Supplemental Methods

Animal Experimental Protocol: Thirteen pigs were anaesthetized with intramuscular Telazol® (tiletamine / zolazepam, 6 mg/kg) and xylazine (2.2 mg/kg) and then intubated. Isoflurane was subsequently administered with forced ventilation to maintain anaesthesia throughout the procedure. The pigs weighed 35-50 kg, were between 3 to 4 months old and either male or female. The lateral ear vein was catheterized for IV fluid administration (0.9% NaCl, 5-10 ml/kg/hr) and both eyes treated with a lubricant for protection. The animal was placed in dorsal recumbency on a surgical table upon a water-filled heating pad. The skin over the medial thigh from the groin to the hock and the entire ventral abdomen was shaved with electric clippers and then treated with a depilatory cream to remove hair. The animal was instrumented for vital sign measurements (SurgiVet® Advisor®; Smiths Medical Inc, Waukesha WI,), i.e. temperature, respiration, SpO_2 levels, non-invasive BP, ECG, and heart rate. The area over the surgical sites was aseptically cleaned and the right kidney exposed using a mid-line or transverse abdominal incision. Invasive procedures were performed by KI and GO (10+ years' experience). Animal handling, microsphere injection, blood reference withdrawal, sample collection and scanning were performed by SZP (8 years' experience), ODK, JMR and JBF (10+ years' experience).

The right femoral artery was catheterized under 2D ultrasound guidance (Philips Sonos 750) insonated using a 15-6L (6-15Mhz) linear array transducer (Phillips Medical Systems, Bothwell, WA). The procedure was performed using a 5Fr gauge catheter (Arrow Percutaneous Sheath Introducer Kit 6Fr sheath (Teleflex Inc, Morrisville, NC) with Cook 5F cardiac straight catheter (Cook Medical, Bloomington)) directed into the left cardiac ventricle under fluoroscopic guidance using a mobile C-arm X-Ray system (Philips BV Endura; Philips, Cambridge, Massachusetts) with positioning confirmed via a contrast injection (Conray 60, Iothalamate Meglumine (0.5-3.0 ml/kg), Liebel-Flarsheim, Cincinnati, OH). The left femoral artery was catheterized for serial blood sampling and invasive BP measurements.

The right renal artery was instrumented with a 4 mm diameter vessel occluder (DOCXS VO-4 Ukiah, CA) and the corresponding vein with an appropriately sized ultrasonic flow sensor connected to a flowmeter (Transonic TS420; Ithaca, NY) previously used as a reference standard for volumetric flow (24, 33). A running average of the volumetric flow rate (ml/min) was displayed on the flowmeter and the continuous volume flow waveform from the flowmeter was recorded using an oscilloscope (Teledyne LeCroy HDO4032; Thousand Oaks, CA) for off-line analysis. Occlusion levels were monitored using a differential pressure manometer (406800; Extech Instruments, MA) to ensure consistent pressure application during each flow state. At the conclusion of the study the animal was euthanized without recovery with barbiturate overdose (sodium pentobarbital, 140-160 mg/kg IV) followed by a bilateral pneumothorax.

At conclusion of each study, both kidneys were harvested for tissue analysis providing 12 samples of the right kidney: 6 samples of both cortex and medulla (top, middle, bottom of lateral and dorsal aspects). Further samples of the contra-lateral un-occluded kidney were acquired (top, bottom of the cortex and medulla). Microsphere perfusion was calculated for each tissue sample using the following equation: $Q_k = (C_k \times Q_r)/C_r$, where Q_k = kidney tissue sample perfusion (mL/min/100g), C_k = microsphere count per 100g for the kidney tissue sample, Q_r = withdrawal rate of a reference blood sample (ml/min), and C_r = microsphere count in reference blood sample.

Supplementary Figure 1

Photos show ultrasound machine setup (A); probe rig and positioning on animal in-situ (B); volumetric blood-flow recording setup, cannula for blood withdrawal and reference blood withdrawal pump (C)

Supplementary Figure 2

Regression using generalized estimating equation (blue) with 95% confidence limits (dotted line) of 3D FMBV using Philips against FMS measured perfusion (A) and volumetric blood flow for absolute values (B). Coefficient of determination (r^2) provided by simple linear regression. 3D three dimensional; FMBV fractional moving blood volume; FMS fluorescent microspheres.

Supplementary Figure 3

Regression using generalized estimating equation (blue) with 95% confidence limits (dotted line) between volumetric flow and FMS (A); 3D-FMBV values acquired by GE and Philips systems (B). Coefficient of determination (r^2) provided by simple linear regression. 3D three dimensional; FMBV fractional moving blood volume; FMS fluorescent microspheres.

Supplementary Table 1 – Perfusion Estimation by fluorescent microspheres (ml/min/100g) from target (right) and contralateral kidney (left) for each pig. 6 samples were acquired for the right cortex and medulla, respectively and 2 for the contralateral. Mean perfusion is displayed with standard deviation in brackets. (* - indicated withdrawal error (Pig 3) and epinephrine administration invalidating measure (Pig 8))

				Flow Condition		
Pig	**Side**	**Kidney Portion**	**Baseline**	**75.00%**	**50.00%**	**25.00%**
3	Right	Cortex	2.16 (0.11)	1.78 (0.09)	*	1.42 (0.07)
		Medulla	0.92 (0.08)	0.80 (0.04)	*	0.65 (0.05)
	Left	Cortex	2.43 (0.00)	2.31 (0.12)	*	2.52 (0.22)
		Medulla	0.98 (0.21)	1.03 (0.18)	*	1.23 (0.28)
6	Right	Cortex	3.07 (0.32)	1.98 (0.90)	1.67 (1.30)	0.93 (0.56)
		Medulla	0.77 (0.15)	0.62 (0.37)	0.71 (0.44)	0.37 (0.14)
	Left	Cortex	2.87 (0.05)	3.07 (0.21)	3.33 (0.19)	4.27 (0.39)
		Medulla	0.41 (0.01)	0.38 (0.03)	0.66 (0.14)	0.59 (0.08)
7	Right	Cortex	3.07 (0.17)	2.57 (0.47)	1.22 (0.22)	0.70 (0.05)
		Medulla	0.87 (0.26)	0.83 (0.53)	1.34 (0.57)	0.26 (0.07)
	Left	Cortex	3.20 (0.00)	2.62 (0.30)	1.45 (0.00)	1.90 (0.00)
		Medulla	0.99 (0.21)	0.98 (0.32)	1.04 (0.29)	0.67 (0.13)
8	Right	Cortex	1.72 (0.17)	1.21 (0.10)	*	*
		Medulla	0.39 (0.10)	0.29 (0.06)	*	*
	Left	Cortex	1.68 (0.43)	1.54 (0.34)	*	*
		Medulla	0.46 (0.06)	0.47 (0.09)	*	*
9	Right	Cortex	3.16 (0.22)	2.28 (0.57)	1.59 (0.90)	0.66 (0.60)
		Medulla	0.71 (0.10)	0.38 (0.13)	0.36 (0.09)	0.10 (0.05)
	Left	Cortex	3.10 (0.07)	2.57 (0.01)	2.57 (0.00)	2.72 (0.05)
		Medulla	0.70 (0.08)	0.72 (0.00)	0.60 (0.06)	0.64 (0.09)
10	Right	Cortex	2.87 (0.64)	1.79 (0.98)	1.27 (0.68)	0.58 (0.32)
		Medulla	0.67 (0.25)	0.38 (0.12)	0.40 (0.08)	0.21 (0.04)
	Left	Cortex	3.35 (0.21)	2.98 (0.16)	2.40 (0.14)	2.31 (0.12)
		Medulla	0.80 (0.15)	0.57 (0.13)	0.48 (0.10)	0.48 (0.08)
11	Right	Cortex	2.65 (0.07)	1.43 (0.08)	1.12 (0.13)	0.35 (0.08)
		Medulla	0.65 (0.13)	0.30 (0.02)	0.41 (0.07)	0.24 (0.11)
	Left	Cortex	2.33 (0.01)	2.21 (0.04)	2.23 (0.00)	2.40 (0.08)
		Medulla	0.56 (0.13)	0.51 (0.10)	0.54 (0.10)	0.51 (0.09)
12	Right	Cortex	1.58 (0.13)	1.44 (0.21)	0.82 (0.10)	0.32 (0.04)
		Medulla	0.50 (0.13)	0.40 (0.10)	0.28 (0.05)	0.12 (0.03)
	Left	Cortex	1.68 (0.06)	2.93 (0.26)	2.30 (0.19)	1.90 (0.12)
		Medulla	0.70 (0.16)	1.06 (0.24)	0.85 (0.21)	0.70 (0.16)
13	Right	Cortex	2.41 (0.11)	1.41 (0.50)	0.72 (0.50)	0.33 (0.29)
		Medulla	0.79 (0.18)	0.39 (0.18)	0.21 (0.10)	0.13 (0.07)
	Left	Cortex	2.47 (0.06)	2.19 (0.16)	2.05 (0.13)	1.92 (0.13)
		Medulla	0.79 (0.02)	0.63 (0.03)	0.54 (0.07)	0.49 (0.03)