Comparability of Plasma Iohexol Clearance Across Population-Based Cohorts

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Rationale & Objective: Glomerular filtration rate (GFR) estimation based on creatinine or cystatin C level is currently the standard method for assessing GFR in epidemiologic research and clinical trials despite several important and well-known limitations. Plasma iohexol clearance has been proposed as an inexpensive method for measuring GFR that could replace estimated GFR in many research projects. However, lack of standardization for iohexol assays and the use of different protocols such as single- and multiple-sample methods could potentially hamper comparisons across studies. We compared iohexol assays and GFR measurement protocols in 3 population-based European cohorts.

Study Design: Cross-sectional investigation.

Setting & Participants: Participants in the Age, Gene/Environment Susceptibility-Kidney Study (AGES-Kidney; n = 805), the Berlin Initiative Study (BIS, n = 570), and the Renal Iohexol Clearance Survey Follow-up Study (RENIS-FU; n = 1,324).

Tests Compared: High-performance liquid chromatography analyses of iohexol. Plasma iohexol clearance calculated using single- versus multiple-sample protocols.

Outcomes: Measures of agreement between methods.

Results: Frozen samples from the 3 studies were obtained and iohexol concentrations were remeasured in the laboratory at the University Hospital of North Norway. Lin's concordance correlation coefficient ρ was >0.96 and C_b (accuracy) was >0.99 for remeasured versus original serum iohexol concentrations in all 3 cohorts, and Passing-Bablok regression did not find differences between measurements, except for a slope of 1.025 (95% CI, 1.006-1.046) for the log-transformed AGES-Kidney measurements. The multiple-sample iohexol clearance measurements in AGES-Kidney and BIS were compared with single-sample GFRs derived from the same iohexol measurements. Mean bias for multiple-sample relative to single-sample GFRs in AGES-Kidney and BIS were −0.25 and −0.15 mL/min, and 99% and 97% of absolute differences were within 10% of the multiple-sample result, respectively.

Limitations: Lack of comparison with an independent gold-standard method.

Conclusions: Agreement between the iohexol assays and clearance protocols in the 3 investigated cohorts was substantial. Our findings indicate that plasma iohexol clearance measurements can be compared across these studies.

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Am J Kidney Dis. XX(XX):1-9. Published online Month X, XXXX.

doi: 10.1053/j.ajkd.2019.10.008

Plasma iohexol clearance is considered a precise and accurate method for measuring glomerular filtration rate (GFR).[1,2] Estimation of GFR from endogenous filtration markers—such as creatinine and cystatin C—may be affected by non-GFR determinants and lacks precision in the high GFR range.[3-6] It has been argued that plasma iohexol clearance should be used in research projects in which unbiased and precise GFR results are necessary. Low cost and easy implementation make plasma iohexol clearance a reasonable option for both clinical trials and epidemiologic studies. However, standardization of iohexol assays is lacking, and different sampling protocols may lead to difficulties comparing results across studies.

We compared iohexol assays and clearance protocols in 3 European population-based cohorts that all used published high-performance liquid chromatography (HPLC) methods and participated in external quality control programs.

Methods

Study Cohorts and Design

This study includes cohorts from the Age, Gene/Environment Susceptibility-Kidney Study (AGES-Kidney), Berlin Initiative Study (BIS), and Renal Iohexol Clearance Survey Follow-up Study (RENIS-FU), which have all been described previously.[7-9] Briefly, the 3 cohorts were recruited from Reykjavik (Iceland), Berlin (Germany), and Tromsø (Norway) and were all population based. RENIS-FU (n = 1,324) included persons aged between 56 and 70 years; AGES-Kidney (n = 805), between 74 and 93 years; and BIS (n = 570), between 70 and 97 years. The 3 studies were approved by the ethics committees of their respective institutions. They all adhered to the Declaration of Helsinki. All participants provided written informed consent.

For the comparison of iohexol assays by remeasurements in the Department of Medical Biochemistry at the University Hospital of North Norway, random plasma and

serum samples were obtained from AGES-Kidney (sample size = 200) and RENIS-FU (sample size = 300). Each randomly selected participant contributed 1 measurement to the sample, which for AGES-Kidney was drawn randomly from all measurements for that person. Because RENIS-FU used a single-sample protocol, persons in RENIS-FU had only 1 measurement. From BIS (sample size = 100), a convenience sample from all 30-minute serum samples was obtained due to lack of frozen material. This explains the generally higher iohexol concentrations in BIS samples.

To compare the single- and multiple-sample protocols for GFR calculation, single-sample GFR was calculated from the series of iohexol measurements in BIS and AGES-Kidney for each person. All persons included in these 2 cohorts were eligible for these comparisons (AGES-Kidney, n = 805; BIS, n = 570).

Iohexol Measurements

Plasma iohexol was originally measured with previously described HPLC assays in the University of Minnesota Core Laboratory (AGES-Kidney), and serum iohexol, in the Department of Nephrology at the Charité Berlin, Germany (BIS) and the Department of Medical Biochemistry at the University Hospital of North Norway (RENIS-FU).[8-10] The methods are described in detail in Item S1. All 3 laboratories participated in the Equalis program for quality control (Equalis AB). In this study, iohexol concentrations in thawed samples from all 3 cohorts were remeasured concurrently at the Department of Medical Biochemistry of the University Hospital of North Norway in Tromsø, Norway, the same laboratory used for the original measurements in RENIS-FU. Median time between the original measurements in RENIS-FU and the remeasurements was 4 years.

Calculation of GFR

AGES-Kidney and BIS used a multiple-sample iohexol protocol with 4 (at 120, 180, 240, and 300 minutes) and 8 (same as AGES-Kidney, with additional samples at 30, 60, 90, and 150 minutes) samples, respectively, whereas the RENIS-FU used the single-sample method.[7-9] BIS calculated GFR using the Schwartz method,[11] AGES-Kidney used the slope-intercept method with the Brøchner-Mortensen correction,[12] and RENIS-FU used a numerical method for calculating GFR from Jacobsson's equations.[9,13] Details of the protocols can be found in Item S2.

To compare single- and multiple-sample protocols, Jacobsson's method was used to calculate single-sample GFRs from the multiple-sample measurements in BIS and AGES-Kidney.[13] For each person, interpolation on a log-scale was used to obtain the iohexol concentration at the optimal time point for the single-sample method. The optimal time point was calculated using Jacobsson's method based on the estimated GFR derived from creatinine level.[13] Estimated extracellular volume (ECV) divided by estimated GFR corresponds to the time for the single sample at which the influence of error from the estimated ECV is minimized.[13] Estimated GFR was calculated from serum creatinine level using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[14] ECV was estimated using Granerus' equation.[15] Participants were excluded if the exponential decay of measured iohexol concentration over time for either the fast or slow component had a Pearson correlation of log iohexol versus time ≥ -0.85 (4 participants in AGES-Kidney and 8 in BIS). Also, participants with missing iohexol data for the slow component were excluded (9 participants in AGES-Kidney and none in BIS). Thus, 792 persons in AGES-Kidney and 562 persons in BIS were included in the analyses.

Statistical Methods

We used Bland-Altman analysis, Passing-Bablok regression, and Lin's concordance correlation coefficient to compare 1 repeated measurement with 1 original measurement of iohexol for each selected person in each cohort.[16-18] In Bland-Altman analysis, bias is expressed as the mean of the differences between the remeasured and original iohexol concentrations, and limits of agreement, as the mean ± 2 times the standard deviation (SD) of the differences. Bland and Altman's method was used for estimating 95% confidence intervals for bias and limits of agreement.[19]

The necessary sample sizes for the Passing-Bablok regressions were calculated using Linnet's method for Deming regression because sample size calculation for Passing-Bablok regression is not readily available, although simulations of power for small sample sizes have been published.[20-22] We assumed constant and identical coefficients of variation (CVs) of 3% for all 3 cohorts. Range ratios (maximum divided by minimum observed original iohexol measurement) were 8.1, 14.1, and 3.0 for AGES-Kidney, BIS, and RENIS-FU, respectively. A higher range ratio requires a lower sample size to detect a deviation with the same power. To obtain sufficiently precise estimates for the calibration equations between cohorts, we aimed to detect a deviation from zero for the intercept of 1, and a deviation in slope from unity of 0.05 with alpha of 0.05 and power of 0.90. Because we assumed that Passing-Bablok regression, which is a nonparametric method, has lower power than a Deming regression, we also assumed that sample sizes should be increased for the Passing-Bablok regressions. By Linnet's method,[21] sample sizes of 200 from AGES Kidney, 100 from BIS, and 300 from RENIS-FU satisfy this requirement by a wide margin when using his method for measurement with proportional SDs. However, because Passing-Bablok regressions with log-transformed measurements were performed after inspection of the data, we recalculated the necessary sample sizes. Using log-transformed data, range ratios were reduced to 1.6, 1.7, and 1.3 for AGES-Kidney, BIS, and RENIS-FU, respectively. The requirements to detect deviations of 1 for the intercept and 0.05 for the slope were recalculated

Table 1. Characteristics of the Population-Based Cohorts

	AGES-Kidney	BIS	RENIS-FU
No. of participants	805	570	1,324
Age, y	80.3 ± 4.0	78.5 ± 6.2	63.6 ± 4.0
Male sex	355 (44.1%)	326 (57.2%)	657 (49.6%)
Body weight, kg	77.0 ± 14.1	77.3 ± 13.9	79.4 ± 14.3
Height, cm	167.7 ± 9.4	166.3 ± 8.5	170.6 ± 8.7
BMI, kg/m^2	27.4 ± 4.3	27.9 ± 4.2	27.2 ± 4.1
BSA, m^2	1.86 ± 0.20	1.85 ± 0.19	1.91 ± 0.19
Absolute GFR, mL/min[a]	66.3 ± 19.1	64.7 ± 19.3	98.5 ± 19.8
BSA-adjusted GFR, mL/min/1.73 m^2[a]			
Mean	61.6 ± 16.3	60.3 ± 16.4	89.1 ± 14.5
≤30	33 (4%)	17 (3%)	2 (0.2%)
30-60	302 (38%)	256 (45%)	33 (2%)
60-75	307 (38%)	203 (36%)	169 (13%)
>75	163 (20%)	94 (16%)	1,120 (85%)

Note: Except when indicated otherwise, data are shown as mean ± standard deviation or count (percent).
Abbreviations: AGES-Kidney, Age, Gene/Environment Susceptibility-Kidney Study; BIS, Berlin Initiative Study; BMI, body mass index; BSA, body surface area; GFR, glomerular filtration rate; RENIS-FU, Renal Iohexol-Clearance Survey Follow-up.
[a]For AGES-Kidney, recalibrated values according to the Passing-Bablok regression in Table 2 are given.

to corresponding requirements at the log scale mean for each cohort. Whereas slope requirements were identical at 0.049 for all 3 cohorts, intercept requirements were now 0.01, 0.005, and 0.02 for AGES-Kidney, BIS, and RENIS-FU, respectively. Using Linnet's table for power of 90% with constant SDs,[21] the necessary sample sizes were found to be lower for the slope but higher for the intercept than those used in our study. This means that the actual power was <90% to detect deviations in the intercepts, but >90% to detect deviations in the slopes.

Bland-Altman analysis, Passing-Bablok regression, Lin's concordance correlation coefficient, and concordance within 5% and 10% were used to study agreement between single- and multiple-sample GFRs in the AGES-Kidney and BIS cohort. Concordance was defined as percentage of absolute differences between the multiple- and single-sample results of less than 5% or 10% of the original multiple-sample result. Differences in concordance across categories of body mass index (BMI) were compared using a logistic regression model with concordance for each participant (yes/no) as the dependent variable and variables for cohort, BMI, and their interaction as independent variables. Statistical significance of the variable for BMI and/or the interaction was taken to indicate a difference across BMI categories.

The same method was used for testing differences in concordance across categories of GFR. GFR categories were based on the mean of the multiple- and single-sample GFR measurements for each person. We also carried out Bland-Altman analysis, Passing-Bablok regression, and estimated Lin's concordance correlation coefficient in the GFR subgroups. Absolute GFR measured in mL/min was used in these

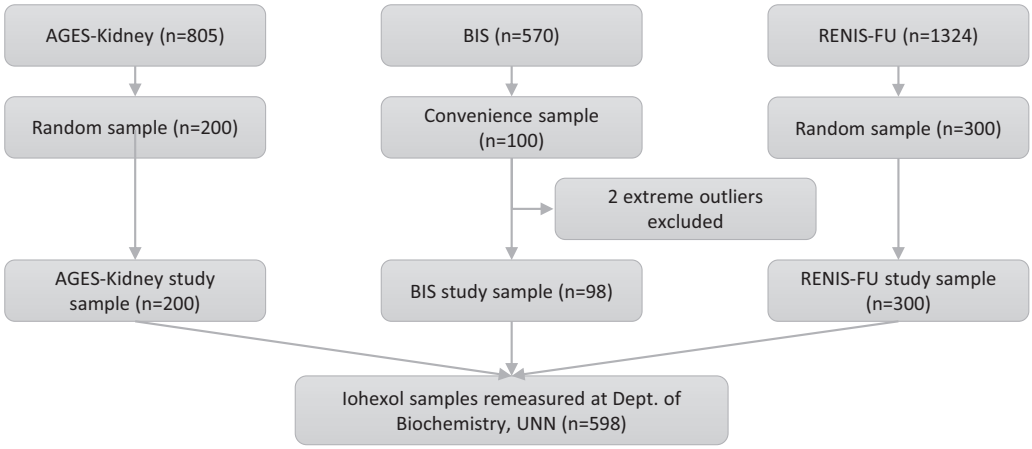

Figure 1. Inclusion of iohexol samples from the Age, Gene/Environment Susceptibility-Kidney Study (AGES-Kidney), Berlin Initiative Study (BIS), and Renal Iohexol-Clearance Survey Follow-up (RENIS-FU) cohorts for remeasurements in the Department of Biochemistry at the University Hospital of North Norway (UNN).

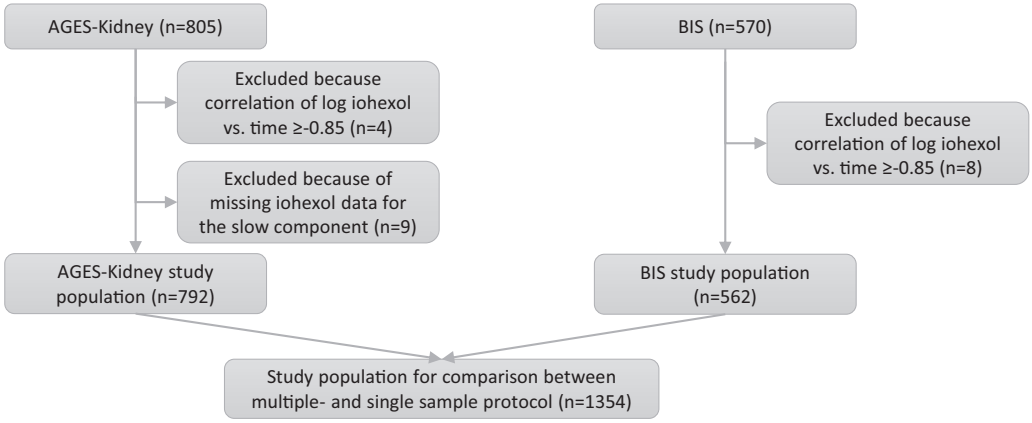

Figure 2. Inclusion of study participants from the Age, Gene/Environment Susceptibility-Kidney Study (AGES-Kidney) and Berlin Initiative Study (BIS) cohorts for comparison between the multiple- and single-sample protocols for measuring glomerular filtration rate as iohexol clearance.

analyses, but GFR adjusted for body surface area calculated using the equation of Dubois is included in Table 1.[23] We used R, version 3.5.1 (R Foundation for Statistical Computing). Statistical significance was set at $P < 0.05$.

Results

Cohort Characteristics

Characteristics of the 3 cohorts are shown in Table 1. Flowcharts of the inclusion of samples and participants for remeasurements of iohexol and for comparison of the multiple- and single-sample protocols are shown in Figures 1 and 2, respectively.

Iohexol Measurements

Two measurements from BIS demonstrated differences between the original and remeasured iohexol-concentrations of 109 and 55 mg/L. These extreme outliers were believed to be results from procedural errors and were excluded from further analyses. Table 2 and Figure 3 show comparisons between the remeasured and original results of the HPLC measurements. Mean CVs between the original and remeasured iohexol values were 3.5%, 1.8%, and 3.2% for AGES-Kidney, BIS, and RENIS-FU, respectively. Because we remeasured a single sample from each individual in AGES-Kidney and BIS, GFR based on remeasured values could not be calculated for these

Table 2. Comparison Between Original and Remeasured Log-Transformed Serum/Plasma Iohexol Concentrations in 3 Population-Based Cohorts

Bland-Altman Analyses of Agreement[a]	N	Bias (95% CI)	Lower Limit of Agreement (95% CI)	Upper Limit of Agreement (95% CI)
AGES-Kidney	200	2.1% (1.3% to 2.9%)	−8.6% (−9.8% to −7.3%)	14.0% (12.5% to 15.6%)
BIS	98	0.5% (−0.2% to 1.2%)	−5.8% (−6.9% to −4.7%)	7.3% (6.1% to 8.5%)
RENIS-FU	300	−0.7% (−1.3% to 0.0%)	−11.5% (−12.5% to −10.4%)	11.5% (10.2% to 12.8%)

Passing-Bablok Regression	Intercept (95% CI)	Slope (95% CI)
AGES-Kidney	−0.091 (−0.181 to 0.001)	1.025 (1.006 to 1.046)
BIS	0.031 (−0.103 to 0.177)	0.995 (0.968 to 1.020)
RENIS-FU	−0.023 (−0.158 to 0.101)	1.005 (0.974 to 1.039)

Lin's Concordance Correlation Coefficient[b]	ρ (95% CI)	C_b
AGES-Kidney	0.991 (0.988 to 0.993)	0.999
BIS	0.994 (0.991 to 0.996)	1.000
RENIS-FU	0.961 (0.952 to 0.969)	0.999

Note: Iohexol was measured in plasma in AGES-Kidney and in serum in BIS and RENIS-FU.
Abbreviations: AGES-Kidney, Age, Gene/Environment Susceptibility-Kidney Study; BIS, Berlin Initiative Study; CI, confidence interval; RENIS, Renal Iohexol-Clearance Survey Follow-up.
[a]Agreement analyzed on the log-transformed scale. Bias is expressed as percent change in remeasured iohexol relative to the original measurement.
[b]Lin's concordance correlation coefficient measures deviations from identity between 2 measurements where ρ expresses precision and C_b expresses accuracy. Values of 1 correspond to perfect precision and accuracy.

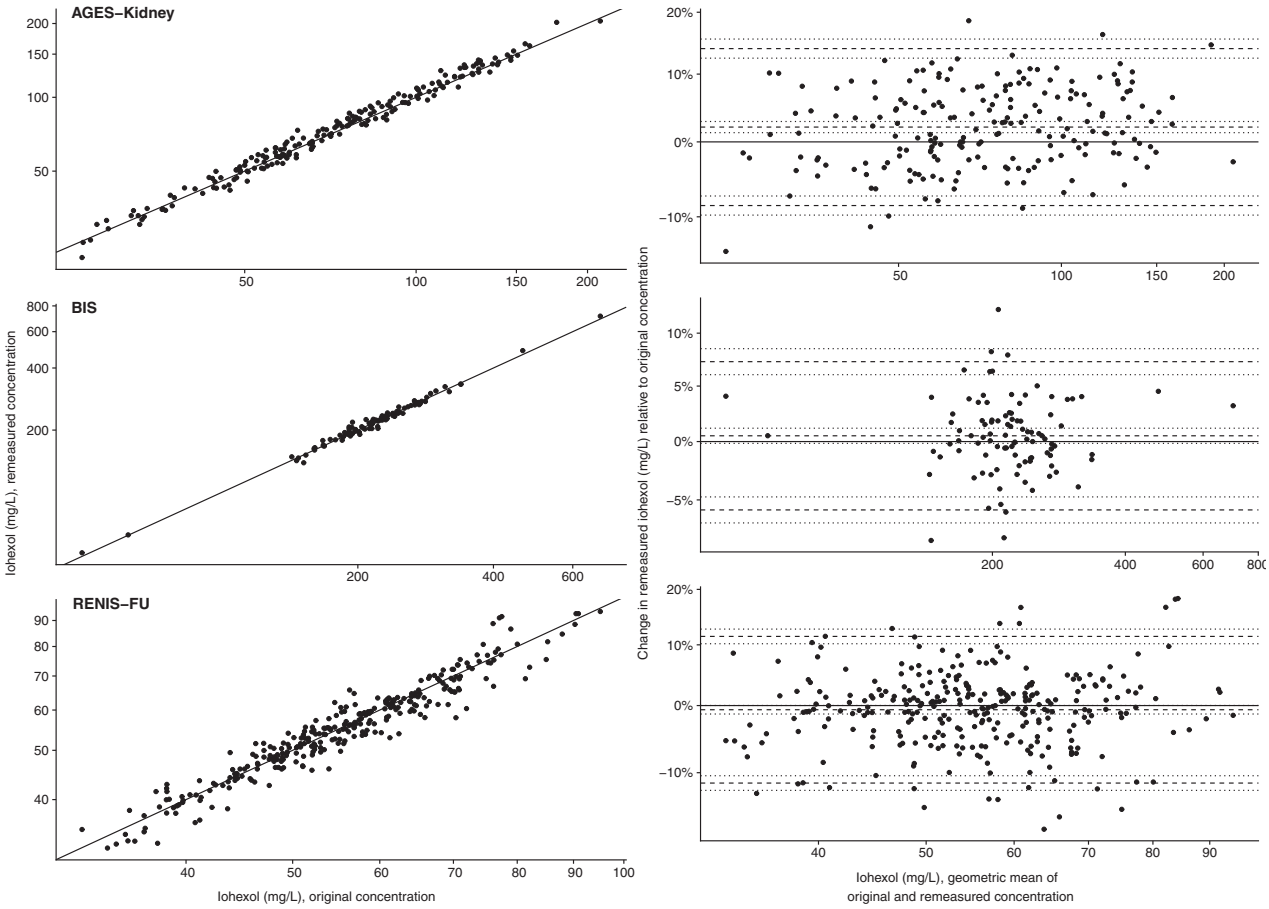

Figure 3. Scatterplots and Bland-Altman plots of original and remeasured log-transformed serum/plasma iohexol concentrations for the Age, Gene/Environment Susceptibility-Kidney Study (AGES-Kidney), Berlin Initiative Study (BIS), and Renal Iohexol-Clearance Survey Follow-up (RENIS-FU) Study cohorts. To the left, the remeasured iohexol concentrations are plotted against the original results with identity indicated as a solid line. To the right, percentage change in remeasured iohexol concentrations relative to the original results are plotted against the geometric mean of the 2. Mean bias and lower and upper limits of agreement are plotted as dashed lines, and 95% confidence intervals, by dotted lines.

cohorts. The CV for recalculated single-sample GFRs in RENIS-FU was 2.6%.

Iohexol concentrations were log-transformed before the Bland-Altman and Passing-Bablok regression analyses because of a skewed distribution. When expressed as percentage changes, Bland-Altman analyses revealed bias of the remeasured values of −0.7% to 2.1%, relative to the original measurements for the 3 cohorts. Limits of agreement for the BIS cohort were narrower than for the other 2 cohorts. In the Passing-Bablok regression, the slope for the AGES-Kidney cohort indicated a significant difference between the remeasured and original values, but there were no significant differences for the other cohorts (Table 2). When we recalculated GFR with a corresponding calibration of the AGES-Kidney iohexol concentrations, the difference between mean values of the original (67.22 mL/min) and recalculated GFRs (66.35 mL/min) was only 0.87 mL/min. Lin's concordance correlation coefficient ρ (precision) was >0.96 and C_b (accuracy) was >0.99 for all 3 cohorts (Table 2). The accuracy statistic C_b measures how far the best-fit line to the data deviates from the line of identity.[18] According to McBride's proposed criteria for ρ, this level of agreement is characterized as substantial.[24]

Multiple- Versus Single-Sample Plasma Iohexol Clearance Protocols

The comparison between multiple- and single-sample protocols is shown in Table 3 and Figure 4. In Bland-Altman analyses, mean bias was −0.25 ± 1.46 (SD) mL/min for the AGES-Kidney and −0.15 ± 3.16 mL/min for the BIS cohort. Passing-Bablok regression demonstrated slight deviations from the line of identity for both cohorts. Lin's concordance correlation coefficients indicated substantial agreement between multiple- and single-sample GFRs in both cohorts (Table 3).[24] The percentage of absolute differences between multiple- and single-sample results within 5% of the multiple-sample results was

Table 3. Comparison Between Multiple- and Single-Sample Iohexol Clearance in 2 Population-Based Cohorts

		GFR, mL/min[a]	
	N	Multiple-Sample	Single-Sample
AGES-Kidney	792	66.3 ± 19.1	66.6 ± 19.3
BIS	562	64.8 ± 19.2	64.9 ± 18.6

Bland-Altman Analyses of Agreement[b]	Bias (95% CI)	Lower Limit of Agreement (95% CI)	Upper Limit of Agreement (95% CI)
AGES-Kidney	−0.25 (−0.36 to −0.15)	−3.11 (−3.29 to −2.94)	2.61 (2.43 to 2.78)
BIS	−0.15 (−0.41 to 0.11)	−6.34 (−6.78 to −5.89)	6.03 (5.59 to 6.48)

Passing-Bablok Regression[c]	Intercept (95% CI)	Slope (95% CI)
AGES-Kidney	0.26 (0.10 to 0.43)	0.99 (0.99 to 1.00)
BIS	−1.08 (−1.81 to −0.39)	1.02 (1.01 to 1.03)

Lin's Concordance Correlation Coefficient[d]	ρ (95% CI)	C_b
AGES-Kidney	0.997 (0.997 to 0.997)	1.000
BIS	0.986 (0.984 to 0.988)	0.999

Concordance Within 5% and 10%[e]	5%	10%
AGES-Kidney	95%	99%
BIS	78%	97%

Abbreviations: AGES-Kidney, Age, Gene/Environment Susceptibility-Kidney Study; BIS, Berlin Initiative Study; CI, confidence interval; GFR, glomerular filtration rate.
[a]Values given as mean ± standard deviation.
[b]GFR measured in mL/min. Bias is defined as the mean of multiple- minus single-sample iohexol clearances.
[c]Dependent variable is multiple-sample GFR, independent variable is single-sample GFR.
[d]Lin's concordance correlation coefficient measures deviations from identity between 2 measurements where ρ expresses precision and C_b expresses accuracy. Values of 1 correspond to perfect precision and accuracy.
[e]Concordance defined as the percentage of absolute differences between the multiple- and single-sample results less than 5% or 10% of the original multiple-sample result.

lower for BIS than for AGES-Kidney (78% vs 95%), but the proportion within 10% was similar (97% vs 99%).

Because variability in the interpolated iohexol value used to calculate single-sample GFR will be lower than in the case of a single measurement, sensitivity analysis was performed in which the iohexol measurement closest to the optimal sampling time was used instead. This yielded mean bias of 1.06 ± 2.51 mL/min for AGES-Kidney and 1.08 ± 3.96 mL/min for BIS.

No statistically significant variation in concordance across BMI categories was observed for the 2 cohorts (Table S1), and Lin's concordance correlation coefficient was >0.99 for AGES-Kidney and >0.97 for BIS across all categories. There was a small statistically significant negative bias for the lowest BMI category for both cohorts (−0.51 mL/min for AGES-Kidney and −1.11 mL/min for BIS). For BIS, there was also a statistically significant positive bias of 1.93 mL/min for the highest BMI category. Passing-Bablok regression demonstrated a corresponding tendency for a deviation from the line of identity for the highest BMI category in BIS, but this was not statistically significant (Table S1).

There were statistically significant differences across GFR categories for samples within 5% concordance, with AGES-Kidney demonstrating a generally high percentage of samples within 5% concordance across all GFR categories, with a trend toward greater concordance at high GFRs, while BIS showed greatest concordance at low GFRs, with a significant decrease in concordant samples at higher GFRs (P for interaction = 0.001; Table S2). This is also reflected in the Bland-Altman analysis, Passing-Bablok regression, and Lin's concordance correlation coefficients for BIS, which indicate somewhat lower agreement for high GFRs. However, bias was <1 mL/min for the 2 highest categories, and there were no statistically significant differences across GFR categories for samples within 10% concordance for any of the cohorts (Table S2).

Discussion

We found substantial agreement when comparing different methods for measuring iohexol concentrations and iohexol clearance in 3 population-based cohorts, which compares favorably with interlaboratory comparisons of common clinical chemistry tests, for example, serum creatinine.[25] The results also indicate that serum or plasma iohexol frozen at −80°C is stable. Our results compare favorably with the 2 other studies of interlaboratory agreement for HPLC iohexol analysis that we are aware of.[26,27] The slightly better outcome observed for the BIS cohort may have resulted from greater precision for the higher iohexol concentrations in the samples. The deviation from unity of the slope in the Passing-Bablok regression for AGES-Kidney resulted in a change in

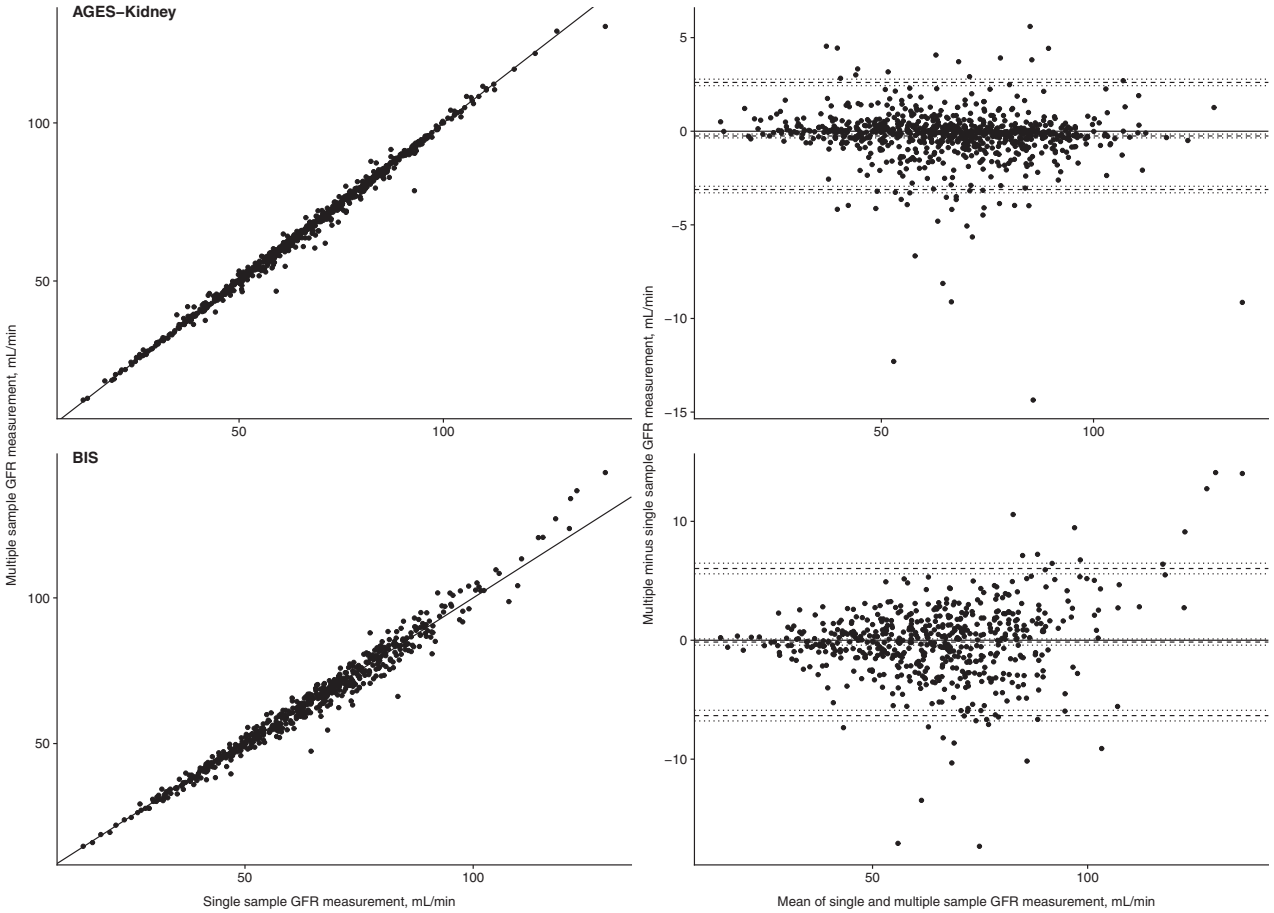

Figure 4. Scatterplots and Bland-Altman plots of multiple- and single-sample glomerular filtration rates (GFRs; mL/min) for the Age, Gene/Environment Susceptibility-Kidney Study (AGES-Kidney) and Berlin Initiative Study (BIS) cohorts. The series of multiple-sample iohexol concentrations have been used for calculating single-sample GFR for each person. To the left, multiple-sample is plotted against single-sample GFR with identity indicated as a solid line. To the right, the difference between multiple- and single-sample GFRs is plotted against the mean of the 2. Mean bias and lower and upper limits of agreement are plotted as dashed lines, and 95% confidence intervals, by dotted lines.

recalculated GFR of only 0.87 mL/min when iohexol concentrations were recalibrated.

Previous comparisons of multiple- and single-sample protocols have also demonstrated good agreement. In comparisons based on the same series of iohexol concentration measurements, bias has been found within the range of −0.2 to 4 mL/min/1.73 m^2 with limits of agreement comparable to our results.[28-31] Two studies compared both the single- and multiple-sample methods with an independent multiple-sample ^{51}Cr-EDTA clearance and observed similar performances for both methods.[31,32] Although very small differences between the 2 methods were found in both the AGES-Kidney and BIS cohorts, the SD of the distribution was greater in BIS than in AGES-Kidney. The explanation could be that Jacobsson's single-sample method is directly derived from the Brøchner-Mortensen method used in AGES-Kidney, whereas a 2-compartment model was used in BIS.

It has been suggested that the single-sample method is questionable in persons with extremes of body weight because it calculates the ECV from weight and sex using the Graneus' equation.[2,33] Although there was a tendency toward smaller 5% concordance for low and high BMI in the present study, this was not statistically significant, and results for 10% concordance were >92% for all BMI categories. Hence, any bias for the single-sample method at the extremes of weight is probably small and can be minimized by using the optimal sampling time, as outlined by Jacobsson.[13] This is the time point at which the influence from error in the ECV estimate is at its minimum.

There was a statistically significant difference in 5% concordance between single- and multiple-sample methods for categories of GFR, but with opposite trends for AGES-Kidney and BIS (Table S2). The lower 5% concordance for high GFRs in BIS can probably be explained by the use of different multiple-sample methods in AGES-Kidney and BIS, whereas comparison with an

independent gold-standard technique would be necessary to decide which of the methods is superior. The 10% concordance was ≥95% across all GFR categories for both methods.

The most important limitation of the present study is the inability to compare original and remeasured GFRs in the AGES-Kidney and BIS cohorts because only 1 iohexol sample from each multiple-sample series was remeasured for each participant. In RENIS-FU, the CV of recalculated GFR from the remeasured single samples can be compared to the CV of interindividual day-to-day variation in GFR (2.6% vs 4.2%), established in a previous study of the same cohort.[3,4] By calculating the corresponding variance from the mean GFR observed in that analysis (94 mL/min),[3,4] we can estimate that ~38% of the total day-to-day variance in GFR can be attributed to measurement error, and the rest, to intraindividual biological variation. This result should nevertheless be interpreted with caution because several authors have reported higher CVs for the total day-to-day variation, in the range of 5% to 10%.[2] Both measurement error and biological fluctuations probably vary between different research settings.

Another limitation of the present study is the lack of comparison to an independent gold-standard method. Although comparable, we cannot exclude the possibility that the methods in all 3 centers are biased. However, because previous comparisons of iohexol clearance with renal inulin clearance have found a median bias of 3% (95% confidence interval, 0%-6%), a common bias for the 3 cohorts is probably small.[1] It should be noted that the present study included few persons with very low GFRs, which may limit the generalizability of results to patients in this category.

We conclude that the HPLC assays in the 3 investigated cohorts yielded almost identical iohexol values and that multiple- and single-sample plasma iohexol clearance methods based on these measurements were highly comparable.

Supplementary Material

Supplementary File (PDF)

Item S1: HPLC for iohexol measurement.

Item S2: Methods for calculating plasma iohexol clearance.

Table S1: Agreement statistics for comparison of multiple and single sample GFR calculations according to cohort and BMI category.

Table S2: Agreement statistics for comparison of multiple and single sample GFR calculations according to cohort and GFR category.

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Authors' Contributions: Development of study concept and design: BOE, ES, NE, RP, OSI, TM; acquisition, analysis or interpretation of data: BOE, ES, NE, RP, OSI, TM, MG, VG, ASL, LKS, ABK, MS; statistical analysis: BOE. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Support: AGES-Kidney was supported by a grant from National Institutes of Health, United States (R01 DK082447), a contract from the National Institute on Aging, United States (N01-AG-1-2100), Hjartavernd, Iceland (Icelandic Heart Association), and the Icelandic Parliament (Althingi). The Icelandic Heart Association provided support for data collection. BIS measurements were funded by the KfH-Foundation of Preventive Medicine, Germany and the Dr. Werner Jackstädt Foundation, Germany. RENIS-FU was funded by the Northern Norway Regional Health Authority and supported by a grant from Boehringer-Ingelheim, Germany. The funding sources did not have any role in study design, analysis, and interpretation of data; writing the report; and the decision to submit the report for publication.

Financial Disclosure: Dr Schaeffner has received honoraria from Siemens Healthcare and Fresenius Medical Care for giving a lecture on kidney function assessment and from Fresenius Kabi for providing lectures and chairing a workshop on critiquing the literature. Dr Karger has received grant funding for collaborations with Siemens Healthcare Diagnostics. Dr Ebert has received honoraria from Siemens Healthineers. The remaining authors declare that they have no relevant financial interests.

Acknowledgements: We thank all participants in the AGES-Kidney, BIS, and RENIS-FU cohorts for their contributions to this investigation.

Peer Review: Received April 24, 2019. Evaluated by 4 external peer reviewers, with direct editorial input from a Statistics/Methods Editor and an Associate Editor, who served as Acting Editor-in-Chief. Accepted in revised form October 5, 2019. The involvement of an Acting Editor-in-Chief was to comply with *AJKD*'s procedures for potential conflicts of interest for editors, described in the Information for Authors & Journal Policies.

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