Glomerular filtration rate via plasma iohexol disappearance: Pilot study for chronic kidney disease in children

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To guide the design of a nation-wide cohort study of chronic kidney disease in children, we determined how iohexol plasma disappearance curves could be used in children to measure glomerular filtration rate (GFR). Iohexol (5 ml) was administered intravenously and blood samples were obtained at 10, 20, 30, 60, 120, 240, 300, and 360 min after injection ($N = 29$) and assayed by high performance liquid chromatography. Four urines were also collected following the injection. Intra-assay coefficient of variation (CV) in serum was 1.3% at 100 mg/l, 2.6% at 15 mg/l, and 3.4% for duplicate unknowns. GFR(9) was computed from iohexol dose and area under the nine-point blood disappearance curve, using double exponential modeling. Only 2.8% of 254 data points deviated by >3 CV from the curves. GFR(4) calculated from 10, 30, 120, and 300 min points correlated well with GFR(9) ($r = 0.999$) and showed no bias (means $\pm$ s.d. of GFR(9) and GFR(4) $= 59.3 \pm 36.3$ and 59.4 ± 36.0 ml/min per 1.73 m^2). Relationship of GFR(9) and one-compartment GFR followed quadratic equation as previously reported by Brochner-Mortensen, allowing GFR to be calculated from 120 and 300 min points. This GFR(2) correlated well with GFR(9) ($r = 0.986$). Estimated GFR from Schwartz height/creatinine formula correlated with GFR(9)($r = 0.934$) but overestimated GFR by 12.2 ml/min per 1.73 m^2. Urine iohexol clearance was poorly correlated ($r = 0.770$) with GFR(9) owing to variability in urine collections (median CV = 24%). GFR can be measured accurately using four-point iohexol plasma disappearance (in most cases, two points suffice); estimated GFR and urinary clearances are less useful.

Kidney International (2006) **69,** 2070–2077. doi:10.1038/sj.ki.5000385; published online 12 April 2006

KEYWORDS: pediatric nephrology; chronic kidney disease; children; glomerular filtration rate; renal function

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Received 3 January 2006; revised 4 February 2006; accepted 7 February 2006; published online 12 April 2006

Glomerular filtration rate (GFR) is the most useful indicator of kidney function and progression of kidney disease. In current practice, GFR is generally determined by creatinine clearance or estimated by an equation, that takes into consideration serum creatinine, age, race, and gender in adults,[1] and serum creatinine, height, and an empirical constant in children.[2] However, creatinine clearance has limited accuracy owing to the secretion of creatinine by the renal proximal tubules.[3] When there is a clinical need to accurately determine GFR, as for planning cancer chemotherapy with nephrotoxic agents or in staging chronic kidney disease (CKiD) in preparation for renal replacement therapy, it may be necessary to utilize a more definitive measurement of GFR. Similarly, in planning for clinical trials or observational research studies in chronic kidney disease, an accurate measure of GFR is required. We sought to determine the feasibility, accuracy, and precision of the plasma disappearance of iohexol as the measure of GFR in a nation-wide cohort study of chronic kidney disease in children. The primary aim of the CKiD study is to define risk factors for decline in GFR in children, and to examine the effect of GFR decline on cardiovascular morbidity, growth, cognition, and behavior.

The gold standard for measuring GFR is the standard inulin clearance, performed by loading and continuously infusing inulin and collecting timed urine samples via an indwelling bladder catheter.[4] Such a method requires extensive technical assistance, the use of a bladder catheter, and a difficult chemical assay. Moreover, inulin is no longer readily available. Accurate collection of urine without bladder catheterization is very difficult in children.

Alternatively, radioactive agents such as ^{51}Cr-EDTA, ^{99m}Tc-DTPA, and ^{125}I or ^{131}I-iothalamate have been used as surrogate markers of GFR. These agents are generally given by a single injection bolus and the disappearance from the blood is monitored (plasma clearance). (It should be noted that we refer to plasma disappearance curve despite the fact that most assays are performed in sera from the subjects. The concept of disappearance from the plasma relates to the fact

that GFR is dependent on renal plasma flow *in vivo*.). From the dose and the area under the curve as a function of time, a determination of GFR can be obtained. Conventional renal clearances are also performed, particularly using iothalamate. However, there are problems with each of these agents: ^{51}Cr-EDTA, although an excellent marker, is not available in the USA; the renal handling of ^{99m}Tc-DTPA can vary with commercial source;[5] and iothalamate is readily secreted by the kidney.[6] Thus, none of these markers can adequately replace the standard inulin clearance. Moreover, the use of radioactive substances and bladder catheterization are strong deterrents to study subject participation, and were therefore deemed unsuitable when planning the CKiD study.

In Scandinavia, iohexol has been used as a satisfactory marker of GFR in adults[7,8] and children.[9,10] Iohexol is readily available as a safe non-ionic low-osmolar contrast agent of molecular weight 821 Da (Omnipaque™) that is used intravenously for radiologic procedures even in the presence of renal disease. It is not secreted, metabolized, or reabsorbed by the kidney.[7,11,12] Iohexol diffuses into the extracellular space, but has less than 2% plasma protein binding,[7,13] and is eliminated exclusively without metabolism by the kidneys.[7,11,12] Extrarenal elimination of iohexol in a setting of reduced GFR is negligible.[14] Most studies indicate close agreement between GFR (measured by inulin clearance) and clearance of iohexol, measured as standard renal clearance or plasma disappearance.[11,12,15–17] There is also a very good correlation between iohexol clearance and ^{51}Cr-EDTA plasma clearance.[8]

Iohexol can be measured in deproteinized serum by high performance liquid chromatography (HPLC), thereby obviating the need for radioactivity. There are two isomers, both of which are handled similarly by the body.[8,11] In practice, the major peak, eluting at about 5 min, is used for plasma disappearance curves. The single-injection approach is technically less demanding than a constant infusion with urine collection and can be accomplished with less technical assistance.

Accordingly, we explored whether iohexol could serve as the GFR marker in the CKiD study. The recruitment goal of this prospective study is to enroll over 500 children and adolescents with mild to moderate chronic kidney disease. By design, GFR is to be measured three times over 5 years of follow-up. We addressed four specific objectives in this pilot study. First, we determined the appropriateness of the two-compartment (double exponential) model for iohexol disappearance from the blood and calculation of GFR and whether it could be approximated by a pair of data points determining each exponential (i.e., four points in total). Second, we investigated whether a one-compartment model of iohexol disappearance yields a satisfactory determination of GFR. Third, we examined whether estimates of GFR (eGFR) using the Schwartz height/creatinine formula[2,18] could closely approximate iohexol GFR based on the double exponential model. Fourth, we compared the two-compartment GFR with the renal clearance of iohexol using four timed collections of urine obtained during the plasma disappearance study.

RESULTS

Iohexol measurement

The intra-assay coefficient of variation (CV) for repeated determinations of iohexol was 1.3% at 100 mg/l and 2.6% at 15 mg/l. The CV for duplicate unknowns was 3.4%.

Two-compartment GFR

The disappearance of iohexol from the blood can be described by a double exponential curve, as seen by the plot of iohexol concentration vs time (Figure 1a). Examination of the logarithm of the iohexol concentrations permits linear curve fitting of the slow (renal) curve (Figure 1b). Subtraction of extrapolated early concentrations from the initial concentrations up to 120 min generates the fast curve, which is also linear on the log scale (Figure 1c). The descriptive statistics of the 29 curves are shown in Table 1, from which it is evident that the half-time of the slow curve (169.9 min) was nearly 10 times longer than that of the fast curve (19.7 min). In addition, the median area of the slow curve (60.83 mg min/ml) was more than 10 times that of the fast curve (4.93 mg min/ml).

A scatterplot of the areas of the slow and fast components for the 29 subjects showed no correlation between the area of the fast component and that of the slow component ($r = 0.17$). The 29 values of GFR(9) ranged from 16.5 to 162.3 ml/min per 1.73 m^2, with four above 100 ml/min per 1.73 m^2, two of which were provided by first and senior authors (GJS and AM) who initiated the study. The median GFR was 56.7 ml/min per 1.73 m^2, with the first and third quartiles being 36.5 and 71.7 ml/min per 1.73 m^2 (Table 1).

From 263 sample points, there were nine low and outlying values (3.4%), which were excluded because they were smaller than the subsequent value. Eliminating these revealed that the goodness of fit was excellent, with only 2.8% of the remaining 254 values deviating by more than 3 CVs from the curves.

Figure 2a shows that GFR(4) was extremely well correlated ($R = 0.999$) with GFR(9). Bland–Altman analysis showed that both the standard deviations (s.d.) and the computed bias were not significantly different between GFR(4) and GFR(9) (Figure 2b). The means and s.d. of GFR(9) and GFR(4) were practically identical (GFR(9): 59.3 ± 36.3 and GFR(4): 59.4 ± 36.0).

One-compartment GFR

Figure 3 shows the scatterplot of the GFR based only on the slow component from the serum concentrations between 120 and 360 min and the GFR(9). This one-compartment clearance, also called a slope–intercept clearance, was well correlated to the two-compartment model. It is evident that the slope–intercept clearance overestimates the two-compartment model GFR, and this overestimate becomes progressively larger at higher GFR. Moreover, the curve fitting of a

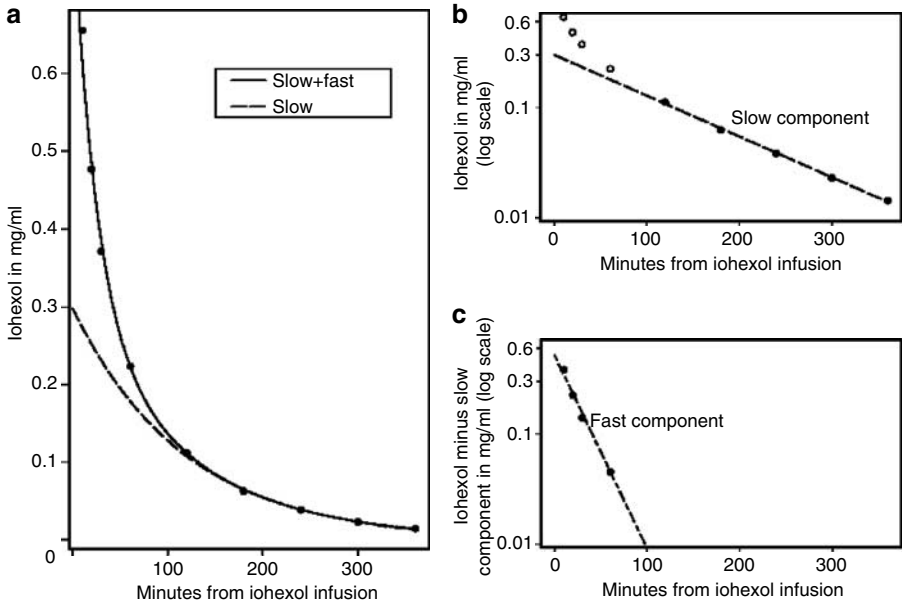

Figure 1 | Iohexol disappearance curves from the blood. (**a**) Plot of iohexol disappearance vs time (min) on the *x* axis. (**b**) Logarithm of iohexol disappearance, showing the linear fitting of the slow (renal) curve to the points from 120 to 360 min. (**c**) Logarithm of iohexol disappearance, showing the linear fitting of the fast curve to the points from 10 to 60 min. This fast iohexol disappearance curve was obtained by subtracting the slow curve from the overall disappearance curve, thus revealing the linear fit of the fast curve.

Table 1 | Descriptive statistics of study population and characteristics of iohexol plasma disappearance curves (*N*=29)

Variable	Median	1st quartile	3rd quartile
Age (years)	14	12	18
Gender	59% male	—	—
Height (cm)	156.2	145.8	168.0
Weight (kg)	58.4	43.2	75.4
BSA (m^2)	1.661	1.313	1.880
Serum creatinine (mg/dl)[a]	1.5	1.1	2.2
eGFR (ml/min per 1.73 m^2)	66.0	43.4	81.0
Iohexol dose (I, mg)	3185.0	3175.4	3223.5
Slow component			
Intercept (mg/ml)=exp *A*	0.245	0.169	0.318
Half-time (min)=log *2/α*	169.9	130.2	240.2
Area (mg min/ml)=exp *A/α*	60.83	39.68	97.53
Fast component			
Intercept (mg/ml)=exp *B*	0.169	0.116	0.238
Half-time (min)=log *2/β*	19.7	16.5	23.4
Area (mg min/ml)=exp *B/β*	4.93	3.44	6.14
Total area=exp *A/α*+exp *B/β*	65.87	44.30	100.72
GFR (ml/min per 1.73 m^2)[b]	56.7	36.5	71.7

BSA, body surface area; eGFR, estimated GFR from Schwartz formula; GFR, glomerular filtration rate.
[a]Values missing from the two adults (*N*=27).
[b]GFR was calculated from (*I*/total area) × 1.73/BSA.

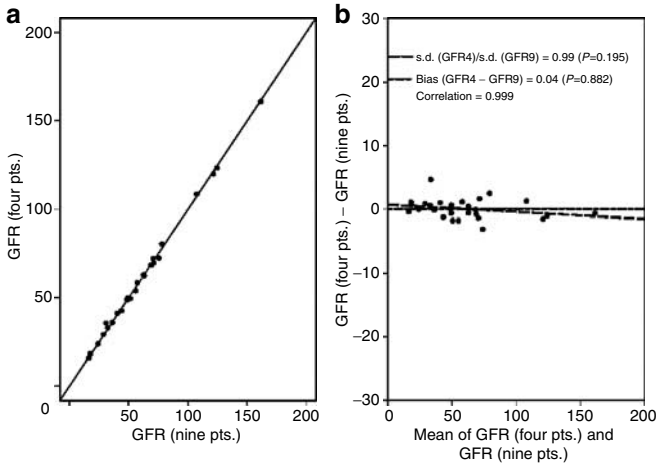

Figure 2 | Two-component GFR comparing nine points against four points. (**a**) Scatterplot of GFR values computed from nine-point iohexol disappearance (*x* axis) vs the four-point GFR computed from samples taken at 10, 30, 120, and 300 min (*y* axis), with line of perfect agreement as reference, showing excellent correlation ($r = 0.999$). (**b**) Bland–Altman plot of GFRs computed from the nine-point GFR (GFR(9)) vs that from the four-point GFR (GFR(4)). The agreement was excellent with no bias or trend towards changing variability at lower GFR values.

linear and squared term was excellent, and the equation was

$$0.9950\mathrm{GFR}(A) - 0.001159(\mathrm{GFR}(A))^2$$

whose coefficients are very similar to those originally published by Brochner-Mortensen.[19] GFR(2) was also calculated solely from the slow (renal) component using the points at 120 and 300 min for the slow component in conjunction with the above equation. The GFR(2) correlated well with that from the 9-point GFR(9) ($r = 0.986$) (Figure 4a). Bland–Altman analysis showed that both the s.d. and the computed bias were not significantly different between the two-compartment and one-compartment GFR values (Figure 4b). One data point was far below zero, indicating significant inaccuracy in the two-point determination of the slow component of that study. This was because

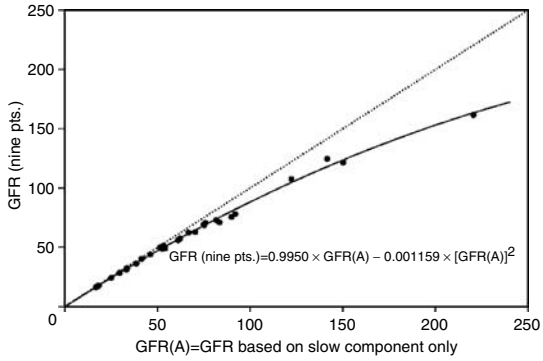

Figure 3 | One-component GFR. Correlation of GFR values computed from nine-point iohexol disappearance (*y* axis) vs GFR computed solely from the iohexol dose divided by the area under the curve of the slow (renal) component that was determined from five points between 120 and 300 min. The one-compartment model progressively underestimated GFR from the two-compartment model.

the concentration at 120 min was unduly low and there were no other points to compensate for this bias, as there is possibly in the presence of four and certainly in the presence of nine points. The two patients with nephrotic syndrome were not outliers on these plots, indicating that edema and ascites fluid did not alter the observed agreement.

Estimated GFR

GFR estimated from the Schwartz formula was also well correlated with the two-compartment GFR ($r = 0.934$) (Figure 5a); however, there was a significant positive bias of 12.22 ml/min per 1.73 m^2, meaning that the eGFR overestimated true GFR. Furthermore, the bias was not significantly different in the four studies performed using Jaffe creatinines. The two adults were not included in this analysis.

Urine GFR

Urine iohexol clearances did not correlate as well as the other clearances with the two-compartment GFR ($r = 0.770$) (Figure 5b). This was owing primarily to large variability in the urine collections. The median CV of the four urine clearances was 24% in the 29 subjects. In addition, the urine clearance significantly underestimated the two-compartment GFR by 13.95 ml/min per 1.73 m^2. One sample showed an underestimation of 97 ml/min per 1.73 m^2 probably reflecting inadequate bladder emptying in a 3-year-old boy. Elimination of that sample yielded an average underestimation of 11.1 ml/min per 1.73 m^2.

DISCUSSION

Use of plasma disappearance for determination of two-compartment GFR

The national CKiD study requires an accurate determination of GFR so that small changes over time can be reliably detected. Whereas inulin is the gold standard for measuring GFR,[20] there are major limitations to using this agent.[21,22]

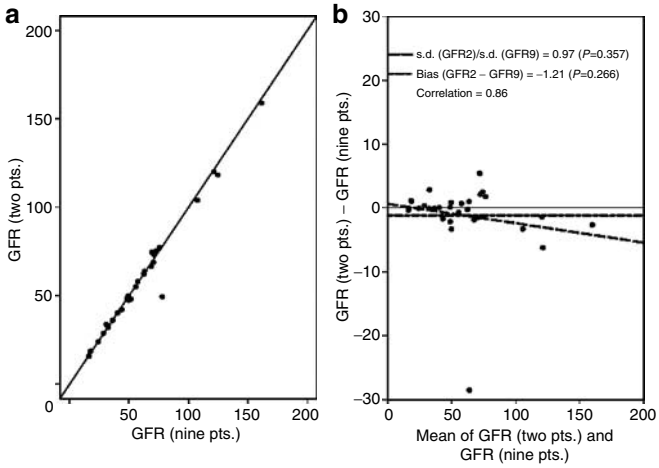

Figure 4 | Two-component GFR based on nine points and one component GFR based on two points (GFR(2)). (**a**) Regression of GFR values computed from nine-point iohexol disappearance ((GFR(9)) vs those computed from the one compartment using the coefficients for a linear and squared term determined from the curve-fitting analysis, which are similar to those used previously by Brochner-Mortensen.[19] The correlation was highly significant at 0.970. (**b**) Bland–Altman plot of GFRs computed from the two compartment model (GFR(9)) vs that from the two-point GFR (GFR(2)). With one exception, the agreement was excellent with virtually no bias and no trend towards changing variability at lower GFR values.

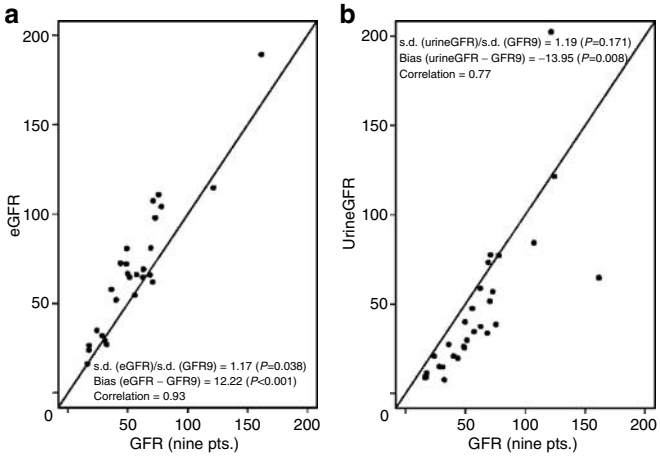

Figure 5 | Comparison of two-compartment GFR (GFR(9)) vs estimated GFR from Schwartz formula and standard urine iohexol clearance. (**a**) Regression of GFR estimated from Schwartz formula (eGFR) vs two compartment GFR(9), showing an excellent correlation coefficient of 0.934. Whereas the correlation was high, the eGFR consistently overestimated GFR(9) by an average of 12.2 ml/min per 1.73 m^2. The variability, while great, did not change as a function of GFR. (**b**) Regression of renal clearance of iohexol (urine GFR) vs the two-compartment GFR(9), showing a weaker correlation coefficient of 0.770. There appeared to be a significant amount of variability at all levels of GFR, and the renal clearance underestimated GFR(9) by 13.95 ml/min per 1.73 m^2.

This has led clinicians to use the plasma disappearance of alternative markers, which diffuse more readily into the extracellular fluids. ^{51}Cr-EDTA (mw 292 Da) serves as an excellent marker for GFR,[23–25] particularly in the UK,[26] but this agent is not available in the USA.

Iothalamate (mw 637 Da) is available as ^{125}I-iothalamate or as a non-radioactive agent. However, iothalamate is actively secreted by renal proximal tubular cells[6] and its clearance exceeds that of inulin or ^{51}Cr-EDTA.[6,27] Thus, this marker is not ideal for measuring GFR, particularly in subjects with reduced kidney function.

Validation of iohexol as a marker for determination of GFR

Based on the experience in Scandinavia, iohexol may be the best alternative available to measure GFR. Iohexol is eliminated exclusively via the kidneys[12,14] and can be completely recovered from the urine within 12–24 h.[12,13] Extrarenal clearance of iohexol does not exceed 2 ml/min per 1.73 m^2.[28] As a non-ionic contrast agent, iohexol causes many fewer adverse reactions than does conventional contrast medium.[13,15] Because the molecular weight of iohexol (821 Da) is somewhat larger than those of the radioactive markers, and the normal elimination half-time is 1.5–2 h,[12,13] the time interval between injection and the final point in plasma sampling should be at least 3 h[9] and longer for children with reduced GFR.

Brandstrom *et al.*[29] examined simultaneous single-injection studies of ^{51}Cr-EDTA and iohexol in 49 adults and found that single-compartment GFRs were highly correlated ($r = 0.922$), and the slope and intercept of the regression were not different from 1 and zero, respectively. These data[29] plus the preceding and comparable study of Krutzen *et al.*[13] in 42 patients ($r = 0.983$) indicate, over a wide range of GFR, that both methods give equivalent results with a mean difference of less than 1 ml/min.

In addition to the excellent agreement of iohexol and ^{51}Cr-EDTA plasma clearances, there is also excellent agreement between the iohexol and inulin clearances. Gaspari *et al.*[11] have shown in 41 patients excellent agreement ($r = 0.982$) between iohexol plasma clearance and inulin renal clearance, and Bland–Altman analysis revealed the mean difference between the two methods to be -1.02 ml/min per 1.73 m^2. Brown and O'Reilly[15] showed in 30 patients an excellent agreement between iohexol plasma clearance and renal inulin clearance ($r = 0.983$) with a mean ratio not significantly different from 1. Similarly, Erley *et al.*[16] showed an excellent agreement in ICU patients between constant inulin infusion clearance and iohexol plasma clearance ($r = 0.980$). In children, Lindblad and Berg[30] showed a good correlation ($r = 0.822$) between iohexol slope clearance and renal inulin clearance. The failure to show a better correlation might have reflected insufficient time for equilibration of the blood samples (1, 2, and 3 h after iohexol injection). (Immediately after intravenous injection, the marker substance leaves the plasma both by glomerular filtration and by diffusion into the extravascular body fluid compartments. The amount of marker substance excreted during this early phase (fast curve) is 'lost' from the body fluids and thus a somewhat smaller dose than injected is distributed in the body fluid compartments. With time, the concentrations in plasma and extravascular fluid equalize, and the plasma elimination rate becomes constant (slow curve). However, because of glomerular filtration, the concentration in the extracellular fluid from then on remains somewhat higher than the plasma concentration). In sum, iohexol plasma clearance appears to yield results that are comparable to those obtained using renal inulin clearance and plasma ^{51}Cr-EDTA disappearance. Indeed, some have suggested that iohexol is the new gold standard measure of GFR.[15,17]

Plasma disappearance of iohexol to measure GFR

The GFR is calculated from the dose of iohexol divided by the area under the plasma disappearance curve (AUC), fitted by a double exponential equation. The two curves can be well approximated by obtaining serum samples at 10, 30, 120, and 300 min, yielding nearly as accurate a measurement as that obtained using the nine points over this time period (see Figure 2). Moreover, for most routine clinical practices, GFR can be satisfactorily estimated by using two blood samples to determine the second exponential (slow, renal curve) in conjunction with the Brochner-Mortensen equation, which we showed here to be very consistent whether iohexol (coefficients: 0.9950 and -0.001159; see Figure 3) or ^{51}Cr-EDTA (coefficients: 0.990778 and -0.001218 for adults;[19] 1.01 and -0.0017 for children[31]) are used. The agreement with the coefficients generated by Brochner-Mortensen is impressive, considering that the original study was performed in adults and the GFR was determined by ^{51}Cr-EDTA. Furthermore, based on preliminary analysis of the first 68 children in the CKiD study, we continue to show excellent agreement (i.e., no bias, same variances and correlation = 0.997) between the GFR based on four points (at 10, 30, 120, and 300 min) and the GFR based on two points (at 120 and 300 min). These additional data strengthen the conclusions based on the pilot study ($N = 29$).

The agreement between two points and nine points in the pilot study and of two points and four points in the preliminary data of CKiD makes it conceivable that the iohexol procedure can be simplified to two points. However, if one uses only two points to measure the slow component, the first of these should be taken no earlier than 2 h after injection to avoid a contribution from the fast exponential.[26] In addition, in children with decreased GFR, the subsequent blood samples should be obtained not earlier than 5 h after injection.

Our pilot study suggests that two points taken at 120 and 300 min after iohexol injection are adequate to determine GFR. However, when using only two points, if one of them is inaccurate, it will have a great impact on the determination of GFR (see outlier in Figure 4). In contrast, when having four points (e.g., at 10, 30, 120, and 300 min), not only have we shown excellent agreement with the nine-point GFR (see Figure 3), but four points allow for the use of crossvalidation methods to make four concentrations mutually consistent. These considerations allow us to recommend a non-radio-

active iohexol-based approach with four points at 10, 30, 120, and 300 min for the nation-wide cohort of children.

Whether or not in the future one simplifies the procedure to utilize the one-compartment approach for all patients depends on the effect of significant edema and ascites on this relation, and this question will be addressed in the first year of the national study. In the pilot study, two patients had significant edema and ascites but the one-compartment GFR was not altered by these physical findings.

Comparison with estimated GFR

Daily clinical practice requires a method for simple and inexpensive yet accurate methods for evaluating GFR. Whereas serum creatinine and estimating equations are widely used in adults for this purpose, they have less utility in pediatrics because of the maturational increase in serum creatinine that occurs concurrently with the increment in muscle mass.[2,32] Since the 1970s, pediatric nephrologists have utilized formulae based on patient height and serum creatinine, because of the close relationship between height and muscle mass.[2,18,33] The formulae were initially developed based on serum creatinine measured by a colorometric reaction with alkaline picrate (Jaffe). More recently, serum creatinine has been measured enzymatically[2] and eGFR formulas have not been generated using enzymatic creatinines. Because enzymatic creatinine determinations yield smaller values than the Jaffe reaction, one would expect the Schwartz-derived eGFR formulae to overestimate true GFR, as seen in this pilot study.

Difficulties with urine collection to measure GFR

It is well known that collection of urine without bladder catheters does not add significant accuracy to the clearance estimate of GFR.[34] Indeed, 24 h urines may vary by as much as 10–15% from one day to another[35,36] and this variability limits the accuracy of renal clearances. Among children with chronic kidney disease, many of whom have bladder dysfunction (e.g., bladder dyssynergia, neurogenic bladder, vesicoureteral reflux), variability in urinary clearances is likely to be even larger than those seen in adult studies.

In our pilot study, the GFR determined from the renal iohexol clearance was poorly correlated with true GFR ($r = 0.770$), and the negative bias indicated that renal iohexol clearance was less than plasma disappearance GFR. Although one might conclude that there is non-renal elimination of iohexol, previous studies have shown minimal non-renal iohexol elimination.[7,12,13,28] It is more likely that the negative bias in the pilot study reflects inadequate bladder emptying. Urologic problems, which occur in a large percentage of children with chronic renal disease, may cause incomplete bladder emptying and hence lead to inaccurate determinations of GFR.

In summary, we have validated the use of iohexol plasma disappearance as a useful measure of GFR in children. Whereas the pilot study utilized the data from nine points obtained during 6 h, we have shown that four points at 10, 30, 120, and 300 min suffice. Furthermore, in most cases, two determinations along the slow curve would be adequate to accurately determine GFR. The main caveat is to allow for complete iohexol equilibration, which may require sampling as late as 5 h after iohexol injection, particularly at low GFR. It is likely that the two- or four-point plasma disappearance of iohexol will be utilized as a standard measure of GFR in situations in which estimates of GFR are likely to be imprecise, as well as in clinical trials and observational studies of chronic kidney disease.

MATERIALS AND METHODS

Subjects

Twenty-nine subjects, including 27 children and two adults, were consented. Each ingested a low-protein diet (19 and 34 g/day for 4–8- and 9–13-year-olds, respectively) on the day before study and no food after midnight. The study was approved by the Research Study Review Boards of the University of Rochester and Johns Hopkins School of Medicine. Fifteen subjects were studied in the General Clinical Research Center of the University of Rochester School of Medicine and 14 in the Pediatric Clinical Research Unit of the Johns Hopkins School of Medicine. At the visit, height, weight, and vital signs were determined, a fluid load of 5 ml/kg given, and two intravenous lines placed, one for blood sampling and one for the iohexol bolus administration. Demographic data showing medians and the first and third quartiles are shown in Table 1. The status of the 27 pediatric subjects at the time of study included 12 with obstructive uropathy-dysplasia, four with kidney transplants, four with chronic glomerulonephrits, and seven with other diseases (including hereditary and cystic kidney disease).

Studies and assays

Before study and as standard of care, blood was obtained for serum creatinine determination; an aliquot was also obtained for HPLC analysis to determine if there were any detectable peak before iohexol infusion. At the University of Rochester, creatinine was determined enzymatically using a Vitros 950 (Ortho Clinical Diagnostics, Rochester, NY, USA) or an Advia 2400 (Bayer Diagnostics, Tarrytown NY, USA) analyzer. At Johns Hopkins, creatinine was determined in 10 of 14 subjects by enzymatic assay (Roche Hitachi Modular P800 (Holliston, MA, USA) and in four by a Jaffe colorimetric assay. Iohexol (5 ml; GE Healthcare, Amersham Division, Princeton, NJ, USA) was administered from a pre-weighed syringe beginning at time zero over a period of 1–2 min. The syringe was then weighed to the nearest tenth gram on the same scale used before the injection. The dose of iohexol (I, in mg) was calculated from the difference in syringe weights multiplied by the concentration of iohexol (647 mg/ml) divided by its density at room temperature (1.345). The median dose of iohexol was 3185 mg (Table 1). Vital signs were taken 10 min after completing the infusion to assure that there were no untoward reactions by the subjects. One subject with shell-fish allergy was given prednisone and diphenhydramine before study. This subject had no allergic reaction to the iohexol. One child had some nausea and vomited once 5 h after the infusion. None of the other 28 subjects had any significant adverse reaction to iohexol.

Blood samples (1 ml) were obtained at 5, 10, 20, 30, 60, 120, 180, 240, 300, and 360 min after starting the iohexol injection. Each sample was placed into a serum separator tube, inverted 5–10 times, and allowed to stand for 30 min, before being centrifuged at 1100 g

for 10 min. The sera obtained were deproteinated by the addition of four volumes of 5% perchloric acid, followed by centrifugation. The HPLC analysis of the supernatant was carried out on an Agilent 1100 HPLC system with variable wavelength ultraviolet detector and a Zorbax Eclipse XBD-C8 4.6 × 150 mm, 5 μm particle size column. The chromatographic conditions included an injection volume of 25 μl, isocratic elution at 1 ml/min with 20 mM potassium phosphate pH 2.5 and 4% acetonitrile; the column was maintained at 30°C, peaks were monitored at 254 nm. The second and larger of the two iohexol peaks, eluting at ~4 min, was used for determination of iohexol concentration (mg/l). Standards were prepared by diluting injectable iohexol in sheep serum. Initial tests adding iohexol to the sera of kidney transplant patients (taking at least 10 different medications) did not show significant losses in iohexol recovery (not shown). In all 29 studies, there was no detectable peak at ~4 min in the blood samples taken before iohexol infusion, indicating no interference with the assay.

After completion of the iohexol bolus, subjects were asked to provide a 'discard' urine sample. The time of this discard was taken to be the beginning of the first urine collection. The volume of the discard was measured and the subjects were asked to drink the equivalent volume of fluids. Four collections of urine were then obtained following this format. Volumes were measured to the nearest 5 ml.

Calculations

Two-compartment model. Following the approach of Sapirstein[37] the disappearance of a glomerular marker can be resolved into two curves using the logarithm of the concentration of iohexol as a function of time. The slow (renal) curve was determined from the concentrations obtained at 120–360 min. The fast curve was determined from 10 to 60 min concentrations. Because the iohexol concentrations at the 5 min point were frequently less than those at 10 min (reflecting ongoing equilibration), the 5 min data were not utilized in any analysis and GFR was determined from the remaining nine points (GFR(9)).

GFR was calculated from

$$\mathrm{GFR} = I/(\exp A/\alpha + \exp B/\beta) \times 1.73/\mathrm{BSA},\ \text{in ml/min per } 1.73\mathrm{m}^2$$

where I is the dose of iohexol (mg), $\exp A$ is the intercept of the slow curve, and α its corresponding slope, $\exp B$ the intercept of the fast curve, and β its corresponding slope; GFR was corrected to 1.73 m^2 body surface area (BSA) by the ratio 1.73/BSA.

Body surface area. Values of BSA were determined from the subject's height and weight using the formula of Haycock *et al.*:[38]

$$\mathrm{BSA}(\mathrm{m}^2) = 0.024265 \times \mathrm{Wt}^{0.5378} \times \mathrm{Ht}^{0.3964}$$

where Wt is the subject's weight in kg and Ht the height in cm.

To reduce the number of necessary serum samples in preparation for the nation-wide study, we compared a four-point GFR (GFR(4)), comprised of the samples taken at 10, 30, 120, and 300 min against GFR(9). These samples are the minimum required to determine both the slow curve (120 and 300 min) and the fast curve (10 and 30 min).

One-compartment model. The one-compartment model is predicated on the observation that most of the GFR can be approximated by the slow (A, α) curve. Consistent with the observation of Brochner-Mortensen,[19]

$$\mathrm{GFR} = C_1 \times \mathrm{GFR}(A) + C_2 \times (\mathrm{GFR}(A))^2$$

where $\mathrm{GFR}(A) = (I/(\exp A/\alpha)) \times 1.73/\mathrm{BSA}$. and C_1 and C_2 are constants determined empirically by curve-fitting against the two-compartment GFR. Specifically, to determine C_1 and C_2, we regressed GFR(9) on GFR(A) based only on the slow curve from the five concentrations obtained at 120–360 min.

To explore the possibility that only two points may suffice to measure GFR, we compared a two-point GFR (GFR(2)), comprised of the samples taken only at 120 and 300 min, to characterize the slow curve and used it in the Brochner-Mortensen-like equation[19] to calculate the GFR. Specifically,

$$\mathrm{GFR}(2) = C_1 \times \mathrm{GFR}(a) + C_2 \times (\mathrm{GFR}(a))^2$$

where GFR(a) is the GFR of the slow curve derived from the concentrations at 120 and 300 min.

Estimated GFR. The eGFR was calculated from:

$$\mathrm{eGFR} = k \times \mathrm{Ht/Scr},\ \text{in ml/ min per } 1.73m^2$$

where $k = 0.55$, except in males above 13-years old ($k = 0.7$),[2,39] Ht (height) in cm, and Scr is serum creatinine in mg/dl. This formula is not valid in adults and hence was not used for the two adult subjects.

Renal clearance of iohexol (GFR_i). The standard renal clearance equation was used to calculate $\mathrm{GFR_i}$ and the four data points were averaged as a geometric mean for each subject:

$$\mathrm{GFR_i} = U_i \times V_i / S_i$$

where U_i is the iohexol concentration in the ith urine sample, V_i is the urine volume of the ith urine sample, and S_i is the area under the serum iohexol disappearance curve while urine is being collected for the ith clearance.

Statistics

Nonparametric statistics (e.g., medians and quartiles) were used to describe the demographics of the study population and the components of the calculations of GFRs.

To describe the agreement between two variables Y (e.g., GFR(9)) and X (e.g., GFR(4)), we depicted the standard plot of X vs Y and the corresponding Bland–Altman[40] plot of the average of X and Y vs the difference of X from Y. The slope of the linear regression of the difference ($X-Y$) on the average ($(X+Y)/2$) from the Bland–Altman plot is directly related to the ratio of the s.d. of X and Y (i.e., the s.d. of X and Y are equal if and only if the slope is zero), and the residual variance is directly related to the correlation between X and Y. The mean of the differences between the two tests is referred to as bias. Testing the null hypothesis of no bias (i.e., mean difference = 0) is accomplished by application of the paired t-test. The testing of no bias and equality of s.d. can be accomplished in a unified analysis by centering $(X+Y)/2$ around its mean and performing the regression as indicated above.

Standard regression methods for Gaussian data were used to derive the estimates of the coefficients of the Brochner-Mortensen equation,[19] which amounts to a quadratic regression model with no intercept with GFR(9) as the dependent variable and both GFR(A) and the square of GFR(A) as the independent variables.

ACKNOWLEDGMENTS

The CKiD is funded by the National Institute of Diabetes and Digestive and Kidney Diseases, with additional funding from the National Institute of Neurological Disorders and Stroke, the National Institute of Child Health and Human Development, and the National Heart, Lung, and Blood Institute (UO1-DK-66143, UO1-DK-66174,

UO1-DK-66116). The CKID website is located at http://www.state-pi.jhsph.edu/ckid. The clinical coordinating centers (Principal Investigators) are at Children's Mercy Hospital and the University of Missouri – Kansas City (Bradley Warady, MD) and Johns Hopkins School of Medicine (Susan Furth, MD, PhD), and the data coordinating center (Principal Investigator) is at the Johns Hopkins Bloomberg School of Public Health (Alvaro Muñoz, PhD), with the Central Biochemistry Laboratory at the University of Rochester (George J Schwartz, MD). We are grateful to GE Healthcare, Amersham Division, for providing us with the iohexol (Omnipaque™). The pilot study was conducted in the General Clinical Research Center at the University of Rochester and the Pediatric Clinical Research Unit of the Johns Hopkins School of Medicine, which were supported by NIH Grants M01 RR00044 and M01 RR00052. We are indebted to Ms Paula Maier for coordinating the study and all of the samples, and to Mr Brian Erway and Dr Tai Kwong for skillfully developing the iohexol HPLC assay at the University of Rochester Medical Center.

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