Changes in blood pressure and arterial hemodynamics following living kidney donation

Running title: The EARNEST study

Anna.M.Price MBChB[a,j], George.H.B.Greenhall MBChB[b,j], William.E.Moody PhD[a,j], Richard.P.Steeds MD[a,j], Patrick.B.Mark PhD[c,j], Nicola.C.Edwards PhD[a,j], Manvir.K.Hayer MBChB[a,j], Luke.C.Pickup MBBS[a,j], Ashwin Radhakrishnan BM[a,j], Jonathan.P.Law MBChB[a,j], Debasish Banerjee MD[d,j] Tunde Campbell MBBS[e,j] Charles.R.V.Tomson DM[f,j], John.R.Cockcroft FRCP[g,j], Badri Shrestha MD[h,j], Ian.B.Wilkinson DM[i,j], Laurie.A.Tomlinson PhD[b,j], Charles.J.Ferro MD[a,j], Jonathan.N.Townend MD[a,j]

a. Birmingham Cardio-Renal Group, Centre for Clinical Cardiovascular Science, Nuffield House, Queen Elizabeth Hospital Birmingham, Birmingham, United Kingdom.
b. London School of Hygiene and Tropical Medicine, London, United Kingdom.
c. British Heart Foundation Cardiovascular Research Centre, Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, United Kingdom.
d. St George's University Hospitals NHS Foundation Trust, United Kingdom.
e. Manchester Royal Infirmary, Manchester, United Kingdom.
f. Southmead Hospital, Westbury-on-Trym, Bristol, United Kingdom.
g. Department of Cardiology, Wales Heart Research Institute, University Hospital, Cardiff, United Kingdom.
h. Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, United Kingdom.
i. Cambridge Clinical Trials Unit, Clinical School, Addenbrooke's Hospital, University of Cambridge, Cambridge, United Kingdom.
j. On behalf of the EARNEST investigators.

Corresponding authors details: Dr Anna Price; annaprice@doctors.org.uk, Clinical Research Fellow, Cardiology Research Team, Room 19 Clinical Research Offices, Old Nuclear Medicine Department, Queen Elizabeth Hospital, Edgbaston, Birmingham, B15 2TH, United Kingdom, +44121 371 4624

Word count: 3064/3000

Abstract word count: 238/300

Number of figures: 2 **Number of tables:** 3

ClinicalTrials.gov identifier (NCT number): NCT01769924.

(https://clinicaltrials.gov/ct2/show/NCT01769924).

Abstract

Background and objectives

The Effect of a Reduction in Glomerular Filtration Rate after Nephrectomy on Arterial Stiffness and Central Hemodynamics (EARNEST) was a multi-center, prospective, controlled study designed to investigate the associations of an isolated reduction in kidney function on blood pressure and arterial hemodynamics.

Design, setting, participants and measurements

Prospective living kidney donors and healthy controls who fulfilled criteria for donation were recruited from centers with expertise in vascular research. Participants underwent office and ambulatory blood pressure measurement, assessment of arterial stiffness and biochemical tests at baseline and 12 months.

Results

A total of 469 participants were recruited and 306 (168 donors and 138 controls) were followed up at 12 months. In the donor group, mean eGFR was 27 mL/min/1.73m^2 lower than baseline at 12 months.

Compared to baseline, at 12 months the mean within group difference in ambulatory day systolic blood pressure in donors was 0.1mmHg (95% CI -1.7, 1.9) and 0.6mmHg (95% CI -0.7, 2.0) in controls. The between group difference was not significant, -0.5mmHg (95% CI -2.8, 1.7), p=0.62. The mean within group difference in pulse wave velocity in donors was 0.3m/s (95% CI 0.1, 0.4) and 0.2m/s (95% CI -0.0, 0.4) in controls. The between group difference was also not significant, 0.1m/s (95% CI -0.2, 0.3) p=0.49.

Conclusions

Changes in ambulatory peripheral blood pressure and pulse wave velocity in kidney donors at 12 months after nephrectomy were small and not different from controls.

Keywords (5-7)**:** Blood pressure; Arterial stiffness; Hypertension; Living kidney donors; Chronic kidney disease; Pulse wave velocity.

Introduction

Chronic kidney disease (CKD) is a major risk factor for cardiovascular disease; there is a graded association, independent of multiple cardiovascular risk factors, between glomerular filtration rate (GFR) and cardiovascular risk.(1) In early stage CKD, mortality from cardiovascular events is more likely than the need for kidney replacement therapy.(2) Hypertension, increased arterial stiffness, chronic inflammation and uremic toxins are thought to be key mediators of the higher cardiovascular risk.(3) In patients with end stage kidney disease (ESKD) increased arterial stiffness as measured by pulse wave velocity is an independent predictor of mortality.(4) Increased arterial stiffness is also highly prevalent in earlier stages of CKD.(5) It is not clear whether increased blood pressure and arterial stiffness in CKD are direct consequences of the reduced GFR or result from multiple co-morbid conditions that tend to accompany CKD.

Living kidney donors provide an opportunity to prospectively examine the cardiovascular consequences of a reduction in kidney function without the confounding effects of co-morbid disease. In the long term, kidney donors lose approximately 30% of their baseline GFR and consequently over 65% have a GFR consistent with stages 2 and 3 CKD.(6) They also have similar biochemical abnormalities to patients with CKD.(7) While the risk of ESKD after nephrectomy is higher compared to controls, absolute risk over a 15 year period, remains low.(8, 9) To date, however, most studies of kidney donors have not shown a higher cardiovascular risk or mortality.(10, 11) Only one study has shown higher cardiovascular mortality compared to controls, which occurred later over a 10 year follow-up period.(8) The aim of this study was to determine the effect of the reduction in kidney function that occurs after kidney donation on arterial stiffness and blood pressure in a sample large enough to detect small differences.

Materials and Methods

Study design

The EARNEST (**E**ffect of **A** **R**eduction in glomerular filtration rate after **NE**phrectomy on arterial **ST**iffness and central hemodynamics) study was a prospective multicenter UK cohort study. We aimed to recruit 440 controls and 440 donors over a two year period from seven centers recognised for performing high numbers of living kidney transplants within the UK. Recruitment began in April 2012; the last follow up patient was studied in May 2016. Recruitment was terminated in May 2015 on pragmatic and financial grounds. This was principally due to unanticipated large numbers of recruited participants dropping out at follow up.

Study population

The inclusion and exclusion criteria for donors and controls were in accordance with national guidelines disseminated by the Joint Working Party of the British Transplantation Society and the Renal Association for living kidney donors.(12) Both donors and controls had to be deemed fit to donate a kidney.

Most healthy controls were individuals undergoing workup for donation but who were ultimately unable to donate due to factors such as immunological mismatch or recipient illness. Alternatively, donor-related family members or volunteers donating blood at local blood donation centers were recruited.

Study protocol

All participants were investigated at baseline (less than 6 weeks prior to nephrectomy for prospective living kidney donors) and at 12 months. A full illustrated and detailed protocol has previously been published with a summary presented below.(13)

1. **Blood pressure measurement:** Office blood pressure was measured three times, from the non-dominant arm after 5 minutes of rest, using a validated automated device. Blood pressure was taken in both a sitting and supine position. Participants underwent 24hr ambulatory blood pressure monitoring using the Mobilo-O-Graph NG; IEM (Stolberg, Germany).(14) Blood pressure recordings were taken every 30 minutes between the hours of 0800-2200, and every 60 minutes between 2201 and 0759.(15, 16)

2. **Pulse wave velocity:** Pulse wave velocity was measured using SphygmoCor (Atcor Medical, Sydney, Australia) by trained personnel after asking the participant to lie supine for 15 minutes. Repeated uniform pressure waveforms were acquired from both the carotid and femoral artery using a high fidelity micromanometer (SPC-301; Millar Instruments, Houston, TX).(17) A 3 lead electrocardiogram was used to determine the time between the R wave and the foot of the pulse at each respective site as previously described.(18) Arterial path distance was inferred using the distance from the sternal notch to the femoral pulse subtracted by the distance between the sternal notch and the carotid pulse. (19)

3. **Pulse wave analysis:** Using the SphygmoCor device; arterial pressure waveforms were obtained from which central waveforms can be calculated. Central blood pressure and augmentation index (AIx) were calculated using transfer functions as previously

described.(15, 20) Augmentation index is the augmentation pressure from the aortic waveform expressed as a percentage of pulse pressure, see **Figure 1**.

4. **Assessment of glomerular filtration rate:** Glomerular filtration rate was determined in all participants using standardised creatinine assays and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine 2009 equation.(21, 22) A subset of living kidney donors underwent isotopic GFR measurement using clearance of ^{51}Cr-EDTA at both baseline and follow up.(23, 24)

5. **Blood and urine:** Biochemistry measurements included serum creatinine, calcium, albumin, phosphate and uric acid and urinary albumin: creatinine ratio.

Primary outcomes

1. Mean change in ambulatory systolic blood pressure.
2. Mean change in pulse wave velocity.

Statement of ethics

Ethical approval for the main study was obtained in February 2013 from the South Cambridge Regional Ethics Committee (Integrated Research Application System Reference: 118797, Research Ethics Committee approval number 13/EE/0015). The EARNEST sub study (CRIB-DONOR) commenced in 2011, ethical approval was obtained from the West Midlands Research Ethics Committee. All participants underwent informed consent in keeping with the principles set out by the Declaration of Helsinki.

Power calculations and sample size

Using data from previous studies, the standard deviation of the within-patient changes was assumed to be 10 mmHg for blood pressure and 1.0 m/s for pulse wave velocity.(17, 25) A sample size of 800 participants (400 subjects per group) was planned in order to provide 80% power to detect a difference of 2.2mmHg in systolic pressure or 0.22 m/s in pulse wave velocity using a 2-sided *t*-test at the 2.5% significance level. Values for a sample size of 400 participants (200 subjects per group) have 92% power to detect a difference of 4 mmHg for systolic blood pressure and 0.4 m/s for pulse wave velocity, allowing for 15% loss to follow-up at a significance level of 5%.

Statistical analysis

Statistical analysis was performed using Stata statistical software (release 15. StataCorp LCC, College Station, TX). Continuous variables at baseline and 12 months were compared using independent *t*-tests. Categorical variables were compared using Chi squared tests. A paired samples *t* test was used to estimate the mean change and 95% confidence interval between baseline and follow-up in each group (within-group change). Change from baseline to 12 months was calculated for both donors and controls. An independent *t* test was used to estimate the mean change and 95% confidence interval between within-group change in donors and within-group change in controls (between-group change). Carotid-femoral pulse wave velocity was adjusted for average mean arterial pressure and average supine heart rate using unstandardized residuals calculated from a linear regression model. In supplementary analyses, we used multivariable linear regression to account for factors which may have confounded the relationship between kidney donation and change in pulse wave velocity (age, sex and smoking status). A p-value of <0.05 was considered significant, no adjustments were made for multiple comparisons. Data presented includes subjects who returned for follow up. We dealt with missing data by performing a complete case analysis. Further sub-analysis of those who remained in the study compared to those who were lost to follow up are detailed in supplement **Table S1, S2 and S3**.

Results

Follow up and events

A total of 469 participants were recruited; 20 were excluded as they lacked the minimal data set required for analysis and two were found to be ineligible after the initial visit (see **Figure 2)**. Recruitment was terminated at 3 years despite the lower than planned sample size due to financial constraints. Of the remaining 447 participants, there were 201 controls and 246 donors. Of these, a total of 38 controls and 46 donors were patients that originally consented into the CRIB-DONOR sub study, who re-consented to allow their data to be included.(26) One hundred and forty-one participants were unable to attend follow up at 12 months leaving 168 donors and 138 controls with complete paired data, who were included in the final analysis. The commonest reasons for lack of study completion by participants were change of address or difficulty attending clinic visits due to travel distance, work and childcare commitments.

For comparison between patients who were lost to follow up and those who continued in the study, see **Table S1**. Minimal differences were observed in those who did not return for follow up at 12 months. Participants who continued in the study, had a marginally lower eGFR and were more likely to be taking anti-hypertensive medications.

In addition, a further 49 donors and 27 controls who returned for follow up had incomplete ambulatory blood pressure recordings.

Patient characteristics

The demographics of living kidney donors and healthy controls who attended for both baseline and 12 month follow up visits were comparable with the exception of tobacco use, see **Table 1**. Baseline hemodynamic and biochemical characteristics are shown in **Tables 2 and 3**. There were no significant differences between donors and controls in any of the

baseline hemodynamic values and no clinically significant differences in biochemical values. At follow up there were 6 living kidney donors whose eGFR fell into stage 3b CKD and one whose eGFR fell into stage 4 CKD according to the Kidney Disease Improving Global Outcome guidelines.

Patient demographics and hemodynamic and biochemical characteristics at baseline for all those recruited (n=447) are shown in supplementary **Tables S2 and S3**. Donors had a higher mean age than controls; (51 yrs. vs 47 yrs., p=0.003) and were more likely to have a history of previous smoking; (46% vs. 33%, p=0.007).

Comparison of hemodynamic variables in living kidney donors and controls

Arterial hemodynamic parameters at baseline and 12 months are given in **Table 2**. There were no significant differences between donors and controls in office or ambulatory blood pressures at 12 months. The changes in office systolic blood pressure from baseline to 12 months in donors and controls were small. The mean change seen in donors (+1.8mmHg) was however, different to that in controls, in whom there was a mean reduction of 1mmHg (difference of 2.8mmHg, 95% confidence interval (CI) 0.3-5.4, p=0.03). Using current American Heart Association ambulatory blood pressure criteria, 13 (9%) in the control group and 15 (9%) in the donor group developed hypertension over the 12 month period with no significant difference between the two groups, p=0.18.(27) The mean change in ambulatory heart rate was significantly greater in donors compared to controls at 12 months (difference of 2.8 bpm, 95% CI 0.1-5.5, p=0.04).

Adjusted pulse wave velocity was not significantly different at 12 months nor was there any difference in changes from baseline. Our supplementary analyses showed no association between kidney donation and change in pulse wave velocity when accounting for factors which may influence this relationship, see **Table S4**. Change in central diastolic blood pressure and

augmentation index adjusted for heart rate were not significantly different in donors compared to controls. When considering changes from baseline, only central systolic blood pressure changes were significantly greater in donors than controls (difference of 3.3mmHg, 95% CI 0.3, 6.3, p=0.03).

Comparison of biochemistry in living kidney donors and controls

Results are shown in **Table 3**. At 12 months, eGFR fell by a mean of 27mL/min/1.73m2 in donors but was unchanged in controls. Although iGFR measurement was part of the protocol for donation, in practice few subjects consented to a 12 month iGFR due to concerns about the duration of the test and exposure to ionising radiation. The mean change from baseline for phosphate in donors was significantly lower compared to controls (difference -0.31 mg/dL, 95% CI -0.31, -0.1, p=<0.001. In contrast a significant increase in uric acid was seen in donors compared to controls (difference 0.9 mg/dL, 95% CI 0.7, 1.0, p=<0.001).

Discussion

This prospective study of ambulatory blood pressure monitoring and arterial hemodynamics in kidney donors provides important findings. There was no difference in office or ambulatory blood pressure in donors compared to controls at 12 months after nephrectomy. Pulse wave velocity also did not differ in these groups. Central systolic blood pressure increased slightly more in donors than controls and at 12 months was higher in the donor group. These results suggest that the risk of a significant rise in blood pressure at 12 months in kidney donors is small. This is in keeping with findings from the smaller sub-study CRIB-DONOR but is surprising in view of the high prevalence of hypertension in patients with CKD and similar levels of GFR.(26) Our data suggest that a simple loss of nephron numbers does not invariably result in an elevated blood pressure and that other aspects of CKD such as inflammation and nephron dysfunction may be required for this key pathophysiological mediator to occur.

Previous data have been contradictory. In a 2006 meta-analysis of 48 studies of office blood pressure in kidney donors, including a total of 5145 patients, there was an increase in systolic blood pressure of 6 (95% CI 2-11) mmHg and an increase in diastolic pressure of 4 (95% CI 1-7) mmHg in donors at 5 years.(28) More recently however, Kasiske et al. found no significant difference in over 300 participants between kidney donors and controls in office blood pressure at any time point up to 36 months.(29) There was also no difference in ambulatory blood pressure in 135 donors and 126 controls at 36 months.(29) Taken together, our data and the study of Kasiske et al. suggest that the risk of a clinically important change in blood pressure in the short term following kidney donation is low.(29) Longer term data are of course required.

Despite the absence of change in peripheral pressure, the mean change in central systolic pressure was greater in donors at 12 months compared to controls (+ 2.1 vs-1.2mmHg, p=0.03). While this small difference may be a chance result due to multiple comparisons it may be

important as central blood pressure is better related to left ventricular mass, carotid intimal thickness and cardiovascular events than peripheral pressure.(30, 31) The small increase in AIx was not accompanied by any rise in pulse wave velocity. Discrepancies between changes in Aix and pulse wave velocity have been found by other observers in a number of situations and remain incompletely explained.(32) Any increase in AIx suggests an increase in wave reflection which might explain the increase in central blood pressure. As pulse wave velocity was unchanged it is possible that this increased reflection occurred due to changes in peripheral, rather than central arterial stiffness. We speculate that this occurred as a consequence of ligation of one of the renal arteries causing amplification of the reflection site without a corresponding change in pulse wave velocity, although to date this has no supportive animal or human evidence. Previous studies examining arterial stiffness in kidney donors have been small and uncontrolled. De-Seigneux et al. studied 21 patients before and one year after nephrectomy and found no change in AIx or pulse wave velocity.(33) Similarly Fesler et al. found no change in pulse wave velocity at 12 months post nephrectomy in 45 donors.(34) A cross sectional study of 101 living kidney donors, however, found that pulse wave velocity was 10% higher than control patients.(35) We cannot exclude a small effect on pulse wave velocity, as the study was not powered to detect a difference of less than 0.4m/s.

Most of the biochemical changes after donation are in accord with previous studies (26, 29) Our finding of lower phosphate levels in donors is perhaps surprising in view of the renal excretion of phosphate but is consistent with a large prospective study of bone metabolism in kidney donors.(36) We speculate that this is a result of an increase in fibroblast growth factor 23 which has a pivotal role in phosphate homeostasis and has been associated with left ventricular hypertrophy.(37, 38)

Conclusions

In summary, this is the largest controlled longitudinal prospective study of hemodynamics in living kidney donors. This study indicates there is no change in ambulatory blood pressure or arterial stiffness at 12 months post nephrectomy despite changes in biochemistry. This has important implications for the future of living kidney donors but also provides valuable insight into the pathophysiology of hypertension and myocardial disease in CKD suggesting that an increase in blood pressure is not an inevitable consequence of a reduced GFR.

Limitations

We did not reach the planned sample size and a substantial proportion of participants did not return for follow up at one year, which limited the study power and introduces the potential for selection bias. Barriers to studies of living kidney donors have been reported by others.(39) They are often geographically remote from the transplant center (in contrast to the recipient) and after donation are usually in full time work. Barriers to ambulatory blood pressure monitoring in this study were in keeping with those previously observed, where one in five patients describe 24-hr monitoring as uncomfortable and nearly 70% are woken from sleep.(40, 41)

These limitations however, do not affect the internal validity of our results. There were only minor differences between participants who did and did not return for follow-up so our results should be generalizable to the wider pool of potential kidney donors. Although not statistically different, the healthy controls were on average 2 years younger, more likely to be male and more likely to have a history of hypertension. There was a greater rate of smoking amongst donors which could be due to in part to social deprivation based on geographical area or reflect health promoting behaviour in healthy controls. In addition, the large number of parameters

measured beyond the pre-specified primary end-points mean that there are issues of multiple testing necessitating caution in interpreting results as some differences may have arisen by chance.

Lack of ethnic diversity has been a notable problem in living kidney donor research.(7) Over 90% of our cohort were Caucasian and this does reflect the vast racial disparity currently facing transplantation.(42) We recognise that while our data at 12 months are reassuring, longer term and more diverse studies are required particularly in light of literature showing higher cardiovascular risk in the long term.(8)

Disclosures

No conflict of interests to declare.

Acknowledgments

The Research was funded by the British Heart Foundation and carried out at the National Institute for Health Research (NIHR)/Wellcome Trust Birmingham Clinical Research Facility. The views are those of the authors. We would like to thank Peter Nightingale for statistical input and support. We would also like to thank the research nurses across all sites who helped carry out study visits.

Source of Funding

This study was funded by the British Heart Foundation (PG/12/35/29403). AM Price is supported by a British Heart Foundation Fellowship (FS/16/73/32314). LAT is funded by a Wellcome Trust intermediate clinical fellowship (101143/Z/13/Z).

Supplemental material-Table of Contents

References

1. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu C-y: Chronic Kidney Disease and the Risks of Death, Cardiovascular Events, and Hospitalization. *New England Journal of Medicine,* 351**:** 1296-1305, 2004
2. O'Hare AM, Choi AI, Bertenthal D, Bacchetti P, Garg AX, Kaufman JS, Walter LC, Mehta KM, Steinman MA, Allon M, McClellan WM, Landefeld CS: Age Affects Outcomes in Chronic Kidney Disease. *Journal of the American Society of Nephrology,* 18**:** 2758-2765, 2007
3. Zanoli L, Lentini P, Briet M, Castellino P, House AA, London GM, Malatino L, McCullough PA, Mikhailidis DP, Boutouyrie P: Arterial Stiffness in the Heart Disease of CKD. *Journal of the American Society of Nephrology,* 30**:** 918-928, 2019
4. Blacher J, Safar ME, Guerin AP, Pannier B, Marchais SJ, London GM: Aortic pulse wave velocity index and mortality in end-stage renal disease. *Kidney Int,* 63**:** 1852-1860, 2003
5. Briet M, Bozec E, Laurent S, Fassot C, London G, Jacquot C: Arterial stiffness and enlargement in mild-to-moderate chronic kidney disease. *Kidney Int,* 69**:** 350-357, 2006
6. Moody WE, Chue CD, Inston NG, Edwards NC, Steeds RP, Ferro CJ, Townend JN: Understanding the effects of chronic kidney disease on cardiovascular risk: are there lessons to be learnt from healthy kidney donors. *J Hum Hypertens,* 26**:** 141-148, 2012
7. Price AM, Edwards NC, Hayer MK, Moody WE, Steeds RP, Ferro CJ, Townend JN: Chronic kidney disease as a cardiovascular risk factor: lessons from kidney donors. *Journal of the American Society of Hypertension,* 12**:** 497-505.e494, 2018
8. Mjoen G, Hallan S, Hartmann A, Foss A, Midtvedt K, Oyen O, Reisater A, Pfeffer P, Jenssen T, Leivestad T, Line P-D, Ovrehus M, Dale DO, Pihlstrom H, Holme I, Dekker FW, Holdaas H: Long-term risks for kidney donors. *Kidney Int,* 86**:** 162-167, 2014
9. Grams ME, Sang Y, Levey AS, Matsushita K, Ballew S, Chang AR, Chow EKH, Kasiske BL, Kovesdy CP, Nadkarni GN, Shalev V, Segev DL, Coresh J, Lentine KL, Garg AX: Kidney-Failure Risk Projection for the Living Kidney-Donor Candidate. *New England Journal of Medicine,* 374**:** 411-421, 2016
10. Garg AX, Meirambayeva A, Huang A, Kim J, Prasad GVR, Knoll G, Boudville N, Lok C, McFarlane P, Karpinski M, Storsley L, Klarenbach S, Lam N, Thomas SM, Dipchand C, Reese P, Doshi M, Gibney E, Taub K, Young A: Cardiovascular disease in kidney donors: matched cohort study. *BMJ,* 344, 2012
11. Segev DL, Muzaale AD, Caffo BS, et al.: Perioperative mortality and long-term survival following live kidney donation. *JAMA,* 303**:** 959-966, 2010
12. British Transplantation Society: Living donor kidney transplantation. Third Ed. The British Transplant Society and Renal Association United Kingdom, 2011
13. Moody WE, Tomlinson LA, Ferro CJ, Steeds RP, Mark PB, Zehnder D, Tomson CR, Cockcroft JR, Wilkinson IB, Townend JN: Effect of A Reduction in glomerular filtration rate after NEphrectomy on arterial STiffness and central hemodynamics: Rationale and design of the EARNEST study. *American Heart Journal,* 167**:** 141-149.e142, 2014
14. Weiss W, Gohlisch C, Harsch-Gladisch C, Tölle M, Zidek W, van der Giet M: Oscillometric estimation of central blood pressure: validation of the Mobil-O-Graph in comparison with the SphygmoCor device. *Blood Pressure Monitoring,* 17**:** 128-131, 2012
15. Weber T, Wassertheurer S, Rammer M, Maurer E, Hametner B, Mayer Christopher C, Kropf J, Eber B: Validation of a Brachial Cuff-Based Method for Estimating Central Systolic Blood Pressure. *Hypertension,* 58**:** 825-832, 2011
16. Wilkinson IB, McEniery CM, Cockcroft JR, Roman MJ, Franklin SS: Central blood pressure: current evidence and clinical importance. *European Heart Journal,* 35**:** 1719-1725, 2014
17. Wilkinson IB, Fuchs SA, Jansen IM, Spratt JC, Murray GD, Cockcroft JR, Webb DJ: Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. *Journal of Hypertension,* 16**:** 2079-2084, 1998
18. Butlin M, Qasem A: Large Artery Stiffness Assessment Using SphygmoCor Technology. *Pulse (Basel),* 4**:** 180-192, 2017

19. Townsend RR, Wilkinson IB, Schiffrin EL, Avolio AP, Chirinos JA, Cockcroft JR, Heffernan KS, Lakatta EG, McEniery CM, Mitchell GF, Najjar SS, Nichols WW, Urbina EM, Weber T: Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness. *Hypertension,* 66**:** 698-722, 2015
20. Savage MT, Ferro CJ, Pinder SJ, Tomson CRV: Reproducibility of derived central arterial waveforms in patients with chronic renal failure. *Clinical Science,* 103**:** 59-65, 2002
21. Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro AF, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J: A New Equation to Estimate Glomerular Filtration Rate. *Annals of internal medicine,* 150**:** 604-612, 2009
22. Hsu C-y, Bansal N: Measured GFR as "Gold Standard"—All that Glitters Is Not Gold? *Clinical Journal of the American Society of Nephrology,* 6**:** 1813-1814, 2011
23. Mårtensson J, Groth S, Rehling M, Gref M: Chromium-51-EDTA Clearance in Adults with a Single-Plasma Sample. *Journal of Nuclear Medicine,* 39**:** 2131-2137, 1998
24. Fleming JS, Zivanovic MA, Blake GM, Burniston M, Cosgriff PS: Guidelines for the measurement of glomerular filtration rate using plasma sampling. *Nuclear Medicine Communications,* 25**:** 759-769, 2004
25. Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlöf B, Sever PS, Poulter NR: Prognostic significance of visit-to-visit variability, maximum systolic blood pressure, and episodic hypertension. *The Lancet,* 375**:** 895-905, 2010
26. Moody WE, Ferro CJ, Edwards NC, Chue CD, Lin ELS, Taylor RJ, Cockwell P, Steeds RP, Townend JN: Cardiovascular Effects of Unilateral Nephrectomy in Living Kidney Donors. *Hypertension*, 67: 368-377, 2016
27. Whelton PK, Carey RM, Aronow WS, Casey DE, Collins KJ, Dennison Himmelfarb C, DePalma SM, Gidding S, Jamerson KA, Jones DW, MacLaughlin EJ, Muntner P, Ovbiagele B, Smith SC, Spencer CC, Stafford RS, Taler SJ, Thomas RJ, Williams KA, Williamson JD, Wright JT: 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Journal of the American College of Cardiology,* 71**:** e127-e248, 2018
28. Boudville N, Ramesh Prasad GV, Knoll G, Muirhead N, Thiessen-Philbrook H, Yang RC, Rosas-Arellano MP, Housawi A, Garg AX: Meta-Analysis: Risk for Hypertension in Living Kidney Donors. *Annals of Internal Medicine,* 145**:** 185-196, 2006
29. Kasiske BL, Anderson-Haag T, Israni AK, Kalil RS, Kimmel PL, Kraus ES, Kumar R, Posselt AA, Pesavento TE, Rabb H, Steffes MW, Snyder JJ, Weir MR: A Prospective Controlled Study of Living Kidney Donors: Three-Year Follow-up. *American Journal of Kidney Diseases,* 66**:** 114-124, 2015
30. de Luca N, Asmar RG, London GM, O'Rourke MF, Safar ME, investigators obotRP: Selective reduction of cardiac mass and central blood pressure on low-dose combination perindopril/indapamide in hypertensive subjects. *Journal of Hypertension,* 22**:** 1623-1630, 2004
31. Boutouyrie P, Bussy C, Lacolley P, Girerd X, Laloux B, Laurent S: Association Between Local Pulse Pressure, Mean Blood Pressure, and Large-Artery Remodeling. *Circulation,* 100**:** 1387-1393, 1999
32. Izzard AS, Grassi G: Alterations in pulse wave velocity and augmentation index in Australian aboriginals: characteristics and mechanisms. *Journal of Hypertension,* 25**:** 511-512, 2007
33. de Seigneux S, Ponte B, Berchtold L, Hadaya K, Martin P-Y, Pasch A: Living kidney donation does not adversely affect serum calcification propensity and markers of vascular stiffness. *Transplant International,* 28**:** 1074-1080, 2015
34. Fesler P, Mourad G, du Cailar G, Ribstein J, Mimran A: Arterial stiffness: an independent determinant of adaptive glomerular hyperfiltration after kidney donation. *American Journal of Physiology - Renal Physiology,* 308**:** F567-F571, 2015
35. Bahous SA, Stephan A, Blacher J, Safar ME: Aortic Stiffness, Living Donors, and Renal Transplantation. *Hypertension,* 47**:** 216-221, 2006

36. Kasiske BL, Kumar R, Kimmel PL, Pesavento TE, Kalil RS, Kraus ES, Rabb H, Posselt AM, Anderson-Haag TL, Steffes MW, Israni AK, Snyder JJ, Singh RJ, Weir MR: Abnormalities in biomarkers of mineral and bone metabolism in kidney donors. *Kidney International,* 90**:** 861-868, 2016
37. Takashi Y, Fukumoto S: FGF23-Klotho axis in CKD. *Renal Replacement Therapy,* 2**:** 20, 2016
38. Faul C, Amaral AP, Oskouei B, Hu MC, Sloan A, Isakova T: FGF23 induces left ventricular hypertrophy. *J Clin Invest,* 121, 2011
39. Kim SH, Hwang HS, Yoon HE, Kim YK, Choi BS, Moon IS, Kim JC, Hwang TK, Kim YS, Yang CW: Long-Term Risk of Hypertension and Chronic Kidney Disease in Living Kidney Donors. *Transplantation Proceedings,* 44**:** 632-634,
40. Viera AJ, Lingley K, Hinderliter AL: Tolerability of the Oscar 2 ambulatory blood pressure monitor among research participants: a cross-sectional repeated measures study. *BMC Medical Research Methodology,* 11**:** 59, 2011
41. Ernst ME, Bergus GR: Favorable patient acceptance of ambulatory blood pressure monitoring in a primary care setting in the United States: a cross-sectional survey. *BMC Family Practice,* 4**:** 15, 2003
42. Purnell TS, Luo X, Cooper LA, Massie AB, Kucirka LM, Henderson ML, Gordon EJ, Crews DC, Boulware LE, Segev DL: Association of Race and Ethnicity With Live Donor Kidney Transplantation in the United States From 1995 to 2014.Trends in Live Donor Kidney Transplantation Receipt by Race/Ethnicity, 1995-2014. *JAMA,* 319**:** 49-61, 2018
43. Stoner L, Young JM, Fryer S: Assessments of arterial stiffness and endothelial function using pulse wave analysis. *Int J Vasc Med,* 2012**:** 903107-903107, 2012

Figure 1: A typical aortic pulse wave form generated from applanation tonometry of the radial artery using the SphygmaCor, taken from Stoner et al (43). The maximum pressure is systolic and the minimum pressure is diastolic. The first peak, the forward wave, indicates ejected blood from the heart. The second peak, the reflected wave, is that returned from peripheral vasculature. The difference between the two is augmentation pressure. Augmentation index is augmentation pressure expressed as a percentage of pulse pressure. As augmentation index is influenced by timing of the reflected wave, augmentation index is corrected for a heart rate of 75 beats per minute.

Figure 2: A flow chart demonstrating those recruited and those lost to follow up.

*Following eligibility assessment there were 22 patients who consented to take part but were ultimately excluded from the study. After baseline blood tests two 'healthy controls' did not meet criteria due to incidental findings; one was diagnosed with diabetes and one had an insufficient kidney function. Consequently, neither met living kidney donation criteria. A further 20 patients consented to take part and withdrew prior to completing baseline assessment. This was usually because of competing appointments during living kidney donor work up.

Table 1: Baseline characteristics of participants in the EARNEST study (**E**ffect of **A** **R**eduction in glomerular filtration rate after **NE**phrectomy on arterial **ST**iffness and central hemodynamics) who completed both baseline and 12-month evaluations.

	Controls n=138	**Donors n=168**
Sex (male) *	57 (41)	78 (46)
Age (years) *	49 ± 14	51 ± 12
Race	Caucasian=127 (92) Non-white=8 (6) Unknown=3 (2)	Caucasian=158 (94) Non-white= 9 (5) Unknown= 1 (1)
History of hypertension	9 (7)	17 (10)
Anti-hypertensive usage*	9 (7)	18 (11)
ACE/ARB usage	4 (3)	5 (3)
Calcium channel blocker usage	4 (3)	6 (4)
Current or ex-smoker *	38 (28)	74 (44)
eGFR, categories (mL/min/1.73m2)	<80= 25 (18) 80 to <90= 23 (17) >90 =88 (65)	<80= 38 (23) 80 to <90= 39 (23) >90= 91 (54)
Normalised isotopic GFR (ml/min/1.73m2)*	89 ± 13	89 ± 12

ACE; Angiotensin Converting Enzyme. ARB; Angiotensin receptor blocker. CKD; Chronic Kidney Disease. eGFR; estimated Glomerular Filtration Rate. GFR; Glomerular Filtration Rate.

*For the following categories: sex, age, anti-hypertensive usage and current or ex-smoker there were n=168 donors and n=137 controls due to an incomplete data set for one healthy control. For isotopic GFR, results from controls were part of the CRIB-DONOR sub study and included n=90 donors and n=22 controls.
Categorical variables are presented as n (%) and continuous data are represented as mean ± standard deviation.

Table 2: Changes in hemodynamic and arterial parameters over 12 months.

Variables	Patient group n=sample size	Single Time point Baseline	12 months	Change Within-group*	Between-group†	p-Value ‡
Weight (kg)	Donors=168	75.4 ± 13.5	77.1 ± 14.7	1.7 (0.4, 3.0)		
	Controls=136	74.7 ± 13.9	74.9 ± 13.8	0.2 (-0.4, 0.8)	1.5 (-0.12, 3.0)	0.07
BMI (kg/m2)	Donors=168	26.2 ± 3.3	26.8 ± 4.6	0.6 (0.1, 1.2)		
	Controls=136	26.0 ± 4.0	26.2 ± 4.0	0.1 (-0.1, 0.3)	0.5 (-0.1, 1.1)	0.09
Seated office systolic BP (mmHg)	Donors=168	125 ± 14	127 ± 12	1.8 (-0.0, 3.6)		
	Controls=135	125 ±17	124 ± 17	-1.0 (-2.8, 0.7)	2.8 (0.3, 5.4)	0.03
Seated office diastolic BP (mmHg)	Donors=168	78 ± 9	80 ± 8	1.7 (0.4, 2.9)		
	Controls=135	77 ±10	78± 9	0.7 (-0.8, 1.9)	1.0 (-0.74, 2.9)	0.25
Ambulatory day systolic BP (mmHg)	Donors=119	124 ± 10	124 ± 10	0.1 (-1.7, 1.9)		
	Controls=111	122 ± 10	123 ± 12	0.6 (-0.7, 2.0)	-0.5 (-2.8, 1.7)	0.62
Ambulatory day diastolic BP (mmHg)	Donors=119	79 ± 8	79 ± 8	0.2 (-0.9,1.4)		
	Controls=111	77 ± 8	78 ± 9	0.9 (0.0, 1.7)	-0.6 (-2.1, 0.9)	0.40
Ambulatory day HR (bpm)	Donors=65	73 ± 9	74 ± 10	1.5 (-0.9, 3.9)		
	Controls=82	72 ± 9	71 ± 10	-1.3 (-2.8, 0.2)	2.8 (0.1, 5.5)	0.04
Ambulatory night systolic BP (mmHg)	Donors=111	111 ± 11	112 ±11	0.9 (-1.1, 3.0)		
	Controls=105	110 ± 10	110 ±12	-0.6 (-2.5,1.3)	1.5 (-1.2, 4.3)	0.27
Ambulatory night diastolic BP (mmHg)	Donors=111	67 ± 9	69 ± 9	1.4 (-0.2, 3.0)		
	Controls=105	66 ± 8	66 ± 9	0.3 (-1.15, 1.7)	1.1 (-0.9, 3.3)	0.27

Central systolic BP (mmHg)	Donors=105	113 ± 14	115 ± 14	2.1 (-0.2, 4.4)		
	Controls=108	111 ± 17	109 ± 17	-1.2 (-3.1, 0.7)	3.3 (0.3, 6.3)	0.03
Central diastolic BP (mmHg)	Donors=105	77 ± 9	78 ± 10	1.3 (-0.7, 3.2)		
	Controls=108	75 ± 10	75 ± 10	-0.3 (-1.9, 1.1)	1.5 (-0.9, 4.0)	0.22
Augmentation index, corrected for HR (%)	Donors=104	22.1 ± 12.0	25.6 ± 12.2	3.4 (1.5, 5.3)		
	Controls=108	20.4 ±12.5	22.3 ± 12.0	1.8 (-0.0, 3.6)	1.6 (-1.0, 4.2)	0.23
Adjusted carotid-femoral pulse wave velocity (m/s)	Donors=168	7.0 ± 1.3	7.3 ± 1.4	0.3 (0.1, 0.4)		
	Controls=138	7.0 ± 1.4	7.2 ±1.4	0.2 (-0.0, 0.4)	0.1 (-0.2, 0.3)	0.49

BPM; Beats per minute. BMI; Body mass index. BP; Blood Pressure, CI; Confidence interval, HR: Heart rate, SD: Standard deviation.

Data is displayed as mean ± SD or mean (95% lower CI, 95% upper CI).

* Within-group change refers to change in values between baseline and follow up in each group i.e. mean weight in donors for baseline was 75.4 kg and at follow up was 77.1 kg giving a within-group change of 1.7kg. The 95% confidence interval was estimated using paired sample *t*-tests.

† Between-group change refers to the difference between donors and controls within-group change i.e. for weight, within group change for donors is 1.7kg and 0.2kg for controls giving a between group-change of 1.5kg. The 95% confidence interval was estimated using independent *t*-tests.

‡ Comparison between controls and donors was made for within-group change [i.e. mean change in weight in donors (1.7kg) vs mean change in weight in controls (0.2kg)] using an independent samples *t*-tests.

Table 3: Changes in biochemical parameters over 12 months.

Variables	Patient group n=sample size	Single time point		Change		
		Baseline	12 months	Within-group*	Between-group†	p-Value ‡
Sodium (meq/L)	Donors=167	140 ± 2	140 ± 2	-0.3 (-0.7, 0.0)	-0.1 (-0.6, 0.4)	0.59
	Controls=137	141 ± 2	140 ± 2	-0.2 (-0.5, 0.2)		
Potassium (meq/L)	Donors=167	4.3 ± 0.3	4.4 ± 0.4	0.1 (0.0, 0.2)	0.1 (0.0, 0.2)	0.02
	Controls=134	4.2 ± 0.3	4.2 ± 0.3	-0.0 (-0.1, 0.1)		
Urea (mg/dL)	Donors=167	31 ± 8	38 ± 10	8.4 (7.2, 9.6)	7.2 (5.4, 9.0)	<0.001
	Controls=136	30 ± 8	31 ± 8	1.0 (0.03, 2.4)		
Creatinine (mg/dL)	Donors=168	0.8 ± 0.2	1.2 ± 0.2	0.3 (0.3, 0.4)	0.3 (0.3, 0.4)	<0.001
	Controls=136	0.8 ± 0.2	0.8 ± 0.2	-0.02 (-0.03, 0.009)		
eGFR (mL/min/1.73 m2)	Donors=168	91 ± 15	64 ± 14	-27 (-29, -26)	-29 (-32, -26)	<0.001
	Controls=136	94 ± 16	96 ± 17	2 (-0.4, 3.8)		
Albumin (g/dL)	Donors=145	4.27 ± 0.39	4.22 ± 0.43	-0.04 (-0.10, 0.01)	-0.07 (-0.15, -0.0)	0.04
	Controls=135	4.16 ± 0.44	4.19 ± 0.48	0.03 (-0.01, 0.08)		
Corrected calcium (mg/dL)	Donors=148	9.2 ± 0.4	9.2 ± 0.4	0.0 (-0.0, 0.0)	-0.0 (-0.0 – 0.0)	0.28
	Controls=136	9.2 ± 0.4	9.2 ± 0.4	0.0 (-0.0, 0.0)		
Phosphate, (mg/dL)	Donors=130	3.4 ± 0.6	3.1 ± 0.6	-0.3 (-0.3, -0.3)	-0.31 (-0.31, -0.1)	<0.001
	Controls=121	3.4 ± 0.6	3.4 ± 0.6	0.0 (-0.0, 0.3)		
Magnesium,	Donors=77	2.2 ± 1.7	2.2 ± 0.2	0.0 (-0.0, 0.0)		

(mg/dL)	Controls=85	2.2 ± 1.7	2.2 ± 0.2	0.0 (-0.0, 0.0)	-0.0 (-0.0- 0.0)	0.94
Uric acid (mg/dL)	Donors=93	5.0 ± 1.2	5.9 ± 1.3	0.9 (0.69, 1.0)	0.9 (0.7, 1.0)	<0.001
	Controls=95	4.8 ± 1.1	4.8 ± 1.1	-0.0 (-0.6, 0.1)		
Urine albumin: creatinine ratio (mg/mmol)	Donors=66	24.6 ± 41.2	23.1 ± 39.8	-1.5 (-9.7, 7.1)	-1.5 (-9.7, 12.4)	0.80
	Controls=69	20.1 ± 32.7	17.1 ± 32.0	-3.0 (-11.5, 5.3)		

CI; Confidence interval, eGFR; estimated glomerular filtration rate, SD: Standard deviation. Data is displayed as mean ± SD or mean (95% lower CI-95% upper CI).

* Within-group change refers to change in values between baseline and follow up in each group i.e. mean potassium in donors for baseline was 4.3 meq/L and at follow up was 4.4 meq/L giving a within-group change of 0.1 meq/L. The 95% confidence interval was estimated using paired sample *t*-tests.

† Between-group change refers to the difference between donors and controls within-group change i.e. for potassium, within group change for donors is 0.1 meq/L and 0.0 meq/L for controls giving a between group-change of 0.1 meq/L. The 95% confidence interval was estimated using independent *t*-tests.

‡ Comparison between controls and donors was made for within-group change [i.e. mean change in potassium in donors (0.1 meq/L) vs mean change in weight in controls (0.0 meq/L)] using an independent samples *t*-tests.