Estimation of renal perfusion based on measurement of Rubidium-82 clearance by PET/CT scanning in healthy subjects

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Abstract

Background: Changes in renal blood flow (RBF) may play a pathophysiological role in hypertension and kidney disease. However, RBF determination in humans has proven difficult. We aimed to confirm the feasibility of RBF estimation based on positron emission tomography/computed tomography (PET/CT) and rubidium-82 ($^{82}$Rb) using the abdominal aorta as input function in a 1-tissue compartment model.

Methods: Eighteen healthy subjects underwent two dynamic $^{82}$Rb PET/CT scans in two different fields of view (FOV). FOV-A included the left ventricular blood pool (LVBP), the abdominal aorta (AA) and the majority of the kidneys. FOV-B included AA and the kidneys in their entirety. In FOV-A, an input function was derived from LVBP and from AA; in FOV-B from AA. 1-tissue compartmental modeling was performed using tissue time activity curves generated from volumes of interest contouring the kidneys, where the renal clearance of $^{82}$Rb is represented by the $K_1$ kinetic parameter. To investigate the correct interpretation of $K_1$, we assumed to first estimate effective renal plasma flow (ERPF) by extrapolating clearance values (ml/min/cm$^3$) to whole kidney values (ml/min) using the estimated total kidney volume. Thereafter, RPF was estimated from ERPF using an assumed extraction fraction (0.89). Lastly, RBF was estimated from RPF using measured haematocrit values. Intra-assay coefficients of variation and inter-observer variation were calculated.

Results: For both kidneys, $K_1$ values derived from AA did not differ significantly from values obtained from LVBP, neither were significant differences seen between AA in FOV-A and AA in FOV-B, nor between the right and left kidneys. For both kidneys, the intra-assay coefficients of variation were low (~ 5%) for both input functions. The measured $K_1$ of 2.04 ml/min/cm$^3$ suggests an estimated total renal perfusion normalized to body surface area of 628 ± 95 ml/min/1.73 m$^2$ and subsequently an estimated RBF of 1091 ± 162 ml/min/1.73 m$^2$. 
Conclusion: RBF estimation based on PET/CT and $^{82}\text{Rb}$ using AA as input function in a 1-tissue compartment model is feasible in a single FOV. The measured $K_1$ clearance values are most likely representative of ERPF rather than estimated RBF values.

Keywords: PET/CT, Rubidium-82, Pharmacokinetic modelling, Renal blood flow, Effective renal plasma flow

Background

Kidney disease and hypertension are major contributors to the overall global disease burden. In the pathogenesis of acute kidney injury (AKI), renal ischemia, as a result of a reduction in total RBF, has been accepted as a significant factor. However, recent studies suggest that renal hypoperfusion may play a less important role (1, 2). In fact, RBF measurements in sepsis-associated AKI have shown much discrepancy; reduced, normal, or even increased RBF have been reported (3-5). In patients with chronic kidney disease (CKD), RBF is reduced compared with controls (6, 7), possibly contributing to the progression of renal dysfunction. In renal circulation studies, most patients with essential hypertension display reduced RBF (8, 9); the greatest reduction demonstrated in malignant hypertension (9, 10). Additionally, renal vasoconstriction has been identified in pre-hypertensive adults, indicating that renal vascular abnormalities could be a cause of hypertension rather than caused by hypertension (11, 12).

Quantification of renal perfusion in humans has proven difficult. Current clearance-based methods estimating ERPF are time consuming and burdensome for patients (13-15) and alternative radiological imaging techniques assessing RBF, such as magnetic resonance imaging and ultrasonography, all have considerable limitations (7, 16) – none of which have been routinely implemented in clinical practise. Dynamic positron emission tomography (PET) using perfusion tracers is currently considered the most accurate, non-invasive method for determination of organ
perfusion. Thus, with good homogeneity and high perfusion rate, the kidneys are well suited for PET studies.

PET scans using $^{82}$Rb are routinely performed to assess myocardial blood flow in patients suspected of ischemic heart disease (17, 18). $^{82}$Rb is a potassium analogue with a short half-life of 75 seconds, produced in a generator by the radioactive decay of strontium-82 ($^{82}$Sr). Due to its high first-pass renal extraction (≈ 90%) and slow wash-out, $^{82}$Rb is well suited for mathematic modelling of RBF using dynamic PET-methods (19).

The first human $^{82}$Rb PET/CT study of renal perfusion showed high image quality, resolution and contrast, as well as demonstrated a high natural $^{82}$Rb renal uptake (20, 21). RBF was evaluated using a 1-tissue compartment model, where the $K_1$ parameter is presumed to represent estimated RBF.

Compartmental modelling requires an input function (IF) described by a blood-pool time activity curve (TAC). In quantification of myocardial blood flow, the LVBP has been validated as an image-derived input function (IDIF) (22, 23), obviating the need for arterial blood sampling.

However, the LVBP is not necessarily ideal for studying renal perfusion, as the LVBP and the kidneys in their entirety may not fit within a single limited axial scanner-FOV. This is especially true for older PET-scanners. In order, to ensure that an IF is estimated as correctly as possible, as well as to minimize radiation dose associated with the scanning, inclusion of the blood-pool and the kidneys in their entirety in the same FOV, is important. This can be accomplished if the AA can replace the LVBP as IF in the model, as suggested by Tahari et al. (21).

To determine whether this method is suitable for clinical, reliable assessment of RBF, this study further explores the substitution of AA as a valid alternative to LVBP by comparing the resulting $K_1$ values obtained from use of the two different IFs. Method precision was evaluated by determination of intra-assay coefficients of variation for both IFs. Furthermore, current literature assumes that the perfusion quantity measured using $^{82}$Rb is estimated RBF. Early investigations into the exchange
rates of radioactive potassium and rubidium between plasma and erythrocytes showed rates of ~ 2% per hour (24, 25), implying that initially, and hence during renal uptake studies, the majority of injected $^{82}$Rb will be almost exclusively present in plasma. We discuss and question whether flow values measured by $^{82}$Rb clearance are actually representative of RBF, or whether they should be interpreted as estimates of ERPF.

**Methods**

**Study design**

This study was performed as a randomized cross-over study (Fig. 1). During a period of approximately 45 minutes, each subject underwent four 8-minute dynamic $^{82}$Rb PET/CT scans in two different bed positions, A and B (FOV-A and FOV-B). In each bed position, duplicate scans were performed.

**Participants**

Healthy participants were recruited through advertisement, primarily at local educational institutions. Prior to inclusion, each participant completed a screening program. Screening consisted of a medical history; a clinical examination including measurements of weight, height, and blood pressure; electrocardiography as well as blood tests to determine electrolytes, creatinine, albumin, alanine aminotransferase (ALAT), leucocytes, haemoglobin, haematocrit and thrombocytes. Urine was screened for leucocytes, glucose, nitrite, ketones, and haemoglobin. In female subjects, pregnancy was ruled out. Inclusion criteria were: men and women aged 18-40 years with a body mass index (BMI) in the range 18.5-30.0 kg/m$^2$. Exclusion criteria were: medical treatment (except hormonal contraceptives); pregnancy or breastfeeding; smoking; substance abuse; alcohol consumption >14 units$^1$ per week for men and >7 units per week for women; signs of clinically relevant kidney disease, heart disease, liver disease or endocrine disease in the history, clinical

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$^1$ Danish alcohol unit = 15 ml (12 g) pure alcohol.
examination, or the paraclinical tests; hypertension; neoplastic disease, and blood donation within 1 month of the examination day. Withdrawal criteria were development of exclusion criteria or withdrawal of consent.

4 Number of subjects

17 subjects are required to detect a 0.40 ml/min/cm$^3$ difference in RBF (standard deviation (SD) 0.38 ml/min/cm$^3$) for a 5% significance level and power of 80%. To allow for dropout, 20 subjects were included.

8 Pre-scan procedure

For 24 hours preceding the acquisition of PET/CT scans, fluid intake was standardized to 35 ml/kg body weight of still water, subjects maintained a free diet and were instructed to avoid strenuous exercise. Subjects arrived at 8 a.m. at the Department of Nuclear Medicine, Herning Hospital, Regional Hospital West Jutland, Denmark after an overnight fast. In female subjects, pregnancy was ruled out.

14 Radiopharmaceutical

On each day of examination, the $^{82}$Sr/$^{82}$Rb generator (Cardiogen-82; Bracco Diagnostics Inc., Monroe Township, NJ, USA) was quality checked according to approved guidelines (Bracco Diagnostics Inc.) including test for breakthrough of $^{82}$Sr/$^{85}$Sr. The generator was calibrated to deliver a dose of 555 MBq (15 mCi) $^{82}$Rb for each injection. $^{82}$Rb was administered as a bolus-injection using a pre-programmed pump and infusion system. The subjects received four doses in total.

PET/CT scanning

All PET/CT scans were performed on the same scanner (Siemens Biograph mCT; 64 slice-4R) with a 22 cm axial FOV. On each day of examination, the PET/CT scanner was quality checked and
calibrated according to system required procedures. A peripheral venous catheter (Venflon) was placed in a cubital vein for $^{82}$Rb injection. Subjects rested in a sitting position for approximately 30 minutes before voiding. They were then placed in a supine position in the PET/CT scanner with arms extended above the head and the generator infusion system connected to the Venflon. All subjects underwent two consecutive duplicate PET/CT scans. The duplicate scans were acquired in bed position A (FOV A), including the LVBP, the AA and as much of the kidneys as possible (Fig. 2a) and bed position B (FOV B), including the AA and the kidneys in their entirety (Fig. 2b). Computer generated randomization determined the acquisition sequence for the two FOVs for each participant. In each bed position, an initial planar scout image was acquired to determine positioning of the scanner over the required FOV. Following positioning, a low-dose CT scan was performed immediately followed by a bolus injection of 555 MBq $^{82}$Rb and a dynamic PET-scan in list-mode for 8 minutes synchronized with the start of injection (21). Sequentially, and 10 minutes after the first PET scan was initiated, a second dose of $^{82}$Rb was administered and a duplicate PET scan performed in list-mode for 8 minutes. The bed position was then shifted, and the procedure repeated for the second FOV.

Low-dose CT scans (25 mAs, 100 kV) were performed for attenuation correction purposes only. PET data were acquired in dynamic list-mode, which was re-binned using 32 frames (20×6 s, 5×12 s, 4×30 s and 3×60 s) and iteratively reconstructed (21 subsets, 2 iterations) using Siemens TrueX and time-of-flight reconstruction in a matrix of 128×128 (voxel size: 6.4 x 6.4 x 3.0 mm$^3$) and post-filtered with a 5.0 mm Gaussian filter to produce attenuation and decay corrected dynamic sequences. We found it unnecessary to correct for motion of the kidneys.

The effective radiation dose associated with the study was < 4 millisievert (mSv): each low-dose CT scan contributed 0.4 mSv and each 555 MBq bolus injection of $^{82}$Rb contributed with 1.26 $\mu$Sv/MBq (20).
Analysis of $^{82}$Rb PET/CT studies

A 1-tissue compartment model was used for flow estimation (21), as illustrated in Fig. 3. The $K_1$ parameter represents the renal clearance of $^{82}$Rb, where $K_1$ (ml/min/cm$^3$) is equal to the product of the blood flow component carrying the $^{82}$Rb (erythrocyte and/or plasma) and its extraction fraction (EF) in the kidneys. Due to $^{82}$Rb having a high first pass extraction (~90%) (19), its uptake rate $K_1$ will be closely related to, and hence can be used as, an estimate of flow (26). Compartmental modelling was performed using the PMOD software (PMOD Technologies Ltd., Zurich, Switzerland, version 4.01).

TACs were obtained by defining relevant volumes of interest (VOIs) in the various anatomical regions-of-interest (Fig. 4), with the LVBP and AA defining IFs for the kinetic modelling. The LVBP was defined in FOV-A using a limiting box and the hot-contour tool with a typical cut-off 45 – 60% of the maximum limiting box activity. Ensuring avoidance of surrounding activity in the right and left ventricular luminae, a background VOI was manually placed centrally in the left ventricular wall and defined on at least 10 contiguous slices. Partial-volume effect (PVE) and spill-over activity from the left ventricular wall was then corrected adopting the method described by Katoh et al. (27).

The AA-VOI was defined in both FOVs using a box (10×10×30 mm$^3$) placed in the lumen of the abdominal aorta cranially to the departure of the renal arteries. An aortic background VOI was defined within a limiting box around the AA-VOI by applying a cold-contour with typical cut-off 10 – 25% and excluding all structures not representing background activity.

Based on the formulation for PVE and spill-over of background activity correction in LVBP (27), AA activity can be similarly corrected using:

$$R_A(t) = \beta \cdot C_A(t) + (1 - \beta) \cdot Bg(t) \quad \text{(Eq. 1)}$$
where \( C_A(t) \) represents the corrected AA activity, \( R_A(t) \) is the measured AA activity, \( C_{Bg}(t) \) is the measured aortic background activity and \( \beta \) represents the recovery coefficient to be determined. To find the optimal \( \beta \)-value for AA activity curve correction, multiple choices for \( \beta \) in the range 0.5 – 1.0 were systematically tested with the optimal subject-specific \( \beta \)-value defined as the value resulting in the corrected AA peak-activity best approximating the \( \beta \)-corrected peak-activity for LVBP for each individual. The mean recovery coefficient was calculated as the average of all individually determined optimal \( \beta \)-values, after which the AA activity for each subject was corrected using both the subject-optimized \( \beta \)-value and the mean \( \beta \)-value.

Tissue-TACs for both kidneys were obtained using hot-contouring in both FOVs as described previously and \( K_1 \) values for each kidney obtained for both LVBP and AA IFs using the 1-tissue compartment model. A blood volume fraction of 10% was assumed to account for activity from the vascular space within the VOIs contouring the kidneys (28).

Kinetic analysis was performed independently by two observers; a medical resident (observer 1) and an experienced nuclear medical physician (observer 2).

**Renal blood flow estimation**

Assuming that, after intravenous injection and throughout the 8-minute duration of the study acquisition \( {^{82}}Rb \) is almost exclusively distributed in the plasma [24, 25], then ERPF can be estimated using the measured \( {^{82}}Rb \) clearance (\( K_1 \)) and the total kidney volume (\( V_{Total} \)) as determined by the renal contour volumes described above:

\[
ERPF = K_1 \cdot V_{Total} \quad \text{(Eq. 2)}
\]

RPF can be estimated using the assumed EF (\( \sim 0.89 \) [19]):

\[
RPF = \frac{ERPF}{EF} \quad \text{(Eq. 3)}
\]

Subsequently, estimated RBF can be calculated from estimated RPF using the haematocrit (Hct) as:
\[ RBF = \frac{RPF}{1 - Hct} \quad (\text{Eq. 4}) \]

Results for estimated RPF and RBF are normalized to body surface area (BSA) using the Dubois formula:

\[ BSA = 0.007184 \cdot \text{height}^{0.725} \cdot \text{weight}^{0.425} \quad (\text{Eq. 5}) \]

**Statistical analysis**

Statistical tests were performed using SPSS Statistics ver. 20 (IBM Corp., Armonk, NY, USA). For each subject, the result for \( K_1 \) was defined as the mean value of the two independent \( K_1 \) values determined for each FOV for both input functions. Values are presented as mean ± SD for all completing subjects. Paired sample t-testing was used for comparison of \( K_1 \) values obtained using LVBP and AA IFs, where \( p < 0.05 \) was considered statistically significant.

Intra-assay coefficients of variation were calculated for each kidney based on the duplicate \( K_1 \) determinations in each FOV. Inter-observer variability was assessed using the intra-class correlation coefficient (ICC) with 95% confidence interval (CI) (29).

**Results**

**Demographics**

The participation flow chart for the study is depicted in Fig. 5. Eighteen healthy subjects completed the study and had scans accepted for analysis. Clinical and biochemical characteristics are shown in Table 1.
Table 1: Clinical and biochemical characteristics (n=18)

| Characteristic                  | Value          |
|--------------------------------|----------------|
| Age (years)                    | 21 ± 4         |
| Gender (women/men)             | 7/11           |
| BMI (kg/m^2)                   | 24.1 ± 2.5     |
| Office SBP (mmHg)              | 127 ± 9        |
| Office DBP (mmHg)              | 73 ± 10        |
| Heart rate (beats/min)         | 71 ± 11        |
| P-alanine aminotransferase (U/L)| 26 ± 11        |
| P-sodium (mmol/L)              | 140 ± 2        |
| P-potassium (mmol/L)           | 3.7 ± 0.2      |
| P-albumin (g/L)                | 43 ± 3         |
| P-creatinine (µmol/L)          | 73 ± 13        |
| eGFR\textsubscript{MDRD} (mL/min/1.73m^2) | 118 ± 11 |
| B-hemoglobin (mmol/L)          | 9.0 ± 0.4      |
| B-leucocytes (x 10^9/L)        | 7.1 ± 1.9      |
| B-thrombocytes (x 10^9/L)      | 275 ± 48       |
| B-haematocrit                  | 0.42 ± 0.02    |

Data are presented as mean ± SD. BMI, Body Mass Index; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR\textsubscript{MDRD}, estimated glomerular filtration rate calculated using the Modification of diet in renal Disease Study equation; EVF, erythrocyte volume fraction

Recovery coefficient

The optimal recovery coefficient for correction of PVE and spill-over in the AA varied for all subjects with the mean optimal $\beta$-value $= 0.69 ± 0.09$ (range 0.53 – 0.80).

Input curves

Fig. 6 illustrates typical TACs generated from VOIs in the LVBP and the myocardium, as well as $\beta$-corrected activity in LVBP. For all curves, the activity peaks rapidly. However, whereas for LVBP the flow-peak is followed by a continuous decline, the myocardial activity plateaus around 1.0 minute post injection (p.i.) after the decline of the initial flow-peak.

Fig. 7 illustrates typical TACs generated from VOIs placed in the AA and the aortic background ($\beta$-corrected as well as uncorrected), with $\beta$-corrected LVBP activity included for comparison. As for
LVBP, AA activities rapidly reach their maximum peak followed by rapid declines, while the aortic background activity rises slowly until reaching a plateau between 0.5 and 3.0 minutes p.i. followed by a slow decline.

Uncorrected AA activity reaches a substantially lower peak than LVBP activity. By definition, AA activity corrected using the subject-specific recovery coefficient $\beta$ and $\beta$-corrected LVBP activity, reach similar peak maxima. For the shown subject, AA corrected activity using the mean $\beta$-value reaches a higher peak than the measured LVBP activity.

Fig. 8 shows typical TACs generated from VOIs in the $\beta$-corrected LVBP activity, the AA (corrected using the mean $\beta$-value) and within the kidney contours. The kidney uptake rises more slowly until reaching a plateau between 1.5 and 4.5 minutes p.i. followed by a slow decline.

**Renal clearance - measurement of $K_1$, estimation of ERPF and RBF**

High renal uptake of $^{82}$Rb was demonstrated with no discernible urinary activity (Fig. 9).

The caudal part of the kidneys was outside FOV-A in 5 out of the 18 completing subjects.

Measurements in FOV-B showed that $K_1$ for the excluded caudal sections did not differ from the global $K_1$ for the kidneys.

Table 2 presents the mean $K_1$ results for all IFs applied in the analysis. $K_1$ values using the uncorrected AA IF were significantly higher than those using LVBP, whereas $K_1$ derived from subject-specific $\beta$-corrected, and those from mean $\beta$-corrected AA IFs, were not significantly different from LVBP values. No significant difference was observed between left and right kidneys.
Table 2 Mean $K_1$ values for all investigated input functions

|                    | FOV-A | FOV-B |
|--------------------|-------|-------|
|                    |       |       |
| **β-corrected**    |       |       |
| LVBP               |       |       |
| Uncorrected        |       |       |
| AA                 |       |       |
| Subject-optimized  |       |       |
| β-value            |       |       |
|                    |       |       |
| **FOV-A**          |       |       |
| Right kidney       | 2.02 ± 0.31 | 2.91 ± 0.48$^a$ | 2.09 ± 0.35$^{bcd}$ | 2.86 ± 0.45$^a$ | 2.05 ± 0.33$^{cef}$ |
| Left kidney        | 2.00 ± 0.31 | 2.88 ± 0.46$^a$ | 2.06 ± 0.33$^{bcd}$ | 2.84 ± 0.42$^a$ | 2.03 ± 0.30$^{cef}$ |

Data are presented as mean ± SD. $K_1$ units are ml/min/cm$^3$. Paired t-test $^a$: $p < 0.001$ vs. $K_1$ values derived from LVBP. $^b$: $p < 0.001$ vs. $K_1$ values derived from uncorrected AA activity in FOV-A. $^c$: NS vs. $K_1$ values obtained from LVBP. $^d$: NS vs. $K_1$ values obtained from subject-specific β-corrected AA activity. $^e$: NS vs. $K_1$ values derived from uncorrected AA activity in FOV-B. $^f$: NS vs. $K_1$ values obtained from corrected AA activity using mean recovery coefficient (mean β-value) in FOVA. LVBP, left ventricular blood pool; AA, abdominal aorta; FOV-A, field of view A; FOV-B, field of view B. NS: Non-significant

Intra-assay coefficients of variation for the duplicate VOIs for each FOV were calculated for $K_1$ derived from LVBP and mean β-corrected AA IFs. As illustrated in Table 3, the intra-assay coefficients of variation were similar (~ 5%) for both IFs.

Table 3 Intra-assay coefficients of variation using β-corrected activity

|                   | LVBP Mean β-value | AA (FOV-A) Mean β-value | AA (FOV-B) Mean β-value |
|-------------------|-------------------|--------------------------|--------------------------|
| Right kidney      | 5.3               | 4.5                      | 4.7                      |
| Left kidney       | 5.3               | 4.2                      | 4.5                      |

Data are presented as percentages. LVBP, left ventricular blood pool; AA, abdominal aorta; FOV-A, field of view A; FOV-B, field of view B. AA are using the mean β-value as recovery coefficient.

Table 4 shows inter-observer variability when using LVBP and mean β-corrected AA IFs for the two FOVs. Using LVBP, ICC was indicative of moderate to excellent reliability for both kidneys.

For AA in FOV-A, ICC was suggestive of excellent reliability for the right kidney and good to excellent reliability for the left kidney. For AA in FOV-B, ICC was indicative of excellent reliability for both kidneys.
Table 4 Inter-observer variability using $\beta$-corrected activity

|               | LVBP | AA (FOV-A) | AA (FOV-B) |
|---------------|------|------------|------------|
| Right kidney  | 0.884 (0.717; 0.955) | 0.965 (0.909; 0.987) | 0.976 (0.936; 0.991) |
| Left kidney   | 0.889 (0.730; 0.957) | 0.948 (0.866; 0.980) | 0.965 (0.908; 0.987) |

Data are presented as ICC estimates with 95% confidence intervals in parentheses.

LVBP, left ventricular blood pool; AA, abdominal aorta; FOV-A, field of view A; FOV-B, field of view B. AA are using the mean $\beta$-value as recovery coefficient.

Under the assumptions described in Methods and using equations 2-4, total flow values were estimated (Table 5). Total RPF and RBF were estimated to be $628 \pm 95$ ml/min/1.73 m$^2$ and $1091 \pm 162$ ml/min/1.73 m$^2$, respectively.

Table 5 Estimation of RPF and RBF based on $^{82}$Rb clearance values ($K_1$) using mean $\beta$-corrected AA activity in FOV-B

| Average $K_1$ (ml/min/cm$^3$) | Total renal volume (cm$^3$) | Total ERPF (ml/min) | Total RPF (ml/min) | Average Hct | Total RBF (ml/min) |
|-------------------------------|------------------------------|---------------------|-------------------|-------------|---------------------|
| 2.04 ± 0.31                   | 296 ± 30                     | 603 ± 90            | 677 ± 102         | 0.42 ± 0.02  | 1176 ± 183          |

Data are presented as means ± SD. Total ERPF is calculated as the product of $K_1$ and total kidney volume. Total RPF is estimated from ERPF using an assumed EF of 0.89 [19]. ERPF, effective renal plasma flow; RPF, renal plasma flow; Hct, haematocrit; RBF, renal blood flow.

Discussion

This study confirms that RBF estimation based on $^{82}$Rb PET/CT using AA as the IF in a 1-tissue compartment model is feasible, as previously demonstrated by Tahari et al. (21). Additionally, our results support the use of the $\beta$-corrected AA-VOI in a single FOV as an alternative IF to the LVBP. The low intra-assay coefficients of variation are acceptable with good to excellent inter-observer reliability, thus allowing estimated RBF to be determined using single FOV assessment of the kidneys in their entirety. However as discussed below, we believe the renal clearance of $^{82}$Rb ($K_1$), to represent ERPF, rather than direct estimation of RBF.

$^{82}$Rb as renal perfusion tracer
There are many advantages to using $^{82}$Rb PET/CT for measurement of renal perfusion: it is non-invasive and does not require blood sampling or urine collection, making the procedure less burdensome for patients; it allows for single kidney blood flow estimation and is readily available from $^{82}$Sr/$^{82}$Rb generators which are already in-situ at sites routinely using $^{82}$Rb for assessment of myocardial blood flow, thus making it cost effective. In comparison, the "ideal tracer" — $^{15}$O-water — can be utilized only in centres with on-site cyclotron access (30). The combination of a short $^{82}$Rb half-life of 75 seconds and short acquisition time allows for repeated scans of the same subject within a short timeframe, presenting unique opportunities to examine acute effects of differing drugs on renal perfusion. For example, $^{82}$Rb PET/CT may be especially suitable for use in cross-over studies exploring interventional effects.

No absolute contraindications exist to the use of $^{82}$Rb, thus patients suffering from all stages of AKI and CKD can undergo the examination without risk of deterioration of renal function.

Since renal $^{82}$Rb accumulation exceeds myocardial $^{82}$Rb accumulation, half the tracer dose of cardiac studies is sufficient to perform good quality renal imaging, resulting in a low effective radiation dose (~1 mSv) for a single scan of the kidneys in their entirety, including the AA for use as IF. Additionally, for modern digital scanners with high sensitivities, even lower tracer doses may be sufficient to perform the examination.
Input functions

Pharmacokinetic modelling requires an IF, where sampling of peripheral arterial blood to produce an arterial TAC is the gold standard method for obtaining an accurate estimation. However, the short half-life of $^{82}$Rb necessitates an alternative to the arterial sampling derived input curve. This can be achieved using image derived curves based on e.g. PET/CT scanning, where LVBP and AA are examples of IDIFs. Accurate IDIF estimation requires calibration of the $^{82}$Rb-tracer injector system and imaging scanner with associated dose calibrators to ensure accurate voxel activity concentrations in the acquired images.

We obtained IDIFs from TACs based on VOIs placed in both LVBP and AA in the dynamic PET images. Use of IDIFs based on large-size vascular structures, combined with the high resolution of modern PET scanners, reduces PVE in activity measurement (31, 32). However, due to the significant uptake of $^{82}$Rb activity in the left ventricular wall, LVBP was corrected for PVE and spill-over by applying the method of Katoh et. al, (27). It is unclear whether LVBP activity was corrected for PVE and spill-over by Tahari et al. (21), however our $K_1$ values obtained from $\beta$-corrected LVBP activity are comparable to their values. Likewise, the uncorrected activity in the AA is observed to be lower than LVBP activity in both studies. Tahari et. al. (21) assessed the effect to arise from PVE and as a first approximation, corrected by scaling AA activity to match the observed maximum LVBP activity. It is uncertain whether the lower AA activity is solely due to PVE, or whether other effects contribute to the observed peak differences. In this study we have adapted the methodology described by Katoh et. al (27), where the effects to be corrected for are combined in the recovery coefficient $\beta$. For isotopes with half-lives that allow for physical blood sampling and well-counter measurement, the blood activity levels can be determined for calculation of the $\beta$-coefficient; this is not possible for $^{82}$Rb.

Thus, estimation of $\beta$ was performed using systematic application of differing $\beta$-values to identify an optimal solution for each subject in the study, with the mean $\beta$-value for all subjects determined.
as $0.69 \pm 0.09$. For comparison, a simple scaling of AA activity with respect to the peak activity or area-under-curve ratios for AA and LVBP, gave estimates for correction factors which were almost identical to $\beta$ estimated using the method applied here. Furthermore, our $K_1$ results using $\beta$-corrected AA activity are comparable to those obtained from AA in the study by Tahari et al.

Overall, good agreement is observed between the estimated activity curves for both IFs and renal curves (Fig. 8) and our results for $K_1$ in this and Tahari's study (21).

For both AA and LVBP, the intra-assay coefficients of variation are acceptably low, indicating that $^{82}$Rb PET/CT is a precise method for evaluation of $K_1$, hence allowing for determination of changes in $K_1$.

The inter-observer variability assessment supports the use of AA as IF as a robust image-derived method for determining renal perfusion, with excellent reliability demonstrated for both kidneys in their entirety and AA in the same FOV (FOV-B) and good to excellent reliability for AA in FOV-A, compared to moderate to excellent reliability using LVBP.
Renal clearance - measurement of $K_1$, estimation of ERPF and RBF

High renal $^{82}\text{Rb}$ uptake and accumulation were confirmed. To avoid errors in uptake estimation caused by regional differences, it is important to measure uptake in the entire kidney. In our study, 13 out of 18 completing subjects (72%) showed both LVBP and the entire kidneys in FOV-A, such that 5 analyses were performed on truncated kidneys. In the article by Tahari et al., only 3 out of 8 subjects (38%) had the LVBP and kidneys in the same FOV (corresponding to FOV-A) and 5 out of 8 subjects had the LVBP and kidneys in separate acquisitions. As a global quality control, we found no significant difference between $K_1$ values derived from AA activity curves in FOV-A and those in FOV-B, supporting the assumption that in the studied population with healthy, lesion-free kidneys, quantitation obtained from truncated images of the kidney tissue is representative of values which would be obtained from imaging the kidneys in their entirety.

The accuracy of $^{82}\text{Rb}$ PET/CT for RBF estimation cannot be evaluated in the present study. Myocardial $^{82}\text{Rb}$ extraction is flow-dependent with a roll-off in extraction observed at increasing flow rates (33, 34). If this is also the case for renal extraction, $K_1$, and hence renal perfusion, may be underestimated; not only with respect to the absolute values, but also relatively compared to flow-values measured below the extraction roll-off point. As such, this requires further investigation, e.g. by comparison with RBF measurements using the non-flow-dependent tracer, $^{15}\text{O}$-water (30).

Using 1 cm$^3$ equivalence to 1 g of tissue, individual kidney volumes can be approximated from the volumes encompassed by the kidney VOIs, allowing conversion of $K_1$ values (ml/min/cm$^3$) to total flow values for both kidneys (ml/min). Total flow values are summarized in Table 5.

Our clearance values expressed as ERPF (Table 5) are low when compared to generally accepted values for RBF: ~1000-1200 ml/min (35); however, they are similar to previously published values for ERPF with values 420-640 ml/min (36, 37). This strongly suggests we may actually be estimating RPF and not RBF as is the current understanding.
Whether we measure estimated RBF or ERPF with $^{82}\text{Rb}$ depends on the distribution of the tracer between plasma and erythrocytes in whole blood. Early studies of potassium permeability showed a very slow exchange of radioactive potassium and rubidium between plasma and erythrocytes amounting to 1.8-2.1% per hour and even less over 8 minutes of study (24, 25). Hence, most $^{82}\text{Rb}$ is present in the plasma during renal uptake studies, implying the measured renal uptake values represent estimated RPF after correction for extraction, if EF is close to unity. Assuming our data represents RPF, estimated RBF can be calculated by correcting with the haematocrit value which is easily measured; the results of which are presented in Table 5.

For canines, EF is estimated to be 0.89 (0.80-0.95) (19), but to our knowledge remains to be determined in humans due to difficulty in calibrating and measuring blood activity for $^{82}\text{Rb}$. However, if we assume the extraction values to be similar for humans, we find an average total estimated RBF value normalized to BSA of $1091 \pm 162 \text{ ml/min/1.73 m}^2$, which is within the expected range for RBF in healthy subjects.

**Study strengths and limitations**

The major strengths of this study are a combination of the randomized cross-over design, the standardization of pre-scan conditions (fluid intake, exercise level, duration of fasting period), and the consecutive acquisition of the four $^{82}\text{Rb}$ PET/CT scans over a short 45-minute period; enabling optimal evaluation of intra-assay coefficients of variation and hereby precision.

The homogeneous study population consisted of healthy adults, providing estimated RBF measurements uninfluenced by age and medical therapy. However, this is also a potential bias as it is not certain results from this study can be directly applied to a population of elderly subjects, nor to subjects suffering from hypertension or renal disease; additional feasibility studies may be needed for these populations. Additionally, before $^{82}\text{Rb}$ PET/CT can be implemented for clinical estimated RBF determination, further evaluation is required of day-to-day variation as well as the
quantitative accuracy of the method; ideally by comparison of $K_1$ determined using $^{82}$Rb and $^{15}$O-water PET/CT.

**Conclusion**

The results presented in this study, for a population of healthy subjects, support the use of an AA IDIF in the 1-tissue compartment model as an alternative to LVBP; it is sufficient to determine estimated RBF using a single FOV including AA and kidneys in their entirety using a single dynamic $^{82}$Rb PET/CT scan. Use of AA gave rise to an acceptably low intra-assay coefficient of variation (~5%) and good to excellent inter-observer reliability.

Our data suggests that the actual flow values measured by $^{82}$Rb most likely represent ERPF rather than RBF which is essential for the correct interpretation of future perfusion studies using $^{82}$Rb.

**Abbreviations**

AA: abdominal aorta; AKI: Acute kidney injury; ALAT: Alanine aminotransferase; BMI: Body mass index; BSA: Body surface area; CI: Confidence interval; CKD: Chronic kidney disease; CT: Computed tomography; EF: Extraction fraction; ERPF: Effective renal plasma flow; FOV: Field of view; ICC: Intra-class correlation coefficient; IDIF: Image-derived input function; IF: Input function; LVBP: Left ventricular blood pool; MBq: Megabecquerel; mCi: Millicurie; mSv: MilliSievert; $^{15}$O water: oxygen-15 labeled water; PET: Positron emission tomography; p.i.: Post injection; PVE: Partial volume effect; $^{82}$Rb: Rubidium-82; RBF: Renal blood flow; $^{82}$Sr: Strontium-82; SD: Standard deviation; TAC: time activity curve; VOI: volume of interest.

**Declarations**

**Ethics approval and consent to participate**

The study was approved by the Regional Scientific Ethics Committee (journal number: 1-10-72-175-16), the Danish Medicines Agency (EudraCT-number: 2016-004080-39), the Danish Data
Protection Agency, and was conducted in agreement with the Declaration of Helsinki 2013.

Informed written consent was obtained from all participants.

**Consent for publication**

Informed consent was obtained from all participants regarding publishing of data.

**Availability of data and materials**

The datasets and trial protocol (Danish) are available from the corresponding author on reasonable request.

**Competing interest**

The authors declare that they have no competing interest.

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**Authors' contributions**

Study concept & design: all authors; Method implementation, creation & validation of analysis tools: JT, CF; Data acquisition: SSL; Data analysis: SSL, JT; Data interpretation: SSL, JT, CF; drafting of manuscript: SSL; Critical revision: SSL, JT, CF, JNB; Approval of final manuscript: all authors.

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Figure legends

**Fig. 1** Study design

**Fig. 2** Anatomical contents within the 22cm axial FOV for an example study image (subject 2): a) FOV-A: bed position includes LVBP, AA and kidneys (body length and kidney size determine whether the kidneys can be seen in their entirety within the FOV); b) FOV-B bed position includes AA and kidneys in their entirety
Fig. 3 I-tissue compartment model used for estimation of RBF. \( k_1 \) is the rate constant for \(^{82}\text{Rb} \) uptake in the kidneys from the vascular space, whereas \( k_2 \) is the rate constant for release of \(^{82}\text{Rb} \) back into the blood. No discernible tracer activity was observed via urinary excretion (21).

Fig. 4 VOIs were drawn in a) myocardium, b) left ventricular blood pool, c) abdominal aorta (orange) and aortic background (purple) and d) contouring the kidneys (green – right; cerise - left).

Fig. 5 Participant flow in the study.

Fig. 6 Typical time activity curves from the left ventricular blood pool and the myocardium from one of the subjects.

Fig. 7 a) Representative time activity curves from the various input organs and the aortic background from one of the study subjects; b) Magnification of activity peaks (highlighted red box), and c) Magnification of content in the highlighted blue box.

Fig. 8 Typical time activity curves from the left ventricular blood pool, abdominal aorta, and the kidneys from one of the study subjects.

Fig. 9 Typical example of a) coronal and b) transaxial PET/CT images of kidneys during maximal \(^{82}\text{Rb} \) uptake from one of the study subjects. Shown example is for FOV-B.
