Novel use of ultrasound to examine regional blood flow in the mouse kidney

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Sullivan JC, Wang B, Boesen EI, D’Angelo G, Pollock JS, Pollock DM. Novel use of ultrasound to examine regional blood flow in the mouse kidney. Am J Physiol Renal Physiol 297: F228–F235, 2009. First published May 6, 2009; doi:10.1152/ajprenal.00016.2009.—Conventional methods used for measuring regional renal blood flow, such as laser-Doppler flowmetry, are highly invasive, and each measurement is restricted to a discrete location. The aim of this study was to determine whether ultrasound imaging in conjunction with enhanced contrast agent (microbubbles; Vevo MicroMarker, VisualSonics) could provide a viable noninvasive alternative. This was achieved by determining changes in renal cortical and medullary rate of perfusion in response to a bolus injection of endothelin-1 (ET-1; 0.6, 1.0, or 2.0 mmol/kg) and comparing these responses to those observed in separate groups of mice with conventional laser-Doppler methods. Intravenous infusion of ET-1 in anesthetized male C57bl/6 mice resulted in a dose-dependent increase in mean arterial pressure and a dose-dependent decrease in total renal blood flow as measured by pulse-wave Doppler. ET-1 infusion resulted in a dose-dependent decrease in regional kidney perfusion as measured by both ultrasound with enhanced contrast agent and laser-Doppler measurements, verifying the use of ultrasound to measure regional kidney perfusion. Noted limitations of ultrasound imaging compared with laser-Doppler methods. In conclusion, ultrasound represents an effective and noninvasive alternative to measure regional blood flow in the mouse kidney.

IMAGING TECHNIQUES, INCLUDING magnetic resonance imaging (MRI), ultrafast X-ray computerized tomography (CT), and nuclear medicine imaging (NM), are being used increasingly both clinically and experimentally and have allowed for a number of recent advances in both physiology and pathophysiology. However, the usefulness or desirability of these techniques for assessing renal tissue perfusion in small animals is limited by characteristics such as the degree of spatial and temporal resolution afforded, or by the use of X-rays, radioisotopes, or potentially nephrotoxic contrast agents (4, 15). As such, recent advancements using high-frequency ultrasound for imaging provide exciting new opportunities for in vivo analysis of blood flow. The use of ultrasound imaging in conjunction with enhanced contrast agents provides a noninvasive means of assessing tissue perfusion in the brain (27), liver (7), heart (7), and kidneys (21, 29). Typically, ultrasound contrast agents are microbubbles designed to enhance the acoustic signal of blood in the circulation. The contrast agent used in this study is composed of gas-filled (nitrogen and perfluorobutane) microbubbles encapsulated by a lipid shell with a diameter range of 2.3–2.9 μm. Previous studies using intravital microscopy have shown that microbubbles <5 μm in diameter are small enough to allow for free movement through the bloodstream (6, 16). The microbubbles remain confined to the vasculature and slowly release their gas into the plasma. Microbubbles will be totally cleared from the blood in ~15 min, with the gas in the plasma eliminated through the lungs, and the shell components removed by the blood, liver, and kidneys (3). These characteristics make microbubbles ideal blood pool markers, allowing for the visualization and quantification of regional microvascular blood flow of the kidney.

There are a number of potential advantages associated with the use of ultrasound in conjunction with contrast agents. First, this is a noninvasive approach to image organs without disrupting tissue dynamics and physiological processes. Second, ultrasound imaging allows for the design of extended longitudinal studies with imaging at multiple time points. Third, ultrasound can measure both perfusion volume and blood flow velocity (rate of contrast entry). Fourth, tissue perfusion can be assessed in a larger region of interest than with laser-Doppler flowmetry, for example allowing for a more accurate representation of blood flow in each region.

The kidney is critical in the regulation of salt and water balance and in the long-term control of blood pressure. Therefore, alterations in renal cortical and medullary blood flow under different physiological and pathophysiological conditions have the potential to profoundly influence blood pressure (18). Laser-Doppler flowmetry is currently the most established and frequently used method to assess regional blood perfusion in the kidney. Laser-Doppler flowmeters emit light at a single wavelength into the tissue through a fiber optic source; the reflected light is collected and directed to a photo detector. There is a frequency shift in light that interacts with moving red blood cells, and the magnitude of the shift is proportional to blood velocity. This technique often requires the kidney to be exposed and for optical fibers or probes to be inserted to the desired depth into the kidney to measure regional tissue perfusion. At the end of the experiment, fiber placement is confirmed by dissecting the kidney. While this method provides for reliable measurements of tissue perfusion, it is highly invasive and difficult to maintain on a chronic basis, especially in mice. Furthermore, measurements are made only in one discrete location, ~1 mm², which cannot be manipulated once the probes are in place (25). While chronic laser-Doppler studies have been performed in rats (19, 20), this requires chronically instrumenting rodents.

The purpose of this study was to determine whether ultrasound imaging in conjunction with enhanced contrast agents could provide a viable noninvasive alternative to measure changes in regional kidney blood perfusion. To assess the
feasibility of this technique, hemodynamic responses to endothelin (ET)-1 were compared using the ultrasound method and laser-Doppler flowmetry. ET-1 is a vasoactive peptide that plays an important role in regulating vascular tone and fluid volume status. ET-1 activation of $\text{ET}_A$ receptors on vascular smooth muscle cells induces a potent vasoconstriction, and endothelial cell $\text{ET}_B$ receptors mediate vasodilation (19, 24, 26). ET-1 has been shown to influence renal blood flow. Specifically, our group and others have shown using single-fiber, laser-Doppler flowmetry in rats, that ET-1 results in a transient increase in medullary perfusion and decreases cortical perfusion (8, 22, 28).

**METHODS**

**Animals.** All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved and monitored by the Medical College of Georgia Institutional Animal Care and Use Committee. Male C57BL/6 mice (22–32 g) were used for all studies. Mice were fasted overnight before imaging to eliminate shadowing and signal dropout due to intestinal movement into the imaging plane. Animals were anesthetized with inhalational anesthetic (isoflurane, Baxter, Deerfield, IL) delivered at 1.5% concentration with air through a nonrebreathing anesthetic delivery system. In a subset of mice, the carotid artery was cannulated for measurement of mean arterial pressure (MAP) and heart rate (Powerlab data-acquisition system).

**Ultrasound imaging.** Mice were imaged in a supine position on a THM100 MousePad with integrated temperature sensor, heater, and ECG electrodes (Indus Instruments, Houston, TX). Appendages were secured to ECG pads to allow constant monitoring of heart rate and body temperature. Body temperature was maintained at 37.5°C. A 27-gauge ½-in. needle (connected to a catheter) was inserted into a tail vein for intravenous (iv) administration of a contrast agent and ET-1. Depilatory cream (Nair, Carter-Horner, Mississauga, ON, Canada) was used to remove fur from the region of interest and medical ultrasound acoustic gel (Other-Sonic, Pharmaceutical Innovations, Newark, NJ) was used as a coupling fluid between the real-time microvisualization (RMV) scanhead and the skin. Ultrasound imaging was performed using the Vevo 770 system (VisualSonics, Toronto, ON, Canada). Using B-mode imaging, the RMV-706 scanhead was positioned and held immobile using the VisualSonics Vevo Integrated Rail System II to view the mouse kidney. The RMV-706 scanhead is a 40-MHz scanhead with a 6-mm focal length and lateral and axial resolutions of 68.2 and 38.5 μm, respectively.

**Pulse-wave Doppler measurement of renal blood flow.** Renal artery blood flow was measured using pulse-wave Doppler (PW-mode). Following a brief stabilization period, a baseline recording of pulse-wave Doppler blood flow was obtained in the renal artery before it enters the kidney to measure total renal blood flow (see Fig. 5A). After several baseline renal artery blood flow measurements, 50 μl of ET-1 (final concentration 0.6, 1.0, or 2.0 nmol/kg; American Peptide, Sunnyvale, CA) was injected into the tail vein at a rate of 800 μl/min for 8 s (total injected volume of 106 μl). Pulse-wave Doppler blood flow was continuously monitored for 20 min following ET-1 or saline injection.

**Ultrasound contrast imaging of the kidney.** In additional mice, experiments were performed using Vevo MicroMarker (VisualSonics) ultrasound contrast agent, which consists of a gas-filled (nitrogen and perfluorobutane) microbubble (2.3- to 2.9-μm diameter) that is injected (iv). Before use, the contrast agent was reconstituted using 0.7 ml sterile saline. The contrast agent was gently agitated by hand for 15 s and then allowed to equilibrate for 10 min. The reconstituted microbubbles have a mean concentration of $2 \times 10^9$ microbubbles/ml. The contrast agent was diluted with saline (1:5) and injected as a bolus via the tail vein catheter at a rate of 800 μl/min for 8 s (total injected dose of $1.5 \times 10^7$ μl microbubbles, total injected volume of 106 μl) using a syringe pump (Harvard Apparatus, Holliston, MA). Using contrast-mode imaging, the RMV-706 scanhead was used to view the mouse kidney, and kidney perfusion was determined using the appropriate VisualSonics software package. Contrast-mode imaging offers image acquisition and analysis tools to detect and quantify vascular structures and dynamics using an enhanced contrast agent. A contrast-
mode image is constructed by comparing data acquired using a contrast agent with referenced background data. Three doses of ET-1 (0.6, 1.0, 2.0 nmol/kg) were studied to determine whether there is a dose-dependent effect of ET-1 infusion on kidney tissue perfusion. Saline-treated mice served as the control. After a 30-min stabilization period, the baseline rate of tissue perfusion was quantified by analyzing contrast agent intensity during a 50-s, 800-frame image recording, referred to as a cine loop, with the contrast agent injected into the middle of the cine loop. Frames are collected at a rate of 14 frames/s. Frames before contrast agent injection indicate background noise in the kidney and serve as a reference cine loop. Figure 1, top left, is a representative example of a reference image. A contrast-mode image is then digitally constructed by anatomically comparing data acquired in the presence of the contrast agent with the reference cine loop for that image. This image can be rendered in either gray-scale (Fig. 1, top right) or with the gray-scale image subtracted showing microbubbles alone in green-scale (Fig. 1, bottom left and bottom right). These rendered images indicate the difference between the acquired image with the contrast agent and the reference image. The mice were then allowed a 20-min reequilibration period during which the time-contrast agent in the circulation degrades. Saline or ET-1 (final concentration: 0.6, 1.0, or 2.0 nmol/kg in 50 μl) was infused into the tail vein at a rate of 800 μl/min for 8 s. After 20 min, kidney perfusion was again quantified as described above. To assess the reproducibility of the experimental protocol, regional tissue perfusion was measured in the same five mice on 4 different days over a 2-wk period: days 1, 5, 9, 13 (Fig. 2). This timeline allowed sufficient time for the tail vein to recover from the infusion of ET-1 (2 nmol/kg) and microbubbles.

Image analysis. Pulse-wave Doppler images and cine loops of kidney perfusion curves were digitally stored and transferred to a personal PC for off-line analysis. To reduce variability, imaging parameters were held constant throughout each experiment, with focus and depth optimized at the beginning of the study for each animal. All studies were done using approximately the same scan plane as determined by anatomic markers. Analysis begins with the construction of a contrast agent time intensity curve generated in a user-defined region of interest in the kidney cortex or medulla. A similar-sized region of interest was selected for each mouse. A representative curve is shown in Fig. 3A and depicts the influx of microbubbles into the kidney following contrast agent injection. Because of the unique organization of the renal microcirculation (1 inlet, the renal artery, and 1 outlet, the renal vein, with a large number of smaller arteries and capillaries), a rising exponential function of signal changes for the influx of microbubbles during the “wash-in curve” was applied (14). An alternative explanation for why the rising exponential function describes the signal changes would be that microbubbles get trapped in the microvasculature. However, this is unlikely since intravital microscopy studies have confirmed the absence of bubble trapping in the microcirculation even if transient trapping can be observed for a small percentage of bubbles (6, 16). The intensity of the contrast agent in each region of interest was defined as the difference between the signal intensity after contrast agent injection and the mean reference value before injection. Data are expressed as intensity per square millimeter and represent the volume of intravascular space. To determine blood velocity, perfusion curves were fitted by the appropriate software to a rising exponential function where contrast intensity = B + A[1 – exp(–βt)], where B is the reference intensity, A is the plateau signal intensity, the exponential rate parameter β is the velocity of the microbubbles entering the imaging plane, and t is a constant for each scanhead. The rate of tissue perfusion was calculated by (A – B) * β.

Laser-Doppler measurements of red blood cell flux. We used identical methods as we have previously published using rats (22, 28, 31). In separate animals, mice were anesthetized with isoflurane, placed on a servo-controlled heating table, and the jugular vein was cannulated for constant infusion of 0.9% NaCl at 6 μl/min throughout the experiment to replace fluid losses. A retroperitoneal incision was made, and the left kidney was placed in a lucte cup to avoid movement artifacts in laser-Doppler measurements (BLF 21D laser-Doppler flowmeter, Transonic Systems, Ithaca, NY). Cortical red blood cell flux was measured using a 24-G needle-type laser-Doppler probe inserted into the proximal pole of the kidney to a depth of 1 mm with the aid of a micromanipulator. Medullary red blood cell flux was measured using a single-fiber laser-Doppler type M probe inserted perpendicularly to the long axis of the kidney to a depth of 3 mm below the lateral surface and secured to the renal capsule using
cycloacrylate adhesive. Following a 10-min stabilization period and a
1-min baseline period, 50 μl of 0.9% NaCl or ET-1 (0.6, 1.0, or 2.0
nmol/kg) was administered via the jugular vein catheter at a rate of
800 μl/min for 8 s (total injected volume of 106 μl), and the response
was recorded. The experimental protocol was identical to that for the
ultrasound studies. The mice were then euthanized by an overdose of
anesthetic, and laser-Doppler measurements were recorded for several
more minutes after the animals were euthanized. The kidney was then
 dissected to ensure appropriate positioning of the laser-Doppler probes.

Statistical analysis. Statistical analysis was performed using
GraphPad Prism version 4.02 for Windows (GraphPad Software, San
Diego, CA). Data were compared using an ANOVA followed by a
Tukey post hoc test. Data are expressed as means ± SE. A P value
<0.05 is considered statistically significant.

RESULTS

Ultrasound imaging with enhanced contrast agent in the
mouse kidney. The VisualSonics ultrasound instrument al-
lowed for detailed imaging of the mouse kidney as indicated by
the representative image in Fig. 1, top left. The same kidney is
also shown in contrast mode in gray-scale (Fig. 1, top right)
and green-scale (Fig. 1, bottom left and bottom right). Contrast-
mode imaging was used to assess renal blood perfusion using
an enhanced contrast agent, and the green color in (top right
and bottom left and right) indicates the presence of an en-
hanced contrast agent.

**MAP and heart rate with ET-1 infusion.** Before assessing the
effects of ET-1 infusion on regional kidney perfusion, we began by verifying the dose-response relationship between increasing doses to ET-1 with blood pressure and heart rate in anesthetized mice. Measurements were taken 20 min following the infusion of saline or ET-1 and reported as percent change from steady-state baseline values before infusion. As expected, ET-1 significantly increased MAP at all doses compared with saline infusion, and the increase in pressure was dose depen-
dent (absolute MAP in mmHg: saline, 79 ± 3; 0.6 nmol/kg ET, 92 ± 2; 1.0 nmol/kg ET, 98 ± 2; 2.0 nmol/kg ET-1, 105 ± 3;
P < 0.05). The percent change in MAP from baseline is shown in Fig. 3A. Similarly, heart rate displayed a dose-dependent
increase with increasing doses of ET (beats/min: saline, 584 ±
8; 0.6 nmol/kg ET, 568 ± 10; 1.0 nmol/kg ET, 558 ± 23; 2.0
nmol/kg ET, 509 ± 21). The percent change in heart rate from
baseline is shown in Fig. 3B.

ET-1 decreases renal blood flow. Ultrasound imaging was
used to locate the renal artery by following the aorta, and blood
flow velocity was measured by pulse-wave Doppler to assess
the effect of increasing doses of ET-1 infusion on total renal
blood flow (Fig. 4). All doses of ET-1 resulted in a large and
rapid fall in total renal blood flow; however, at 0.6 nmol/kg
ET-1 renal blood flow was returned to normal by 20 min
postinfusion. In contrast, 1 nmol/kg ET-1 resulted in a signif-
icant attenuation of renal blood flow at 20 min and at the
highest dose of ET-1 utilized, total renal blood flow remained
significantly attenuated even after 20 min.

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Measurements of regional tissue perfusion using ultrasound. In the renal cortex, blood velocity and the rate of tissue perfusion decrease in a dose-dependent manner with increasing doses of ET-1 (Fig. 5). In the renal medulla, increasing doses of ET-1 resulted in a decrease in blood volume, blood velocity, and the rate of tissue perfusion. In both the cortex and medulla, changes in renal hemodynamic properties reached significance at 2 nmol/kg ET-1 (Fig. 6).

To assess the experimental variability and reproducibility of the protocol, regional tissue perfusion was measured using an enhanced contrast agent in the same five mice on 4 different days over a 2-wk period (Fig. 7). Day-to-day variability within the same mouse was minimal, supporting the use of ultrasound in longitudinal studies. Intermouse variability was slightly higher, although still within an acceptable range. There were no significant differences with
either day-to-day or mouse-to-mouse variability in either the renal cortex or medulla.

Measurements of regional red blood cell flux using laser-Doppler. To assess the viability of using ultrasound to measure regional tissue perfusion in the kidney, the same experimental protocol was performed using the conventional method of laser-Doppler flowmetry (Fig. 8). Similar to what was observed using ultrasound with enhanced contrast agent, infusion of ET-1 resulted in a dose-dependent decrease in renal cortical and medullary tissue perfusion, as assessed by laser-Doppler measurement of red blood cell flux. In both the cortex and medulla, changes in renal perfusion reached significance at 1 nmol/kg ET-1. Although changes in regional perfusion tended to be greater when measured by ultrasound compared with laser-Doppler, these data suggest that ultrasound imaging in conjunction with an enhanced contrast agent serves as a noninvasive alternative to laser-Doppler to assess regional changes in tissue perfusion.

DISCUSSION

Recent advances in ultrasound imaging technology have provided powerful new tools for in vivo tissue visualization in rodents. Real-time high-resolution imaging in combination with the ability to collect functional information on tissue perfusion using an enhanced contrast agent and blood flow via pulse-wave Doppler has the potential to significantly affect the investigation of physiology and disease progression. While it has previously been shown that ultrasound imaging can be used to detect gross changes in rat kidney morphology (29), tumor progression in mice (10), and the characterization of polycystic kidney disease in mice (23), this is the first study to assess regional blood flow in the mouse kidney. The primary finding of this study was that ultrasound imaging in conjunction with an enhanced contrast agent yielded results similar to standard laser-Doppler flowmetry, supporting the validity of this technique. Both methods found that a bolus injection of ET-1 results in a dose-dependent decrease in blood perfusion in the renal cortex and medulla.

Noninvasive imaging techniques such as MRI, ultrafast X-ray CT, NM, and ultrasound imaging have been making strides to improve their capability to measure tissue perfusion. All these technologies measure time-intensity curves for the passage of an intravascular contrast medium injected in the circulation. However, in terms of measuring regional tissue perfusion, ultrasound has some distinct advantages compared with these other approaches. While MRI can efficiently measure blood volume and microvascular density, it is very challenging to accurately quantify arterial input and measure tissue perfusion (4, 15). Moreover, the currently approved agents for MRI are freely diffusible. Therefore, vascular volume measurements and tissue perfusion assessments using MRI are less accurate than those derived from a contrast agent in conjunction with ultrasound imaging, where the contrast agent is strictly confined in the vasculature. CT can be used to estimate tissue perfusion; however, the iodinated contrast media used in CT is freely diffusible in nature and so suffers from the same limitation as MRI. Moreover, lack of ionizing radiation and relatively high resolution in ultrasound compared with CT and NM make ultrasound an attractive alternative (2).

To date, laser-Doppler flowmetry has been the method of choice to assess regional tissue perfusion. However, the use of ultrasound imaging in conjunction with an enhanced contrast agent has a number of advantages compared with laser-Doppler flowmetry. First and foremost, ultrasound imaging is a
noninvasive procedure. The kidney can be imaged and blood perfusion assessed without any physical disturbance to the kidney. To assess red blood cell flux and blood perfusion using laser-Doppler, it is necessary to surgically expose and immobilize the kidney. Under these conditions, the kidney is damaged by the insertion of the fibers, and extra precautions must be made to ensure that the temperature of the kidney is maintained. We found that the changes in tissue perfusion tended to be greater when measured by ultrasound compared with laser-Doppler. We hypothesize that the surgical exteriorization of the kidney for the implantation of the laser-Doppler fibers results in a depression of blood flow and thus relative responsiveness. Second, by imaging the kidney to determine the region of interest for the study, the user can use anatomic markers, allowing for a higher degree of certainty that a similar region of the kidney is being examined in each study. In contrast, with laser-Doppler flowmetry, fiber placement cannot be confirmed until the end of the study. The ability to reposition the recording target region without damaging the kidney is an advantage of ultrasound over laser-Doppler. Third, ultrasound imaging allows for extended longitudinal studies without chronic instrumentation where the same animal can be followed over time to assess how either aging or the progression of a disease regulates tissue anatomy and blood perfusion.

Despite these advantages, there were certain limitations that must be noted with the use of ultrasound to assess regional kidney blood perfusion. One limitation is the lower sensitivity in assessing regional renal blood perfusion compared with the more conventional laser-Doppler approach. The initial intent of this study was to quantitate renal cortical, outer medullary, and inner medullary hemodynamic properties following the infusion of ET-1 as a means of validating ultrasound as a method for determining these variables in vivo. However, we found the degree of variability was very large when the user-defined region of interest was small. Attempts were made to assess more specific regions of the medulla, that is, the inner medulla, but as one might expect, the contrast signal was quite low and resulted in a considerable degree of variability such that reliable data were unobtainable. In addition, we did not have the means to verify changes in inner medullary blood perfusion using standard techniques. Even when large regions of interest were studied by ultrasound, we found a large degree of animal-to-animal variation associated with the quantification of contrast agent intensity. Therefore, our experience is that the user must be very careful and consistent when defining the region of interest. As a result, laser-Doppler flowmetry appears to be more sensitive (lower SD) to changes in tissue perfusion following ET-1 compared with ultrasound. An additional limitation associated with ultrasound is its ability to report the time course of the response. We attempted to assess the effects of ET-1 on renal hemodynamic responses at 2 and 20 min post-ET-1 infusion. However, due to the robust hemodynamic response to ET-1 infusion, we were not able to detect reliable changes in blood perfusion at 2 min. This is illustrated in Fig. 2, which shows a typical time-intensity curve under baseline conditions (A) and at 2 min post ET-1 infusion (B). At 2 min following the infusion of ET-1, there is not an apparent influx of microbubbles, making quantification of tissue perfusion impossible at this time point. We suspect this is related to the potent vasoconstrictor properties of ET-1 and the robust decrease in blood flow immediately following infusion. Moreover, contrast-mode imaging relies on an anatomic alignment of data acquired during contrast agent influx with a referenced set of background data. Therefore, if the anatomy shifts due to the mouse moving, changes in respiration, or large hemodynamic changes during the injection of contrast agent, a time-intensity curve cannot reliably be generated. Currently, ultrasound is not the best option to measure transient changes in tissue perfusion, and its utility is limited to fairly steady-state conditions. For applications requiring quantification of rapid or transient changes in tissue perfusion or continuous data acquisition, laser-Doppler flowmetry has a clear advantage over ultrasound imaging in conjunction with an enhanced contrast agent.

For this study, regional tissue perfusion was assessed using ultrasound by calculating the influx of an enhanced contrast agent into the tissue following a bolus injection. An alternative approach to measure perfusion would be to allow the contrast agent to come to equilibrium, and then use a destruction frequency to disrupt the contrast agent in the region of interest and measure the influx of agent back into the tissue. While this method has been shown to be effective in other experimental protocols (17, 30), we were unable to effectively remove the contrast agent in the kidney even with multiple destruction sequences. We hypothesize that this is related to the size of our region of interest and the relatively high degree of perfusion in kidney tissue. While this approach may work with a more-focused region of interest, the renal cortex and medulla may be too large in terms of physical size and volume of blood to...
effectively remove enough of the contrast agent to visualize fresh contrast agent entering the tissue. Interestingly, while the use of the destruction sequence may not be effective in the determination of regional tissue perfusion, ultrasound in conjunction with specialized contrast agent destruction has been shown to be an efficient means of gene transfer in the kidney (12, 13).

Using a similar bolus iv injection of ET-1 in the rat, Gurbano et al. (8) observed a prolonged decrease in renal cortical blood perfusion with a transient increase in medullary blood perfusion lasting only a few minutes. Furthermore, we have previously published that in response to a slow iv infusion in rats on a normal-salt diet, ET-1 produces a significant decrease in renal cortical blood perfusion but not medullary blood perfusion, as measured by laser-Doppler (28). Therefore, we reasoned that an iv bolus infusion of ET-1 would be an ideal way of validating the ultrasound technique due to potential differential effects on regional blood flow in the kidney. While our studies suggest that ultrasound would not have been able to detect a transient change in medullary blood flow even if present, studies with laser-Doppler flowmetry did not detect an increase in medullary blood flow even with continuous measurement (See Supplementary Figure; all supplementary material for this article is accessible on the journal web site). While it is possible that our dose range and time frame may not have allowed us to observe a transient renal medullary vaso-dilation, additional studies are needed to fully examine a potential species difference in regional renal responses to ET-1.

In conclusion, the use of ultrasound imaging is of growing interest as new technologies become available for in vivo analysis of blood flow. Despite some limitations including a lower degree of sensitivity to changes in tissue perfusion and the inability to assess rapid or transient changes in tissue perfusion, we found that the ultrasound method can be used successfully to examine regional tissue perfusion in the kidney. This application will be especially useful for extended longitudinal studies in small rodents. As this method does not involve a surgical procedure, ultrasound offers a rapid, noninvasive way to obtain hemodynamic measurements with little risk to the experimental subject.

GRANTS
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REFERENCES