Serial determination of glomerular filtration rate in conscious mice using FITC-inulin clearance

Zhonghua Qi, Irene Whitt, Amit Mehta, Jianping Jin, Min Zhao, Raymond C. Harris, Agnes B. Fogo, and Matthew D. Breyer. Serial determination of glomerular filtration rate in conscious mice using FITC-inulin clearance. Am J Physiol Renal Physiol 286: F590–F596, 2004. First published November 4, 2003; 10.1152/ajprenal.00324.2003—Two nonradioactive methods for determining glomerular filtration rate (GFR) in conscious mice using FITC-labeled inulin (FITC-inulin) were evaluated. The first method measured GFR using clearance kinetics of plasma FITC-inulin after a single bolus injection. Based on a two-compartment model, estimated GFR was 236.69 ± 16.55 and 140.20 ± 22.27 μl/min in male and female C57BL/6J mice, respectively. Total or 5/6 nephrectomy reduced inulin clearance to 0 or 32.80 ± 9.32 μl/min, respectively. Conversely, diabetes mellitus induced by streptozotocin was associated with increased GFR. The other approach measured urinary inulin clearance using intraperitoneal microosmotic pumps to deliver FITC-inulin and metabolic cages to collect timed urine samples. This approach yielded similar GFR values of 211.11 ± 26.56 and 157.36 ± 20.02 μl/min in male and female mice, respectively. These studies demonstrate the feasibility of repeated nonisotopic measurement of inulin clearance in conscious mice.

THE FORMATION OF URINE BEGINS AT the glomerulus with the generation of a plasma ultrafiltrate. The rate of formation of this ultrafiltrate [glomerular filtration rate (GFR)] has become a mainstay for evaluating renal function and monitoring the progression of kidney disease in humans (27, 35).

An ideal tracer for determining GFR should be biologically inert, freely filtered through the glomerular filtration barrier and neither reabsorbed nor secreted by the renal tubule. A variety of tracers have been used for the measurement of GFR, including endogenous markers such as creatinine and cystatin C (7, 16, 27), or exogenous substances, including inulin, radioactive labeled EDTA, and iothalamate (6, 12, 16, 27), or exogenous substances, including inulin, radioactive labeled EDTA, and iothalamate (6, 12, 16, 27). In larger animals including humans, inulin clearance has become a gold standard for measuring renal function (17, 27, 30, 43). GFR can be determined by two major approaches: 1) determination of a tracer’s urinary excretion rate using timed urine collections (7, 26, 27, 44) or 2) determination of a tracer’s elimination kinetics from plasma after a single bolus injection (10, 14, 17, 19, 45).

Within the past decade, the development of techniques allowing manipulation of the mouse genome combined with the complete sequencing of the mouse genome have made the mouse a uniquely tractable animal model with which to study diseases (29, 35, 39a). In contrast, the ability to define renal disease in this species has not progressed as rapidly and has been significantly hampered by the lack of the routine and easy methods to determine GFR. Physiological characteristics of mice, including limited blood volume and the presence of high levels of noncreatinine chromagens in the blood, complicate the use of endogenous creatinine clearance as an index of GFR (35, 37). For example, using HPLC, calculated creatinine levels in mice are ~9 μM (<0.2 mg/dl) (37). These values are ~<20% of typical values of ~45 μM obtained with the widely used alkaline picate method (37). It is likely that cross-reacting noncreatinine chromagens account for the discrepancy between the two methods. Although similar chromagens exist in humans, these generally contribute to <10% of measured circulating creatinine levels, using similar techniques (37). This circumstance mandates the use of more rigorous methods to determine GFR in conscious mice. In the present studies, we examined the feasibility of serially measuring GFR in conscious mice using FITC-inulin clearance.

MATERIALS AND METHODS

Materials

C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME). Swiss-Webster mice were from Charles River Laboratories (Wilmington, MA). FITC-inulin and streptozotocin were the products of Sigma. Microosmotic pumps (Alzet, model 1007D, with a release rate of 0.5 μl/h for 7 days) were purchased from Durect (Cupertino, CA). Metabolic cages were purchased from Braintree Scientific ( Braintree, MA). The low-salt diet (0.03% Na+) and high-salt diet (3.15% Na+) were the products of TestDiet (Richmond, IN). All protocols were approved by the Institutional Animal Care and Use Committee of Vanderbilt University.

Microosmotic Pump Method

Preparation of FITC-inulin solution. FITC-inulin (5%) was dissolved in 0.9% NaCl by heating the solution in boiling water. To remove residual FITC not bound to inulin, the solution was dialyzed in 1,000 ml of 0.9% NaCl at room temperature for 24 h using a 1,000-Da cutoff dialysis membrane (Spectra/Pro 6, Spectrum Laboratories, Rancho Dominguez, CA). The dialysis bottle was wrapped with aluminum foil. After 24 h of dialysis, the fluorescence detected in the dialysate accounts for ~2.76% of total fluorescence inside the membrane. The FITC-inulin concentration decreased from 5 to ~3% after dialysis due to oncotic movement of fluid into the dialysis membrane. Before use, the dialyzed FITC-inulin solution was sterilized by filtration through a 0.22-μm filter (Costar, Corning, NY).

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Implantation of microosmotic pumps. Mice were anesthetized with ketamine (50 μg/g body wt) and xylazine (5 μg/g body wt) and placed on a warming pad. The midline fur was shaved, and the skin was sterilized with iodine and ethanol. Two microosmotic pumps filled with ~100 μl of 3% FITC-inulin were inserted into the peritoneal cavity through a small midline incision (~0.5 cm). The incision was closed with sterilized suture. After their complete recovery from anesthesia, the mice were housed individually in metabolic cages. Pilot studies revealed plasma FITC levels were inadequate to measure GFR when a single microosmotic pump was used. Using two pumps, a plasma fluorescence level two to six times higher than background was achieved.

Blood and urine collection. Urine was collected over 24 h on day 7 post-pump implantation. To harvest the residual fluorescence remaining on the cages, each cage was rinsed twice using 10 ml of 500 mM HEPES (pH 7.4) each time. The fluorescence harvested by rinsing the cages accounts for 26.2 ± 2.2 and 36.7 ± 5.0% of total urinary fluorescence detected in male and female mice, respectively. The total fluorescence counts, including that in the urine and in the harvested rinse solution, were ~96 ± 7% of estimated fluorescence released from the minipump. Blood was sampled through the saphenous vein at the end of a 24-h urine collection, using the method of Hem et al. (25). Briefly, the conscious mice were restrained inside a saphenous vein at the end of a 24-h urine collection, using the method of Hem et al. (25). Briefly, the conscious mice were restrained inside a heparinized capillary tube (Fisher Scientific) by venipuncture using a 23-gauge syringe needle. On average, this yielded 30 μl of plasma after centrifugation (4,000 rpm, 10 min). Preliminary data showed 10 μl of blood were collected with a heparinized capillary tube (Fisher Scientific) by venipuncture using a sterile 23-gauge syringe needle. On average, this yielded 30 μl of plasma after centrifugation (4,000 rpm, 10 min).

Measurement of FITC-inulin in plasma and urine. Because pH significantly affects FITC fluorescence value (32), both plasma and urine samples were buffered to pH 7.4 with 500 mM HEPES. Preliminary data showed 10 μl of HEPES maintain pH at 7.4 in 40 μl of plasma or urine. The titrated samples were then loaded onto a 96-well plate, 50 μl sample/well. Fluorescence was determined using a Fluoroscan Ascent FL (FIN-00811, Labsystems, Helsinki, Finland) with 485-nm excitation and read at 538-nm emission. The relationship between fluorescence and inulin concentration for both plasma and urine, confirmed the utility of fluorescence to measure FITC-inulin.

Stability of FITC-inulin fluorescence. The dissociation rate of FITC from inulin was determined by quantifying the rate of appearance of fluorescence in the dialysate. Dialyzed FITC-inulin was redialyzed at 37°C over 7 days. The fluorescence detected in the dialysate accounts for 0.84, 1.20, 1.66, 2.68, and 3.12% of total fluorescence inside the dialysis membrane on days 1, 2, 3, 5, and 7, respectively. Photo-bleaching or oxidative degradation of fluorescence was also evaluated by examining the change in FITC-inulin fluorescence occurring in 0.9% NaCl, wrapped with aluminum foil after 1, 2, 3, 5, and 7 days of incubation at 37°C. There was no difference in fluorescence counts between days 0 and 7.

Calculation of GFR. GFR was evaluated on day 7 post-pump implantation using two methods. One is to calculate inulin clearance using the 24-h urinary FITC-inulin excretion rate (urinary fluorescence counts/24 h) divided by the concentration of plasma FITC-inulin (fluorescence counts/μl). The other method is using the equation:

\[ \text{inulin clearance} = \text{inulin infusion rate/steady-state blood inulin concentration} \]

as previously described (10, 45). GFR was expressed in microliters per minute.

Experimental groups. GFR was first determined in C57BL/6J male and female mice. The effects of increased water intake on GFR were then explored in C57BL/6J mice provided either a high-salt diet or sugar water containing 1–4% glucose.

Single Bolus Injection Method

Introduction of FITC-inulin and blood collection. Dialyzed FITC-inulin (3.74 μg body wt) was injected retroorbitally under light anesthesia induced using isoflurane (Baxter Pharmaceutical Products, Deerfield, IL). The anesthesia lasted ~20 s. Approximately 20 μl of blood were collected via the saphenous vein at 3, 7, 10, 15, 35, 55, and 75 min postinjection of FITC-inulin, yielding 10 μl of plasma for the determination of FITC concentration by fluorescence, as described above.

Calculation of GFR from plasma clearance. Both one- and two-compartment clearance models were employed for the calculation of GFR. In the two-compartment model used, depicted in Fig. 1B, the initial, rapid-decay phase represents redistribution of the tracer from the intravascular compartment to the extracellular fluid. Systemic elimination also occurs, but the distribution process is relatively dominant during this initial phase. During the later phase, slower decay in tracer concentration predominantly reflects systemic clearance from the plasma. At any given time (t), the plasma concentration of the tracer (Y) equals \( A e^{-\alpha t} + B e^{-\beta t} \) (http://curvesit.com/0205.htm) (10, 45). In the present studies, plasma fluorescence data were fit to a two-phase exponential decay curve using nonlinear regression (GraphPad Prism, GraphPad Software, San Diego, CA). Because FITC-inulin was injected as a bolus, and the plasma fluorescence decreased to nearly zero at 180 min postinjection (Fig. 1A), the plateau was set to zero, and GFR was calculated using the equation GFR = \( I/(A/\alpha + B/\beta) \) (10, 45), where I is the amount of FITC-inulin delivered by the bolus injection, A and B are the y-intercept values of the two decay rates, and \( \alpha \) and \( \beta \) are the decay constants for the distribution and elimination phases, respectively (10, 45).
For GFR calculation using the one-compartment model, we adapted the equation described by Catlin (10) and used the plasma
FITC-inulin data obtained for two-compartment analysis at 10, 15, 35,
and 75 min post-bolus injection. The elimination rate constant (K)
and the volume of distribution (Vd) were obtained by a semilogarithmic
plot of plasma fluorescence concentration at each time point
(logarithmic) vs. time (arithmetic), and GFR was calculated using
the equation GFR = Vd × K.

Experimental groups. GFR measurement using a single bolus
injection (iv) of FITC-inulin was conducted in normal male and
female C57BL/6J mice and in male Swiss-Webster mice. Three
additional groups of C57BL/6J male mice with either total or %
nephrectomy and early-stage diabetes mellitus (at 5 wk posthypergly-
cemia) induced by injection of streptozotocin were also studied.

Nephrectomy. C57BL/6J male mice were anesthetized using ket-
amine (50 μg/g body wt) and xylazine (5 μg/g body wt) to achieve the
desired depth of anesthesia for 45 min. A 0.5-cm incision parallel to
the spinal column was cut on each flank of the back under aseptic
conditions. For total nephrectomy, both kidneys were removed
through the incisions. For % nephrectomy, the right kidney and upper
and lower thirds of the left kidney were removed. The incisions were
sutured (6.0 thread, Ethicon, Somerville, NJ), and the mice were
allowed to recover from anesthesia. Determination of GFR was
conducted in mice 2 wk after % nephrectomy or immediately after full
recovery from anesthesia in mice with total nephrectomy.

Streptozotocin-induced diabetes mellitus model. At 8 wk of age,
C57BL/6J male mice received daily streptozotocin injections intra-
peritoneally (50 mg/kg, made fresh in 0.1 M citrate buffer, pH 4.5) for
5 consecutive days. The fasting level of blood glucose was examined
weekly with a B-Glucose Analyzer. GFR was measured after 5 wk of
documented hyperglycemia.

Statistics

All data are expressed as the means ± SE. A paired t-test was used
for analysis of data from different time points within the same group,
and an unpaired t-test for the comparison of data between two groups.
A P value <0.05 was considered significant.

RESULTS

Measurement of GFR by a Single Bolus Injection
of FITC-Inulin

GFR was examined in male and female C57BL/6J mice after
a single bolus injection of FITC-inulin. The representative
plasma elimination rate of FITC-inulin, plotted on arithmetic
coordinates (Fig. 1A), exhibits characteristics typical of a
two-compartment system, with a rapid, initial decay rate and a
slower linear terminal segment (10, 45). Using nonlinear
regression to curve fit the data, the second-phase plasma elimi-
nation kinetics of FITC-inulin yielded an estimated GFR of
149.97 ± 20.86 μl/min in female mice and 240.11 ± 21.37
μl/min in male mice (Fig. 2). Subsequent redetermination of
GFR in these same mice 3 wk later was not significantly
different (130.43 ± 25.28 μl/min in female and 233.26 ±
29.32 μl/min in male mice, P = not significant compared with
week 1). The combined average GFR (weeks 1 + 3) are
presented in Table 1. Male C57BL/6J mice exhibit a significa-
cantly greater inulin clearance rate compared with female
mice.

GFR was also measured in male Swiss-Webster mice. In-
terestingly, the GFR in Swiss-Webster mice was significantly
higher than C57BL/6J mice (Table 1). This difference remains
significant even after GFR was corrected for body weight.

Because in previous reports of radioisotopic determination
of GFR in conscious mice GFR was calculated using only later
time points and fit to a single-compartment model, we recal-
culated GFR using the present data according to the same
single-compartment analysis. As shown in Table 1, the GFR
based on the one-compartment model was significantly higher
than that based on the two-compartment model for both male
and female C57BL/6J mice.

Effect of nephrectomy. To verify that inulin clearance as
determined by the plasma decay rate reflects renal function, we
compared the clearance in mice after the removal of both
kidneys or % nephrectomy. The elimination curve for FITC-
inulin shows that the decay rate of the second phase of plasma
fluorescence was markedly attenuated in mice after subtotal
nephrectomy and abolished in mice without kidneys. This
resulted in plasma fluorescence values remaining dramatically
higher at the end of the experiment compared with values
determined in normal control mice (Fig. 3B). Accordingly, the
inulin clearance rate was 0 in mice with total nephrectomy and
32.80 ± 9.32 μl/min (25.35 ± 0.84 g body wt) after %
nephrectomy (Fig. 3A).

Hyperfiltration in diabetic mice. Inulin clearance was also
examined in diabetic C57BL/6 male mice 5 wk after the onset

![Fig. 2. Repeated determination of glomerular filtration rate (GFR) was performed in both conscious male (n = 6) and female C57BL/6 mice (n = 6) using the single bolus injection approach. The GFR values were not different in either gender when determined at different time points 2 wk apart. *P < 0.05 male vs. female mice at the same time point.](http://ajprenal.physiology.org/)
of streptozotocin-induced hyperglycemia (500–800 mg/dl). Inulin clearance was significantly higher in diabetic mice than in control, nondiabetic male C57BL/6 mice (303.03 ± 17.75 vs. 236.69 ± 16.55 µl/min, P < 0.05; Fig. 3A).

**GFR Determined by Urinary FITC-Inulin Clearance**

FITC-inulin clearance was also measured under steady-state (equilibrium) conditions, achieved using osmotic pumps. After implantation of microosmotic pumps and the housing of mice in metabolic cages, the animals experienced significant weight loss over the first few days and then gradually recovered preoperative weight by day 7. Therefore, inulin clearance determined on day 7 post-pump implantation was used. Inulin clearance values obtained using this method were comparable to those determined using the bolus two-compartment model in both male and female C57BL/6J mice but lower than the bolus method when analyzed using a one-compartment model (Table 1).

GFR was also estimated from plasma inulin levels on day 7 and the known pump infusion rate. This yielded GFR values of 233.96 ± 42.96 µl/min in male C57BL/6 mice and 170.01 ± 18.18 µl/min in female mice, which are comparable to those based on the urinary FITC excretion rate.

Volume depletion may have contributed to the postoperative weight loss, so two maneuvers, including a high-salt diet and use of sugar water, were used to increase water intake. When provided an 8% NaCl diet (3.15% Na⁺), the mice drank significantly more water than when provided a control diet. Interestingly, a high-salt diet accentuated the weight loss compared with mice on control diet (Table 2). This occurred despite the fact that food intake was comparable in the two groups (2.67 ± 0.08 vs. 2.75 ± 0.02 g/24 h). GFR was not different in mice on a high-salt vs. a normal-salt diet.

Increased water intake was also achieved by providing mice with sweetened water containing 1–4% glucose. Blood glucose concentration was not different from that in mice ingesting tap water. A dramatic increase in both water intake and urine excretion was observed in mice provided sugar water compared with mice ingesting tap water or a high-salt diet (Table 2). Weight loss in mice provided sugar water was also significantly greater than in the other groups. In addition, GFR was significantly lower in mice provided sugar water than the other groups.

### Table 1. **GFR in conscious mice determined using FITC-inulin clearance**

<table>
<thead>
<tr>
<th></th>
<th>Minipump Method, Day 7 (C57BL/6J)</th>
<th>Bolus Method (2-Compartment Model)</th>
<th>Bolus Method (1-Compartment Model)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>GFR, µl/min</td>
<td>211.1 ± 26.6</td>
<td>236.7 ± 16.6</td>
<td>494.9 ± 25.8*</td>
</tr>
<tr>
<td>GFR, µl·min⁻¹·g BW⁻¹</td>
<td>9.1 ± 1.2</td>
<td>8.7 ± 0.6</td>
<td>12.1 ± 1.0*</td>
</tr>
<tr>
<td>GFR, µl·min⁻¹·g KW⁻¹</td>
<td>883.5 ± 140.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, wk</td>
<td>10</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>GFR, µl/min</td>
<td>157.4 ± 20.0</td>
<td>140.2 ± 22.3</td>
<td>181.5 ± 20.6*</td>
</tr>
<tr>
<td>GFR, µl·min⁻¹·g BW⁻¹</td>
<td>8.3 ± 1.0</td>
<td>6.3 ± 0.9</td>
<td>8.2 ± 0.9</td>
</tr>
<tr>
<td>Age, wk</td>
<td>10</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of rats; GFR, glomerular filtration rate; BW, body weight; KW, kidney weight. *P < 0.05 vs. GFR obtained from bolus method using 2-compartment model in C57BL/6J male mice. †P < 0.05 vs. GFR obtained by minipump method.
Innovative Methodology

INULIN CLEARANCE IN CONSCIOUS MICE

Table 2. Effects of high-salt diet or sugar water on GFR in female C57BL/6J mice

<table>
<thead>
<tr>
<th>Status</th>
<th>GFR on Day 7, µl/min</th>
<th>ΔBW on Day 7 vs. Day 0, %</th>
<th>Water Intake, ml/24 h</th>
<th>Urine Volume, ml/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 9)</td>
<td>157.36 ± 20.02</td>
<td>4.14 ± 1.62</td>
<td>6.55 ± 0.69</td>
<td>1.16 ± 0.26</td>
</tr>
<tr>
<td>High-salt diet (n = 5)</td>
<td>175.50 ± 19.26</td>
<td>−6.05 ± 2.67*</td>
<td>10.30 ± 0.72*</td>
<td>6.16 ± 0.25*</td>
</tr>
<tr>
<td>Sugar water (n = 10)</td>
<td>70.29 ± 13.17†</td>
<td>−14.66 ± 1.54†</td>
<td>23.28 ± 2.96†</td>
<td>14.50 ± 2.01†</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of rats. GFR was determined using microosmotic minipump method. Water intake and urine volume are the averaged data over 7 days. ΔBW means changes in body weight on day 7 over day 0. GFR are the values on day 7. *P < 0.05 vs. control. †P < 0.05 vs. high-salt diet.

DISCUSSION

Determination of GFR is integral to the assessment of renal function. Its measurement in mice presents unique technical hurdles, including limited total blood volume and the presence of high levels of endogenous noncreatinine chromagens, limiting the utility of creatinine clearance. Furthermore, use of sensitive radioisotopic methods are complicated by convenience and safety issues. Inulin has been widely used to determine GFR and is considered a “gold standard” marker of glomerular filtration, being freely filtered and neither reabsorbed nor secreted by the tubule (19, 27). FITC labeling of inulin has made it possible to detect minute quantities of inulin in blood (32) and has previously been used successfully to determine GFR in anesthetized mice (8, 32, 33, 38). The present studies utilized FITC-inulin to monitor GFR in conscious mice.

Two approaches to measure FITC-inulin clearance in conscious mice were tested. One approach was based on the plasma clearance kinetics of FITC-inulin after a single bolus injection. A major advantage of this approach is that it does not require timed urine collections or an operative procedure. The other approach determined the urinary excretion rate of FITC-inulin, using intraperitoneal implantation of microosmotic pumps to continuously deliver FITC-inulin. This approach required less blood sampling but also requires the acquisition of timed urine collections from mice housed in metabolic cages.

To determine GFR based on plasma inulin elimination kinetics, multiple timed blood collections are necessary. After a bolus injection of FITC-inulin, the kinetics of plasma FITC fluorescence decay followed a biphasic profile. The highest fluorescence detected in saphenous venous blood occurred within 3 min. Fluorescence then fell rapidly within the first 10 min, followed by a more gradual decline over the next hour, consistent with a biexponential decay. We therefore analyzed FITC-inulin clearance using a two-compartment model composed of a distribution phase and an elimination phase (10, 45). Calculation of GFR, based on a two-compartment clearance model after a single bolus injection, has also been widely employed in larger animals, including rats and humans (14, 43, 45). Using this approach, the absolute GFR was ~236 µl/min in male C57BL/6 mice and 140 µl/min in female mice. These values were serially reproducible for both male and female mice and were comparable to values obtained from the microosmotic pump method performed in the present studies.

Comparison of these GFR values to previously published results for GFR in mice is complicated by the varied units used to report GFR, including microliters per minute per gram

Table 3. Published GFR values in mice

<table>
<thead>
<tr>
<th>Status</th>
<th>GFR, µl/min</th>
<th>BW, g</th>
<th>Gender</th>
<th>Strain</th>
<th>Tracer</th>
<th>Reference No.</th>
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<tr>
<td>Conscious</td>
<td>330</td>
<td>27.54</td>
<td>M</td>
<td>C57BL/6</td>
<td>[3H]inulin</td>
<td>22</td>
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<tr>
<td>Conscious</td>
<td>570</td>
<td>32.72</td>
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<td>B6D2F1</td>
<td>[3H]inulin</td>
<td>22</td>
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<tr>
<td>Conscious</td>
<td>497</td>
<td>36.3</td>
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<td>Swiss-Webster</td>
<td>[3H]inulin</td>
<td>24</td>
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<td>Conscious</td>
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<td>Swiss-Webster</td>
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<td>Conscious</td>
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<td>BALB/c</td>
<td>[3H]inulin</td>
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<tr>
<td>Conscious</td>
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<td>22.5</td>
<td>M</td>
<td>BALB/c</td>
<td>[3H]inulin</td>
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<tr>
<td>Conscious</td>
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<td>MLeprab/+</td>
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<td>C57BL/6</td>
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<td>C57BL/6</td>
<td>FITC-inulin</td>
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<td>CD-1</td>
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<td></td>
<td>M</td>
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<td>[3H]inulin</td>
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<td>C57 × 129</td>
<td>[131I]iodohippurate</td>
<td>46</td>
</tr>
</tbody>
</table>

Summarized data from the published literature are shown. M, male; F, female. Some of the parameters are not available in the publications. *One-compartment model.
kidney weight, per gram body weight, or per surface area (m²). In the present studies, we used microliters per minute per mouse, because serial GFR determination in conscious, viable mice does not allow measurement of kidney weight. Furthermore, studies of obese strains of mice (e.g., ob/ob or db/db) may invalidate correction of GFR by body weight. Thus the GFR from published literature was reexpressed using microliters per minute per mouse (Table 3). In addition, GFR values determined in the present studies are expressed after correction for body weight (per gram) and kidney weight (Table 1).

It is evident from this summary that significant variation in GFR exists in mice and may be due to several factors (Table 3). Gender differences in GFR have been previously reported in many species including humans and mice (21, 24, 36, 40). In the present studies, GFR was also greater in male vs. female mice. Because this difference disappeared when GFR is corrected for body weight, it may be attributable to differences in kidney size between genders. Mouse strain appears to be another factor influencing the levels of GFR. For example, C57BL/6J mice have previously been found to exhibit a relatively low GFR compared with other inbred strains (22). GFR obtained using 51Cr-EDTA elimination kinetics in conscious C57BL/6J male mice was only 57.9% of that in B6D2F1 hybrid mice (Table 3), a difference that could not be explained by difference in body or kidney weight between these strains (22). In the present studies, we also observed a significant difference in GFR between Swiss-Webster and C57BL/6J mice (Table 1), supporting the existence of a significant variation in GFR between mouse strains.

Published GFR studies in C57BL/6J mice are limited, especially in conscious mice. The previously published data were based on plasma elimination kinetics of 51Cr-EDTA and calculated using a single-compartment model (13, 18, 22, 24). This single-compartment model had been more recently shown to overestimate GFR in rats (45). Recalculating our FITC bolus data using a single-compartment model yields an estimated GFR of 317 μl·min⁻¹·mouse⁻¹ in male mice, nearly identical to the value of 330 μl·min⁻¹·mouse⁻¹ previously reported by Hackbarth and Hackbarth (22) using one compartment (Table 3). However, this value is significantly higher than that from the microosmotic pump method (P < 0.05), suggesting it overestimates GFR in mice, as was found in rats (45).

Other previous studies examined GFR in anesthetized animals. Anesthesia may significantly influence GFR. The values of GFR in conscious rats (4) were only ~60% of that obtained by the same group in anesthetized rats (1). GFR data from anesthetized C57BL/6J mice (provided in Table 3) averaged ~288 μl/min (11, 28, 41, 47), which is only 19% higher than GFR in conscious mice obtained in the present studies.

GFR is a dynamic parameter that can be regulated by a variety of physiological factors. Changes in hemodynamics associated with the early stages of diabetes mellitus, or decreased renal mass after nephrectomy, were found to significantly alter GFR in both the present and previous studies (5, 13, 39).

An alternative approach to measuring GFR utilized an approach previously used to determine GFR in conscious rats (26). In mice, loss of body weight was observed during the first few days. This may be associated with the stress of an altered living environment, as well as postoperative stress. Over time, the animals’ body weight gradually recovered. By 7 days post-pump implantation, body weight had returned to levels comparable to that before the operation (Table 2), supporting the validity of GFR measurements on that day. At that time, GFR was not different from that obtained by the bolus technique.

The extent of early postoperative weight loss may be partially due to volume depletion. Efforts to mitigate weight loss due to volume depletion, including use of a high-salt diet and provision of sweetened water, were attempted. An increase in water intake achieved using sweetened water increased urine flow dramatically; however, this was also associated with suppressed GFR. An inverse correlation between water intake and GFR has also been demonstrated in conscious rats (4). The mechanisms contributing to decreased GFR in this setting are uncertain; however, decreased plasma vasopressin levels during diuresis may play a role (3, 4, 49). Vasopressin potentially regulated GFR, and vasopressin-deficient Brattleboro rats failed to hyperfilter after hyperglycemia induced by streptozotocin (3). Conversely, chronic administration of vasopressin elevated GFR (4, 20). Antiuretic mice ingesting tap water exhibited a significantly higher GFR than did the diuretic mice (Table 2).

The minipump method was also validated by measuring steady-state plasma FITC levels. Theoretically, when FITC-inulin is infused at a constant rate, the amount of inulin in the body will accumulate until the rates of elimination and infusion are equal. Therefore, inulin clearance can be estimated using the inulin infusion rate under the steady state of plasma inulin concentration (10, 45). Recalculation of GFR using plasma inulin concentration on day 7 and the pump infusion rate yields GFR values comparable to those based on the urinary excretion rate. The similar values indicate that an equilibrium in plasma inulin concentration was achieved and support the conclusion that excreted urinary FITC-inulin was completely collected.

In summary, the present studies characterized two approaches to monitor GFR in conscious mice using FITC-inulin. These methods yielded internally consistent results that sensitively reflect changes in renal function and agree with previous values for mice. The single bolus FITC-inulin method may be preferable for serial GFR measurements in mice because it does not require surgery or metabolic cages compared with the osmotic pump method. The osmotic pump approach may be suitable for studies addressing renal electrolyte metabolism but is less useful for long-term monitoring of renal function because of the repeated surgery required. These two methods will hopefully facilitate studies of renal function in mice.

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GRANTS

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REFERENCES

INULIN CLEARANCE IN CONSCIOUS MICE


