Compartmental-modelling-based measurement of murine glomerular filtration rate using $^{18}$F-fluoride PET/CT

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Accurate measurement of glomerular filtration rate (GFR) is essential for optimal decision making in many clinical settings of renal failure. We aimed to show that GFR can be accurately measured using compartmental tracer kinetic analysis of $^{18}$F-fluoride dynamic PET/CT. Twenty-three male Sprague-Dawley rats of three experimental groups (cyclosporine-administered [n = 8], unilaterally nephrectomized [n = 8], and control [n = 7]) underwent simultaneous $^{18}$F-fluoride dynamic PET/CT and reference $^{51}$Cr-EDTA GFR ($\text{GFR}_{\text{creDTA}}$) test at day 0 and post-intervention day 3. $^{18}$F-fluoride PET GFR ($\text{GFR}_{\text{f-pet}}$) was calculated by multiplying the influx rate and functional kidney volume in a single-tissue-compartmental kinetic model. Within-test repeatability and between-test agreement were evaluated by intraclass correlation coefficient (ICC) and Bland-Altman analysis. In the control group, repeatability of $\text{GFR}_{\text{f-pet}}$ was excellent (ICC = 0.9901, repeatability coefficient = 12.5%). $\text{GFR}_{\text{f-pet}}$ significantly decreased in the renally impaired rats in accordance with respective $\text{GFR}_{\text{creDTA}}$ changes. in the pooled population, $\text{GFR}_{\text{f-pet}}$ agreed well with $\text{GFR}_{\text{creDTA}}$ with minimal bias (−2.4%) and narrow 95% limits of agreement (−25.0% to 20.1%). These data suggest that the single-compartmental kinetic analysis of $^{18}$F-fluoride dynamic PET/CT is an accurate method for GFR measurement. Further studies in humans are warranted.

The glomerular filtration rate (GFR) is a widely accepted measure of global renal function, and accurate measurement of GFR is essential for optimal decision making in many clinical settings of renal failure. The GFR has been typically measured as the urinary clearance of an ideal filtration marker such as inulin. Alternatively, plasma clearance of a filtration marker, such as $^{51}$Cr-ethylenediamine-tetraacetic acid (EDTA), has been advocated for GFR measurement because of its acceptable accuracy without the necessity for tricky urine handling. However, its drawbacks include the requirement for multiple blood samplings and a time-consuming procedure.

Nuclear medicine imaging techniques offer various means of GFR quantitation. Planar renal scintigraphy using $^{99m}$Tc-diethylenetriamine-pentaacetic acid (DTPA) can provide imaging-based estimation of GFR via Gates’ method. However, the GFR calculated from the Gates’ formula was reported to be less accurate than measured or estimated GFR, probably due to the potential errors in the correction of background and kidney depth, inherent limitations of two-dimensional images. Positron emission tomography (PET) enables dynamic 3-dimensional imaging of GFR.

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imaging, allowing accurate measurement of input function and tissue concentration of radiotracers, therefore has the potential for quantitative renal imaging. Several proof-of-concept studies produced promising results. 68Ga-1,4,7-triaza-cyclononane-1,4,7-triacetic acid (68Ga-NOTA) or 68Ga-EDTA have been investigated for GFR measurement but the results are yet to be validated. To date, there is no accepted methodological standard of PET for GFR measurement.

18F-fluoride is an established skeletal PET radiopharmaceutical, but it could also be used for renal imaging because fluoride is not bound to plasma protein and thus is freely filtered through glomeruli. However, fluoride clearance is always lower than GFR due to significant tubular reabsorption. Therefore, the previous 18F-fluoride dynamic PET/CT study reported a moderate correlation of fluoride clearance with a broad range of renal function parameters; the direct measurement of GFR was beyond the scope.

Compartmental tracer kinetic modelling enables the measurement of rate constants as parameters of important physiological processes in vivo. Dynamic PET is suited for this purpose due to its accurate and non-invasive quantification ability. We hypothesized that because the compartmental modelling allows the separate quantification of influx and efflux rates, we might be able to quantify GFR using 18F-fluoride influx rate despite the presence of tubular reabsorption. In this study, we showed that GFR could be accurately measured in rats via compartmental modelling of dynamic 18F-fluoride PET/CT. Neither urine handling nor blood sampling was necessary in this imaging-based approach. Validity of the compartmental model was independently tested by calculating GFR using dynamic PET/CT scans of 68Ga-NOTA.

Results

Within-test repeatability. The single-tissue-compartmental model provided excellent goodness-of-fit to the 18F-fluoride renal cortical time-activity curve (TAC) (median $R^2 = 0.9674$ [inter-quartile range (IQR) = 0.9538–0.9763]). The results of the parameter estimation are summarized in Table 1. The renal cortical volume $V_C$ between paired measurements was highly concordant (intraclass correlation coefficient [ICC] = 0.9846 [95% confidence interval (CI) = 0.9802–0.9946], repeatability coefficient = 3.1%), which suggests the reproducibility of the manual drawing of the volumes of interest (VOIs).

The repeatability of 18F-fluoride PET GFR ($\text{GFR}_{\text{PET}}$) was excellent (ICC = 0.9901 [95% CI = 0.9501–0.9982], repeatability coefficient = 12.5%), whereas the repeatability of 51Cr-EDTA GFR ($\text{GFR}_{\text{CrEDTA}}$) was slightly lower than that of $\text{GFR}_{\text{PET}}$ (ICC = 0.9372 [95% CI = 0.7155–0.9887], repeatability coefficient = 22.2%; Fig. 1).

Between-test agreement. $\text{GFR}_{\text{PET}}$ and $\text{GFR}_{\text{CrEDTA}}$ (Table 2) fell near the reported range of 51Cr-EDTA plasma clearance in rats (1.50–3.0 mL/min). Body surface areas (BSAs) of the rats were estimated as 413 ± 16 cm² (range = 380–455 cm²). The BSA-normalized $\text{GFR}_{\text{PET}}$ (range = 41.2–140.2 mL/min/1.73 m²) and $\text{GFR}_{\text{CrEDTA}}$ (range = 44.2–127.6 mL/min/1.73 m²) were well-matched with BSA-normalized human GFR.

Table 1. Model parameters. The figures are expressed as mean ± standard deviation. $V_C$, renal cortical volume; $v_B$, blood volume fraction; $K_I$, influx constant; $k_2$, efflux constant.

| Group          | Number of kidneys | $V_C$ (cm³)  | $v_B$       | $K_I$ (ml/cm³/min) | $k_2$ (min⁻¹) |
|----------------|-------------------|--------------|-------------|-------------------|---------------|
| Cyclosporine   | Baseline          | 1.022 ± 0.074| 0.111 ± 0.034| 1.109 ± 0.259     | 0.802 ± 0.179 |
|                | Post              | 1.021 ± 0.074| 0.125 ± 0.036| 0.978 ± 0.244     | 0.764 ± 0.215 |
| Nephrectomy    | Baseline          | 1.150 ± 0.072| 0.106 ± 0.027| 0.967 ± 0.175     | 0.783 ± 0.221 |
|                | Post              | 1.178 ± 0.057| 0.133 ± 0.039| 1.043 ± 0.100     | 0.958 ± 0.024 |
| Control        | 1st               | 1.067 ± 0.135| 0.093 ± 0.026| 1.009 ± 0.269     | 0.787 ± 0.215 |
|                | 2nd               | 1.065 ± 0.135| 0.105 ± 0.029| 1.043 ± 0.277     | 0.820 ± 0.215 |

Figure 1. Bland-Altman plots for repeatability of (A) $\text{GFR}_{\text{PET}}$ and (B) $\text{GFR}_{\text{CrEDTA}}$. The solid lines represent biases, and the dashed lines represent 95% limits of agreement. Difference (%) = $100 \times (\text{GFR}_{\text{1st}} - \text{GFR}_{\text{2nd}})/\left(\text{mean of }\text{GFR}_{\text{1st}} \text{ and }\text{GFR}_{\text{2nd}}\right)$.
The baseline GFR F-PET and GFR CrEDTA were not significantly different among the experimental groups (P = 0.830 and 0.686, respectively; Table 2). After cyclosporine intake or nephrectomy, GFRF-PET and GFRCrEDTA were significantly decreased (Supplementary Fig. 1), whereas in the control group, there was no such decrease (Supplementary Fig. 2). In each of the three groups, GFR F-PET and GFR CrEDTA were in good agreement (Supplementary Fig. 3). In the pooled population (46 measurements), GFR F-PET agreed well with GFR CrEDTA (ICC = 0.937 [95% CI = 0.889–0.965]), with minimal bias (−2.4% [relative difference]; −0.027 ml/min [absolute difference]) and narrow 95% limits of agreement (LOA) (−25.0% to 20.1% [relative difference]; −0.401 to 0.347 ml/min [absolute difference]) (Fig. 2, Supplementary Fig. 4). P30 and P10 (see Statistics in the Methods section) were 97.8% (45/46) and 60.9% (28/46), respectively. The accuracy statistics of the GFR F-PET were summarized in the Table 3.

GFRF-PET-15min showed almost perfect agreement with GFR F-PET (ICC = 0.998 [95% CI = 0.997–0.999], bias = 0.1%, and 95% LOA = −3.3% to 3.5%; Supplementary Fig. 3), which suggests that the two could be used interchangeably and therefore that imaging time could be shortened to 15 min without loss of accuracy.

**Dynamic 68Ga-NOTA PET/CT.** Overall, 68Ga-NOTA showed poorer goodness-of-fit (median $R^2$ = 0.5223 [IQR = 0.2295–0.6528] for the 20 kidneys) than did 18F-fluoride. The discrepancy between the model curve and kidney TAC was particularly large at later time points (>about 15–20 min). The goodness-of-fit was improved when only the first 15 min of data was used for fitting (median $R^2$ = 0.8557 [IQR = 0.8238–0.9001]). Thus, we used 68Ga-NOTA PET GFR using first 15 min of data (GFRNOTA-PET-15min) for the subsequent analysis.

| Subgroup   | GFR F-PET (ml/min) Baseline | Post | P   | GFR CrEDTA (ml/min) Baseline | Post | P   |
|------------|----------------------------|------|-----|-----------------------------|------|-----|
| Cyclosporine | 2.01 ± 0.43               | 1.73 ± 0.33 | 0.0113 | 2.08 ± 0.35               | 1.82 ± 0.38 | 0.0300 |
| Nephrectomy | 1.98 ± 0.34               | 1.06 ± 0.08 | 0.0001 | 1.97 ± 0.35               | 1.21 ± 0.07 | 0.0009 |
| Control    | 1.98 ± 0.69               | 2.01 ± 0.69 | 0.4415 | 1.93 ± 0.68               | 1.90 ± 0.62 | 0.7603 |

Table 2. GFR in subgroups.

| Group     | ICC  | 95% CI for ICC | Bias | LOA          | Bias | LOA          | $P_{30}$ | $P_{10}$ |
|-----------|------|----------------|------|--------------|------|--------------|---------|----------|
| Total     | 0.937| 0.889–0.965    | −2.4 | −25.0 to 20.1| −0.027| −0.401 to 0.347| 97.8 (45/46)| 60.9 (28/46) |
| Cys       | 0.898| 0.740–0.963    | −4.5 | −22.9 to 13.8| −0.080| −0.396 to 0.236| 100 (16/16)| 81.3 (13/16) |
| Nx        | 0.939| 0.839–0.978    | −6.5 | −28.5 to 15.5| −0.073| −0.395 to 0.248| 93.8 (15/16)| 50.0 (8/16)  |
| Control   | 0.941| 0.833–0.981    | 4.6  | −17.9 to 27.2| 0.086 | −0.329 to 0.502| 100 (14/14)| 57.1 (7/14)  |

Table 3. Accuracy statistics. ICC, intraclass correlation coefficient between 18F-fluoride PET GFR and 51Cr-EDTA GFR; CI, confidence interval; LOA, limits of agreement; Cys, cyclosporine; Nx, nephrectomy.
Because 68Ga-NOTA GFR calculation using whole-blood input function produced significant bias, conversion to plasma input function was essential (Supplementary Fig. 6A). After conversion using measured haematocrit, GFRCrEDTA showed a good agreement with GFRCrEDTA (ICC = 0.9664 [95% CI = 0.8787–0.9914]) with minimal bias (−2.4%) and narrow 95% LOA (−25.9% to 21.1%; Supplementary Fig. 6B). GFRCrEDTA using a fixed haematocrit of 0.45 showed far wider LOA (−46.8% to 55.5%) than those using measured haematocrit (Supplementary Fig. 6C).

Discussion

In this study, we developed a compartmental tracer kinetic model for PET-based GFR measurement and applied it to 18F-fluoride, which is not a GFR tracer under the conventional concept of urinary or plasma clearance measurement. According to the model, the influx rate $K_1$ can be considered as GFR per unit extravascular renal cortical volume for any tracer that is freely filtered through glomeruli but does not undergo tubular secretion. Previous reports suggest that 18F-fluoride has such properties. GFRF-PET was in good agreement with gold-standard GFRCrEDTA, in conditions of nephrotoxic drug use and post-nephrectomy with minimal bias and narrow LOA. P30 and P10 were 97.8% and 60.9%, respectively, which suggests that GFRF-PET possesses sufficient accuracy (P90 > 80% and P10 > 50%) compared with other GFR markers such as iohexol, iothalamate and DTPA. Furthermore, the accuracy of GFRF-PET was preserved with a reduction in imaging time to 15 min, which bears practical importance.

Good within-test repeatability is a prerequisite for assessing between-test agreement. The repeatability of GFRF-PET was excellent with repeatability coefficient (half-width of the LOA) of 12.6%. GFRCrEDTA measured in this study showed slightly poorer repeatability coefficient of 22.2%, which is somewhat large compared to the reproducibility figures previously reported in humans (7.4–9.0%)18. This might have been caused by technical difficulties of the small animal experiment. We speculate that the agreement between GFRF-PET and GFRCrEDTA might be even better in humans, considering the expected increase in the precision of GFRCrEDTA. To our knowledge, approaches of measuring GFR by using a compartmental rate constant have not been attempted in the field of nuclear medicine. In contrast, various types of compartmental modelling approach have been employed in magnetic resonance imaging (MRI) or CT studies. However, a critical literature review suggested that these MRI- or CT-based methodologies are not adequately accurate to be used as routine clinical or research tools19. Among the MRI-based methods, the cortical compartment model proposed by Annet et al. is similar to ours20. The differences are that Annet's method used two-dimensional regions of interest (ROIs) and abdominal aortic input function and that the dispersion and time delay from aorta to renal vasculature were accounted for. Many MRI-based methods use two-dimensional single-slice ROIs for better temporal resolution, and this acts as a limitation because a single slice or a slab cannot be representative of a whole kidney20–22. In this respect, the inherent 3-dimensional capability of PET is an advantage. The use of dispersion- and time-delay-corrected aortic input curves might be a merit of Annet's method in their rabbit experiment. However, we do not think that the non-correction for dispersion and time-delay caused any significant biases in our rat experiments because of smaller animal size. If this PET/CT analysis is implemented in humans, a proper selection of site for arterial input function measurement may become an important issue.

There may be a concern about the spill-out from the renal pelvic radioactivity into the renal cortical ROIs, considering small size of the rat kidneys. However, the scatter from the renal pelvic radioactivity turned out to be negligible compared with the renal cortical uptake. No significant amount of spill-out activity from the renal pelvis reached the renal cortical ROIs because the renal cortex and renal pelvis are intervened by the renal medulla and because the spatial resolution in terms of full-width half-maximum of the micro PET system used in our study was 0.7 mm that was much smaller than the thickness of the renal medulla (more than 3 mm).

We conducted another set of experiments using 68Ga-NOTA. The results also showed good agreement with GFRCrEDTA (Supplementary Fig. 6B). However, the goodness-of-fit to the 68Ga-NOTA data was not as good as that for 18F-fluoride. The cause of the poor fit is unclear. We speculate that the urination process might not follow first-order (exponential) kinetics and therefore that the process might not be appropriately described by an exponential rate constant $k_\text{e}$. For 68Ga-NOTA, the rate constant $k_\text{e} = k_3 + k_\text{reab}$ becomes $k_3$, because $k_\text{reab} = 0$, and according to the above speculation, $k_3$ also becomes an inappropriately modelled parameter. This could hamper the validity of the model equations. In contrast, 18F-fluoride is reabsorbed through the lipid bilayer of tubular cells via passive diffusion23, and passive diffusion follows first-order kinetics. The reabsorption of fluoride is approximately 60% of glomerular filtrate, but it could increase up to 90%11,12. This implies that $k_\text{reab}$ comprises a major portion of the efflux constant $k_2$, causing the efflux process to roughly follow first-order kinetics. Therefore, the model fit becomes better for 18F-fluoride, which would be a paradoxical advantage of nonzero reabsorption.

Measurement of haematocrit was essential for the calculation of 68Ga-NOTA plasma input function because the fixed plasma fraction produced imprecise GFR (Supplementary Fig. 6C). In contrast, a fixed plasma fraction of 1.23 produced accurate GFR for 18F-fluoride. It is likely that the plasma fraction of 18F-fluoride is relatively stable irrespective of haematocrit because 18F-fluoride permeates into the RBC24, whereas the plasma fraction of 68Ga-NOTA is more affected by haematocrit because 68Ga-NOTA cannot enter in the RBC. The high accuracy of GFRF-PET under a fixed plasma fraction is an advantage because haematocrit need not be measured, eliminating the need for blood sampling.

Given the high accuracy of the GFR measurement using dynamic 18F-fluoride PET, translational application to humans may be promising for appropriate indications. Using the expensive PET technology for GFR measurement could only be justified in clinical situations where accurate measurement of GFR is critically necessary. Such situations might include nephron-sparing surgery for malignant lesions in patients with marginal renal function, determination of overall and split renal function before abdominal radiotherapy, and monitoring of renal function during nephrotoxic drug use25.
The present study has limitations. First, the range of the measured GFR was not sufficiently wide. The normalized GFRcPET measured in this study fell within 41.2–140.2 mL/min/1.73 m2 BSA. Further validation is needed for low GFR values because chronic kidney disease stage grades 4 and 5 (GFR <30 mL/min/1.73 m2) were not included in the tested range26. Second, manual drawing of ROIs is too laborious for future clinical application. Automatic segmentation of renal cortex might have to be implemented.

In conclusion, dynamic 18F-fluoride PET/CT in conjunction with a single-compartmental modelling approach holds promise as a reliable and accurate method for GFR measurement. The difficulties in urine handling and blood sampling in the measurement of conventional urinary and plasma clearance of ideal filtration markers may be overcome by pure image-based analysis. A quick assessment of GFR (within 15 min) is another practical advantage of this approach. Further studies in humans are warranted.

Materials and Methods

Tracer kinetic modelling. The compartmental tracer kinetic modelling is a mathematical framework that originated from the field of pharmacokinetics and is a commonly used model for analysing PET data27. In the modelling, it is assumed that there are physiologically separate pools, or compartments, of a tracer substance27. Each compartment has its own influx and efflux rate constants, and the model fitting procedure allows to quantify them. We devised a compartmental tracer kinetic model in which the rate constant of a certain compartment could be interpreted as GFR.

In the model, extravascular renal cortex (EVRC), which contains Bowman’s capsule, the renal tubule, and the interstitium, serves as a functional kidney volume. A tracer enters the EVRC via glomerular filtration and tubular secretion and moves out via reabsorption and urinary outflow (Fig. 3A). The rate of change in the tracer amount within the EVRC can be described by the following equation:

\[
\frac{dA_{EC}(t)}{dt} = GFR \times C_p(t) + k_{secr} \times C_p(t) - k_u \times A_{EC}(t) - k_{reabs} \times A_{EC}(t)
\]

where \(A_{EC}(t)\) = tracer amount within EVRC, \(C_p(t)\) = tracer concentration in plasma, \(k_{secr}\) = rate constant of tubular secretion, \(k_u\) = rate constant of tracer loss due to urinary outflow from the cortex, and \(k_{reabs}\) = rate constant of tubular reabsorption.

Because no tubular secretion occurs for the 18F-fluoride11,12, \(k_{secr} = 0\) (Fig. 3A), the Equation (1) becomes as follows:

\[
\frac{dA_{EC}(t)}{dt} = GFR \times C_p(t) - (k_u + k_{reabs}) \times A_{EC}(t)
\]

(2)

Dividing the equation by EVRC volume \(V_{EC} = V_C \times (1 - vB)\) gives

\[
\frac{d}{dt}(A_{EC}(t)/V_{EC}) = GFR \times C_p(t) - (k_u + k_{reabs}) \times \frac{A_{EC}(t)}{V_{EC}}
\]

\[
\frac{dC_{EC}(t)}{dt} = K_1 \times C_p(t) - K_2 \times C_{EC}(t)
\]

(3)

where \(V_C\) = renal cortical volume, \(vB\) = vascular volume fraction, \(C_{EC}(t)\) = tracer concentration within the EVRC, \(K_1 = GFR/V_{EC}\) and \(K_2 = k_u + k_{reabs}\) (Fig. 3B).

The solution to Equation (3) can be expressed as follows:
\[
C_{EC}(t) = K_1 \times \int_0^t C_{p}(\tau) e^{-k_1(t-\tau)}d\tau = K_1 \times C_{p}(t) \otimes e^{-k_1 t}
\]

(4)

where \(\otimes\) = convolution integral.

The model function \(C_{model}(t)\) can be expressed as a superposition of \(C_{EC}(t)\) and \(C_{p}(t)\) according to their respective volume fractions in the kidney:

\[
C_{model}(t) = C_{EC}(t) \times (1 - vB) + C_{p}(t) \times vB
\]

The \(C_{model}(t)\) is fitted to the renal cortical TAC with \(K_1\), \(k_2\), and \(vB\) as fitting parameters. Single-kidney GFR is obtained by multiplying \(K_1\) and \(V_C \times (1 - vB)\), and total GFR is the sum of the GFR values of both kidneys.

We applied the above model to \(^{18}\text{F}\)-fluoride dynamic PET/CT to measure the GFR and compared the values with gold-standard \(^{51}\text{Cr}\)-EDTA GFR. Additionally, we tested the model using \(^{68}\text{Ga}\)-NOTA. \(^{68}\text{Ga}\)-NOTA was recently reported as a promising GFR tracer with no tubular reabsorption and secretion, and minimal binding to RBCs and serum protein\(^8\).

Radiopharmaceutical preparation. \(^{18}\text{F}\)-fluoride was produced by proton irradiation to the \(\text{H}_2^{18}\text{O}\) target using an in-house cyclotron (KOTRON-13, Samyoung Unitech). \(^{68}\text{Ga}\)-NOTA was produced by labelling NOTA (ChemaTech) with \(^{68}\text{Ga}\) eluted from a \(^{68}\text{Ge}/^{68}\text{Ga}\) generator (IGG100; Eckert & Ziegler) as previously described\(^8\).

Protocol of \(^{18}\text{F}\)-fluoride dynamic PET/CT Imaging and the \(^{51}\text{Cr}\)-EDTA Test. Imaging was performed from the thorax to the abdomen in the prone position on a dedicated small-animal PET/CT scanner (NanoScan micro PET/CT 122S; Mediso) under general anaesthesia through isoflurane inhalation (2–3% in 2–5 L/min of oxygen). In each PET/CT imaging sessions, \(^{18}\text{F}\)-fluoride (3.7 MBq/100 g rat weight in 200 \(\mu\text{L}\) solution) and \(^{51}\text{Cr}\)-EDTA (GE Healthcare; 0.19 MBq in 500 \(\mu\text{L}\) solution) were simultaneously injected via the tail vein after the acquisition of the contrast-enhanced CT scan. Immediately following the injection of the radiopharmaceuticals, dynamic \(^{18}\text{F}\)-fluoride PET images were obtained in the list mode for 60 min with varying frame durations (5 s \(\times\) 6, 10 s \(\times\) 3, 15 s \(\times\) 4, 30 s \(\times\) 16, 60 s \(\times\) 20, and 300 s \(\times\) 6) (please see the Supplementary Methods for PET/CT parameters for acquisition and reconstruction).

After the dynamic PET acquisition, at 60 and 100 min post \(^{51}\text{Cr}\)-EDTA injection, 1 mL of blood was withdrawn via tail-tip cutting (Fig. 4A). Following each blood withdrawal, 1 mL of saline was flushed to replenish the volume. Plasma samples obtained by centrifugation (3,000 rpm for 8 min) were divided into two aliquots for
duplication, and the radioactivity of the plasma aliquots was measured for 20 min using a well counter (Wizard 1480, Perkin Elmer) 24 h after the blood withdrawal to ensure full decay of the PET radiopharmaceuticals. The plasma clearance of $^{51}$Cr-EDTA was calculated from the mean values of the duplicate counts after background correction using the two-sample slope-intercept method\(^2\). The slope-intercept plasma clearance was corrected for neglecting the fast exponential in the bi-exponential plasma curve, generating the GFR$_{\text{CrEDTA}}$ (please see the Supplementary Methods for details)\(^2\).

Animal experiment protocol. For the $^{18}$F-fluoride PET/CT experiment, 23 male Sprague-Dawley rats (age: 8 weeks; weight: $280 \pm 12$ g) were used. The rats were divided into three experimental groups. Eight rats were administered with cyclosporine (Sandimmun INJ, Novartis) 30 mg/kg orally from day 0 to 2 to induce renal impairment medically. Another eight rats underwent left total nephrectomy at day 1 to form a surgical renal impairment group. The remaining seven rats were fed 1 mL/day olive oil from day 0 to 2 and served as controls. Each rat underwent two $^{18}$F-fluoride PET/CT imaging sessions at an interval of 3 days, at baseline (day 0) and after the renal impairment or control procedures (day 3) (Fig. 4B).

For the $^{68}$Ga-NOTA PET/CT experiment, 10 male naïve Sprague-Dawley rats ($334 \pm 52$ g) underwent dynamic PET/CT and a $^{51}$Cr-EDTA test. The experimental protocol was the same for the $^{68}$Ga-NOTA experiment, except for the haematocrit measurement (please see Supplementary Methods) and $^{68}$Ga-NOTA (3.7 MBq/100 g rat weight) injection.

Image analysis. We performed PET/CT data analysis and tracer kinetic modelling using PMOD software (version 3.8; PMOD Technologies). ROIs were manually drawn over the renal cortices on the coronal CT images (Fig. 5A), and the ROIs over the same kidney were integrated to form a VOI. A 3-mm-diameter spherical VOI was placed in the left ventricular cavity to obtain whole-blood input function (Fig. 5B). The ROIs was overlaid on the co-registered dynamic PET images to obtain renal cortical TACs (Fig. 5C). In order to convert whole-blood
input function to plasma input function, we adopted a fixed plasma fraction of 1.23 for $^{18}$F-fluoride because it permeates into RBCs with its intracellular concentration stable with about half in plasma. In contrast, we adopted a plasma fraction of 1/(1–hematocrit) for $^{68}$Ga-NOTA because it does not distribute into RBCs. To test whether the measurement of haematocrit is mandatory for the calculation of $^{68}$Ga-NOTA plasma input function, we calculated another set of plasma input functions by assuming a fixed haematocrit of 0.45.

The single-tissue-compartmental model curve using the plasma input function was fitted to the renal cortical TACs to obtain GFR$_{\text{PET}}$ and $^{68}$Ga-NOTA PET GFR (GFR$_{\text{NOTA-PET}}$) (Fig. 5D). Additionally, we calculated PET GFR only using the first 15 min of data (GFR$_{\text{PET-15min}}$ and GFR$_{\text{NOTA-PET-15min}}$) to test the feasibility of reducing imaging time.

Statistics. The goodness-of-fit of the model was assessed using the coefficient of determination ($R^2$). We used the control group data to test for repeatability. Within-test repeatability and between-test agreement were assessed by means of the ICC and the Bland–Altman analysis. Accuracy of GFR$_{\text{PET}}$ was expressed by P$_{30}$ and P$_{10}$, which are defined as the percentages of the measurements that lie within the ±30% and ±10% ranges from reference GFR$_{\text{EDTA}}$, respectively. The paired-samples t-test was performed to analyse the difference between paired observations. The Kruskal-Wallis test was performed for group comparisons. Two-sided $P < 0.05$ was considered as significant. All statistical tests were performed using MedCalc statistical software (version 18.5; MedCalc Software bvba).

Study approval. The rats were cared for in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. The study protocol was approved by the Institutional Animal Care and Use Committee of Seoul National University Bundang Hospital (IACUC No. BA1705-223/041-01). All experiments were performed in accordance with relevant guidelines and regulations.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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**Author Contributions**
W.L. and H.S.L. designed research. Y.K. and H.J. conducted experiments. H.S.L., Y.K., H.L., H.J. and B.M. acquired data. H.S.L., Y.K. and W.L. analysed data. S.B., D.C. and K.K. gave critique. H.S.L., Y.K., H.J. and W.L. wrote the manuscript.

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