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ORIGINAL ARTICLE

Living kidney donation does not adversely affect serum calcification propensity and markers of vascular stiffness

Sophie de Seigneux,[1,*] Belen Ponte,[1,*] Lena Berchtold,[1] Karine Hadaya,[1] Pierre-Yves Martin[1] and Andreas Pasch[2]

1 Service of Nephrology, University Hospital of Geneva, Geneva, Switzerland
2 Department of Clinical Chemistry, University Hospital and University of Bern, Bern, Switzerland

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Correspondence
Sophie de Seigneux, Service of Nephrology, Department of Medicine Specialties, University Hospital of Geneva, 4 rue Gabrielle Perret Gentil, 1211 Genève 4, Switzerland.
Tel.: +41223729762;
fax: +41223729769;
e-mail: sophie.deseigneux@hcuge.ch

*These authors contributed equally to this work.

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Summary

Living kidney donors (LKDs) experience a decline in glomerular filtration rate (GFR) after donation. Calcification propensity (T_{50}) can be determined by a blood test predicting all-cause mortality in patients with chronic kidney disease. We studied the impact of kidney donation on T_{50} and markers of arterial stiffness. We analyzed T_{50} prospectively before and 1 year after kidney donation in 21 LKDs along with fetuin-A, mineral metabolism markers, kidney length, pulse wave velocity (PWV), augmentation index (AI), and renal resistive index (RRI) as markers of arterial stiffness. We studied the impact of kidney donation on these parameters. LKDs were 54 ± 10 years old and had a GFR of 101 ± 18 ml/min/1.73 m^2 before donation, decreasing to 67 ± 8 ml/min/1.73 m^2 after donation ($P < 0.001$). Despite this, T_{50} improved after donation (290 ± 53 to 312 ± 38 min, $P = 0.049$). This change was inversely related to plasma phosphate ($P = 0.03$), which declined after donation ($P = 0.002$). Fetuin-A levels increased after donation ($P = 0.01$). Upon donation, the length of the remaining kidney increased ($P < 0.001$) while PWV, AI, and RRI remained unchanged. Calcification propensity was not adversely affected by kidney donation. This indicates that T_{50} is independent from GFR in LKDs and that kidney donation does neither worsen calcification propensity nor markers of vascular stiffness at 1 year.

Introduction

Living donation has become an important source of kidneys for transplantation. Due to organ shortage, the number of living kidney donation is increasing worldwide. In 2012, according to the global observatory on donation and transplantation (http://www.transplant-observatory.org), living donation represented 42.3% of 77 818 transplanted kidneys. An improved understanding of the complications potentially associated with donation is crucial, as this technique is currently widely promoted to decrease waiting list time in renal transplantation.

Kidney donation is currently considered as a safe procedure. However, the main problem of assessing the fate of living kidney donors (LKDs) is to find an adequate and comparable control group of non-LKDs. Recent reports indicate that kidney donors may have, in the long term, a slightly increased risk of end-stage renal disease and mortality compared to a matched population [1,2], whereas their overall prognosis and mortality are better or equal to those of the general population [3]. Although a decrease in glomerular filtration rate (GFR) is associated with increased mortality in epidemiological studies on patients with CKD, the question of cardiovascular risk is still a matter of debate in LKDs [4,5]. While in a large earlier study, cardiovascular mortality was not increased after donation [6]; in a recent study, global mortality and cardiovascular death appeared to be higher in LKDs compared to potential kidney donors [1].

It has recently been demonstrated that the changes observed in mineral metabolism in LKDs 1 year after dona-

tion are different from those observed in patients with CKD with comparable GFR [7,8]. Indeed, while PTH increased, serum phosphate decreased and FGF23 remained unchanged in LKDs. FGF23 is a circulating, osteocyte-derived phosphatonin acting on the proximal tubule with its cofactor klotho. Modification of FGF23 production is an early marker of altered mineral metabolism. Of note, FGF23 and phosphate levels are predictive of mortality risk [9,10].

Lately, a novel nanoparticle-based test was developed to measure overall calcification propensity (T_{50}) in the serum of patients [11]. Major determinants of this test include serum phosphate, calcium, magnesium, albumin, bone osteoclastic activity, pyrophosphate, and fetuin-A levels (a liver-derived regulator of extracellular matrix mineralization). When determined in stage 3 and 4 patients with CKD, high calcification propensity (i.e. a low T_{50} value) was closely associated with progressive aortic stiffening and increased all-cause mortality in a long-term follow-up [12]. When T_{50} was considered as a continuous variable, each 1 SD reduction (95 min) was associated with a 39% (95% CI, 10–87%) increase in the risk of all-cause mortality independent of demographic, renal, phosphate, and other baseline determinants. According to these findings, the T_{50} test may be performed serially to evaluate the evolution of calcification propensity in a given patient. There are to our knowledge no available data on the evolution of calcification propensity after kidney donation in LKDs.

Here, we investigated the impact of kidney donation on the evolution of calcification propensity 1 year after donation and on indirect markers of arterial stiffness, such as fetuin-A, pulse wave velocity (PWV), and renal resistive index (RRI).

Patients and methods

Patients

From 2010 to 2012, we enrolled, at the University Hospital of Geneva, 28 LKDs in a prospective study on mineral metabolism adaptation with 6 months and 1-year follow-up [8]. All adults suitable for donation, willing to participate and to return for the follow-ups, were included in the study. All of them signed an informed consent. The study was approved by the local ethical committee for human studies of the canton of Geneva and performed according to the Declaration of Helsinki principles.

End points

Our primary end points in this study were to analyze the change after kidney donation in T_{50}, RRI, PWV, and augmentation index (AI).

Our secondary outcome was to correlate T_{50} with parameters related to arterial stiffness such as RRI, PWV, and AI but also mineral metabolism (calcium, phosphate, PTH, FGF23, fetuin-A, Magnesium, circulating Klotho) and renal function.

Collected variables and laboratory data

The data and laboratory collection, including circulating Klotho and FGF23, has been detailed previously [8]. Briefly, in the morning before (baseline) and 1 year after the nephrectomy, fasting blood was drawn and urine collected. Standard laboratory parameters were analyzed in fresh blood and urine on the same day locally by the University Hospital Clinical Laboratory. Additional plasma, serum, and urine samples were taken, centrifuged, and stored at −80 °C for further analyses.

Glomerular filtration rate was measured using the CKD-EPI equation and EDTA clearance before and after donation. Patients had 24 h blood pressure (BP) measurement using a validated device to ensure that hypertension was controlled before and 1 year after donation. Hypertension was defined as known treated hypertension or new office BP ≥140/90 mmHg confirmed by 24 h ambulatory BP measurement [13].

T_{50} measurement

T_{50} was measured from batched serum samples. The methodology has been described previously [11,12]. In short, in this study, serum samples were measured in triplicate in 384-well plates at 37 °C over 600 min in a Nephelostar® nephelometer (BMG Labtech, Ortenberg, Germany) in a blinded manner. For the measurements, calcium solution was mixed with serum, and then the phosphate solution was added. Data analyses of nonlinear regression curves were performed using Microsoft Excel software to determine the half-maximal precipitation time (T_{50}). The analytical coefficients of variation of standards precipitating at 120, 260, and 390 min were 7.8%, 5.1%, and 5.9%, respectively.

Fetuin-A

Serum fetuin-A was measured by an in-house developed precipitation assay using polyclonal rabbit anti-human fetuin-A antibody. In short, serum fetuin-A was measured as described previously [14] with a nephelometric assay. Particles agglutinated to increase the intensity of scattered light proportionally to the amount of fetuin-A in the sample and were compared to standardized curves.

PWV and AI

Arterial stiffness was assessed by PWV and AI, according to a previously reported procedure [15]. Briefly, patients were

instructed not to smoke or drink coffee before the measure and all measurements were performed in the morning. BP was measured directly before the recording and used to calibrate the PWV. The same experienced nephrologist assessed the arterial waveform at the right radial, carotid, and femoral artery by applanation tonometry, after 15 min of rest in supine position. We used a high-fidelity SPC-301 micromanometer (Millar Instruments, Inc., Houston, TX, USA), interfaced with a laptop computer running the SPHYGMOCOR software (AtCor Medical Pty. Ltd., West Ryde, NSW, Australia). The carotid-to-femoral and carotid-to-radial distances were obtained with a ruler using indirect measurement. The PWV was recorded from the carotid-to-femoral measurement. To obtain the AI, the transfer function was used from the radial signal.

RRI

The renal ultrasound was performed right after the PWV measurements. First, the maximal longitudinal length of each kidney was measured in a sagittal plane using gray scale B-mode ultrasound (Aplio XG device from Toshiba Medical Systems, Volketswil, Switzerland). The assessment of the intrarenal vessels was then made by duplex Doppler sonography to obtain the RRI. The technique and reproducibility of these methods have been described in details elsewhere [16,17]. Briefly, RRI was measured on three segmental arteries (superior, middle, and inferior) in each kidney. The values were then averaged to obtain one mean value for each kidney in all LKDs. After donation, kidney length and RRI of the remaining kidney were measured in the same conditions.

For all patients, all the measurements including PWV, AI, and RRI were performed under the same medications, by the same experienced nephrologist (BP), in the morning at baseline and at 1 year.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation or median and interquartile range according to the distribution. Categorical variables are expressed as numbers and percentages. Normality was tested using the Shapiro–Francia test for normal data. Although most of the data were normally distributed according to this test, we preferred to use nonparametric tests for comparisons in order not to overestimate the changes or associations and be more conservative. We report then *P* values from the Wilcoxon signed-rank test, used for paired comparisons between baseline and 1-year measures, instead of paired *t*-test. For correlation analyses between calcification propensity and other parameters, we performed Pearson's tests. We report *R* coefficients and *P* values after controlling the linear associations with scatter plots. We also compared changes using three categories (no change, decrease, and increase) in calcification propensity and other variables of interest using chi-square test. Due to the small numbers of patients, we did not conduct multivariate analyses. Statistical analyses were performed using STATA 13.0 (StataCorp, College Station, TX, USA).

Results

From 28 patients included, seven patients had missing serum samples either at baseline or at 1 year after donation and were excluded, leaving 21 patients for the present T_{50} analysis. The results from mineral metabolism adaptation have been detailed elsewhere [8]. Here, we present only the results obtained from patients in whom calcification propensity could be measured.

Baseline parameters are shown in Table 1. Donors had a preserved renal function and normal baseline electrolytes before donation. RRI is reported at baseline in the kidney that remained in the donor. There was no difference between the left and right RRI measurement before donation ($P = 0.34$). Left and right kidney length was also similar before donation: 10.7 ± 0.9 and 10.7 ± 0.7 cm, respectively ($P = 0.8$). In the majority of LKDs ($n = 18$, 85.7%), the left kidney was donated.

After 1 year, none of the donors had developed a cardiovascular complication. The five LKDs with previous hypertension were controlled by their usual medication (four on AT2 receptor blockers, one on a calcium channel blocker) and none had developed new hypertension or changed his antihypertensive medications. BMI did not increase significantly after 1 year ($P = 0.8$). No patient was taking calcium supplements at baseline or 1 year. The evolution from baseline to 1 year of measured renal function, $T_{50,}$ and bio-

Table 1. Baseline characteristics of the living kidney donors ($n = 21$).

Variables	Results
Age (years)	54.1 ± 10.2
Gender (male/female), *n* (%)	9 (42.9)/12 (57.1)
Body mass index (kg/m^2)	25.1 ± 3.5
24 h SBP/DBP (mmHg)	116.6 ± 13.2/76.0 ± 7.3
Hypertension, *n* (%)	5 (23.8)
Smoking, *n* (%)	7 (33.3)
EDTA GFR (ml/min/1.73 m^2)	101.0 ± 17.5
T_{50} (min)	290.2 ± 53.3
Augmentation index (%)	25.7 ± 11.8
Pulse wave velocity (m/s)	8.4 ± 1.5
Renal resistive index	0.64 ± 0.04

Continuous variables are expressed as mean ± SD when normally distributed or median and (IQR).

logical parameters is depicted in Fig. 1 and Table 2. At 1-year follow-up, there was a decline in renal function (median = −34; IQR −24 to −43 ml/min/1.73 m^2), circulating klotho (median = −68.4; IQR −119.6 to 2.735 pg/ml), 1.25 Vitamin D (median = −60; IQR −87 to −22 pmol/l), and phosphate (median = −0.22; IQR −0.33 to −0.08 mmol/l), but an increase in PTH (median = 1.4; IQR 0.8–2.5 pmol/l) and fetuin-A (median = 0.037; IQR 0.01–0.07 g/l) and no changes in calcium, albumin, magnesium, or FGF23 levels. Calcification propensity improved (i.e. T_{50} increased) slightly, yet significantly, at 1 year after donation (median improvement = 27 min; IQR 17–57 min). Similar observations were made at 6 months (data not shown), where the significance level of the change in T_{50} was higher ($P = 0.02$).

The courses of AI, PWV, RRI, and kidney length are illustrated in Fig. 2. AI, PWV, and RRI remained unchanged after kidney donation. On the contrary, kidney length of the remaining kidney significantly increased (median: 0.7; IQR 0.5–1.2 cm).

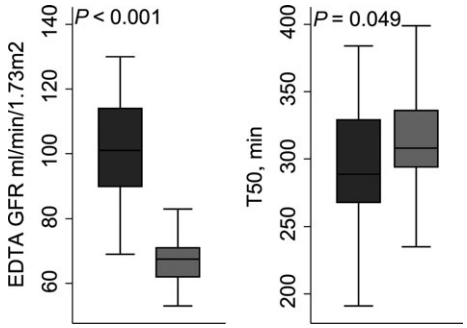

Figure 1 Boxplots representing EDTA clearance and T_{50} before and 1 year after nephrectomy. Baseline is in dark gray and 1-year follow-up in light gray. *P* values are given by paired Wilcoxon tests.

Table 2. Baseline and one year laboratory values ($n = 21$).

	Baseline	One-year postdonation	*P* value
Urea, mmol/l	4.67 ± 1.2	6.00 ± 1.64	**<0.01**
Creatinine, µmol/l	69 ± 14	105 ± 24	**<0.01**
eGFR (CKD-EPI), ml/min/1.73 m^2	95 ± 10	61 ± 11	**<0.01**
Total calcium, mmol/l	2.28 ± 0.1	2.33 ± 0.1	**0.02**
Calcium corrected, mmol/l	2.3 ± 0.1	2.35 ± 0.1	0.10
Serum phosphate, mmol/l	1.25 ± 0.2	1.05 ± 0.2	**<0.01**
Serum albumin, g/l	38 ± 2	39 ± 2	0.31
Serum magnesium, mmol/l	1.00 ± 0.1	1.00 ± 0.1	0.14
Fetuin-A, g/l	0.49 ± 0.14	0.52 ± 0.16	**0.01**
1.25 OH vitamin D, pmol/l	145 ± 53	81 ± 18	**<0.01**
25 OH vitamin D, nmol/l	91 ± 88	79 ± 36	0.60
PTH, pmol/l	4.9 ± 1.9	6.3 ± 2.2	**<0.01**
Klotho, pg/ml	554 ± 177	486 ± 163	**0.02**
FGF23, pg/ml	50 ± 14	51 ± 16	0.90
Beta-crosslaps, ng/l	460 ± 199	363 ± 151	0.10

Bold values are values with significant changes.

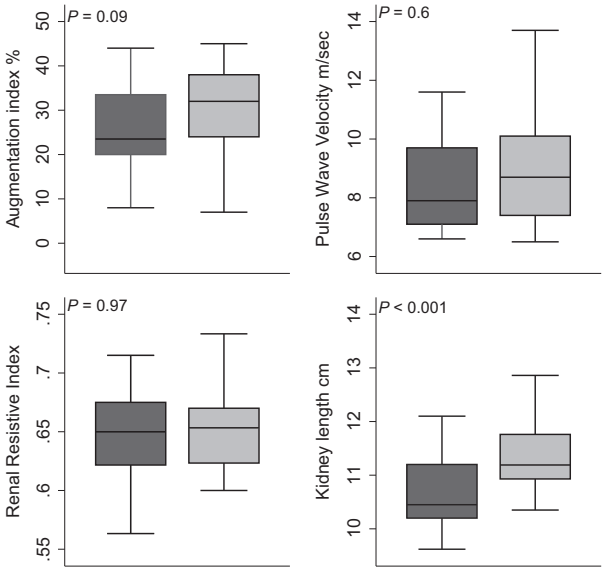

Figure 2 Boxplots representing augmentation index, pulse wave velocity, renal resistive index and kidney length before and 1 year after nephrectomy. Baseline is in dark gray and 1-year follow-up in light gray. *P* values are given by paired Wilcoxon tests.

Correlation analyses revealed no significant associations between T_{50}, AI, RRI, and PWV (all $P > 0.09$), neither at baseline nor at 1 year. Regarding the biochemical parameters, we observed neither a correlation at baseline nor at 1 year between T_{50} and calcium, FGF23, PTH, albumin, magnesium, klotho, fetuin-A, and measured GFR. T_{50} was however linearly and inversely correlated to serum phosphate level (Fig. 3).

Classifying the changes in three categories (no change, decrease, and increase), we found that T_{50}, PWV, AI, RRI, and kidney length increased in 15, 9, 13, 10, and 20 patients, respectively. Again the changes in calcification propensity were inversely associated with the changes in phosphate (χ^2 $P = 0.01$), but not with other parameters. The same results were obtained when considering the correlations between absolute changes in calcification propensity and phosphate ($R = -0.49$, $P = 0.03$).

Discussion

In this prospective study, we observed that calcification propensity (T_{50}) improved slightly after 1 year in most of the LKDs. This improvement in calcification propensity was linearly associated with a decrease in phosphate levels. Fetuin-A levels also increased without correlation with T_{50}. We did not observe any significant changes in parameters

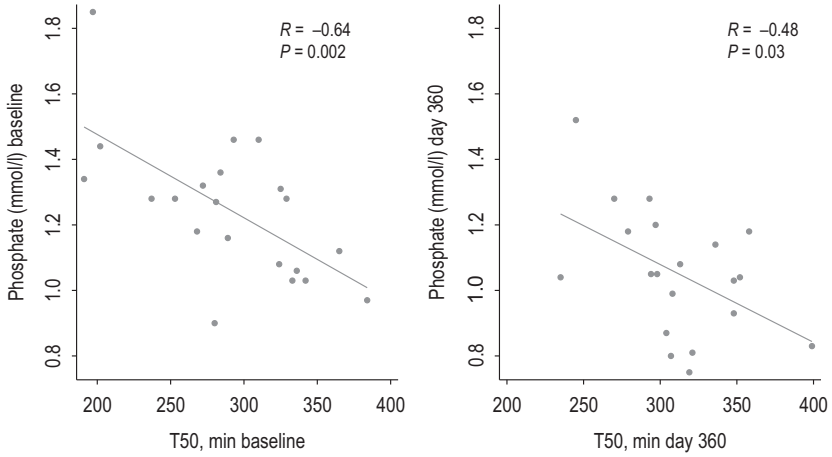

Figure 3 Scatter plot showing the association between T_{50} and phosphate before and 1 year after nephrectomy. Coefficient *R* and *P* values are obtained by Pearson correlation tests. The line represents the linear fit.

used to measure indirectly arterial stiffness such as PWV, AI, or RRI.

Although LKDs experience a loss of GFR in the absence of kidney disease, the effect of the renal mass loss on cardiovascular risk is not well characterized. Calcification propensity (T_{50}) is a novel and unique functional test, which integrates the activities of known and unknown calcification inhibitors and promoters present in serum into a single readout. The T_{50} test is a composite of established nontraditional cardiovascular risk factors, which have a proven *direct* effect on its readout (e.g., serum levels of calcium, phosphate, magnesium, pyrophosphate, fetuin-A, albumin). Although T_{50} was highly predictive of cardiovascular prognosis in CKD 3&4, its single components were not. This demonstrated the added value of the T50 test beyond its single components [12]. T_{50} has not been measured previously in LKDs. In our study, we observed rather than a decrease, a slight increase (i.e., improvement) in its levels after donation. This observation contrasts with what has been shown in patients with CKD.

T_{50} is inversely correlated to phosphate levels in LKDs, as observed in CKD stage 3–4 patients. The pathophysiology of the decreased serum phosphate after donation appears to be related to increased urinary phosphate excretion [8]. This excretion appears to be driven by an increase of PTH post-donation, likely caused by a decrease of 1.25 vitamin D levels (Table 2). The reduction in 1.25 vitamin D levels, although not fully explained yet, might be secondary to a reduction of the renal mass and a decrease in 1-α-hydroxylation efficiency [8]. Correlations with other parameters were not significant, likely due to the low number of patients included into this study. The principle of the T50 test is to measure a biomineralization phenomenon in vitro. As a functional test, it reflects the regulated interplay of various substances to inhibit calcification, as it is the case for clotting factors in a clotting test, and has provided some important hints toward the mechanisms involved in cardiovascular disease [12]. By nature, considering only one factor involved in an orchestrated system will yield weaker associations with relevant end points than considering the entire system.

Fetuin-A is a hepatokine with multiple functions including its inhibitory action on vascular calcifications. Lower levels of fetuin-A have been observed in patients with CKD and correlated with vascular calcifications. Increased levels of fetuin-A might be a risk factor for diabetes, and LKDs have been described to be more prone to insulin resistance [18,19]. Evolution of fetuin-A levels has never been described previously in LKDs. We demonstrate here the fetuin-A levels increase 1 year after kidney donation. Although we found no association with T_{50}, the increase in fetuin-A levels after donation is very likely to contribute to the increase in T_{50}, as previously described [11,12]. The causal mechanism underlying the increased fetuin-A levels after kidney donation is intriguing and presently unclear. Fetuin-A production is reduced in inflammatory conditions and will increase upon reduction of inflammation, a mechanism for which we did not have evidence in our population. In addition, changes in fetuin-A have been correlated to changes in BMI. We observed no such changes in our population, but increases in BMI have been recently described on longer follow-up in kidney donors [20]. However, the causal link between fetuin-A and BMI is debated and may be more related to an effect of fetuin-A on BMI than the opposite [21]. More studies will be needed to understand the regulation of fetuin-A in kidney donors.

In addition, we observed no change in parameters measuring arterial stiffness such as AI or PWV. PWV (rather than AI) is considered to be the best indirect indicator of aortic stiffness, and its measurement is recommended by the European Society of Hypertension to assess target organ

damage [13]. It has been associated to mortality and cardiovascular events in various populations [22,23] including early-stage patients with CKD [24] and apparently healthy individuals [25]. The progression of aortic stiffness has recently been associated with a high calcification propensity in patients with CKD [26]. We found no significant increase in PWV 1 year after kidney donation consistent with the improved calcification propensity.

Finally, the length of the remaining kidney increased significantly 1 year after donation. This increase is expected given the previous literature, even in older and hypertensive donors, and confirms that in adults the remnant kidney is still able to adapt [27]. At the vascular level, we observed no change in RRIs 1 year after donation. It is currently unclear whether the RRI reflects renal vascular resistance and pathological modifications in renal structure and/or systemic arterial stiffness [28]. RRI has been associated with adverse renal [29,30] but also cardiovascular outcomes [31]. One study found a significantly increase of RRI in 41 LKDs, 90 days after donation [32]. RRI levels were still in the normal range in this study and probably reflect the recent loss of renal function and subacute kidney adaptation. One year after the nephrectomy, the renal function is expected to be more stable. Therefore, the absence of significant increase of RRI in our cohort is also in line with an unchanged renal and systemic vascular stiffness.

Altogether, our results indicate that a loss of GFR related to a reduction in nephron mass in the absence of kidney disease does not per se increase at 1 year the calcification risk. These data are in line with recent observations demonstrating that coronary calcium imaging scores are not enhanced and do not progress rapidly in LKDs [33,34] and confirm the observation that uninephrectomy in donors is not equivalent to CKD despite the reduction in GFR and kidney mass [35].

Our study has some limitations. The number of LKDs is small, and some significant associations or changes might be missed because of the lack of power. However, we found a significant improvement in the calcification propensity both at 6 and 12 months, rendering a type I error very unlikely. In addition, phosphate and fetuin-A were modified in a significant and coherent fashion to T_{50}. Although the modest magnitude of these changes are of undetermined clinical significance, our small cohort is sufficiently large to demonstrate that calcification propensity does not worsen but rather improves 1 year after donation. The power is also sufficient to detect clinically relevant changes of PWV and RRI (>1 m/s and >0.05, respectively). Our findings are therefore consistent with the reported good cardiovascular outcome in LKDs. The same phenotypes were measured 1 year apart and each patient acted as his/her own control thus limiting the potential role of confounding factors. All measurements were performed in the same center, by the same operator, limiting the interobserver or interlaboratory variability. One-year follow-up might be considered as too short to be able to detect any cardiovascular event. Most of the cardiovascular events are indeed expected to happen after 5–10 years. T_{50} has however been studied prospectively in a study with a median follow-up of 5 years, where it was shown to be a valuable predictive tool for future events [12]. Our data therefore demonstrate that calcification propensity changes are not directly related to changes in GFR in the absence of renal disease.

We conclude that kidney donation does not worsen calcification propensity and markers of the progression of vascular stiffness measured at 1 year after donation. These results are well in line with previous observations demonstrating that the loss of GFR associated with kidney donation does per se not enhance cardiovascular risk or has a very low direct impact. Longer-term and larger prospective studies are needed to confirm this finding.

Authorship

SdS: designed study, performed study, wrote the paper, collected data. BP: analyzed data, performed study, wrote the paper, collected data. LB: analyzed data, collected data. KH: collected data, wrote the paper. PYM: designed study, wrote the paper. AP: designed study, performed study, wrote the paper.

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