Rapid determination of glomerular filtration rate by single-bolus inulin: a comparison of estimation analyses

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Sturgeon, Cord, Albert D. Sam II, and William R. Law. Rapid determination of glomerular filtration rate by single-bolus inulin: a comparison of estimation analyses. J. Appl. Physiol. 84(6): 2154–2162. 1998.—Rapid measurement of glomerular filtration rate (GFR) by an inulin single-bolus technique would be useful, but its accuracy has been questioned. We hypothesized that reported inaccuracies reflect the use of inappropriate mathematical models. GFR was measured in 14 intact and 5 unilaterally nephrectomized conscious male Sprague-Dawley rats (mean weight 368 ± 12 g) by both single-bolus (25 mg/kg) and constant-infusion techniques (0.693 mg·kg⁻¹·min⁻¹). The temporal decline in plasma inulin concentration was analyzed through biexponential curve fitting, which accounted for renal inulin loss before complete vascular and interstitial mixing. We compared our mathematical model based on empirical rationale with those of other investigators whose studies suggest inaccuracy of single-bolus methods. Our mathematical model yielded GFR values by single bolus that agreed with those obtained by constant infusion [slope = 0.94 ± 0.16 (SE); y intercept = 0.23 ± 0.64; r = 0.82]. In comparison to the data obtained by constant inulin infusion, this method yielded a very small bias of -0.0041 ± 0.19 ml/min. Two previously reported models yielded unsatisfactory values [slope = 1.46 ± 0.34; y intercept = 0.47 ± 1.5; r = 0.72; and slope = 0.17 ± 1.26; y intercept = 17.15 ± 5.14; r = 0.03]. The biases obtained by using these methods were -2.21 ± 0.42 and -13.90 ± 1.44 ml/min, respectively. The data indicate that when appropriate mathematical models are used, inulin clearance after single-bolus delivery can be used to measure GFR equivalent to that obtained by constant infusion of inulin. Attempts to use methods of analysis for simplicity or expediency can result in unacceptable measurements relative to the clinical range of values seen.

mathematical modeling

ESTIMATES OF CREATININE CLEARANCE based on plasma and urine creatinine concentrations do not reflect true renal function in many circumstances (3, 10, 13, 15, 22). For example, creatinine secretion is not constant in acute renal failure (1). Furthermore, indirect evaluations of glomerular filtration rate (GFR) such as blood urea nitrogen and plasma creatinine levels do not become significantly elevated until 50–75% of kidney function is lost (8). As such, these indexes are relatively insensitive measures of impending renal failure.

Calculation of endogenous creatinine clearance is still the most widely used method to evaluate GFR in clinical settings. This method requires timed plasma and 24-h urine samples and is inaccurate if the urine collection (bladder evacuation) is not complete. In the laboratory, determination of inulin clearance is a standard method for estimating GFR. Inulin is biologically inert, unbound by plasma proteins, freely filtered at the glomerulus, and is neither reabsorbed, metabolized, nor secreted by the kidneys (21). Consequently, inulin clearance is an excellent measure of GFR. However, inulin is only occasionally used in the clinical setting for this purpose. When it is used, inulin is infused at a constant rate until steady state is reached. This alone may require several hours. Timed blood and urine samples are then taken to determine inulin clearance (2, 12, 16, 24). Additionally, in oliguric patients, methods requiring urine collection are not feasible. Measurement of steady-state plasma inulin concentrations and the inulin infusion rate is an accurate alternative to obtaining precise urine volumes and urine inulin concentrations, but it still often requires several hours of inulin infusion (9) and may be prone to other errors, such as an inability to attain steady-state plasma concentrations when clearance is very high or low (23).

Single-bolus methods of GFR measurement rely solely on analysis of the plasma-elimination curves and, therefore, take less time and do not require urine collection. Despite findings of good correlation between methods, animal studies have suggested inaccuracy (lack of agreement in values) or difficulty associated with this method (5, 25). Attempts to evaluate single-bolus inulin administration in humans (17) have suggested that it is accurate only 2–3 h after inulin administration, suggesting that this method does not expedite GFR determination. However, inaccuracies may have been introduced by the mathematical modeling used in these analyses. It was our hypothesis that the method of estimating GFR by calculating total body clearance with a single bolus of inulin would be equivalent to a constant inulin-infusion method if the proper mathematical modeling were applied. Moreover, single-bolus analyses are easier and faster to perform and can be completed in <1 h. As part of our study, we compared various mathematical analyses to explain our findings in the context of previous work.
METHODS

Nineteen male Sprague-Dawley rats (mean weight 368 ± 12 g) were anesthetized with 40 mg/kg ip pentobarbital sodium. A 1.5-cm incision was made from the manubrium to the angle of the mandible. The right common carotid artery and a branch of the right internal jugular vein were catheterized with PE-50 tubing. The incision was closed with a running stitch of 3-0 silk suture. In five of the rats, a laparotomy was performed via a 2.5-cm transverse incision. The underlying pannus was dissected, bluntly exposing the rectus musculature. The peritoneum was then entered, and segments of small and large intestines were exteriorized from the abdomi- nal cavity through the laparotomy incision and displaced laterally onto a sterile saline gauze. After obtaining adequate exposure of the kidney, the vascular pedicle was gently dis- sected from its perirenal adipose attachments, and a 3-0 silk ligature was secured around the vascular supply to the renal hilum. A nephrectomy was then performed. After assessment of the region to ensure complete hemostasis, the bowel was gently returned to the peritoneum, and the abdominal and skin incisions were closed separately with 3-0 silk sutures.

Rats were allowed to recover from anesthesia until they regained the righting reflex (~2 h). After the recovery period, each rat was randomly assigned to have either the single- bolus or the constant-infusion method of inulin administration first. A 10 mg/ml solution of inulin (Sigma Chemical, St. Louis, MO; lot no. 120H7155) dissolved in 0.9% saline was administered via the internal jugular catheter, and subsequent serial blood samples were drawn from the carotid catheter. Rats were allowed to recover for 90 min after the last blood sample was taken (which represents at least six plasma inulin half-lives), before the second protocol was initiated. After this recovery period, rats underwent whichever method of inulin administration they did not receive first.

In the single-bolus method, inulin (25 mg/kg) was adminis- tered as a single-bolus infusion that lasted 60 s. Arterial blood samples (300 µl each) were taken before the injection and at 3, 5, 7, 10, 15, 20, 30, and 40 min postinjection.

In the constant-infusion method, a loading dose of inulin (20 mg/kg) was administered, followed by 210 min of inulin infusion at a constant rate of 0.693 mg·min⁻¹·kg⁻¹ (4.16 ml·h⁻¹·kg⁻¹; Harvard Apparatus, South Natick, MA). A 300-µl blood sample was drawn before the loading dose was administered as well as 30, 45, 60, 75, 90, 120, 150, 180, and 210 min after the constant infusion was begun.

Arterial blood pressures and heart rates were measured through the indwelling carotid catheter when it was not being used for obtaining blood samples. These variables were monitored and recorded on a personal computer by the CODAS data-acquisition system (DATAQ, Akron, OH).

Within 1 min from being collected, each blood sample was centrifuged at 10,000 g for 5 min, and the plasma was removed and deproteinized with 8% trichloroacetic acid. After an additional 5 min of centrifugation at 10,000 g, the superna- tant was removed and stored overnight at 4°C for use in the standard anthrone colorimetric assay for inulin (6). At the end of experiments, all rats were euthanized by an overdose of pentobarbital sodium (100 mg/kg iv).

Inulin Clearance Determination From the Constant-Infusion Method

Plasma inulin concentrations measured during the con- stant-infusion technique were used to calculate inulin clear- ance ($C_i$; in ml/min) by the following equation

$$C_i = \frac{R}{[I]_{ss}}$$

where $R$ is the rate of inulin infusion in mg/min and $[I]_{ss}$ is the plasma inulin concentration at steady state in mg/ml. Steady state was defined as conditions of <5% change in plasma inulin concentration over a 30-min period.

Inulin Clearance Determined From the Single-Bolus Inulin Technique

Model A. This model assumes that after single-bolus delivery, inulin is distributed across two compartments, the plasma and interstitial fluid volumes, and is only eliminated from the plasma compartment. The changing concentration of a substance (inulin) introduced into one compartment (plasma) of a two-compartment model (plasma and interstitial fluid) with one site of removal (renal clearance) is described by the following equation

$$P_x = A e^{-\alpha (t_x - t_0)} + B e^{-\beta (t_x - t_0)}$$

where $P_x$ is the concentration of inulin in the plasma at any given time ($t_x$), and $t_0$ is the time of administration of the bolus. $A$ and $B$ are the y-intercept values, and $\alpha$ and $\beta$ are the decay constants of the distribution and elimination phases, respectively. The concentration of inulin in the plasma at any given time can be calculated by this equation. The distribution com-ponent of the decay curve is designated by the exponential term

$$A e^{-\alpha (t_x - t_0)}$$

This represents the inulin loss from the intravascular space that occurs because of the distribution between intravascular and interstitial fluid compartments. The elimination compo- nent of the decay curve is designated by the exponential term

$$B e^{-\beta (t_x - t_0)}$$

and represents the slower rate of inulin loss that occurs as a result of renal elimination. We used the Levenberg-Mar- quardt curve-fitting algorithm in the SlideWrite program (version 6.0, Advanced Graphics, Carlsbad, CA) to analyze the sequential plasma inulin concentration data after single bolus, according to Eq. 2 above.

The regression coefficients from Eq. 2 were used in the following empirically derived equation for calculation of inulin clearance

$$GFR = \frac{C_i}{(t_x - t_0)B} = \frac{1}{\alpha [\ln B - \frac{A}{[P_x]}(e^{[I]_{ss} - t_0} - 1)]}$$

where $I$ is the dosage of inulin delivered in the single bolus. Derivation of this equation is presented in the APPENDIX.

Comparisons with Other Single-Bolus Models

We compared the results from model A and three published equations for GFR determination after single-bolus indicator delivery with the results from the standard constant-infusion method.
Model B. Equation 4 below is an adaptation of that used in an early model described by Sapirstein et al. (19) to calculate GFR in dogs after single-bolus injection of mannitol. The assumption of this model was that when clearance is calculated only trivial error is introduced by neglecting the distribution component of the plasma inulin concentration decline. Therefore, this model calculates clearance based on a one-compartment model

\[
C_i = \frac{I}{B \ln \left( \frac{B}{P_x} \right)} \frac{1}{(T_a - t_0)}
\]  

(4)

For this comparison, Eq. 2 was used to generate \( P_x \) and the regression coefficient \( B \).

Model C. A more recent method for GFR determination after single-bolus inulin delivery used by Prescott et al. (17) fits inulin plasma concentration data to a curve defined by the following equation

\[
P_x = \frac{A[1 - e^{-\alpha (T_a - t_0)}]e^{-\alpha (t_0 - t_a)}T}{\alpha T} + \frac{B[1 - e^{-\beta (T_a - t_0)}]e^{-\beta (t_0 - t_a)}}{\beta T}
\]  

(5)

where \( T \) is the duration of the single-bolus infusion. This model assumes that inulin follows two-compartment elimination kinetics. However, in comparison with models A and B, the equation that describes the plasma inulin concentration time curve is quite different and introduces an additional variable, \( T \), to correct for the changing plasma inulin concentration during inulin infusion (compare Eqs. 5 and 2). For analyses with our data, we used \( T = 1 \) min, the duration of our bolus injection.

Prescott et al. (17) calculated renal clearance of inulin by dividing the amount of inulin excreted in urine during a given time period by the corresponding area under the plasma inulin concentration curve. Inulin clearance was then reported in blocks of discrete time intervals coinciding with these time periods.

For the purpose of our study, we evaluated only the plasma inulin concentration curve equation and not the other methods by which Prescott et al. (17) calculated renal clearance values. We calculated inulin clearance with plasma instead of urine inulin concentrations. Additionally, we calculated inulin clearance only on the basis of area under the entire plasma inulin concentration curve (standard pharmacokinetic Eq. 6 below), instead of calculating values over discrete time periods.

Model D. This model, like model A, assumes that after single-bolus delivery inulin distributes across only two compartments, the plasma and interstitial fluid volumes, and concomitantly undergoes renal elimination from the plasma compartment. It also assumes that the plasma inulin concentration curve is described by Eq. 2. However, in contrast to model A, inulin clearance was calculated by using a standard pharmacokinetic equation for clearance from a two-compartment model (11).

\[
C_i = \frac{I}{A + B}
\]  

(6)

Equation 2 was used to generate the regression coefficients \( A \), \( B \), and \( \beta \) for Eq. 6.

Exclusion Criteria

Blood pressures, heart rates, hematocrits, and plasma inulin concentrations were evaluated to determine the hemodynamic status of each rat during the course of the experiments. In accordance with criteria set before these studies were initiated, rats were excluded from the study if there was a blood pressure change of >5% over any 30-min period (data displayed in Fig. 1) during either the single-bolus or constant-infusion experiments. For the constant-infusion study, average plasma inulin concentrations were calculated only after steady state was reached. Lack of attention to this detail can lead to erroneous results.

Data Analysis

Data from the single-bolus techniques were correlated with those from the constant-infusion technique. Two statistical
methods were used to evaluate the equivalency of each model to the continuous-infusion reference value. First, linear regression was used to compare each single-bolus mathematical model with values for GFR obtained by using the inulin constant-infusion technique. Pearson coefficients were also examined to determine goodness of fit for each regression. The regression coefficients (slopes and intercepts) of each regression analysis were compared with a slope of 1.0 and a y intercept of 0.0 (identity) by using paired t-test. The analysis of method comparison, as described by Altman and Bland (1, 4), was used to determine the bias and estimated limits of agreement between data from each bolus method and data from the continuous inulin-infusion method. A probability value \( P < 0.05 \) was chosen to indicate significant difference from identity.

RESULTS

Model A

Results from applying our data to model A for GFR calculation (plasma inulin concentration curve described by Eq. 2 and clearance calculated by Eq. 3) plotted against GFR values determined by constant inulin infusion yielded a slope of 0.94 ± 0.16 and a y-axis intercept of 0.23 ± 0.64. The Pearson coefficient for these data was 0.82. These data are shown in Fig. 2A. The regression coefficients were not significantly different from identity (slope = 1; intercept = 0; dashed line). By using analysis of method comparison (Fig. 2B) model A analysis yielded a bias (−0.0041 ± 0.19 ml/min), which is small relative to the range of values measured. These data indicate that use of this model accurately estimated GFR as measured by the continuous inulin-infusion method.

Model B

Results from applying our data to an early model (19) for GFR calculation (plasma inulin concentration curve described by Eq. 2 and clearance calculated by Eq. 4) plotted against GFR values determined by constant inulin infusion yielded a slope of 1.46 ± 0.34 and a y-axis intercept of 0.47 ± 1.50, with a Pearson coefficient of 0.72. These data are shown in Fig. 3A. The slope deviated significantly from identity, suggesting that this model did not closely estimate clearance, as measured with the constant-infusion method. Consistent with this analysis, the analysis of method comparison yielded an unacceptable bias of −2.21 ± 0.42 ml/min (Fig 3B). In addition, the distribution of the data indicated increasing inaccuracy with higher GFR values.

Model C

Results from applying our data to a more recent model (17) for GFR calculation (plasma inulin concentration curve described by Eq. 5 and clearance calculated by Eq. 6) plotted against GFR values determined by constant inulin infusion yielded a slope of 0.17 ± 1.26 and a y-axis intercept of 17.15 ± 5.14. For these data, the Pearson coefficient was 0.03. These data are shown in Fig. 4A. Both regression coefficients deviated significantly from identity, indicating that this model did not estimate clearance as described by the constant infusion of inulin. The results of our analysis of methods comparison were consistent with this conclusion, yielding a bias of −13.90 ± 1.44 ml/min, which exceeded the highest GFR value obtained by the continuous-infusion method (6.16 ml/min).

Model D

Results from GFR calculation by standard pharmacokinetics (inulin concentration curve described by Eq. 2 and clearance calculated by Eq. 6) plotted against GFR values determined by constant inulin infusion yielded a slope of 0.94 ± 0.16 and a y-axis intercept of 0.23 ± 0.64. The correlation coefficient for these data was 0.82. These values are statistically identical to those gener-
Our goals were twofold. First, we wanted to test the hypothesis that GFR determinations when a single bolus of inulin is used were the same as those obtained from inulin concentrations of timed plasma collections during a constant infusion of inulin (steady state). We also chose to compare an equation empirically derived by us with that derived by other laboratories. We started with the assumption that the changing concentration of inulin in plasma after single-bolus administration conforms to a two-compartment model, with inulin introduction into the plasma (1st compartment), movement between the plasma and interstitial fluid (2nd compartment), and simultaneous clearance from the plasma via the kidneys. Although equations that accurately define the kinetics of inulin clearance after single-bolus administration have been outlined in theoretical treatises (11, 14, 20), they are not consistently used in determining GFR. The relationships between the coefficients in these equations and physiological phenomena, while theoretically accurate, are not easily recognized. Some investigators have tried to use empirical equations based on the temporal decay of inulin concentrations in plasma (17). Attempts to measure inulin clearance after single-bolus administration have

![Diagram](image-url)
been confounded by lack of uniformity regarding methods of pharmacokinetic analyses, in particular curve fitting and equations used to calculate total body clearance. In addition, linear regression is often the only statistical analysis used, and despite good correlation of methods, the relationship is rarely parity (line of identity), indicating poor agreement. As a result, published attempts to employ the single-bolus technique (5, 15–17, 25) have suggested that its use may be limited in clinical or experimental medicine due to inaccuracies. Consequently, this technique has not been widely adopted for clinical or experimental use. Our inability to consistently and accurately obtain timed urine volumes and urinary inulin concentrations prevented us from comparing the single-bolus inulin method with the “gold standard” of urinary inulin clearance. However, the results of our study indicate that, when appropriate techniques and equations are applied, inulin clearance measurements after single-bolus delivery provide GFR estimates based on total body clearance measurements that are equivalent to those obtained by constant-infusion measurements.

In our experiment, the various methods of GFR calculation following single-bolus inulin delivery were compared with values for GFR derived by a constant-infusion technique. Values for GFR calculated by model A (Eqs. 2 and 3) after single-bolus delivery of inulin were highly correlated with the GFR values obtained by constant inulin infusion and also yielded statistically equivalent values (Fig. 2), with a bias that was not statistically different from zero. This robust analysis of correlation and agreement of values was used in evaluating all of the models. Single-bolus GFR values calculated by standard pharmacokinetics (model D; Eqs. 2 and 6) also correlated highly with those values from the constant-infusion technique as well as those from single-bolus model A (Eqs. 2 and 3). These relationships suggest that the three methods (model A, model D, and constant infusion) all measure the same physiological phenomena. The primary difference between these two equations is the requirement of a time frame across which an area (concentration × time) is calculated. This is explained, as is the rationale underlying our derivation, in APPENDIX.

Values for GFR calculated by model B after single-bolus delivery of inulin correlated well with those obtained by constant inulin infusion, but the relationship was statistically different from the line of identity and there was a significant bias associated with the method. This disagreement of values indicated that there was some inaccuracy associated with this method of GFR determination. The most likely source of error is that Eq. 4 is based on the assumption that the time required for inulin distribution between intravascular and interstitial compartments is negligible (19). In actuality, this mixing time is not negligible. Equilibrium of inulin concentrations between the plasma and interstitial spaces does not occur immediately, and, consequently, a higher plasma inulin concentration is presented for renal filtration than exists in the interstitial space during this period. As a result of this high initial plasma concentration, some amount of inulin injected into the vascular volume is always immediately filtered and removed before it has an appreciable affect on the pharmacokinetics of distribution to the interstitial space. Equation 4 is valid only when the term I represents only the amount of the injected inulin that ultimately does distribute between the two compartments (inulin injected minus “excess” inulin filtered during the distribution phase). Without accounting for the excess inulin lost during the distribution period (see APPENDIX), one would expect this equation to consistently overestimate GFR in direct proportion to the duration of this distribution period. The data are consistent with this expectation.

Values for GFR calculated by using model C (Eqs. 5 and 6) (17), correlated poorly with those from the constant-infusion technique, deviated significantly from identity, and had a significant bias. These data suggest that the assumptions used in model C are also inaccurate. We evaluated the reported equation for the inulin decay curve after single-bolus administration (Eq. 5). The regression coefficients of plasma inulin decay curves fit to Eq. 5, when used to calculate inulin clearance by Eq. 6, yielded GFR values that correlated poorly with the constant-infusion values. A potential source of error in model C is a correction for the time required to administer the bolus of inulin (variable T in Eq. 5). This should not be necessary, since the distribution kinetics are accounted for in the calculation of GFR. It was also suggested that single-bolus inulin administration is not expedient for evaluation of GFR because inulin clearance values change as the time after bolus administration increases (17). Results from our study did not agree with this finding. This suggestion was based on results obtained by using Eq. 5. We found no evidence that inulin undergoes a shift in elimination kinetics over time. It is conceivable, however, that in some investigations curve-fitting methods have been employed that did not result in an accurate delineation of the distribution and elimination components of the inulin decay curve. For example, reports that inulin clearance rates drop as the length of time after the single-bolus increases (17) can be accounted for either if the number of blood samples drawn were insufficient for analysis of the early (distribution) component of the decay curves or if there were insufficient data for accurately determining the total area under the curve (AUC). Dividing indicator dose by the AUC of indicator concentration over time is a common method of determining clearance (5, 7, 9, 17, 24). Indeed, standard pharmacokinetic (11) analysis (Eq. 6) does just this, taking the time variable (t0) to the limit of infinity (see APPENDIX). However, it is only because the limit of infinity is used that this equation is accurate. For any dose divided by AUC method to be accurate, the time course of the curve for which area is calculated must be inclusive from the time when the single bolus was given through a time when plasma indicator concentrations fall to undetectable values, as was done in our empirically derived
model A. Use of an insufficient period of time, or discrete segments of time (e.g., 0–2 h, 2–4 h, etc.) in the calculation of AUC would explain the reported inaccuracy of some single-bolus methods (17, 18). The use of these methods would result in higher calculated values for GFR during the early time periods and may explain the reported overestimation of GFR when these techniques are used (9). Our data based on using these analyses are consistent with these expectations.

We arrived at an equation by constructing arguments based on physiological principles from the known characteristics of inulin removal from the circulatory system and standard pharmacokinetics (see APPENDIX). We can show (APPENDIX) that Eq. 6 (from standard pharmacokinetics) is mathematically equivalent to the equation we derived (Eq. 3) in model A. It is not surprising then that results from comparisons of our method (Eqs. 2 and 3) and standard pharmacokinetics (Eqs. 2 and 5) with the constant-infusion method indicate that single-bolus inulin delivery can be used to provide estimates of GFR in the rat that are not statistically different from clearance values based on constant inulin infusion.

We believe erroneous analyses of single-bolus GFR measurements can be avoided with some simple precautions. Because elimination of inulin follows two-compartment kinetics, when single-bolus inulin methods are used, several data points must be taken during both the distribution and elimination phases to clearly delineate them in any curve-fitting algorithm. In addition, equations that correctly describe the plasma inulin concentration curve and renal clearance rate (e.g., Eqs. 2 and 5, respectively) must be used. One must subsequently use both of the exponential functions of the plasma inulin decay curve to account for excess inulin filtered during the distribution phase and plasma-interstitial compartment interactions throughout the procedure. Furthermore, when using a dose divided by the AUC method of analysis of plasma clearance, such as Eq. 3, which requires a discrete time variable, an end-point time (t_e) must be chosen at which sufficient time has elapsed for the plasma inulin concentration to reach undetectable levels. This results in using the area under the entire measurable plasma inulin concentration curve when calculating inulin clearance. Analyses that do not estimate the area under the entire curve will not reflect true GFR when using time-based assessments. Alternatively, the standard pharmacokinetic equation provides accurate values independent of time, assuming the first two precautions are observed. In addition, more care must be taken when assessing the accuracy of new methods, avoiding sweeping conclusions based solely on Pearson coefficients.

Another study by Florijn et al. (9) compared single-bolus GFR estimations based on total body inulin clearance with those calculated by constant inulin infusion and found better correlation than did we (Pearson r = 0.95). The lower correlation we observed may have been due to our need to perform the two analyses on the same day. While randomization of the order of single-bolus vs. constant-infusion approaches reduced the impact of interactions between the procedures on the slope and intercept of the line, it would still lend itself to a poorer correlation. Despite this, the correlation we observed was very good and, according to the work of Florijn et al., may underestimate the true correlation. The apparent discrepancy between their findings and ours may also be related to the nature of the data sets used to obtain correlation. The study by Florijn et al. was conducted in patients and run three separate times in most patients, which would increase the number of data sets and reduce variability with repetitions. These findings indicate that the single-bolus approach yields results that are not statistically different from those obtained by using a constant-infusion approach.

Regarding usefulness, the single-bolus inulin injection method might be a better option than what is currently available for the determination of GFR, especially under circumstances when acute assessment of renal function is needed. The most commonly used index of GFR is creatinine clearance, or plasma creatinine concentration. Although creatinine has an advantage over inulin because it is produced endogenously, it is a less desirable substance for evaluation of kidney function because it is secreted by the kidney to a variable degree. Additionally, plasma creatinine concentration does not appreciably rise until 50–75% of kidney function is lost (8), obviating its use as an index of acute renal function change. Furthermore, recent reports indicate that estimates of creatinine clearance in humans do not reflect true renal function in many circumstances; i.e., declining renal function, body fluid expansion, spinal cord injury, normal aging, renal transplant, and following administration of certain drugs (3, 10, 13, 15, 22). The constant-infusion technique is time consuming, especially when evaluating acute renal function changes. The single-bolus inulin technique, however, could be used to rapidly estimate GFR.

APPENDIX

Standard pharmacokinetics (11) dictate that calculating clearance requires only the volume of distribution (V_d) and the elimination constant β

$$C_i = \frac{V_d}{\beta}$$

(A1)

If one were to assume monoexponential decay and negligible mixing time between plasma and interstitial compartments, the equation for clearance of inulin after single-bolus administration, as described previously (19, 20), would be

$$C_i = \frac{1}{B} \ln \left( \frac{B}{P_x} \right) \frac{I}{(t_e - t_0)}$$

(A2)

where I is the inulin dose (in mg, injected in a single bolus), and the regression coefficient B (the y intercept of the elimination curve) is the plasma inulin concentration extrapolated back to the time of administration (assuming that decay
is monoexponential and mixing time is negligible. Accordingly
\[ \frac{I}{B} = V_d \]  
(A3)

when mixing is instantaneous. Additionally, since the change in plasma inulin concentration divided by the change in time is the definition of the elimination rate constant
\[ \frac{\ln \left| \frac{B}{P_x} \right|}{(t_x - t_0)} = \beta \]  
(A4)

Therefore, by substituting the elimination constant \( \beta \) from Eq. A4 into Eq. A2 and substituting \( V_d \) from Eq. A3 into Eq. A2, one can see that Eqs. A1 and A2 are equivalent under conditions when mixing is instantaneous after single-bolus delivery of inulin.

However, mixing of inulin within the extracellular fluid volume is never instantaneous, and, therefore, inulin is eliminated via two-compartment (biexponential) kinetics. Thus, during the period immediately following the single bolus, when plasma inulin concentration is significantly higher than interstitial concentration, more inulin is lost through glomerular filtration than would be if mixing were instantaneous. To apply clearance to Eq. A2 above, the quantity I should have an amount subtracted from it to account for the inulin lost to glomerular filtration (\( I_L \)) before mixing is complete between intravascular and interstitial fluid volumes. Thus
\[ I_A = I - I_L \]  
(A5)

We will refer to the resultant value as actual inulin (\( I_A \)). This value can be divided by B to calculate the actual volume of distribution.

After a single bolus, the initial plasma decay of inulin, which is greater than that which occurs from renal elimination alone, is represented by the first part of Eq. 2 (see METHODS), the distribution phase
\[ y = Ae^{-\alpha (t_x - t_0)} \]  
(A6)

The inulin cleared by renal elimination (or inulin lost; \( I_L \)) during this distribution phase can be calculated by the following
\[ I_L = (C_i)(AUC_1) \]  
(A7)

where \( AUC_1 \) is the area under the distribution phase of the plasma inulin concentration curve (Eq. A6). Therefore, by substituting the value of \( I_L \) from Eq. A7 into Eq. A5
\[ I_A = I - (C_i)(AUC_1) \]  
(A8)

Substituting \( I_A \) for I in Eq. A2
\[ l - (C_i)(AUC_1) \ln \left| \frac{B}{P_x} \right| \frac{1}{(t_x - t_0)} = B \]  
(A9)

This equation can then be solved for \( C_i \), yielding
\[ C_i = \frac{1 - (t_x - t_0)B}{\ln \left| \frac{B}{P_x} \right| + AUC_1} \]  
(A10)

\( AUC_1 \) is the integral of the inulin distribution decay curve (Eq. A6)
\[ AUC_1 = \frac{-A}{\alpha} \left[ e^{-\alpha (t_x - t_0)} - 1 \right] \]  
(A11)

Thus, the final equation we derived for calculating GFR was
\[ GFR = C_i = \frac{1}{\frac{(t_x - t_0)B}{\ln \left| \frac{B}{P_x} \right|} - \frac{A}{\alpha} \left[ e^{-\alpha (t_x - t_0)} - 1 \right]} \]  
(A12)

For \( t_x \) we chose 45 min, a time when plasma inulin had reached undetectable concentrations after single-bolus injection in our experiments.

Standard pharmacokinetics (Eq. 6), as well as the equation we derived, accurately calculated GFR in our rat model. This is not surprising because, in fact, these two equations can be shown to be equivalent. Part of the denominator of Eq. A12 is equivalent to \( \beta \) (see Eq. A4); thus \( \beta \) can be substituted into Eq. A12 with the following result
\[ GFR = C_i = \frac{1}{\frac{(t_x - t_0)B}{\ln \left| \frac{B}{P_x} \right|} - \frac{A}{\alpha} \left[ e^{-\alpha (t_x - t_0)} - 1 \right]} \]  
(A13)

From Eq. A11, as \( t_x \) approaches infinity, the value of \( e^{-\alpha (t_x - t_0)} \) approaches 0; thus at the limit of \( t_x = \infty \), \( AUC_1 \) (Eq. A11) can be written
\[ AUC_1 = \frac{A}{\alpha} \]  
(A14)

Equation A13 can then be replaced with
\[ GFR = C_i = \frac{1}{\frac{(t_x - t_0)B}{\ln \left| \frac{B}{P_x} \right|} + \frac{A}{\alpha} \left[ e^{-\alpha (t_x - t_0)} - 1 \right]} \]  
(A15)

Equation A12 is, therefore, equivalent to Eq. 6, the standard pharmacokinetic equation for calculation of clearance from a two-compartment model when a \( t_x \) is chosen to reflect a time when there is a negligible plasma inulin concentration.

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