy [27]. All of the patients in this study have developed a rapid decrease in total plasma calcium. The hypocalcemia then stimulates PTH secretion, allowing for progressive correction of calcium levels. We therefore attribute the observed transient elevation of PTH and decrease in calcium mainly to hydration, fasting and surgery. During the same time, phosphate levels progressively decrease after nephrectomy while urinary phosphate excretion increases. Decreased phosphatemia can be attributed to decreased intake and hydration, as well as increased urinary
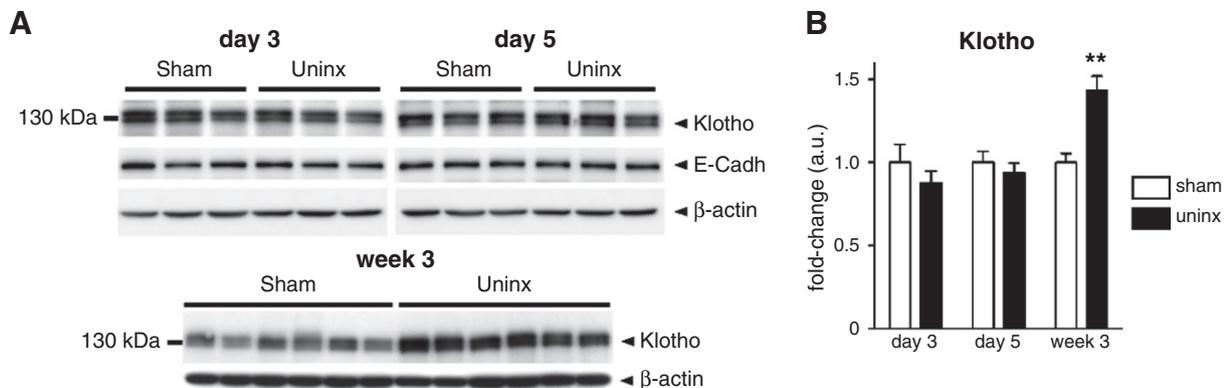

Fig. 2. Representative western blotting (A) and quantification (B) of cortex protein expression of α-Klotho in rat's kidney 3 and 5 days, or three weeks after nephrectomy (Unix) or Sham operation (Sham). E cadherin and Beta Actin are shown as loading controls.

excretion. We demonstrate that the enhanced phosphaturia, as assessed by decreased TmPO$_4^-$/eGFR, persists for three days after the nephrectomy despite normal PTH and low FGF23, and that change in phosphate levels is highly correlated to tubular reabsorption. This observation is most likely in relation to kidney donation itself, as already described after experimental nephrectomy in the rat [28]. The acutely increased phosphaturia has been described to be independent of PTH and sodium load experimentally [28] and, since FGF23 decreases in our observation, is likely also to be independent of this hormone. The positive association between phosphate levels and TmPO$_4^-$/eGFR (even when adjusting for PTH or FGF23) is consistent with this hypothesis. Circulating Klotho levels abruptly decrease by almost half which is consistent with the decreased renal mass. FGF23 levels also rapidly decrease after nephrectomy which is less expected as an increase has been observed when the renal function declines [29]. Known regulators of FGF23 secretion are phosphate, PTH, 1,25(OH)$_2$D [11] and calcium levels [30]. In our setting, FGF23 probably decreases in response to decreased serum phosphate related to decreased intake [31] and increased urinary losses. This is supported by the observation that phosphate levels are positively associated with FGF23 levels.

At six and twelve months, the observed changes in mineral metabolism in our study are probably related to renal mass loss only. LKDs have lost approximately one third of their kidney function and most of them have an eGFR between 60 and 90 ml/min/1.73 m^2. An adaptation of mineral parameters is observed in LKDs: 1,25(OH)$_2$D plasmatic levels are lower, PTH is higher, and serum phosphate, TmPO$_4^-$ and urinary Ca/creat are less than pre-donation levels. Circulating Klotho is lower than before donation, but higher than after nephrectomy, while FGF23 levels are unchanged despite the reduced kidney function. Our interpretation is that the loss of 50% of kidney mass results in an increased single nephron phosphate clearance and decreased 25(OH)D hydroxylation. This induces a decrease in intestinal calcium absorption [32], confirmed by reduced 24 hour urinary calcium excretion [33]. Decreased 1,25(OH)$_2$D likely induces a secondary rise in PTH levels [32]. Enhanced fractional excretion of phosphate may be attributed in part to PTH elevation, although we cannot exclude a direct tubular adaptation, or the intervention of another phosphaturic factor. Enhanced phosphate excretion in the remnant kidney results in decreased plasma phosphate levels.

The absence of a significant elevation in FGF23 and the decreased phosphate levels at 6 and 12 months, whereas eGFR is reduced and PTH and phosphaturia have risen, contrast with what has been described in CKD patients [5,34]. Although these findings may also contrast with the observation by Young et al. [9], the elevation of FGF23 in donors compared to controls at a later time point (five years) observed in this study was very modest (38.1 versus 29.7 pg/ml). We can therefore not exclude that in a larger cohort of patients, we would observe a small difference of FGF23. However given the interindividual variation of FGF23, comparison to controls may be misleading even if perfectly matched, which was not the case in Young's paper. Our observation suggests that a rise in FGF23 may not be necessary for 1α-hydroxylase inhibition during the isolated nephron reduction of LKDs, as opposed to what has been suggested in a model of CKD in rats [5]. Interestingly, in CKD patients, 1,25(OH)$_2$D also decreases before the rise of FGF23, suggesting that 1,25 hydroxylation may be regulated by factors other than FGF23 [34]. Furthermore our observations suggest that an increase in FGF23 level is not needed for the enhanced phosphate excretion of LKDs. Finally, in contrast to what is known in CKD patients [35], the decreased nephron number in LKDs results in decreased plasma phosphate levels at six and twelve months. This difference is probably due to non-damaged residual nephrons in the remnant kidney of LKDs, in opposition to CKD patients, allowing optimal adaptive mechanisms.

In LKDs, decreased circulating Klotho levels after six months probably do not reflect transmembranous Klotho expression in the remnant kidney. Indeed Klotho levels abruptly decrease after nephrectomy and

progressively rise afterwards in LKDs. This suggests that some compensation mechanisms may exist. In line with this, we have observed an increase of Klotho expression in the remnant kidney of uninephrectomized rats. Although we cannot be certain that the same phenomenon occurs in LKDs, this suggests that kidney hypertrophy occurring both in experimental and human nephrectomy may be associated with increased Klotho expression [36,37]. This contrasts with the early decrease in Klotho expression previously described in various types of experimental and human CKDs [10,38,39]. The stability and increase of intrarenal Klotho, an obligatory FGF23 co-factor, may alleviate the need for FGF23 upregulation after nephrectomy and maintain phosphate excretion. Intrarenal α-Klotho expression is indeed more relevant than circulating levels in the regulation of FGF23 [12,40]. These major differences in Klotho expression and FGF23 regulation in LKDs compared to CKD patients demonstrate that mineral metabolism adaptation is different in patients with normal remnant nephrons compared with diseased nephrons.

Finally, bone remodeling markers indicated a tendency to bone formation in line with increased PTH and decreased Ca/Creat at six months in LKDs. The decrease in beta-crosslaps suggests a decrease in bone resorption over time but we have no clear explanation for this. It would be interesting to follow these markers over time and perform serial bone assessments in LKDs as, although fracture rates are not known to be increased [41], milder changes in bone structure may occur.

Our study is limited by the small size of the population and our results may not be generalized to non-Caucasian donors. The lack of measurement of 1,25(OH)$_2$D and 24 hour urine electrolytes in the days following surgery may have slightly modified our interpretation. Given the discrepancy of total versus corrected calcemia, obtaining ionized calcium would have been interesting although it has been demonstrated that both total and ionized calcium decrease after surgery [27]. Furthermore, ionized calcium measurement presents pre-analytical pitfalls that preclude its routine utilization in our institution [42]. Finally, due to the lack of a control group undergoing other kinds of surgery, we cannot exclude that part of altered acute mineral metabolism (days 1–3) is not only due to the nephron loss but also due to additional surgical factors, such as hydration. However such control group matching LKDs' condition is difficult to find not only in the acute setting but also in the chronic observational phase, as donors are a much healthier population than the general population. In any case, our findings in the long term follow-up cannot be due to perioperative considerations. The strength of our study is that we have measured the main co-factors of phosphate handling at repeated time points with consistent results at 6 and 12 months in a very homogenous population. We have also provided experimental data to support our findings. Although changes may be considered as small in terms of number, the physiological effects studied are important for our understanding of adaptation of mineral metabolism to nephron loss and may have long term implications. The specific adaptation of FGF23 and Klotho expression in LKDs has never been showed previously but further studies are needed to understand whether the preserved handling mechanisms in LKDs may help to explain their better cardiovascular outcome when compared with CKD patients that have a similar decline in kidney function.

Conclusion

We demonstrate in this study that mineral metabolism parameters are modified in LKDs, both acutely and at six and twelve months with different regulatory mechanisms. These modifications are characterized in the long-term by decreased phosphate levels and unchanged FGF23 levels. These differences may be related to the fact that remnant nephrons in LKDs are intact, in contrast to most types of CKDs and that kidney Klotho expression does not decrease after nephrectomy in contrast to experimental CKD. FGF23 elevation is therefore not a primary event in nephron loss induced by uninephrectomy, and 1,25(OH)$_2$D and fractional excretion of phosphate are regulated independently of increased

FGF23 in nephron loss without structural lesions. The present study suggests that mineral metabolism adaptation mechanisms differ in LKDs compared with those described in CKD patients. This differential adaptation reinforces the idea that LKDs should not be classified as CKD patients.

Disclosures

Acknowledgments

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