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Feasibility and Impact of the Measurement of Extracellular Fluid Volume Simultaneous with GFR by ^{125}I-Iothalamate

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The feasibility, validity, and possible applications of the assessment of extracellular fluid volume (ECFV) simultaneous with glomerular filtration rate (GFR) were assessed in a series of validation studies using the constant infusion method of ^{125}I-iothalamate (IOT). In 48 subjects with a broad range of GFR, distribution volume (V_d) of IOT corresponded well with V_d bromide (16.71 ± 3.0 and 16.73 ± 3.2 l, respectively, not significant), with a strong correlation ($r = 0.933$, $P < 0.01$) and without systematic deviations. Reproducibility assessment in 25 healthy male subjects showed coefficients of variation of 8.6% of duplicate measurement of V_d IOT during strictly standardized (50 mmol Na^+/d) sodium intake. An increase in dietary sodium intake (200 mmol Na^+/d) induced a corresponding rise in V_d IOT of 1.11 ± 1.5 l ($P < 0.01$). In 158 healthy prospective kidney donors, the impact of indexing of GFR to ECFV was analyzed. Age, gender, height, and body surface area (BSA) were determinants of GFR. Whereas GFR, GFR/BSA, and GFR/height were gender-dependent, GFR/ECFV was gender-independent and not related to height or BSA. This supports the potential of normalizing GFR by ECFV. Thus, ECFV can be simultaneously assessed with GFR by the constant infusion method using IOT. After appropriate validation, also other GFR tracers could be used for such a simultaneous estimation, providing a valuable resource of data on ECFV in renal studies and, moreover, allowing GFR to be indexed to the body fluid compartment it clears: the ECFV.

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The gold standard for measuring glomerular filtration rate (GFR) is by specific tracers, such as inulin, Cr-EDTA, iothalamate, and iohexol (1). Because the distribution volume (V_d) of these tracers for GFR ideally equal extracellular fluid volume (ECFV) (2–9), measuring GFR with such tracers could potentially be used for simultaneous assessment of ECFV.

Simultaneously measuring ECFV and GFR has several advantages. First, it will allow better insight into the (patho)physiologic and clinical role of ECFV in renal disease and its complications, such as hypertension and left ventricular hypertrophy. Second, it has been proposed that the best way to normalize GFR between different individuals would be by ECFV rather than by body surface area (BSA) (6,10,11). Whereas normalizing GFR for ECFV would be attractive from a theoretical perspective, it has not gained acceptance in practice because it is considered too cumbersome (12). Validation of GFR measurement protocols for simultaneous assessment of ECFV would greatly increase the feasibility of normalizing GFR for ECFV.

Various GFR tracers were used for measuring ECFV (2–9), but their validation, reproducibility, and calibration against a gold standard for ECFV are not well documented. In our center, accurate GFR measurement is performed as the clearance of ^{125}I-iothalamate (IOT) by the constant infusion method, simultaneously with the determination of effective renal plasma flow (ERPF) (13). This renal function measurement is used in top-clinical care and for clinical research and allows estimating GFR with a day-to-day variability of only 2.5% (14). The aim of the current study was to validate this renal function protocol for assessing ECFV and to use the combined assessment for normalizing GFR to ECFV. To this purpose, we studied first, the agreement of V_d IOT with V_d of bromide, the golden standard for ECFV measurements, over a wide range of renal function. Second, we assessed the reproducibility of ECFV measurements by assessing V_d IOT under conditions of standardized sodium intake in healthy volunteers. Third, in these volunteers we tested the sensitivity of V_d IOT to detect a change in ECFV. Finally, we analyzed the impact of indexing GFR to ECFV in the above volunteers and in a cohort of 158 potential kidney donors.

Materials and Methods

All experiments were performed in adherence to the Declaration of Helsinki, approved by the Medical Ethics Committee of the University Medical Centre Groningen, and all participating subjects gave written informed consent for participation.

Measurement of Renal Function

Renal function measurements were performed using the constant infusion method with IOT and ^{131}I-Hippuran as described before (13–15). After drawing a time point-0 blood sample, a priming solution

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containing 20 ml infusion solution (0.04 MBq of IOT and 0.03 MBq of ^{131}I-Hippuran) plus an extra amount of 0.6 MBq of IOT is given at 08.00 h, followed by a constant infusion ranging from 6 ml/h in subjects with impaired renal function to 12 ml/h (based on previously known serum creatinine). Plasma concentrations of both tracers are allowed to stabilize during 1.5 h equilibration, which is followed by two 2-h periods for simultaneous clearances of IOT and ^{131}I-Hippuran. The latter are calculated as $(U^*V)/P_{iot}$ and $(I^*V)/P_{hipp}$, respectively. U*V represents urinary excretion of the tracer; I*V, the infusion rate of the tracer, which equals clearance from plasma during steady state; P, tracer values in plasma at the end of each clearance period. The plasma clearance $(I^*V)/P_{hipp}$ equals its urinary clearance because there is no extrarenal clearance of this tracer. Thus, when plasma levels are in steady state, ERPF equals $I^*V/P_{hipp.}$ GFR is calculated as the urinary clearance of IOT, corrected for voiding errors: $(U^*V/P)_{corr}$. As urinary clearance of ^{131}I-Hippuran equals plasma clearance in case of perfect urine collection, we routinely use the ratio of plasma-to-urinary clearance of ^{131}I-Hippuran to correct urinary clearance of IOT for voiding errors and dead space. By this method, coefficient of variation for GFR is 2.5% and for ERPF 5% (13).

Calculation of ECFV as V_d IOT

V_d IOT is calculated as: amount of IOT in the patient divided by P_{IOT} during steady state. The amount of IOT in the patient is calculated as: $[IOT_{infused} - IOT_{excreted}]$.

The latter is calculated as: [bolus + Sum (I*V)] − [urinary + extrarenal excretion of IOT]. Urinary excretion of IOT is measured as Sum (U*V), corrected for voiding errors as described above. Extrarenal excretion of IOT is calculated as described below.

Taken together, V_d IOT is calculated as:{[Bolus + Sum (I*V)] − [Sum (U*V) + extrarenal excretion]}/P.

Calculation and Validation of Extrarenal Clearance of IOT

IOT is not exclusively cleared by the kidney, as some biliary excretion occurs as well. The latter is negligible when renal function is normal but is considerable in patients with impaired renal function 13,14,16. To calculate V_d IOT as proposed above, extrarenal clearance has to be accounted for. In our setup, extrarenal clearance can be calculated from the difference between plasma and urinary clearance. Extrarenal excretion of IOT (% of input) is calculated during steady state as: [(I*V) − (U*V)]/(I*V) * 100%. Subsequently, total extrarenal excretion is calculated as: %ER excretion* [bolus + Sum (I*V)]

In the experiment calibrating V_d IOT against V_d bromide, we validated this calculation of extrarenal clearance of IOT.

Calibration of V_d IOT against V_d bromide

A total of 24 males and 24 females (age, 49 ± 13 yr; median with range of GFR was 54 [17 to 133] ml/min per 1.73 m^2 with 8 subjects with a GFR <30 ml/min per 1.73 m^2, 22 subjects with a GFR between 30 and 60 ml/min per 1.73 m^2; and 18 subjects with a GFR >60 ml/min per 1.73 m^2), routinely visiting our Medical Centre for renal function measurements were included in this experiment. To include subjects with a wide GFR range, we included potential kidney donors (n = 13), renal transplant recipients (n = 32), and subjects with chronic renal disease (n = 3). Renal function and V_d IOT was assessed as described above. First, we tested whether a steady state was reached by comparing the serum IOT levels on the different time points. Second, to validate the calculation of extrarenal clearance of IOT, 31 subjects (median GFR, 52 ml/min per 1.73 m^2; range, 17 to 127 ml/min per 1.73 m^2; age, 52 ± 12 yr, 68% males, 7 potential kidney donors and 24 patients) collected urine during 24 h directly after iothalamate infusion and recovery of IOT was calculated.

On the same day as assessing V_d IOT, subjects received oral NaBr in an approximate dose of 50 mg/kg. Blood samples were, simultaneously to blood samples for IOT, drawn after 4.5 and 5.5 h and V_d bromide was calculated (17) as: Br dose/$[Br]_{serum} \times 0.90 \times 0.95 \times 0.94$.

In the latter formula, 0.90 is the fraction of bromide that is assumed to be distributed in nonextracellular sites (principally erythrocytes), 0.95 is the Donnan equilibrium factor, and 0.94 is the assumed amount of water in serum.

The ECFVs assessed by V_d IOT and V_d bromide were compared by investigating agreement between the two measures as recommended by Bland and Altman (18) with calculation of 95% limits of agreement as mean ± 2 SD of the difference between V_d IOT and V_d bromide.

Reproducibility and Sensitivity to Detect Changes in EFCV over Time

A total of 25 healthy normotensive men (age, 22.7 ± 2.6 yr) were studied. Renal function and ECFV were measured four times, on day 7 and day 14 of two 14-d study periods, with a washout of at least 3 wk in between. Each study period consisted of two 7-d periods with a different dietary sodium content, *i.e.*, low sodium (50 mmol Na^+/d) and high sodium diet (200 mmol Na^+/d), in randomized order. Potassium intake was standardized at 80 mmol/d. Otherwise, the subjects continued their usual food habits. For assessment of dietary compliance, 24-h urine was collected at day 6 during each 7-d period. During all periods, subjects were ambulant and continued normal activities.

During study days, subjects reported at the research unit at 8:00 h, after having abstained from food and alcohol overnight. Height and body weight were measured, after which renal function and V_d IOT was assessed.

Reproducibility was assessed by examining repeated measurements under the same sodium intake for bias and calculating the coefficient of variation (COV). Bias was investigated by examining the mean difference between repeated estimates. The COV was calculated as the within-subject variation expressed as a percentage of the sample mean.

The averaged value of the duplicate measurements was further used to assess the sodium-induced change in ECFV. Additionally, creatinine-based renal function was calculated according to the simplified MDRD formula (19) and 24-h creatinine clearance. Creatinine in blood and urine were determined by Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, NY), which was validated according to the Cleveland protocol.

Between-subjects Normalization of GFR by ECFV

We assessed the impact of normalization of GFR by ECFV in 158 healthy subjects screened as potential kidney donors (Table 1). In this population, we assessed the impact of normalization by ECFV on the difference in renal function between men and women. BSA was calculated according to DuBois and DuBois (20). Serum creatinine based renal function was calculated according to the simplified MDRD formula (19). To analyze the separate contributors to differences in GFR, we performed linear regression, with, respectively, GFR, GFR per 1.73 m^2 BSA, GFR/height, and GFR/ECFV as independent variables, and age and gender as dependent variables. BSA or height was added as independent variable when appropriate. GFR, GFR per 1.73 m^2 BSA, and GFR/height are expressed in ml/min, ml/min per 1.73 m^2 BSA, and ml/min per m, respectively. GFR/ECFV is expressed as $\%*h^{-1}$, corresponding to the % of the ECFV that is cleared per hour. This unit follows from dividing ml/min (GFR) by ml (ECFV) * 60 (transforming min^{-1} in h^{-1}) and * 100 (expression in %).

Table 1. Characteristics of potential donor population ($n = 158$)

	Potential kidney donors	
	Male ($n = 73$)	Female ($n = 85$)
Age (yr)	51 ± 11	52 ± 10
Body weight (kg)	91 ± 13	73 ± 11*
Height (cm)	183 ± 7	169 ± 6*
BSA (m^2)	2.07 ± 0.22	1.80 ± 0.20*
GFR (ml/min)	132 ± 30	105 ± 20*
ECFV as V_d IOT (L)	22.9 ± 4.6	17.4 ± 2.5*
GFR/ECFV (%/h)	35.0 ± 6.3	36.4 ± 6.0
ECFV/GFR (h)	2.9 ± 0.6	2.8 ± 0.5

*$P < 0.01$, male *versus* female.

Data Analyses

Normally distributed data are expressed as mean ± SD in text and tables and plotted as mean ± SEM in figures. T test or paired t test was used for comparing means, and correlations were assessed as Pearson's correlation coefficient. Analysis of variance was used to test whether steady-state levels of IOT were reached. Skewed distributed data were expressed as median with range; with subsequently using the Kruskal-Wallis test for subgroup comparisons. For analyzing the determinants of GFR, GFR/BSA, GFR/height, and GFR/ECFV, we built linear regression models with the mentioned variables as dependent variables and, when appropriate, adding age, gender, BSA, and height as independent variables by forward regression method. All analyses were performed using the Statistical Package SPSS 14.0.

Results

Calibration of V_d IOT against V_d bromide

In the experiments comparing V_d IOT and V_d bromide, the GFR of the 48 included subjects ranged from 20 to 147 ml/min (mean, 79 ± 41 ml/min). After equilibration time, serum levels of IOT were 279 ± 67, 279 ± 76, 276 ± 83, 280 ± 90, and 284 ± 97 counts/ml per min (analysis of variance, $P > 0.9$), respectively, at 1.5, 2.5, 3.5, 4.5, and 5.5 hours after start of the protocol. Thus, plasma levels of IOT were in steady state, a prerequisite for calculation of ECFV during the constant infusion method. To validate the algorithm we used for calculation of extrarenal clearance of IOT, we measured urinary recovery of IOT in 24-h urine collected during the 24 h following IOT infusion. Urinary recovery of IOT (% of input) was strongly and negatively correlated with calculated extrarenal clearance ($r = -0.80$, $P < 0.001$). Mean total urinary recovery was 87% ± 8%, mirrored by a calculated extrarenal clearance of 14% ± 12% of total input, supporting the validity of our calculation of extrarenal clearance of IOT.

In Figure 1, the results of the simultaneous assessments of V_d IOT and V_d bromide are shown. The ECFV obtained as V_d bromide was 16.73 ± 3.15 l, and the ECFV obtained as V_d IOT was 16.71 ± 2.96 l (not significant). V_d IOT and V_d bromide were strongly correlated (upper panel, $r = 0.933$, $P < 0.001$). In the Bland-Altman plot (lower panel), no systematic error was apparent, with 95% limits of agreement ranging from −2.3 to 2.2 l. Similar results were obtained in the 30 subjects in which GFR was below 75 ml/min, *i.e.*, subjects with a substantial extrarenal clearance of IOT, demonstrating the adequacy of our correction for extrarenal clearance of IOT.

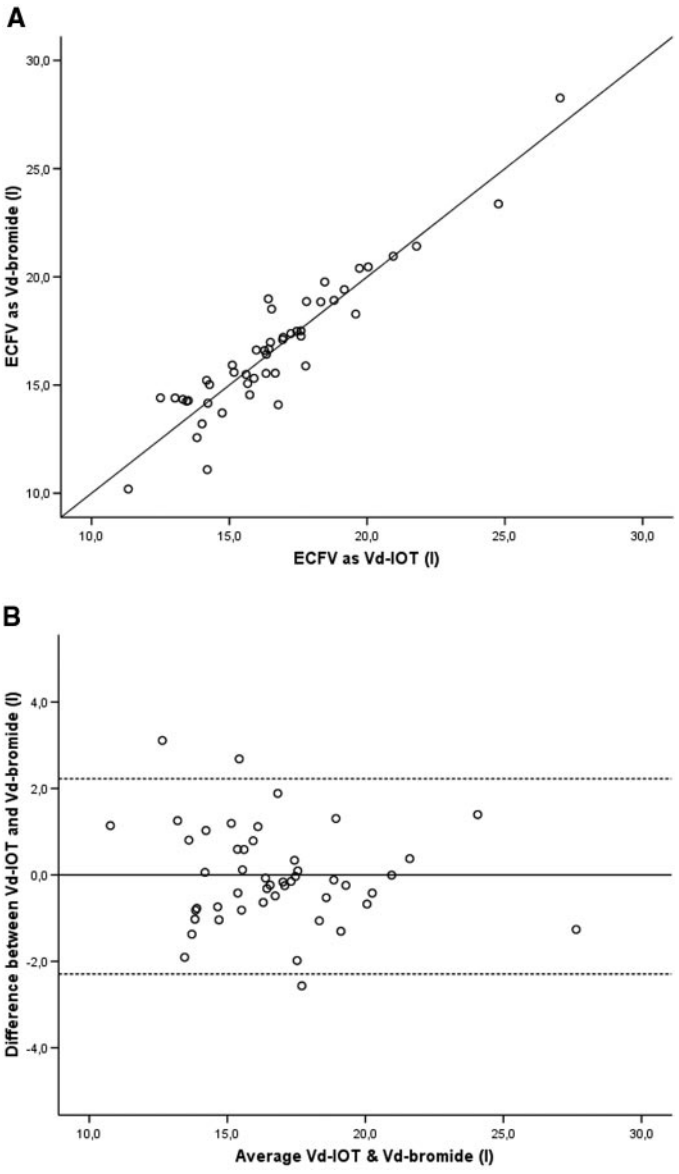

Figure 1. (A) Scatterplot of extracellular volume (ECFV) simultaneously obtained as distribution volume (V_d) of bromide (y-axis) and V_d of ^{125}I-iothalamate (IOT) (x-axis) with line of identity. (B) Bland-Altman plot for V_d bromide and V_d IOT with 95% limits of agreement.

Reproducibility and Sensitivity to Detect Changes in ECFV

Data from the reproducibility experiment are given in Table 2, showing the duplicate measurements on low (50 mmol Na^+/d) and high sodium (200 mmol Na^+/d). Dietary compliance was good during all four periods, as shown by 24-h urinary sodium excretion. ECFV, body weight, and GFR were virtually identical during the duplicate measurements on low and high sodium, respectively. Reproducibility of the ECFV

Table 2. Measurements in healthy young men ($n = 25$) on four separate occasions

	Low sodium diet		High sodium diet	
	Period 1	Period 2	Period 1	Period 2
Na^+ excretion (mmol/24 h)	35 ± 22	39 ± 16	250 ± 68	251 ± 65
Body weight (kg)	80.1 ± 11.0	80.2 ± 11.0	81.8 ± 11.2	81.7 ± 11.3
GFR (ml/min)	127 ± 20	129 ± 18	138 ± 21	137 ± 20
ECFV as V_d IOT (L)	19.7 ± 2.7	20.0 ± 2.4	20.8 ± 2.8	21.1 ± 3.2
MAP (mmHg)	85 ± 6	86 ± 8	87 ± 6	87 ± 7

measurement was assessed separately for the two low-sodium periods and the two high-sodium periods. During low sodium, the mean difference in ECFV (bias) was 1.3%, with a COV of 8.6%. During high sodium, bias was 1.1% with a COV of 13.1%.

In both periods, as anticipated, the shift from low- to high-sodium intake resulted in a rise in ECFV (period 1, 1.09 ± 2.2 l, $P < 0.05$; period 2, 1.13 ± 2.0 l, $P < 0.01$) without statistical differences between the two periods. The mean rise in ECFV induced by high sodium was 1.11 ± 1.49 l ($P < 0.01$), corresponding with a mean rise in body weight of 1.60 ± 0.85 kg ($P < 0.01$), as indicated in Figure 2. The individual changes in body weight were significantly and positively correlated to the individual changes in ECFV ($r = 0.552$, $P < 0.01$).

GFR increased significantly from low to high sodium (128 ± 18 *versus* 137 ± 18 ml/min; $P < 0.01$, Figure 3). The sodium-induced rise in renal function was also significant for GFR/BSA (109 ± 13 *versus* 116 ± 12 ml/min per 1.73 m^2, $P < 0.01$), as well as for GFR/BSA estimated by the MDRD formula (89 ± 12 *versus* 94 ± 12 ml/min per 1.73 m^2, $P < 0.01$) and for 24-h creatinine clearance/BSA (94 ± 20 *versus* 106 ± 21 ml/min per 1.73 m^2, $P < 0.01$). However, renal function expressed as GFR/ECFV remained unchanged during the shift from low to high sodium: 39.0 ± 3.6%*h^{-1} and 39.6 ± 4.2%*h^{-1} (not significant) as the rise in GFR was matched by a corresponding rise in ECFV.

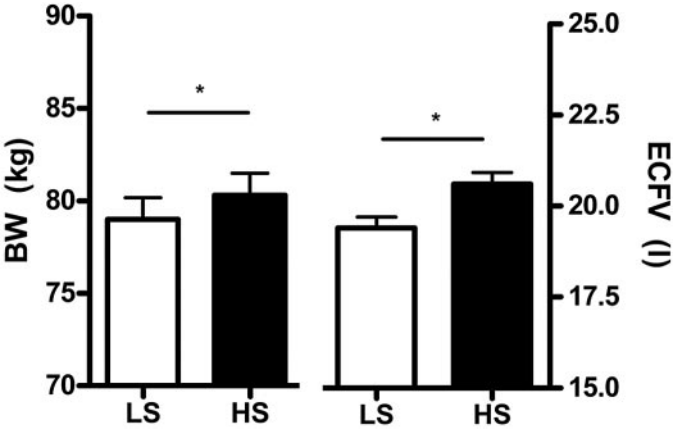

Figure 2. Mean ± SEM for body weight and extracellular volume (ECFV), respectively, in subjects ($n = 25$) in balance on low sodium (LS) and high sodium (HS). *$P < 0.001$.

Between-subject Normalization of GFR by ECFV

To address the impact of expressing renal function as GFR/ECFV, we analyzed data from 158 potential kidney donors. Their characteristics are given in Table 1. We analyzed for the determinants of GFR, GFR/BSA, GFR/height, and GFR/ECFV by multivariate modeling and found the models shown in Table 3. As shown in the left column, age, BSA, and gender are all independent determinants of GFR. Second, in line with the first model, age and gender are both independent determinants of GFR/BSA, and of GFR/height. However, when GFR is indexed to ECFV, only age is an independent determinant for GFR. Thus, in healthy subjects, indexing to ECFV appears to nullify gender and BSA- and height-related differences in GFR.

The impact of the various ways of indexing on the differences in renal function between men and women is illustrated in Figure 4. Uncorrected GFR was higher in men (130 ± 28 *versus* 102 ± 25 ml/min, $P < 0.01$). Body weight and height, and consequently BSA, were higher in men (Table 1), but indexing to BSA did not nullify differences in GFR between men and women (GFR/BSA 105 ± 20 *versus* 96 ± 22 ml/min per 1.73 m^2, $P < 0.01$), and neither did indexing to height (GFR/height 72 ± 15 for men *versus* 62 ± 11 ml/min per m for women, $P < 0.01$). The same was true for GFR estimated by the MDRD (78 ± 12 and 67 ± 9 ml/min per 1.73 m^2, $P < 0.01$). However, GFR indexed to ECFV was similar for men and women, being 35% ± 6%/h and 36% ± 6%/h (not significant). The variance of GFR indexed to ECFV in this healthy population is demonstrated in Figure 5, the average value being 36%/h with a 95% confidence interval of 24% to 48%/h.

Additionally, we expressed renal function as GFR/ECFV in %/h and as ECFV/GFR (h) in the 48 subjects studied in the calibration experiment, dividing these subjects based on GFR/BSA (respectively, a GFR <30, 30 to 60, and >60 ml/min per 1.73 m^2). Median (range) GFR/BSA was 23 (17 to 29), 49 (32 to 59), and 102 (60 to 133) ml/min per 1.73 m^2, respectively ($P < 0.01$). Median (range) GFR/ECFV was 10 (7 to 13), 22 (17 to 25), and 41 (20 to 60) %/h, respectively ($P < 0.01$ between subgroups). Finally, median (range) ECFV/GFR was 9.6 (7.6 to 14.4), 4.6 (4.0 to 6.0), and 2.4 (1.7 to 5.0) h, respectively ($P < 0.01$ between subgroups). These values show that the proportion of the ECFV cleared per unit of time increases with increasing GFR; conversely, the time required to clear the whole ECFV decreases.

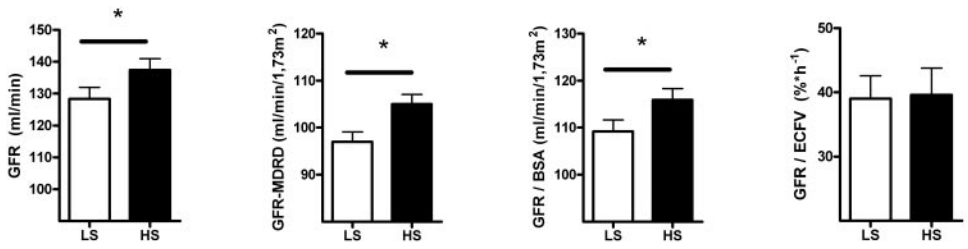

Figure 3. Within-individual values: population of healthy young men ($n = 25$), shown for subjects on low sodium diet (LS) and high sodium diet (HS), respectively. Mean GFR ± SEM for raw data, MDRD, indexed for BSA and indexed for ECFV. *$P < 0.01$, paired analyses.

Table 3. Linear regression models with glomerular filtration rate (GFR) (r of model = 0.702, $P < 0.001$), GFR/body surface area (BSA; $r = 0.518$, $P < 0.01$), GFR/height ($r = 0.580$, $P < 0.01$), and GFR/extracellular volume (ECFV; $r = 0.568$, $P < 0.01$) as the dependent variable

	GFR		GFR/BSA		GFR/h		GFR/ECFV	
	β	P	β	P	β	P	β	P
Age	−0.412	<0.01	−0.485	<0.01	−0.465	<0.01	−0.536	<0.01
Gender	−0.280	<0.01	−0.181	<0.01	−0.328	<0.01	0.058	NS
BSA	0.329	<0.01	—	—	—	—	−0.146	NS
Height*	0.342	<0.01	—	—	—	—	−0.092	NS

NS, not significant; —, not applicable.
*Height is replacing BSA in the models; all other β values are given for the models including BSA.

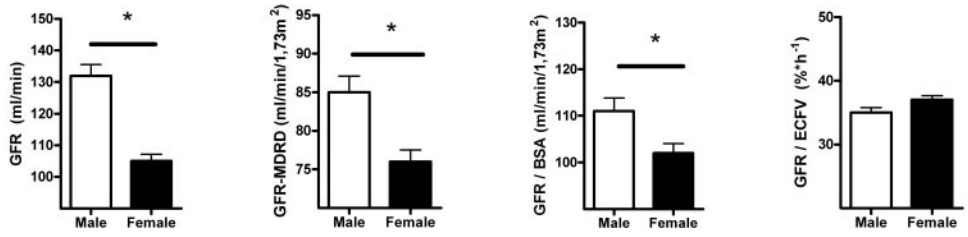

Figure 4. Between-individual values: population of potential kidney donors shown by a breakdown by men ($n = 73$) and women ($n = 85$). Mean GFR ± SEM for raw data, MDRD, indexed for BSA and indexed for ECFV. *$P < 0.01$.

Discussion

Our study demonstrates the validity and reproducibility of assessing ECFV simultaneously with GFR by the constant infusion method with IOT. This provides not only a useful tool for pathophysiologic studies on ECFV in renal conditions but also allows the indexing of GFR to ECFV without need for additional procedures or parameters. As the V_d of other GFR tracers also equals ECFV, renal function protocols with other tracers could similarly be validated for simultaneous assessment of ECFV. This will increase the diagnostic yield of renal function measurements by specific tracers.

This is the first study to validate V_d IOT as a measure for ECFV in human subjects. V_d IOT was in good agreement with V_d bromide as a gold standard for ECFV over a wide range of renal function. Moreover, the day-to-day variation of V_d IOT was low, at least during standardized sodium intakes in healthy subjects, *i.e.*, conditions in which the biologic variation in ECFV is low. Finally, a change in ECFV induced by a shift in sodium intake could be adequately detected by the assessment of V_d IOT.

Other GFR tracers, such as inulin, ^{99}mTc-DTPA, ^{51}Cr-EDTA, and iohexol (2–9), have also been used for simultaneous assessment of GFR and ECFV. ECFV assessed as V_d of ^{51}Cr-EDTA had a day-to-day variation of 11.4% (7), comparable to the reproducibility of V_d IOT in our study. Most studies report that ECFV can be adequately assessed by the GFR tracer studied, without however calibrating V_d of the tracer against a gold standard for ECFV (4,6–8). V_d bromide is considered the gold standard for ECFV assessment because its tissue content and serum distribution are well documented (21). Only one small study, in 10 healthy subjects, calibrated tracer V_d against V_d bromide. In this study, V_d iohexol correlated well with V_d bromide, but V_d iohexol was on average 0.7 L lower than V_d bromide (2). For use in renal populations, however, the calibration in subjects with renal function impairment is needed, as renal function impairment can affect several fac-

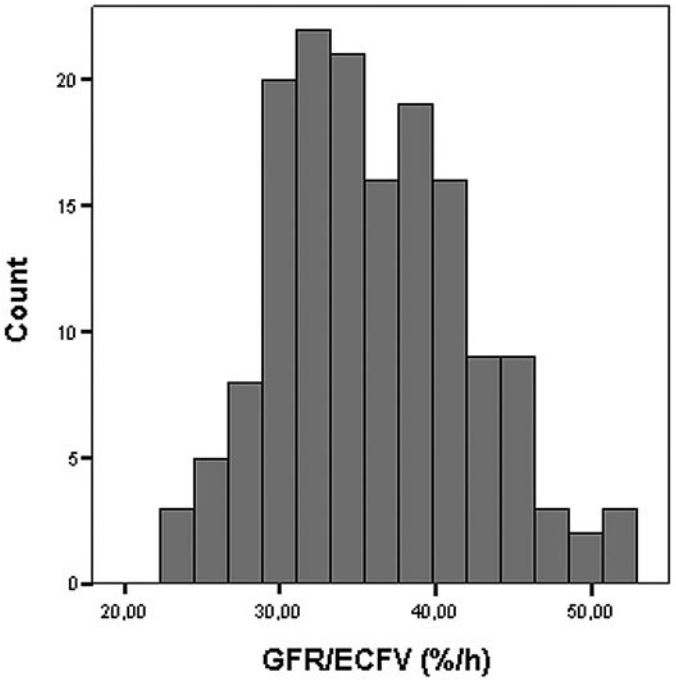

Figure 5. Histogram of GFR/ECFV values in the population of 158 potential kidney donors. Number of subjects given for categories of GFR/ECFV expressed in %/h.

tors relevant to the validity of the ECFV assessment, such as steady-state kinetics and possible extrarenal clearance. Our study is by far the largest to provide calibration against V_d bromide and moreover is the only to include subjects with renal function impairment, thus allowing conclusions on the use of combined measurements of GFR and ECFV in renal populations.

The clearance of specific tracers, such as iothalamate, iohexol, or Cr-EDTA, provides the gold standard for GFR measurement. Because such measurements are relatively laborious and expensive, their use is mainly limited to specialized nephrologic settings, such as screening of potential kidney donors, and research applications. Our data show that the yield of GFR measurements can be increased by additionally providing an estimate of ECFV. Because abnormalities in volume status are important in renal disease and its complications, this might prove a valuable tool for future studies.

Body dimensions differ between individuals. GFR is usually indexed to account for differences in body dimension, generally to BSA (22). However, the use of BSA for indexing has been criticized by several authors (23–25). Indexing to height or ECFV has been recommended, but neither gained broad acceptance despite studies supporting their superiority over indexing to BSA (11,26,27). In our study, GFR was gender dependent. This is in line with data by Turner and Reilly, showing a difference in GFR per 1.73 m^2 BSA between men and women, which could be traced back to a fallacy of indexing for BSA (27). The difference in renal function between men and women, as found for GFR per 1.73 m^2 BSA, was annihilated when GFR was indexed to ECFV. Indexing for height, as recommended by others (26,28), did not annihilate the difference between males and females in our study either, providing an argument against indexing to height.

It has been pointed out recently that no gold standard is available to determine which indexing factor is best (12) and that the best indexing factor for GFR would be the one that provides the best clinical validity. Final proof whether ECFV is indeed the best indexing factor for GFR should therefore be provided by long-term follow-up studies in which superiority of GFR/ECFV as predictor of renal outcome is studied.

When GFR is expressed per liter of ECFV, its unit reflects is the virtual volume of ECFV, which is cleared per unit of time. By indexing GFR (ml/min) to ECFV (l), the volume expression drops out of the equation; thus, GFR/ECFV reflects the proportion of the ECFV cleared per unit of time. The other way around, ECFV/GFR reflects the time needed to clear the complete ECFV. This way of considering renal function may help to appreciate renal function relative to the metabolic and homeostatic requirements of the body. For instance, in our healthy volunteers, GFR/ECFV was approximately 40%/h, implicating that 40% of the ECFV is cleared per hour, and, the other way around, that the kidneys need 2.5 h to clear the complete ECFV. The data in the subjects with renal function impairment, as studied in the calibration experiment, show that GFR/ECFV is lower in those with renal function impairment: a smaller proportion of the ECFV is cleared per unit of time.

ECFV is regulated within relatively narrow boundaries, but it is not fixed. It adapts to altered sodium intake, as also confirmed here. It could be argued that this hampers its suitability as indexing parameter. On the other hand, considering GFR in relation to the prevalent ECFV may allow better interpretation of changes in GFR, by distinguishing between changes in GFR secondary to altered volume status and changes in GFR dissociated from changes in ECFV. In the current study, we induced a modest change in ECFV by a shift in sodium intake to investigate whether the estimate by V_d IOT was sufficiently sensitive to detect the change in ECFV. Indeed, V_d IOT was significantly higher during high-sodium intake. The rise in ECFV matched the anticipated rise in GFR and accordingly, GFR indexed to ECFV was similar during low and liberal sodium intake. It would be of interest, therefore, to have the information on simultaneous values of ECFV and GFR also in disease conditions where GFR, ECFV, or both are disturbed. In diabetes, for instance, elevated GFR and volume expansion occur in incipient diabetic nephropathy (29,30). Although, in a small cohort, hyperfiltration seemed to be independent of ECFV expansion (30), studies in large cohorts are lacking. Routinely implementing ECFV assessment in tracer-based GFR assessment potentially yields large study-cohorts, which have an enormous explanatory potential for studying not only GFR, but also ECFV.

Recently, Titze *et al.* (31) provided innovative insights in the association between sodium balance and ECFV regulation. In rat models, they found that an increase in sodium intake went along with a less than anticipated rise in ECFV, which could be attributed to nonosmotic storage of sodium in tissues as the skin (32). Whether such a nonosmotic storage exists in human has not been established yet. In our study, the mean rise in

ECFV elicited by the shift from low- to high-sodium intake was 1.1 l, where in theory a rise of 1.43 l could be calculated. It is tempting to speculate that this difference reflects nonosmotic storage of sodium, but so far, we have no tools to support this assumption.

Several limitations should be mentioned. First, our assessment of ECFV was not tested in subjects with overt pathology of the ECFV, with fluid overload and/or abnormalities in body fluid distribution, such as the nephrotic syndrome and ascites. Therefore, generalization of our findings to populations with gross abnormalities of ECFV and/or abnormal distribution of body fluid compartments is not warranted and requires more extensive validation in appropriate study populations. Second, P_{IOT} is used for the calculation of both GFR and ECFV, so the two are not arithmetically independent. As a consequence, a correlation between GFR and ECFV cannot simply be interpreted as a biologic association. It should be emphasized, however, that this does not invalidate the use of GFR/ECFV, as in this ratio P_{IOT} falls out of the equation. For this reason, as also pointed out by others, assessment of GFR/ECFV is less sensitive to procedural errors than assessment of GFR alone (5,11). Third, this is a single-center study, investigating one tracer and one measurement protocol only. Thus, for other settings, separate validation and calibration are warranted. Yet, from a theoretical perspective and supported by several studies, single infusion methods using plasma disappearance curves are well suited for ECFV assessment as well (4). Finally, we used a radio-labeled tracer for this study, which obvious has limitations in case of handling, storage, and disposal of radio-labeled materials and exclusion of certain patients as pregnant women and children. Non–radio-labeled tracers as iohexol could be a valid alternative here.

In clinical practice, creatinine-based approaches, be it renal function equations or creatinine clearance, are the usual way to estimate renal function (33,34). Creatinine-based renal function estimates are also generally indexed to body dimensions, usually BSA. However, the V_d of creatinine cannot directly be established, so our strategy is unfortunately not applicable to creatinine-based renal function measurements.

Conclusion

Our study demonstrates the feasibility and validity of measuring ECFV simultaneously with assessing GFR by ^{125}I-iothalamate clearance, without need for adapting the GFR protocol. This not only enables indexing of GFR to ECFV but also provides information on ECFV in studies on renal function. ECFV is a major physiologic parameter, and disturbances are common in renal patients. By our approach, which could also be implemented for other GFR tracers, information on ECFV can be conveniently obtained as an additional parameter in subjects in whom GFR is measured. This will increase the yield of measurements of true GFR.

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Disclosures

None.

References

1. Gaspari F, Perico N, Remuzzi G: Measurement of glomerular filtration rate. *Kidney Int Suppl* 63[Suppl]: S151–S154, 1997
2. Zdolsek JH, Lisander B, Hahn RG: Measuring the size of the extracellular fluid space using bromide, iohexol, and sodium dilution. *Anesth Analg* 101: 1770–1777, 2005
3. Poulsen HL, Jensen HA, Parving HH: Extracellular fluid volume determined by a single injection of inulin in men with untreated essential hypertension. *Scand J Clin Lab Invest* 37: 691–696, 1977
4. Peters AM, Allison H, Ussov WY: Simultaneous measurement of extracellular fluid distribution and renal function with a single injection of 99mTc DTPA. *Nephrol Dial Transplant* 10: 1829–1833, 1995
5. Gunasekera RD, Allison DJ, Peters AM: Glomerular filtration rate in relation to extracellular fluid volume: similarity between 99mTc-DTPA and inulin. *Eur J Nucl Med* 23: 49–54, 1996
6. Brochner-Mortensen J: A simple single injection method for determination of the extracellular fluid volume. *Scand J Clin Lab Invest* 40: 567–573, 1980
7. Brochner-Mortensen J: The extracellular fluid volume in normal man determined as the distribution volume of [51Cr] EDTA. *Scand J Clin Lab Invest* 42: 261–264, 1982
8. Bird NJ, Peters C, Michell AR, Peters AM: Extracellular distribution volumes of hydrophilic solutes used to measure the glomerular filtration rate: comparison between chromium-51-EDTA and iohexol. *Physiol Meas* 28: 223–234, 2007
9. Amici G, Carniato A, Zoli P, Da RG, Boccaletto F, Bocci C, Palermo F: Total clearance and extracellular volume with 125I-iothalamate in peritoneal dialysis. *Adv Perit Dial* 12: 147–150, 1996
10. Bird NJ, Henderson BL, Lui D, Ballinger JR, Peters AM: Indexing glomerular filtration rate to suit children. *J Nucl Med* 44: 1037–1043, 2003
11. Peters AM: The kinetic basis of glomerular filtration rate measurement and new concepts of indexation to body size. *Eur J Nucl Med Mol Imaging* 31: 137–149, 2004
12. Geddes CC, Woo YM, Brady S: Glomerular filtration rate: what is the rationale and justification of normalizing GFR for body surface area? *Nephrol Dial Transplant* 23:4–6, 2008
13. Donker AJ, van der Hem GK, Sluiter WJ, Beekhuis H: A radioisotope method for simultaneous determination of the glomerular filtration rate and the effective renal plasma flow. *Neth J Med* 20: 97–103, 1977
14. Apperloo AJ, de Zeeuw D, Donker AJ, de Jong PE: Precision of glomerular filtration rate determinations for long-term slope calculations is improved by simultaneous infusion of 125I-iothalamate and 131I-hippuran. *J Am Soc Nephrol* 7: 567–572, 1996
15. Zietse R, Blankestijn PJ, Pos B, Balk AH, Derkx FH, Weimar W, Schalekamp MA: Optimising glomerular filtration rate

and effective renal plasma flow measurements using a simple pharmacokinetic model. *Clin Nephrol* 43: 29–34, 1995
16. Welling PG, Mosegaard A, Dobrinska MR, Madsen PO: Pharmacokinetics of 125I-iothalamate and 131I-o-iodohippurate in man. *J Clin Pharmacol* 16: 142–148, 1976
17. Bell EF, Ziegler EE, Forbes GB: Corrected bromide space. *Pediatr Res* 18: 392–393, 1984
18. Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1: 307–310, 1986
19. Levey AS, Greene T, Kusek J, Beck GJ, Group MS: A simplified equation to predict glomerular filtration rate from serum creatinine [Abstract]. *J Am Soc Nephrol* 11: A0828, 2000
20. DuBois D, DuBois EF: A formula to estimate the approximate surface area if height and weight be known. *Arch Int Med* 17: 863–871, 1916
21. Wong WW: Influence of ovariectomy on extracellular fluid volume in rats: assessment of extracellular fluid volume by means of bromide. *J Lab Clin Med* 135: 298–299, 2000
22. McIntosh JF, Moller E, Van Slyke DD: Studies of urea excretion: III: The influence of body size on urea output. *J Clin Invest* 6: 467–483, 1928
23. Delanaye P, Radermecker RP, Rorive M, Depas G, Krzesinski JM: Indexing glomerular filtration rate for body surface area in obese patients is misleading: concept and example. *Nephrol Dial Transplant* 20: 2024–2028, 2005
24. Wang J, Hihara E: Human body surface area: a theoretical approach. *Eur J Appl Physiol* 91: 425–428, 2004
25. Verbraecken J, Van de Heyning P, De Backer W, Van Gaal L: Body surface area in normal-weight, overweight, and obese adults: a comparison study. *Metabolism* 55: 515–524, 2006
26. Schmieder RE, Beil AH, Weihprecht H, Messerli FH: How should renal hemodynamic data be indexed in obesity? *J Am Soc Nephrol* 5: 1709–1713, 1995
27. Turner ST, Reilly SL: Fallacy of indexing renal and systemic hemodynamic measurements for body surface area. *Am J Physiol* 268: R978–R988, 1995
28. Bosma RJ, van der Heide JJ, Oosterop EJ, de Jong PE, Navis G: Body mass index is associated with altered renal hemodynamics in non-obese healthy subjects. *Kidney Int* 65: 259–265, 2004
29. Ortola FV, Ballermann BJ, Anderson S, Mendez RE, Brenner BM: Elevated plasma atrial natriuretic peptide levels in diabetic rats: potential mediator of hyperfiltration. *J Clin Invest* 80: 670–674, 1987
30. Brochner-Mortensen J, Ditzel J: Glomerular filtration rate and extracellular fluid volume in insulin-dependent patients with diabetes mellitus. *Kidney Int* 21: 696–698, 1982
31. Titze J, Bauer K, Schafflhuber M, Dietsch P, Lang R, Schwind KH, Luft FC, Eckardt KU, Hilgers KF: Internal sodium balance in DOCA-salt rats: a body composition study. *Am J Physiol Renal Physiol* 289: F793–F802, 2005
32. Titze J, Lang R, Ilies C, Schwind KH, Kirsch KA, Dietsch P, Luft FC, Hilgers KF: Osmotically inactive skin Na+ storage in rats. *Am J Physiol Renal Physiol* 285: F1108–F1117, 2003
33. Bosma RJ, Doorenbos CR, Stegeman CA, van der Heide JJ, Navis G: Predictive performance of renal function equations in renal transplant recipients: an analysis of patient factors in bias. *Am J Transplant* 5: 2193–2203, 2005
34. Bostom AG, Kronenberg F, Ritz E: Predictive performance of renal function equations for patients with chronic kidney disease and normal serum creatinine levels. *J Am Soc Nephrol* 13: 2140–2144, 2002