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Review Article

Transcutaneous measurement of glomerular filtration rate in small rodents: Through the skin for the win?

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SUMMARY AT A GLANCE

This methods in renal research article describes a new non-invasive technique to measure glomerular filtration rate in small animals. Furthermore, this technology has potential for broader use in renal research, such as in measuring the rate of clearance of albumin from the circulation in animals with proteinuria.

ABSTRACT:

Rodent models of renal physiology and pathology are crucial to our understanding of the molecular, histological and functional sequelae that contribute to kidney diseases. One of the most important measures of renal function is glomerular filtration rate (GFR). While the accurate determination of GFR is pivotal to understanding the progression of disease and/or the benefits of treatment strategies, in rodents the conventional methods for assessment of GFR are inconvenient and cumbersome, not the least because they involve stress and often anaesthesia. The legitimacy of assay-based assessment of plasma and urine markers of GFR in mice has also been heavily scrutinized for their insensitivity to minor declines in GFR and inaccurate detection of renal biomarkers. While infusion-based clearance methods of GFR assessment are thus the gold standard in terms of accuracy, they are limited by the fact that they are primarily non-recovery procedures. This presents a dilemma when trying to document the progression of renal disease, as these measures cannot be taken in the same experimental subject. Here we review a technique of transcutaneous measurement of fluorescein isothiocyanate-labelled sinistrin to calculate GFR in small rodents, using a non-invasive clearance device (NIC-Kidney Device). This is a recently validated non-invasive technique for measuring GFR in small rodents that allows for the real-time measurement of GFR in conscious animals, without the need for plasma and urine assays.

Glomerular filtration rate (GFR) is the key clinical measure of renal function and an essential measurement in the analysis of renal pathology and disease in both clinical and experimental settings. Many studies in rodents employ the measurement of end-products of metabolism such as creatinine to estimate GFR, necessitating the collection of a timed urine (usually over 24 h) and collection of blood which, in addition to being costly, are time consuming and requiring further assay and analysis, which necessarily creates some stress for the animals. Further, not all methods are accurate: for example, non-creatinine chromogens have been shown to interfere with the commonly used Jaffè assay for measuring plasma creatinine, leading to an overestimation of clearance and estimated GFR in the order of 35–50%.[1] While measurement of creatinine by high-performance liquid chromatography overcame many of the issues around the measurement of plasma creatinine in mice,[2] recent studies by Eisner *et al*. have identified tubular secretion as a significant contributor to urinary creatinine in mice, calling into question the validity of creatinine clearance to measure GFR in mice.[1,3]

Infusion-based methods of measuring GFR (e.g. ^{3}H-inulin) are currently the gold standard for accurate assessment of GFR in rodents.[4–6] However, these techniques involve

anaesthesia, which is known to reduce GFR.[7] These techniques also require analysis of blood and urine samples. Radiolabelled markers are often used for infusion-based methods of GFR, thus requiring specialized equipment and technical training to safely perform these procedures and dispose of waste. The major drawback to this technique is that for the most part, the small rodent is unable to recover from the procedure, thus serial measurements within the same animal to characterize the progress of renal pathology cannot be obtained.

The transcutaneous measurement of fluorescein isothiocyanate-labelled sinistrin (FITC-sinistrin) is a new technique that allows for the real-time assessment of GFR in conscious rodents.[8] The method does not require the serial sampling of urine or plasma, or the need to perform laboratory-based assays of renal biomarkers. Moreover, it can be used with conscious rodents and can provide repeated measures of GFR in the same animal. Here we present the detailed general methodology of this technique and principles of GFR calculation in mice. We also present recommendations and adaptations for optimizing this technique in different species of mice, the conventional C57BL/6 mouse and the spiny mouse (*Acomys cahirinus*), a precocial rodent of comparable size with the laboratory mouse, but a rodent with a much higher activity rate and desert-adapted renal physiology.[9]

FITC-SINISTRIN AND THE NIC-KIDNEY DEVICE

FITC-sinistrin is a commercially available exogenous marker of renal function. Sinistrin, like inulin, is freely filtered by the glomerulus, but is not reabsorbed or secreted by the renal tubules, making it an ideal marker of renal clearance. The NIC-Kidney Device is a miniaturized optical device equipped with an internal memory and the ability to record the fluorescent emission of FITC-sinistrin through the skin (Mannheim Pharma and Diagnostics, Mannheim, Germany). Data collected and stored on the device over an experimental period can then be used to generate an elimination kinetics curve of FITC-sinistrin. For more details on the design and functionality of the device, refer to Schreiber *et al*.[10] The half-life of FITC-sinistrin ($t^{1/2}$) can be determined by the relative emission signal detected by the device and the single exponential excretion phase of the elimination kinetics curve. As sinistrin is exclusively filtered by the glomerulus, the $t^{1/2}$ of sinistrin can be used to calculate GFR, with the use of a pre-established conversion factor (see section Determining FITC-Sinistrin Conversion Factor for GFR Calculations). A rechargeable lithium battery powers the NIC-Kidney Device, thus it can be used repeatedly. This makes the NIC-Kidney Device a highly economical choice for the assessment of GFR, with the only consumables being the FITC-sinistrin solution, injecting materials and adhesive tape. The initial expected outlay to set up the NIC-Kidney technique at this time is approximately US$1750, with cost per measurement at ~US$10. The NIC-Kidney Device also requires minimal experimental set-up and is highly portable. This allows for the relatively quick assessment of GFR and allows for the measurement to be taken in various environments (i.e. specific animal housing facilities).

MATERIALS AND METHODS (ADAPTED FROM NIC-KIDNEY DEVICE USER MANUAL, MANNHEIM PHARMA AND DIAGNOSTICS, AND OPTIMIZED FOR C57BL/6 MICE)

Materials

- NIC-Kidney Device*
- Lithium polymer rechargeable battery (4.2 V, 50 mAh) and battery recharger*
- Double-sided adhesive patch*
- NIC-Kidney Device partner software*
- FITC-sinistrin (Fresenius Kabi Austria GMBH, Linz, Austria) and physiological buffer
- Apparatus for inhalation of anaesthesia (e.g. Univentor 400 anaesthesia unit, Univentor Ltd, Zejtun, Malta)
- Electrical shaver
- Depilatory cream
- Adhesive tape (e.g. Leukosilk® tape, width 2.5 cm)
- 1 mL syringe
- Needle (25G–30G, depending on route of injection)

*NIC-Kidney Device, batteries and adhesive patches, supplied by Mannheim Pharma Diagnostics, Mannheim, Germany

Methods

For a flow chart summarizing the NIC-Kidney Device experimental procedure and GFR calculation, refer to Figure 1.

Fur depilation (usually 24 h prior to GFR measurement)

1 The mouse is lightly and briefly anaesthetized (<5 min under 2.1–2.4% isoflurane). A small area of fur on the flank of the mouse (of a size slightly bigger than the size of the NIC-Kidney Device) is removed using an electric shaver followed by application of depilatory cream. When using a mouse strain prone to having pigmented areas on the skin, check that the area is clear of dark colouration after the shave. If pigmentation is present, move to the opposite flank of the mouse, shave, and then apply the depilatory cream. Thoroughly wash the area clean of depilatory cream after ~2 min.

Preparation of NIC-Kidney Device and FITC-sinistrin solution

1 Place one side of the double-sided adhesive patch onto the NIC-Kidney Device, positioning the light-emitting diodes

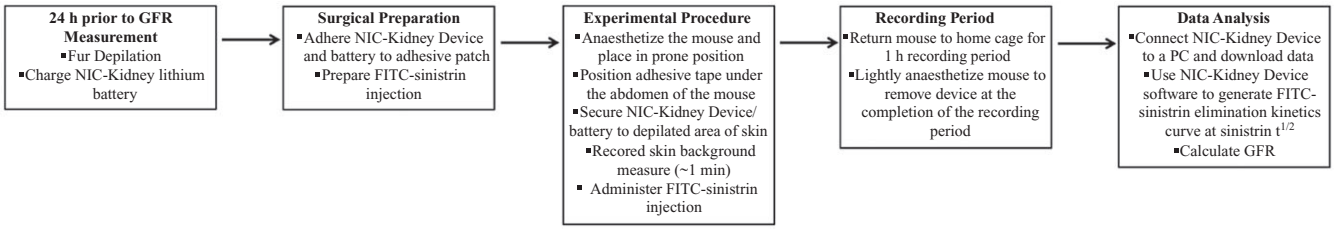

Fig. 1 Flow chart summarizing NIC-Kidney experimental procedure and glomerular filtration rate (GFR) calculation. FTIC-sinistrin, fluorescein isothiocyanate-labelled sinistrin.

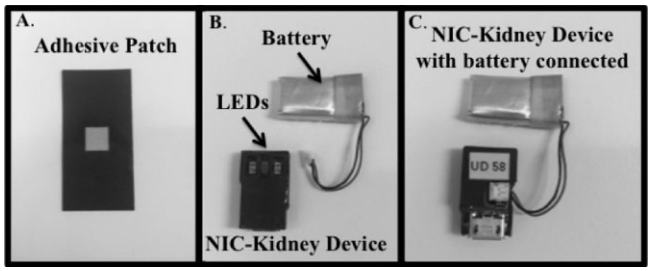

Fig. 2 NIC-Kidney Device and accessories: (A) adhesive patch, (B) NIC-Kidney Device and battery and (C) NIC-Kidney Device with battery connected.

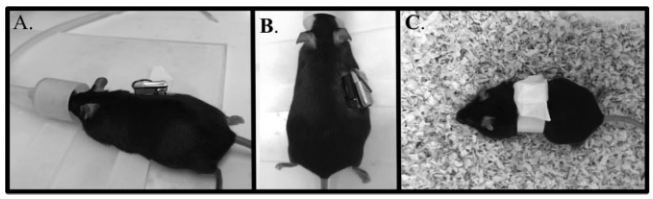

Fig. 3 Adhering the NIC-Kidney Device. (A,B) Mouse lightly anaesthetized with isoflurane. NIC-Kidney Device adhered to depilated patch of skin on the flank of the abdomen. Battery is connected and mounted on top of the device with double-sided adhesive patch. (C) Conscious mouse in home cage for the 1 h recording period.

exactly above the transparent window of the adhesive patch (Fig. 2A,B). With the opposite side of the double-sided adhesive patch intact, cut around the NIC-Kidney Device, leaving the adhesive patch approximately 2–3 mm wider than the edges of NIC-Kidney Device.

2 Adhere the battery to a double-sided adhesive patch of matching size.

3 Dissolve FITC-sinistrin in physiological saline to make stock solution of 10–15 mg/mL. Working solution of FITC-sinistrin is determined according to body weight (BW) of the mouse (4–7 mg/100 g BW for C57BL6 mice). Note: FITC-sinistrin solutions can be prepared in advance. Store aliquots at −20°C away from light.

Experimental procedure

1 Lightly anaesthetize the mouse using isoflurane (2.1–2.4%) and place the animal in the prone position.

2 Place a strip of adhesive tape (Leukosilk® tape, 10–15 cm long) under the abdomen of the mouse.

3 Connect the battery to the NIC-Kidney Device (Fig. 2C) and adhere it to the top of the device via the double-sided adhesive patch. Note: A fully charged battery has a running time of ~2 h, thus connection of the device to the battery should only take place just prior to the device being adhered to the mouse.

4 Adhere the NIC-Kidney Device and battery to the depilated skin on the back of the mouse (Fig. 3A,B).

5 Wrap the adhesive tape around the mouse's body to secure the device in place (Fig. 3C). This minimizes movement artefact and prevents the mouse from being able to chew at wires.

6 Leave the device in place on the rodent for a period of at least 1 min before administering the FITC-sinistrin injection. This is to obtain a background reading through the skin.

7 Inject FITC-sinistrin intravenously (tail vein, jugularis externa via catheter) or by direct injection (retro-orbital, intra-cardiac). For details, see section Methodological Considerations, Route of FITC-Sinistrin Administration.

8 Return mouse to home cage (Fig. 3C). It is recommended that environmental enrichment and wire lids with food and water be removed for the duration of the recording period, to reduce the possibility of the device being knocked or caught on objects.

9 At the end of the 1 h recording period, the tape and device can be removed without anaesthesia; however, we have found it less stressful for the mouse and less damaging on the skin for mice to be lightly anaesthetized so the tape can be gently peeled off. The mouse is returned to home cage for recovery.

10 The experimental procedure can be repeated in the same mouse 24 h after the initial read. Depending on the strain of mouse, adequate readings through the same patch of depilated skin are achievable for around 1 week.

Data analysis

1 The battery is removed from the device. Note: The data are stored on the device until a battery is once more connected.

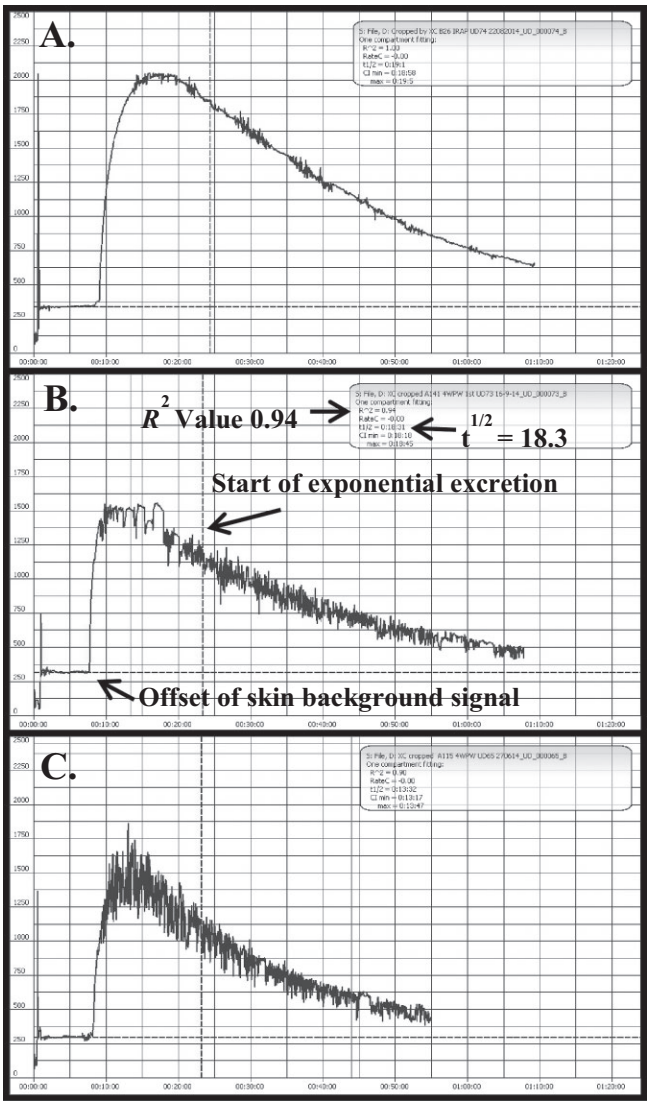

Fig. 4 Representation images of elimination curve of fluorescein isothiocyanate-labelled sinistrin (FITC-sinistrin). Examples (A–C) of readable ($R^2 > 0.8$) elimination kinetics curves of FITC-sinistrin, generated from the emission signal of FITC-sinistrin detected transcutaneously and stored on the NIC-Kidney Device. Panel (B) indicates the points on the curve where the background signal is offset and the beginning of the exponential excretion phase of the curve used to calculate the $t^{1/2}$ of sinistrin.

If the battery is reconnected before the data are downloaded from the device, data from the previous reading will be deleted (there is a 10 s grace period).

2 The device is connected to a PC via a micro USB cable.

3 Data are downloaded from the device as a .csv file, which can be opened and modified in Microsoft Excel, and are readable by NIC-Kidney Device partner software.

4 NIC-Kidney Device software generates the elimination kinetics curve of FITC-sinistrin (Fig. 4), which can then be used to calculate GFR.

$$\text{GFR}\,(\mu\text{L/min/100gBW}) = \frac{\text{Conversion Factor}}{t_{1/2}(\text{FITC-sinistrin})(\text{min})}$$

To analyse the generated curve, first offset the background skin signal detected prior to FITC-sinistrin injection. Then mark on the curve the beginning of the exponential excretion phase of FITC-sinistrin (Fig. 4B). In mice, this is usually ~15 min after the bolus injection. You then select the software to analyse the curve, at which point an R^2 value and $t^{1/2}$ of FITC-sinistrin are calculated. The R^2 value can be used to validate the linearity of the curve. Readings with an $R^2 < 0.8$ should not be included as they may be compromised by movement artefact. Our known studies have an average $R^2 = 0.97$ (range 0.93–1.0) Note: The detachment of the battery from the device at the conclusion of the experiment can cause a spike in signal, and that can skew both the R^2 and $t^{1/2}$ generated from the curve. By opening the .csv file in Microsoft Excel, the signal obtained after the completion of the 1 h test period can be deleted, prior to generating the $t^{1/2}$ of FITC-sinistrin and calculating GFR.

Methodological considerations

Route of FITC-sinistrin administration

The most commonly used route of administration of FITC-sinistrin in rodents is intravenous injection into the tail vein. To generate a readable elimination kinetics curve, it is important that the entire bolus of FITC-sinistrin is administered intravenously. It can be difficult to visualize the tail vein of C57BL/6 mice, hence we found the placement of a latex glove filled with 50–60°C water on top of the tail to warm it up prior to the intravenous injection of FITC-sinistrin very effective in making the vein more visible. This step can be completed while the NIC-Kidney Device is recording the skin background reading, thus minimizing the time the mouse is under anaesthetic. A delivery volume of 0.1–0.2 mL via tail vein is recommended for C57BL/6 mice.

If effective tail vein administration is hard to obtain, as is the case for the spiny mouse, a species prone to dropping their tail and whose tail vein is narrow and located under a thick cartilage sheath,[11] FITC-sinistrin can be administered retro-orbitally[12] or by intra-cardiac injection.[13] For administration of FITC-sinistrin in the spiny mouse, the animal is turned from the prone position, used to adhere the NIC-Kidney Device to the cleared patch of skin, onto its back for injection. FITC-sinistrin is then administered by passing a 27G needle through the chest wall and into the left ventricle of the heart. For this route of administration, it is recommended that the concentration of FITC-sinistrin stock solution be increased to limit the injection volume to 0.03–0.04 mL.

Reducing skin irritation following depilation

Fur removal with shaving and depilatory cream can cause skin irritation in some strains of mice, particularly C57BL/6

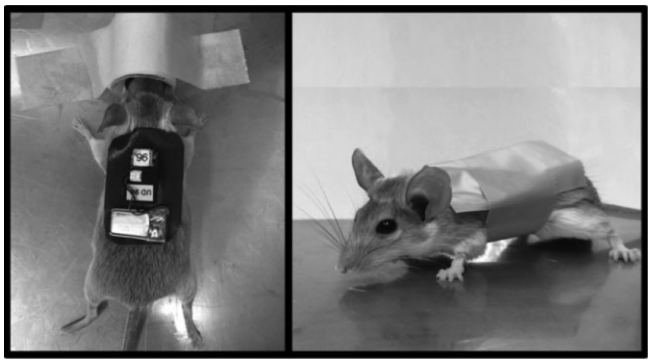

Fig. 5 NIC-Kidney Device adhered to the centre of the back. Spiny mouse with the NIC-Kidney Device and battery adhered in a linear fashion across the back on the rodent.

mice.[14] Minimizing the area of fur removal is thus recommended. Depilation of an area 3–4 mm wider than the size of the NIC-Kidney Device should suffice for adequate adhesion of the device to the skin. It is also recommended that depilatory cream remains on the skin for no more than 2 min and is thoroughly washed off the skin and surrounding area. If scratching of the area is of concern, we recommend that the toenails of the lower limbs be trimmed. This prevents the progression of irritation and promotes the resolute of the lesion. A steroid cream in conjunction with nail trimming may also improve outcomes.

Reducing movement artefact and minimizing access to device circuitry

For rodents that are highly active, adhering the NIC-Kidney Device to the centre of the back, with the battery placed next to the device instead of on top of the device, may aid in reducing movement artefact. For example, in studies of the highly active spiny mouse, the NIC-Kidney Device was placed squarely on the middle of the back (Fig. 5). This positioning may also be of use if the strain of mice under experimentation is prone to chewing at the tape and wires of the device. If movement artefact is still of concern, algorithms for artefact removal from obtained signals can be used (see Shmarlouski *et al*. for detailed discussion).[15]

Analysis and interpretation of data

Determining FITC-sinistrin conversion factor for GFR calculations

Experiments to establish the semi-empirical conversion factor needed to calculate GFR from the $t^{1/2}$ of FITC-sinistrin obtained by the NIC-Kidney Device have been previously described.[8,10] For rats, the semi-empirical conversion factor was determined by measuring *plasma* FITC-sinistrin clearance enzymatically in 20 healthy, awake, male Sprague Dawley (SD) rats with a weight range of 300–500 g, thus covering a wide range of adult SD BWs. GFR was calculated in these experiments based on dose of FITC-sinistrin and the area under the excretion curve (AUC), using a one-compartmental model to determine AUC. Multiplying the mean half-life of FITC-sinistrin and mean GFR generated by this experiment resulted in a conversion factor of 31.26.[8] Similar experiments to establish the semi-empirical conversion factor by measuring *plasma* FITC-sinistrin have been carried out in mice, but with a two-compartment model being used to calculate the AUC. These experiments established the semi-empirical conversion factor for mice to be 14 616.8. It should be noted that strong movement artefact is often common in mice in the recovery phase (initial 1–3 min) from anaesthesia. This makes the use of a two-compartment model for assessment of *transcutaneously* measured FITC-sinistrin difficult. Under these circumstances, a one-compartment model is thus generally used with the established 14 616.8 conversion factor.[10]

Selection of compartment models of analysis

Before calculating GFR using the NIC-Kidney Device, consideration should be given to the distribution of administered FITC-sinistrin in any particular animal model. Whether the bolus injection of FITC-sinistrin will remain predominately in the plasma and extracellular fluid before being excreted or whether it is also likely to defuse into a deeper compartment (e.g. the placenta during pregnancy) needs to be taken into consideration when calculating GFR. For detailed explanation of compartment models and equations, refer to Frennby and Sterner.[16]

VALIDATION OF NIC-KIDNEY DEVICE AND TRANSCUTANEOUS MEASUREMENT OF FITC-SINISTRIN TO DETERMINE GFR

For detailed validation experiments, refer to Schock-Kusch *et al*. and Schreiber *et al*.[8,10,17,18] Briefly, in addition to studies in SD rats, validation of the NIC-Kidney Device has been carried out in numerous strains of mice, including both healthy and disease models of renal function.[10,18] Initial studies in mice, described by Schreiber *et al*., compared GFR measured enzymatically from serial plasma collections and transcutaneous measures of FITC-sinistrin after an intravenous bolus injection of the fluorescent renal marker. These experiments were conducted in C57BL/6 mice ($n = 8$) before and 3–4 days after unilateral nephrectomy (UNX; $n = 8$), and in *pcy* mice (nephronophthisis; $n = 6$). This study concluded that measured GFR was highly comparable in each of the three experimental groups, when calculated enzymatically from plasma excretion and via the transcutaneous measurement of FITC-sinistrin using the NIC-Kidney Device. In addition, the C57BL/6 who had undergone UNX had a GFR that was 70% of GFR pre-UNX. This is in line with previously

Table 1 Published NIC-kidney device use in small rodent models of renal pathology

Species	Strain	Renal pathology	References
Mouse	C57BL/6	Healthy/UNX	Schreiber *et al*.,[10] Schock-Kusch *et al*.,[18] Shmarlouski *et al*.[15]
	pcy	Nephronophthisis	Schreiber *et al*.[10]
	NMRI	Healthy	Shmarlouski *et al*.[15]
	CD1	Healthy	Shmarlouski *et al*.[15]
	Balb/c	Healthy	Schock-Kusch *et al*.,[18] Shmarlouski *et al*.[15]
	FVB/N	$Tg^{umodwt/wt}$	Trudu *et al*.[19]
	ACE 10/10, ACE 3/3	ACE knockouts	Giani *et al*.[20]
Rat	Dahl salt sensitive	Hypertension	Cowley *et al*.[21]
	SD	Healthy/UNX	Schock-Kusch *et al*.,[8] Zöllner *et al*.,[22] Shmarlouski *et al*.[15]
	PCK	Polycystic kidney disease	Sadick *et al*.[23]
	PKD/mhm	Polycystic kidney disease	Sadick *et al*.,[23] Schock-Kusch *et al*.[17]

ACE, angiotensin-converting enzyme; SD, Sprague Dawley rat; $Tg^{umodwt/wt}$, transgenic uromodulin knockout; UNX, unilateral nephrectomy.

reported declines in GFR in UNX rat models and demonstrates the ability of the NIC-Kidney Device to detect changes in GFR in a mouse model of disease.[10] In a second study by Schock-Kusch *et al*., within-animal reproducibility of GFR measured using the NIC-Kidney Device was assessed in four different strains of mice (Balb/c, C57BL/6, SV129 and NMRI). This study found that GFR measures, recorded in the same animals within 3 days, of one another had coefficient of variances between 3.0 and 6.2%, depending on the strain of mice (n = 6–15/strain assessed).[18] This is consistent with our own studies that have demonstrated coefficient of variances of 1–6% for C57BL/6 and 4–5% for spiny mice (unpublished observations). Together, these experiments indicate that the NIC-Kidney Device can accurately calculate conscious GFR from the transcutaneous measurement of FITC-sinistrin, and highlights its usefulness in obtaining sequential, within-animal measures of GFR.

To date, numerous studies have employed the use of the NIC-Kidney Device to measure GFR (for a summary of rodent models of renal pathology that have used the NIC-Kidney Device, refer to Table 1.). In a study by Trudu *et al*. published in *Nature Medicine*, the NIC-Kidney Device was used in conjunction with extensive genetic profiling in a transgenic mouse strain to establish a genetic link between uromodulin overexpression and the development of hypertension and chronic kidney disease.[19] The NIC-Kidney Device has also been used in angiotensin-converting enzyme (ACE) knockout mice to establish that renal tissue levels of ACE necessitate angiotensin II accumulation, GFR reduction, sodium retention and hypertension in response to nitric oxide synthase inhibition.[20] In a third study published by Cowley *et al*., the NIC-Kidney Device was used to correlate GFR and mean arterial pressure during the development of salt-sensitive hypertension in Dahl salt-sensitive rats.[21] This study detected a fall in GFR using the NIC-Kidney Device within 14 days of commencing a high-salt diet. Highlighting the sensitivity of the NIC-Kidney Device, the authors of this study were unable to detect a significant reduction in GFR using creatinine clearance.[21] Indeed, studies aimed at correlating optical detection of GFR using the NIC-Kidney Device with magnetic resonance imaging (MRI) techniques have been completed to determine whether the NIC-Kidney measurement of FITC-sinistrin could be used to validate methods that calculate GFR in rodents by means of MRI.[22,23] While transcutaneous measurement of FITC-sinistrin by the NIC-Kidney Device to determine GFR in small rodents remains a relatively new technique, the wide variety of studies published to date using this technique clearly indicates the scope for which this method could be applied throughout a large range of small rodent models of renal pathology.

CONCLUSIONS

Transcutaneous measurement of GFR using the NIC-Kidney Device allows for the real-time measurement of renal function in conscious small rodents. The technique is fast, requires minimal consumables and so far has been validated in a number of different mouse and rat models where compromised renal function is suspected. The transcutaneous measurement of FITC-sinistrin using the NIC-Kidney Device may therefore be considered as a highly useful and cost-effective tool for the accurate assessment of GFR in rodents.

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REFERENCES

1. Eisner C, Faulhaber-Walter R, Wang Y *et al*. Major contribution of tubular secretion to creatinine clearance in mice. *Kidney Int.* 2009; **77**: 519–26.
2. Yuen PS, Dunn SR, Miyaji T, Yasuda H, Sharma K, Star RA. A simplified method for HPLC determination of creatinine in mouse serum. *Am. J. Physiol. Renal Physiol.* 2004; **286**: F1116–F9.
3. Dunn SR, Qi Z, Bottinger EP, Breyer MD, Sharma K. Utility of endogenous creatinine clearance as a measure of renal function in mice. *Kidney Int.* 2004; **65**: 1959–67.
4. Silkensen J, Kasiske B. Laboratory assessment of kidney disease: Clearance, urinalysis, and kidney biopsy. *Kidney* 2004; **16**: 1079–106.
5. Dickinson H, Moritz KM, Kett MM. A comparative study of renal function in male and female spiny mice, sex specific responses to a high salt challenge. *Biol. Sex. Differ* 2013; **4**: 21–8.
6. Cullen-McEwen LA, Kett MM, Dowling J, Anderson WP, Bertram JF. Nephron number, renal function, and arterial pressure in aged GDNF heterozygous mice. *Hypertension* 2003; **41**: 335–40.
7. Qi Z, Whitt I, Mehta A *et al*. Serial determination of glomerular filtration rate in conscious mice using FITC-inulin clearance. *Am. J. Physiol. Renal Physiol.* 2004; **286**: F590–96.
8. Schock-Kusch D, Sadick M, Henninger N *et al*. Transcutaneous measurement of glomerular filtration rate using FITC-sinistrin in rats. *Nephrol. Dial. Transplant.* 2009; **24**: 2997–3001.
9. Dickinson H, Moritz KM, Wintour ME *et al*. A comparative study of renal function in the desert-adapted spiny mouse and the laboratory-adapted C57BL/6 mouse: Response to dietary salt load. *Am. J. Physiol. Renal Physiol.* 2007; **293**: F1093–8.
10. Schreiber A, Shulhevich Y, Geraci S *et al*. Transcutaneous measurement of renal function in conscious mice. *Am. J. Physiol. Renal Physiol.* 2012; **303**: F783–F8.
11. Dickinson H, Walker D. Managing a colony of spiny mice (*Acomys cahirinus*) for perinatal research. *Aust. N. Z Council Care Anim. Res. Train (ANZCCART) News* 2007; **20**: 4–11.
12. Yardeni T, Eckhaus M, Morris HD, Huizing M, Hoogstraten-Miller S. Retro-orbital injections in mice. *Lab. Anim.* 2011; **40**: 155–60.
13. Murry CE, Soonpaa MH, Reinecke H *et al*. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature* 2004; **428**: 664–8.
14. Hampton AL, Hish GA, Aslam MN *et al*. Progression of ulcerative dermatitis lesions in C57BL/6Crl mice and the development of a scoring system for dermatitis lesions. *J. Am. Assoc. Lab. Anim. Sci.* 2012; **51**: 586–93.
15. Shmarlouski A, Shulhevich Y, Geraci S *et al*. Automatic artifact removal from GFR measurements. *Biomed. Signal Proc. Control* 2014; **14**: 30–41.
16. Frennby B, Sterner G. Contrast media as markers of GFR. *Eur. Radiol.* 2002; **12**: 475–84.
17. Schock-Kusch D, Xie Q, Shulhevich Y *et al*. Transcutaneous assessment of renal function in conscious rats with a device for measuring FITC-sinistrin disappearance curves. *Kidney Int.* 2011; **79**: 1254–8.
18. Schock-Kusch D, Geraci S, Ermeling E *et al*. Reliability of transcutaneous measurement of renal function in various strains of conscious mice. *PLoS One* 2013; **8**: e71519.
19. Trudu M, Janas S, Lanzani C *et al*. Common noncoding UMOD gene variants induce salt-sensitive hypertension and kidney damage by increasing uromodulin expression. *Nat. Med.* 2013; **19**: 1655–60.
20. Giani JF, Janjulia T, Kamat N *et al*. Renal angiotensin-converting enzyme is essential for the hypertension induced by nitric oxide synthesis inhibition. *J. Am. Soc. Nephrol.* 2014; **25**: 12pp. ASN. 2013091030.
21. Cowley AW, Ryan RP, Kurth T, Skelton MM, Schock-Kusch D, Gretz N. Progression of glomerular filtration rate reduction determined in conscious Dahl salt-sensitive hypertensive rats. *Hypertension* 2013; **62**: 85–90.
22. Zöllner FG, Schock-Kusch D, Bäcker S, Neudecker S, Gretz N, Schad LR. Simultaneous measurement of kidney function by dynamic contrast enhanced MRI and FITC-sinistrin clearance in rats at 3 Tesla: Initial results. *PLoS One* 2013; **8**: e79992.
23. Sadick M, Attenberger U, Kraenzlin B *et al*. Two non-invasive GFR-estimation methods in rat models of polycystic kidney disease: 3.0 Tesla dynamic contrast-enhanced MRI and optical imaging. *Nephrol. Dial. Transplant.* 2011; **26**: 3101–8.