

Measurement and Estimation of GFR in Children and Adolescents

George J. Schwartz and Dana F. Work

Department of Pediatrics, University of Rochester School of Medicine, Golisano Children's Hospital at Strong, Rochester, New York

GFR is the best indicator of renal function in children and adolescents and is critical for diagnosing acute and chronic kidney impairment, intervening early to prevent end-stage renal failure, prescribing nephrotoxic drugs and drugs cleared by a failing kidney, and monitoring for side effects of medications. Renal inulin clearance was the gold standard for GFR but is compromised by lack of availability, difficult assays, and problems of collecting timed urine samples, especially in children with vesicoureteral reflux or bladder dysfunction. Creatinine clearance-based estimates of GFR are often used in pediatrics. The addition of cimetidine to eliminate creatinine secretion permits accurate measurement of GFR in those who can completely empty their bladders to provide timed urine collections. Radioisotopes are used in plasma disappearance GFR determinations; however, these are not ideal for use in children, especially for repeated studies. The plasma disappearance of iohexol serves as a promising alternative GFR marker, because it is safe and not radioactive, easily measured, not metabolized or transported by the kidney, and excreted primarily by glomerular filtration. GFR estimating equations, based on serum concentrations of creatinine or cystatin C, are popular clinically and in research studies. Efforts are ongoing to improve these estimating equations for children and make the results readily available to clinicians obtaining standard chemistry profiles, as is being done for adults. However, at this time, there is no dependable substitute for an accurately determined GFR, and iohexol plasma disappearance offers the best combination of safety, accuracy, and reproducible precision.

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In children and adolescents with early chronic kidney disease (CKD) and a well-maintained fluid and electrolyte balance, the urinalysis may be entirely normal. Therefore, a reduced GFR may serve as the only clinical sign of kidney damage in these individuals. Having an accurate means of determining GFR is critical for determining optimal doses of fluids and medications, monitoring for nephrotoxicity caused by antibiotics and chemotherapeutic agents, and assessing progression of renal disease. Early intervention in the course of renal impairment offers the best chance of preventing ESRD in children, adolescents, and young adults.

Determination of GFR

The level of GFR is the product of the single-nephron GFR multiplied by the number of functioning nephrons in both kidneys. In the case of CKD, GFR can be decreased because of a reduction in filtration rate of each nephron and/or a drop in nephron number. Factors leading to decreased renal perfusion may cause a drop in the single nephron GFR. The total GFR serves as the most reliable marker of functioning renal mass.

The most common method of measuring GFR is based on the

concept of clearance. The renal clearance of substance x (C_x) is calculated as:

$$C_x = U_x V / P_x$$

where V is the urine flow rate (ml/min), U_x is urine concentration of substance x, and P_x is the plasma concentration of substance x. C_x is expressed in milliliters per minute. If the substance is freely permeable across the glomerular capillary and is not synthesized, transported, or metabolized by the kidney, C_x is equal to GFR.

To compare GFR measurements among infants, children, and adults of different sizes, it is necessary to scale for a standard of reference. Although kidney weight would be the most direct, such data are not available. However, kidney weight does bear a rather constant relation to body surface area (BSA) in humans and in a variety of animal species (1). In addition, the measurement of BSA has a small coefficient of variation, and scaling GFR to BSA shows that infants reach adult GFR values by 6 to 24 mo of age (2–5). Because absolute GFR is strongly correlated with BSA (1) and the regression coefficient for BSA was not significantly different from 1, adjusting GFR for BSA essentially removes the variability of GFR that is caused by the variation in pediatric body size (6). BSA in children and adolescents can be determined from the formula of Haycock *et al.* (7):

$$BSA(m^2) = 0.024265 \times \text{Weight}^{0.5378} \times \text{Height}^{0.3964},$$

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Correspondence: Dr. George J. Schwartz, Professor of Pediatrics and of Medicine, Chief, Pediatric Nephrology, Box 777, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642. Phone: +585-275-9784; Fax: +585-756-8054; E-mail: George_Schwartz@urmc.rochester.edu

where weight is measured in kilograms and height in centimeters. GFR is scaled to a BSA of 1.73 m² with unit of measurement milliliters per minute per 1.73 m².

The renal clearance of a substance is essentially equal to the plasma clearance of that substance provided that its nonrenal clearance is negligible. When this is the case, GFR can be calculated using a single-injection clearance technique by monitoring the substance's rate of disappearance from the plasma after the injection (8). Some examples of agents used in single injection plasma disappearance curves to measure GFR include radioisotopes such as ^{99m}Tc-DTPA (diethylene triamine pentaacetic acid), ⁵¹Cr-EDTA (ethylene diamine tetraacetic acid), and ¹²⁵I-iothalamate, as well as nonradioactive iothalamate, inulin, and iohexol.

Inulin Clearance and Measurement of GFR in Children

The renal clearance of inulin remains the gold standard for the evaluation of GFR in children and adults. Inulin is not protein bound and is freely filtered by the glomerulus. It is not secreted, metabolized, or reabsorbed by the renal tubules, making this exogenous substance an ideal GFR marker (1). Table 1 shows normal values of GFR determined by inulin clearance in infants, children, adolescents, and young adults.

Classic (standard) inulin clearance requires an intravenous priming dose of inulin followed by a constant infusion to establish a steady-state inulin plasma concentration. The process requires the maintenance of a steady-state concentration for about 45 min, followed by serial urine samples collection every 10 to 20 min (10). Urine can be collected with a urinary catheter or voluntarily. In children who have not yet mastered toilet training or have conditions such as neurogenic bladder, dysfunctional voiding, or vesicoureteral reflux (11), the collection of timed urine specimens will not be accurate (G. J. Schwartz, unpublished observations). Winterborn *et al.* (12) found that in children with vesicoureteral reflux, there is a significant reduction in the correlation of inulin clearance with

single injection ⁵¹Cr-EDTA plasma disappearance compared with individuals without vesico-ureteral reflux disease.

Inulin clearance can also be determined by a constant infusion technique measuring plasma clearance, without collecting urine (13). After the inulin reaches equilibrium in its distribution space, the excretion rate equals the infusion rate and the clearance of inulin can be calculated:

$$C_{In} = I_{In} \times R/S_{In}$$

where I_{In} is the infusion concentration of inulin, R is the infusion rate (ml/min), S_{In} is the serum concentration of inulin, and C_{In} is the clearance of inulin in milliliters per minute per 1.73 m². There is a great deal of technical difficulty associated with this method, primarily because it is difficult to obtain constant inulin plasma or serum concentrations during intravenous infusion (14). In addition, inulin has a high molecular weight, and this takes time to fully equilibrate. If the steady state is not reached, unequilibrated samples will show lower concentrations, and this would lead to an apparent overestimation of GFR (14).

In an effort to simplify GFR determination using inulin plasma clearance, it would be important to optimize the blood sampling, particularly in children. Swinkels *et al.* (15) aimed to optimize the method for calculating GFR in children through the use of serum inulin clearance by using the least number of possible blood samples. The study analyzed 117 serum inulin time-decay curves in 59 children with renal disease. The two-compartment exponential inulin plasma disappearance model was compared with the one-compartment model. According to the two-compartment model, the initial rapid fall in plasma inulin is caused by the distribution of inulin from the intravascular to the interstitial fluid, and the more gradual fall occurring later is mainly caused by the renal excretion of inulin. The optimum number of sampling points needed was found to be seven (at times 0, 10, 20, 30, 65, 120, and 240 min) to maintain a good fit to the plasma disappearance curve using the two-compartment model (15). That is, four points were needed to describe the distribution phase and two points to describe the renal elimination phase; a blank is routinely obtained at time zero. In general, the distribution phase did not contribute significantly to the elimination phase more than 65 to 90 min after injection (15). We recently confirmed these findings for the plasma disappearance of iohexol (16), but we concluded that only five sampling points were needed (at times 0, 10, 30, 120, and 300 min), such that two points each were used to describe the distribution and renal excretion curves without any significant loss of accuracy.

Whereas plasma disappearance of most GFR markers is better modeled with three to four exponential functions (17–19), particularly with sampling times beyond 4 h, two compartments simplify the analysis, reduce the number of blood samplings, and provide reasonable accuracy (8,20,21). For iohexol and ⁵¹Cr-EDTA, two-compartment plasma disappearance models seem to correlate well with renal clearance data (22,23). The one-compartment model examines only renal excretion. As expected, the GFR found by Swinkels *et al.* using the one-

Table 1. Glomerular filtration rate in normal children and young adults without renal disease determined by inulin clearance

Age (yr)	GFR (ml/min/1.73 m ²) (mean ± SD)
3–4	111.2 ± 18.5
5–6	114.1 ± 18.6
7–8	111.3 ± 18.3
9–10	110.0 ± 21.6
11–12	116.4 ± 18.9
13–15	117.2 ± 16.1
2.7–11.6	127.1 ± 13.5
9–12	116.6 ± 18.1
16.2–34	112 ± 13

Data compiled from references 2 and 9.

compartment model was higher than that obtained using the two-compartment model, reflecting the smaller area under the curve obtained using only the single compartment curve.

Brochner-Mortensen (17) published a similar result with $^{51}\text{Cr-EDTA}$ plasma disappearance. By curve fitting, he showed that the one-compartment clearance exceeded the three to four exponential plasma clearance, and the relative difference increased with increasing clearance values. His correction for plasma clearance in adults based on the renal excretion curve was:

$$\text{Plasma clearance} = \text{GFR} = 0.990778 C_1 - 0.001218 C_1^2,$$

where C_1 is the one-compartment model clearance or renal excretion curve.

Chantler *et al.* (24) compared the monoexponential plasma disappearance of $^{51}\text{Cr-EDTA}$ with the renal clearance of inulin and found a relatively constant correction factor of 0.93 should be multiplied by the one-compartment plasma disappearance to yield an accurate measure of GFR. This factor would reduce the overestimation of GFR by the one-compartment plasma disappearance curve but, unlike Brochner-Mortensen (17) and Brochner-Mortensen *et al.* (25), they found the correction was relatively constant through the whole range of GFR. In any case, because of the delay in equilibration with the extravascular fluid space, one-compartment plasma disappearance curves should not be used in patients with significant edema or ascites (24,26).

Recently, by measuring GFR in children using a two-compartment iohexol plasma disappearance curve (16), we found that the plasma clearance could be well approximated by generating coefficients according to the method of Brochner-Mortensen (17):

$$\text{GFR} = 0.9950 C_1 - 0.001159 C_1^2.$$

These coefficients are remarkably similar to those generated by Brochner-Mortensen (see above), thereby validating iohexol as an excellent marker for determination of GFR.

Whereas the single injection method is much less technically difficult and time consuming, the continuous infusion method is generally thought to be more accurate. Van Rossum *et al.* (20) compared the plasma clearance of inulin using a single injection method with the continuous infusion method in 24 pediatric patients with a median GFR of 42 ml/min per 1.73 m². The advantage of the single injection method is that it takes about 4 h compared with a much longer period of time to reach a complete equilibrium of inulin when using a continuous infusion (27). Using the single injection method (20), inulin was infused, and blood samples were taken at 0, 10, 30, 90, and 240 min after injection. For the continuous infusion method, inulin was infused overnight, and three capillary blood samples were taken the next day. The results of the study showed an inulin plasma clearance that was on average 9.7 ml/min per 1.73 m² higher than that determined by the use of a continuous infusion, a statistically significant difference. Moreover, this difference was found to decrease for individuals with GFR values less than 50 ml/min per 1.73 m² and to increase for those with

GFR values greater than 50 ml/min per 1.73 m². The study did not compare either plasma method with the renal (urinary) clearance of inulin, which remains the gold standard for GFR determination.

Hellerstein *et al.* (27) showed that the infusion clearance of inulin (after appropriate time for equilibration) consistently exceeded the renal inulin clearance by 13.8 ml/min per 1.73 m². The reason for this difference was probably that the inulin infusion rate exceeded the inulin excretion rate, although plasma inulin levels were constant, suggesting that full equilibration of inulin had not yet occurred, even after an overnight infusion. The issue of the timing of equilibration, plus the inequality with renal clearances, has prevented infusion clearances from becoming the gold standard measure of GFR. Indeed, given the lack of availability, the difficulty in measuring its concentrations, and the slow rate of equilibration, the general use of inulin in pediatric nephrology is currently limited.

Iohexol Clearance

The use of iohexol clearance serves as a promising alternative to inulin for accurate GFR determination. Iohexol is a nonionic low osmolar contrast agent that is used intravenously at much higher doses for radiologic procedures even in the presence of renal disease and is not reabsorbed, metabolized, or secreted by the kidney (22,28,29). Iohexol is excreted completely unmetabolized in the urine (22,28,30,31). There is close agreement between GFR values obtained with iohexol clearance compared with inulin clearance (14,22,28,32,33). Iohexol may equilibrate faster than inulin within the various body compartments based on its smaller size. Iohexol has been found to have an extremely low toxicity even when used in radiographic doses which are 10 to 50 times higher than those used for GFR determination (32,34,35). Indeed, no serious adverse events have been noted in more than 15 yr of experience in Scandinavia (34) or in more than 900 GFR determinations performed in the NIH-supported North American Chronic Kidney Disease in Children (CKiD) cohort study (6).

To test the use of iohexol in advance of recruiting subjects for the CKiD study, we used an HPLC method to assay sera for iohexol concentration and performed a pilot study (16) at the University of Rochester Medical Center and at Johns Hopkins Hospital. We showed that GFR could be measured accurately by collecting, in addition to a zero-time blank, nine blood samples at 10, 20, 30, 60, 120, 180, 240, 300, and 360 min after a single injection of iohexol. When the concentration of iohexol was plotted as a function of time, the GFR could be calculated from the iohexol dose and the area under the curve as a function of time in a two-compartment model. Furthermore, we found that the two compartments could be well described from only four points, at 10, 30, 120, and 300 min, resulting in a high correlation with the nine point plasma disappearance curve ($r = 0.999$). The clearance of iohexol could also be determined from the slow (renal) curve using the mono-exponential (Brochner-Mortensen) method ($r = 0.986$). In the CKiD pilot study, it was found that the one-compartment clearance overestimated the GFR compared with the two-compartment model. However, the coefficients that were generated to correct for this overes-

timation were very similar to the quadratic equation published previously by Brochner-Mortensen (17), thus showing the (1) usefulness and overall applicability of a one-compartment model of iohexol disappearance, (2) similarity of iohexol plasma disappearance to that of ^{51}Cr -EDTA, and (3) adequacy of the two-compartment model to describe iohexol disappearance over a 5-h observation period.

Although single injection clearances lead to an equilibrated state more quickly than infusion studies, adequate time must be taken to monitor this disappearance. In children, because of the lower absolute GFR compared with adults, an accurate plasma disappearance curve should be based on blood samples taken over at least 5 h (31,36). In the setting of CKD and severely reduced GFR, or in premature infants, late sampling should be performed 8 to 24 h after injection (36,37).

In an effort to use plasma disappearance as an epidemiologic tool, Niculescu-Duvaz *et al.* (38) compared fingerprick blood sampling with standard intravenous blood draws for determining GFR measurements. Excellent agreement was found between clearances based on blood spots collected on filter paper after fingerpricks and those based on serum samples. This finding suggests that subjects could be injected in a treatment center and then sent home with a timer, filter paper, and lancets to obtain the blood samples at home. Longer time sampling could be used to improve accuracy of the mono-exponential slope clearance. With iohexol being stable for at least 1 wk at room temperature (G. J. Schwartz, B. Erway, and T. Kwong, unpublished observations), the blood spots on filters can be sent back to the laboratory by mail for analysis without the need for specialized shipping or handling. We believe this fingerprick approach will facilitate the use of iohexol-based GFR measurements in epidemiologic studies and elective outpatient renal screening tests.

Use of Radioisotopes

Because of the ease of assaying concentrations, radioisotopes are commonly used for the determination of GFR. The most frequently used radioisotope in the United States is $^{99\text{m}}\text{Tc}$ -DTPA. GFR can be determined by measuring the uptake of $^{99\text{m}}\text{Tc}$ -DTPA by each kidney with a scintillation camera or, more accurately, through the use of a single intravenous injection with subsequent monitoring of timed serum samples (39). Although the plasma clearance of $^{99\text{m}}\text{Tc}$ -DTPA has been shown to correlate well with the renal clearance of inulin (40), the accuracy of $^{99\text{m}}\text{Tc}$ -DTPA has been shown to differ depending on the commercial source used, which may be caused by variabilities in protein binding (41).

^{51}Cr -EDTA and ^{125}I iothalamate are two other radioisotopes used to measure GFR. The plasma clearance of ^{51}Cr -EDTA has been shown to correlate well with the renal clearance of inulin (40); however, it is not available in the United States. Table 2 shows normal values for ^{51}Cr -EDTA clearance in infants and children. ^{125}I iothalamate has been used extensively to measure GFR in children (43–45), both by standard renal clearance and by constant infusion and plasma disappearance; however, there are problems with this agent. The use of renal iothalamate clearances with the collection of urine result in major inaccuracies,

Table 2. Plasma ^{51}Cr -EDTA clearance in normal infants and children

Age (mo)	Mean GFR \pm SD (ml/min/1.73 m ²)
≤ 1.2	52.0 \pm 9.0
1.2–3.6	61.7 \pm 14.3
3.6–7.9	71.7 \pm 13.9
7.9–12	82.6 \pm 17.3
12–18	91.5 \pm 17.8
18–24	94.5 \pm 18.1
>24	104.4 \pm 19.9

Data from reference 42.

especially in children, because of the inability to assure quantitative emptying of the bladder (43,46,47). Indeed, urine collection adds to the uncertainty of measuring GFR in children and adults (16,48–51). Our pilot study with iohexol (16) showed that collection of multiple samples of urine resulted in a median coefficient of variation of the renal clearance of 24% in the measurement. More importantly, Odland *et al.* (52) studied the renal clearance of unlabeled iothalamate in six healthy volunteers. It was found that the renal clearance of iothalamate equaled that of creatinine but exceeded that of inulin by 38% ($P < 0.01$). When the plasma clearance of ^{51}Cr -EDTA was compared with iothalamate using the single injection technique in 19 patients, the average plasma clearance of iothalamate was 13% higher than that of EDTA ($P < 0.001$). This difference was reduced by pretreatment of the patients with probenecid. This study showed that iothalamate is subject to significant tubular secretion by renal proximal tubular cells, making it an inferior marker for measuring GFR.

Creatinine-Based Measurement of GFRs

Because of the expense and time needed to use exogenous markers for the determination of a true GFR, the estimation of GFR from the renal clearance of creatinine has been used frequently in the pediatric patient population. Creatinine is secreted by renal tubular cells and filtered by glomerular capillaries. The extrarenal clearance of creatinine in people with normal kidney function is relatively small. However, in patients with CKD, as much as two thirds of total daily creatinine excretion can occur by extrarenal elimination (53). Therefore, falsely elevated GFR values can be associated with the use of creatinine clearance, especially in individuals with chronic renal failure (54).

Cimetidine, a histamine receptor antagonist, has been found to inhibit tubular creatinine secretion in patients with renal disease, leading to a creatinine clearance value more representative of the true GFR (46,55). To evaluate the reproducibility of timed-urine collections for renal clearance studies, Hellerstein *et al.* (46) examined 222 cimetidine creatinine clearance studies done on 32 pediatric patients from 4.8 to 21 yr of age. According to the protocol, cimetidine was taken twice daily for 2 d before the study and once in the morning on the day of the study. The

total daily dose of cimetidine was 20 mg/kg for individuals with a previously measured clearance of 75 ml/min per 1.73 m² or greater, with a maximum daily dose of 1600 mg. A sliding scale dose reduction was applied to patients with a decreased GFR. To ensure that residual urine was less than 10 to 15 ml, bladder scans were done before and after a 2-h urine collection period. The coefficient of variation of the rate of creatinine excretion was found to be approximately 10%. Note that the use of any renal clearance method for the determination of GFR is problematic in many children because it requires, in addition to toilet training, complete bladder emptying. Bladder scans are not routinely available. The use of bladder catheters for performing such clearances is not routinely recommended for clinical use and is contraindicated for research studies.

Estimation of GFR without Urine Collection

To estimate GFR without collecting urine, we advanced the concept of height, as a measure of muscle mass, divided by serum creatinine as being a strong surrogate marker of GFR in children (56). Taking into account the relationship between creatinine production and muscle mass, we derived a formula (56), which has become popularized as the Schwartz formula and used to estimate GFR: $eGFR = k \times L/S_{Cr}$, where eGFR is estimated GFR in milliliters per minute per 1.73 m², L is height in centimeters, S_{Cr} is serum creatinine in milligrams per deciliter, and k is an empirical constant determined by comparing the L/S_{Cr} ratio against measured GFR (57). The value of k is 0.45 for term infants throughout the first year of life (58), 0.55 for children and adolescent girls (56), and 0.7 for adolescent boys (59), using Jaffe creatinine methodology (Table 3). Much of the success of this approach depended on the precision of the creatinine assay, which is difficult to maintain at values less than 1 mg/dl but was very precise when coupled with a dialysis step and using blanks spaced between samples, as described for our renal laboratory at Albert Einstein College of Medicine (57,60,61).

Creatinine values determined by enzymatic creatinine assays are more specific and thus differ from the Jaffe method in which serum creatinine is measured by a colorimetric reaction with alkaline picrate. Especially at low levels of serum creatinine, enzymatic creatinine values tend to run lower than those determined by the Jaffe method (57,62), resulting in overestimation of GFR if used with the same “k” values as described above. Indeed, in our pilot study, the original Schwartz formula overestimated iohexol-based GFR by approximately 20%. Updated values for “k” must be determined to estimate GFR with enzymatic serum creatinine assays measured using isotope dilution mass spectrometry–based reference standards (63,64). It is likely that the revised values for “k” would be 20 to 30% lower using such an approach (see below).

Whereas the Schwartz formula is the most popular formula for the estimation of GFR in children, the Modification of Diet in Renal Disease (MDRD) formula is the most widely used estimate for adults (65):

$$eGFR \text{ (ml/min/1.73 m}^2\text{)} = 186 \times S_{Cr}^{-1.54} \times \text{age}^{-0.203} \\ \times 1.21^{\text{African-American}} \times 0.742^{\text{female}}.$$

However, several studies have shown that this formula is grossly inaccurate in children (66–68).

The Counahan-Barratt formula (69) has been used in children, primarily in Europe.

$$eGFR \text{ (ml/min/1.73m}^2\text{)} = 0.43 \times (L)/Scr \text{ (see Table 3).}$$

This formula has the same format as the Schwartz formula, and the “k” is reduced by 31% because “true” creatinine concentration was determined using the Jaffe reaction after adsorption onto an ion-exchange resin to remove noncreatinine chromogen. This approximated a “true” creatinine in the 1970s, before the advent of enzymatic creatinine analyzers. To generate this formula, GFR was measured using a single exponential ⁵¹Cr-EDTA plasma disappearance with a linear correction for computing GFR (23), which may be a less accurate determination compared with the Brochner-Mortensen equation (17).

The Cockcroft-Gault equation is a formula used in adults (70):

$$eGFR \text{ (ml/min)} = [140 - \text{age (yr)}] \times [\text{weight (kg)/72} \\ \times \text{serum creatinine (mg/dl)}] \times 0.85^{\text{female}}.$$

The Cockcroft-Gault equation has been shown to give an appropriate estimate of creatinine clearance in children older than 12 yr of age (67). However, the use of a weight-based formula that does not take into account BSA presents a major problem in growing children. Filler *et al.* (66) showed the unsuitability of the Cockcroft-Gault formula for use in children. The study compared GFR values determined by ^{99m}Tc-DTPA in 262 children 1 to 18 yr of age with renal disease to GFR values found by using a cystatin C–based formula, the Cockcroft-Gault formula, and the Schwartz formula. The Cockcroft-Gault formula showed the greatest amount of bias compared with the other two formulas. Of note, although the formula determined by the MDRD study has been found to be more accurate than the Cockcroft-Gault formula, this formula has not been validated in individuals less than 18 yr of age (65).

To improve the precision of creatinine-based estimates of GFR, Leger *et al.* (71) sought to determine the most appropriate equation to relate ⁵¹Cr-EDTA, body weight, height, and plasma creatinine in 64 children with renal disease by using the Non-linear Mixed Effects Model program. This is a program used in drug development for studying pharmacokinetic parameters and patient covariates. The determined equation was validated in 33 additional children. The most predictive equation was $GFR \text{ (ml/min)} = [55.5 \times \text{body weight (kg)} + 0.147 \times \text{length (cm)}^2] / \text{plasma creatinine } (\mu\text{M})$ (see Table 3, in which the equation is rewritten for creatinine in mg/dl units). The correlation between the GFR determined through the use of ⁵¹Cr-EDTA and the estimated GFR from the above equation was 0.83.

When using creatinine-based estimating formulas, it is important to know which creatinine assay is being used, because the coefficients of each equation are critically dependent on assay methodology (see Table 3) (6). As the effort spreads across the United States to reference all creatinine methods to isotope dilution mass spectroscopy standards, it may become possible to generate more universal equations. Clearly in pediatric screening equations, assay instruments must be capable of

Table 3. Equations using serum biomarkers for estimating GFR in children and adolescents

Equation	Patient Population	N	Age (yr)	Reference Method	Median GFR or GFR Range	Creatinine Assay	Cystatin C Assay	Equation
Creatinine based								
Schwartz (56)	CKD	77	1–21	Cin	3–220	Jaffe	NA	$0.55 \times \text{Ht}/\text{Scr}$
Counahan (69)	CKD	103	0.2–14	$^{51}\text{Cr-EDTA}^{\text{P1}}$	4–200	Jaffe	NA	$0.43 \times \text{Ht}/\text{Scr}$
Leger (71)	CKD, Tx	97	1–21	$^{51}\text{Cr-EDTA}^{\text{P1}}$	97	Jaffe	NA	$(0.641 \times \text{Wt})/\text{Scr} + (0.00131 \times \text{Ht}^2)/\text{Scr}$
Schwartz (6)	CKD ^a	349	1–17	Iohexol ^{P2}	41	Enzymatic	NA	$0.413 \times \text{Ht}/\text{Scr}$ $40.7 \times (\text{HT}/\text{Scr})^{0.640} \times (30/\text{BUN})^{0.202}$
Cystatin C based								
Filler (87)	CKD, Tx	85	1–18	$^{99\text{m}}\text{Tc-DTPA}^{\text{P1}}$	103	NA	Neph	$91.62 \times (\text{cysC})^{-1.123}$
Grubb (97)	CKD	85	3–17	Iohexol ^{P1}	108	NA	Turb	$84.69 \times (\text{cysC})^{-1.680} \times 1.384^{\text{if } <14 \text{ yrs}}$
Zappitelli (45)	CKD, Tx	103	1–18	Iothalamate ^{Cl}	74	NA	Neph	$75.94 \times (\text{cysC})^{-1.17} \times 1.2^{\text{if Tx}}$
Creatinine and cystatin C based								
Bouvet (95)	CKD	100	1–23	$^{51}\text{Cr-EDTA}^{\text{P1}}$	92	Jaffe	Neph	$63.2 \times (\text{Scr}/1.086)^{-0.35} \times (\text{cysC}/1.2)^{-0.56} \times (\text{Wt}/45)^{0.30} \times (\text{years}/14)^{0.40}$
Zappitelli (45)	CKD, Tx	103	1–18	Iothalamate ^{Cl}	74	Enzymatic	Neph	$43.82 \times e^{0.003 \times \text{Ht}} \times (\text{cysC})^{-0.635} \times (\text{Scr})^{-0.547}$
Schwartz (6)	CKD ^a	349	1–17	Iohexol ^{P2}	41	Enzymatic	Turb	$39.1 \times (\text{HT}/\text{Scr})^{0.516} \times (1.8/\text{cysC})^{0.294} \times (30/\text{BUN})^{0.169} \times (1.099)^{\text{if male}} \times (\text{HT}/1.4)^{0.188}$

^aMulticenter study.

CKD, chronic kidney disease; Tx, renal transplant; Scr, serum creatinine (mg/dl); Ht, height (cm); HT, height (m); BUN, blood urea nitrogen; cysC, cystatin C; Wt, weight (kg); Cin, inulin renal clearance; P1, plasma disappearance, one compartment; P2, plasma disappearance, two compartment; Cl, constant infusion; Neph, particle-enhanced nephelometric immunoassay; Turb, particle-enhanced turbidometric immunoassay; NA, not applicable.

reproducibly measuring down to the 0.2- to 0.3-mg/dl range, and universal standards in that range need to be developed (63).

Improving Creatinine-Based Estimates of GFR: Cystatin C

The inaccuracies of creatinine-based estimates of GFR in children are well known, especially in children with reduced muscle mass (45). Recent studies have addressed the use of other endogenous markers, such as cystatin C, a ubiquitous nonglycosylated cysteine protease inhibitor protein that is produced at a relatively constant rate and is freely filtered by the kidneys (72). This constancy of production is apparently independent of inflammatory conditions, muscle mass, sex, body composition, and age (after 12 mo) (73,74). Because cystatin C is catabolized and almost completely reabsorbed by renal proximal tubular cells, little is excreted in the urine, so that it cannot be used to measure GFR by standard urinary clearance techniques (75). In healthy individuals, a blood level of cystatin C is approximately 0.8 to 1 mg/L (76,77), and men and whites may show slightly higher levels than women and blacks, respectively (76). Finney *et al.* (73) measured cystatin C and creatinine in 291 children 1 d to 17 yr of age and found that cystatin C concentrations in children reached adult levels by 1 yr of age, implying that a single reference range for plasma cystatin C could be used from 1 yr of age and beyond. They also found that in premature infants, cystatin C was significantly elevated with concentrations between 1.10 and 2.06 mg/L.

Interindividual variations of cystatin C account for 25% of its biologic variability compared with 93% for creatinine (78). Thus, the upper limit of the population reference interval for cystatin C is seldom more than 3 to 4 SD from the mean value of any healthy individual (compared with 13 SD for creatinine). However, there are also discrepancies in the determination of cystatin C in the same blood samples between the Dako turbidimetric (light absorbing) method and the Dade-Behring nephelometric (automated light scattering) method (79), suggesting differential sensitivities to the assay conditions and different reactivities to the antibodies against the cystatin C molecule.

In children, the concentration of serum cystatin C in some studies is better correlated with GFR than is serum creatinine (73,80). Moreover, subtle decrements in GFR are more readily detected by changes in serum cystatin C than by serum creatinine (80), in part because of the shorter half-life of cystatin C. Thus, although cystatin C is not a conventional marker of GFR, reciprocal values of serum cystatin C levels are reasonably well correlated with GFR in adults (81,82) and in children (62,83–85).

In some studies, the serum concentration of cystatin C may be superior to serum creatinine in distinguishing normal from abnormal GFR (86), and a definitive numerical estimated GFR has been derived from its plasma concentration (79,83,87) (see Table 3). However, cystatin C levels may underestimate GFR in renal transplant patients, which may be a result of inflammation or the use of immunosuppressive therapy (88). Other factors, such as high C-reactive protein, smoking status (89), steroids (90), diabetes with ketonuria (91), and thyroid dysfunction

(92), may influence serum cystatin C levels. Therefore, caution must be used when estimating GFR in these situations (89). In view of the recent findings of cystatin C being found in the urine during glomerular injury with heavy proteinuria (93,94), there is some doubt as to whether serum cystatin C alone can accurately estimate GFR.

Stickle *et al.* (83) measured plasma cystatin C, serum creatinine, and inulin clearance in a population of pediatric patients with renal disease 4 to 19 yr of age. For estimating GFR in this patient population, these investigators showed that cystatin C concentration was reciprocally related to GFR and was broadly equivalent to serum creatinine for estimation of GFR in pediatric patients. Bouvet *et al.* (95) generated equations combining serum cystatin C and creatinine and measured GFR by ⁵¹Cr-EDTA plasma disappearance. The results of this study showed a more precise estimate of GFR when cystatin C was added to serum creatinine- and demographic-based formulas (see Table 3). Zappitelli *et al.* (45) generated two cystatin C–based estimating equations incorporating serum creatinine, height and weight, and diagnosis against ¹²⁵Iothalamate renal clearances (see Table 3). The equations were less biased and more precise than other serum creatinine based estimates for patients with kidney transplants or spina bifida and comparable to these equations for other forms of renal disease. These are the first studies to incorporate both cystatin C and creatinine in GFR estimating equations for children.

One of the main objectives of the NIH-sponsored CKiD study was to develop an equation to estimate GFR using demographic variables and endogenous biochemical markers of renal function including creatinine, cystatin C, and blood urea nitrogen (BUN) (6). The subjects recruited for the study in more than 40 participating sites around the United States and Canada were between the ages of 1 and 16 yr of age and had known mild to moderate kidney disease as shown by an estimated GFR of 30 to 90 ml/min per 1.73 m² based on the original Schwartz formula. The median iohexol-based double exponential plasma disappearance GFR in 349 children was 41.3 ml/min per 1.73 m² (95% of the values were between 21.1 and 75.9) and median serum creatinine was 1.3 mg/dl. The original Schwartz formula was found to overestimate the GFR by approximately 12 ml/min per 1.73 m² (29%) compared with the GFR determined by the plasma disappearance of iohexol. BSA, weight, and height were most strongly correlated with iohexol plasma disappearance. Height/serum creatinine was highly correlated with measured GFR ($r = 0.81$), as was cystatin C ($r = 0.69$) and BUN ($r = 0.62$). Using multiple linear regression analyses of log-transformed variables, we generated several estimate formulas (see Table 3). The CKiD study is the first multicentered study to generate estimating equations in children. The best equation was:

$$\begin{aligned} \text{eGFR} = & 39.1 \times [\text{height (m)}^2 / S_{\text{cr}}(\text{mg/dl})]^{0.516} \\ & \times [1.8 / \text{cystatin C (mg/L)}]^{0.294} \times [30 / \text{BUN (mg/dl)}]^{0.169} \times [1.099^{\text{male}}] \\ & \times [\text{height (m)} / 1.4]^{0.188}. \end{aligned}$$

This formula yielded 87.7% of estimated GFR within 30% of the iohexol-based GFR, and 45.6% within 10%. Interestingly, the formulas by Bouvet *et al.* (95) and Zappitelli *et al.* (45), when applied to this dataset, required the generation of coefficients that differed from those published by these authors, probably reflecting different assays of the endogenous variables and/or different measurements of GFR. When this equation was applied to a test group of 168 children being studied 1 yr later, it showed the highest accuracy and correlation and the narrowest 95% limits of agreement with measured GFR compared with the other published cystatin C- and creatinine-based equations, using original coefficients or those derived from the CKiD population.

We also found, when height is measured in centimeters, that a bedside calculation of $0.41 \times \text{ht}/S_{\text{cr}}$ provides a good approximation (and a continuation of the same format as the original Schwartz formula; see below and Table 3) of the estimated GFR based on enzymatic serum creatinine determinations referenced to isotope dilution mass spectroscopy standards (63).

Further studies need to be done that also take into account serum creatinine, cystatin C, and BUN in children with more normal kidney function and body habitus before such equations can be applied universally to pediatric patients. Because cystatin C is presently not readily available at many clinical laboratories, it cannot be used to routinely estimate GFR; making this test readily available to clinicians should help reduce kidney and cardiovascular morbidity. In the interim, and in keeping with the NKF recommendations to stage CKD (96), it is important for clinicians to have a ready estimate of GFR from the standard Basic Metabolic Profile or Chem 8 laboratory panel, which includes a BUN and creatinine in addition to electrolytes, glucose, and calcium. Indeed, two of our model equations are available for this purpose and should be able to distinguish children with CKD 1 from those with CKD 2 or 3, because they have been generated from GFRs ranging from 21 to 76 ml/min per 1.73 m² (6) (see Table 3):

$$\text{eGFR} = 40.7 \times [\text{height (m)}/S_{\text{cr}} \text{ (mg/dl)}]^{0.640} \times [30/\text{BUN (mg/dl)}]^{0.202}$$

or

$$\text{eGFR} = 0.41 \times \text{height (cm)}/S_{\text{cr}} \text{ (mg/dl)}$$

[updated Schwartz “bedside” formula].

Indeed the equation incorporating BUN and S_{cr} showed in the original training set (6) that 83.7% of values were within 30% of measured GFR and 38.4% were within 10% with a root mean squared error of 0.196. When applied to the test data set, the correlation was 0.85 and the bias was -1.60 ml/min per 1.73 m² (Figure 1). The updated Schwartz ht/ S_{cr} “bedside” formula showed 79.4% of values within 30% of measured GFR and 37.0% within 10%, with a root mean squared error of 0.223. When the updated Schwartz formula was applied to the test data set, the correlation was 0.84 and the bias was -1.75 ml/min per 1.73 m² (Figure 2).

These two foregoing equations may be used to estimate GFR. For example, if the clinician specifies a measured height, the

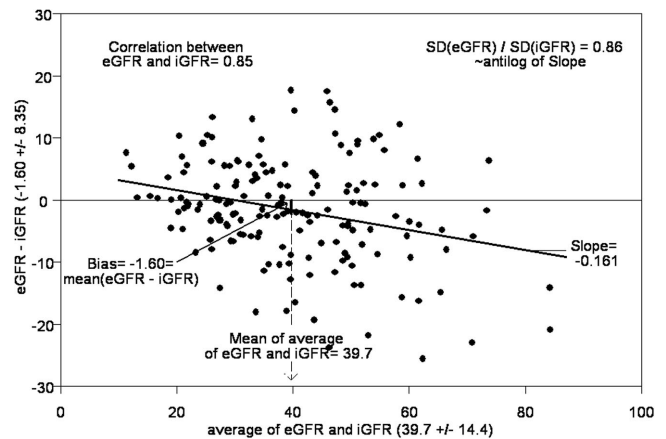


Figure 1. Bland-Altman analysis of comparison of $\text{eGFR} = 40.7 \times [\text{height (m)}/S_{\text{cr}} \text{ (mg/dl)}]^{0.640} \times [30/\text{BUN (mg/dl)}]^{0.202}$ compared with iohexol-based plasma disappearance GFR in 168 children in the CKiD study comprising the test data set. The estimate formula appears to underestimate measured GFR by 1.6 ml/min per 1.73 m² at the mean GFR. Note also that the overestimation appears to be larger at higher GFR.

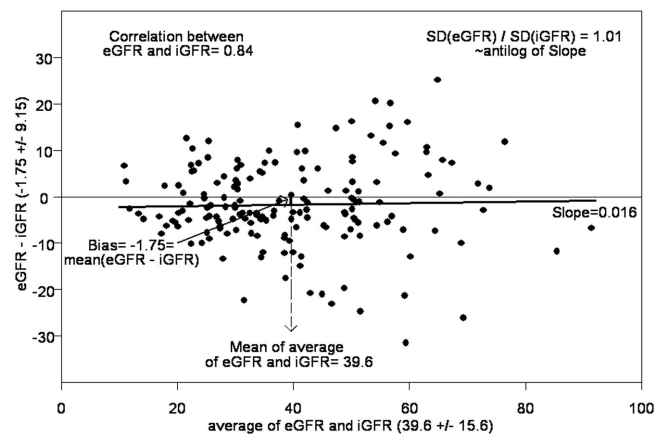


Figure 2. Bland-Altman analysis of comparison of $\text{eGFR} = 0.413 \times [\text{height (cm)}/S_{\text{cr}} \text{ (mg/dl)}]$ compared with iohexol-based plasma disappearance GFR in 168 children in the CKiD study comprising the test data set. The estimate formula underestimate measured GFR by 1.75 ml/min per 1.73 m² at the mean GFR, and there does not appear to be any additional underestimation at higher GFR.

laboratory computer can be programmed to calculate an estimated GFR from the child’s age, height, and Basic Metabolic Profile or Chem 8 analytes. If there is no specification of the child’s height, we suggest that estimated GFRs be presented by the laboratory for the age-related height percentiles (3rd, 50th, and 97th). The spread of height from the 3rd to 97th percentile is approximately 15 to 20%, and this critically affects the GFR estimate. That is, for the same BUN and serum creatinine in an 11-yr-old girl (Table 4) or boy (Table 5), the ratio of eGFR for the 97th height percentile is 12% higher than that for the 3rd percentile (as seen in the rightmost column of each table). The influence of height percentile on GFR using the updated

Table 4. Age-related height percentiles for girls

Age (yr)	Height at 3rd Percentile (m)	Height at 50th Percentile (m)	Height at 97th Percentile (m)	Ratio of eGFR for Height in the 97th:3rd Percentiles
3	0.88	0.96	1.04	1.11
7	1.13	1.22	1.34	1.12
11	1.33	1.47	1.59	1.12
15	1.51	1.62	1.76	1.10

Height percentile data from reference 98.

Table 5. Age-related height percentiles for boys

Age (yr)	Height at 3rd Percentile (m)	Height at 50th Percentile (m)	Height at 97th Percentile (m)	Ratio of eGFR for Height in the 97th:3rd Percentiles
3	0.89	0.97	1.05	1.11
7	1.13	1.23	1.35	1.12
11	1.32	1.45	1.57	1.12
15	1.56	1.71	1.86	1.12

Height percentile data from reference 98.

Schwartz formula would be by as much as 20%. In our preliminary experience, both equations work well within the limits of 15 to 75 ml/min per 1.73 m², but the updated ht/S_{cr} formula seems to be more accurate for GFR values greater than 90 ml/min per 1.73 m² (unpublished observations; compare Figures 1 and 2). However, because these equations were generated in children with most GFR values less than 75 ml/min per 1.73 m², estimated values falling above that range should be specified as “greater than 75 ml/min per 1.73 m²,” at least until data are generated for GFRs measured above this level.

Conclusion

In clinical practice, having an accurate yet practical means to monitor renal function is extremely important to protect the kidneys from toxic damage and to prescribe adequate fluid, antibiotic, and chemotherapeutic regimens. GFR cannot be accurately measured using timed urine collections in children who are not toilet trained or have conditions such as vesicoureteral reflux, bladder dyssynergia, or neurogenic bladder. The use of iohexol plasma disappearance serves as a promising alternative to inulin for precise, accurate, and relatively rapid determination of GFR. In addition to repeatedly measuring the GFR of each subject, one of the goals of the CKiD study is to eventually provide clinicians with a means to estimate GFR in the children who have a serum chemistry panel performed. GFR estimating equations need to be adapted to correct for differences in body habitus, sex, puberty, infancy, and prematurity. As a result of recent studies, the utility of estimated GFR formulas continues to improve with the addition of previously unmeasured endogenous markers. There currently exists no equation for monitoring acute changes in GFR. However, the equations developed in the CKiD study (6) may be able to determine longitudinal changes in GFR over time. Thus, in the

near future, it may be possible to predict subsequent GFR values in children through the use of serum biomarkers without the need for formal plasma disappearance clearances. Multicenter studies in healthy children, as well as those with CKD, are needed to generate such widely applicable estimation equations.

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Disclosures

None.

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