Metabolic MRI with hyperpolarized [1-^{13}C]pyruvate separates benign oligemia from infarcting penumbra in porcine stroke

Supplementary Materials and Methods

Animals and ET-1 injection

The pigs were obtained from a conventional farmer and acclimatized for 14 days before inclusion in the study. Before transport (15 min) to the experimental facilities, the pigs were fed as normal and sedated with 2.5 mg/kg intramuscular tiletamine and zolazepam. Upon arrival, an ear vein catheter was placed and the pigs were anaesthetized with propofol (3 mg/kg followed by 4-8 mg/kg/h IV) and fentanyl (0.01 mg/kg followed by 0.035 mg/kg/h IV), intubated and ventilated. Catheters (8F) were placed in the femoral vein, the femoral artery and the internal jugular vein using ultrasound guided Seldinger technique. Throughout the experiment, blood pressure, heart rate and end-tidal CO_2 was monitored. Arterial blood was sampled every hour and analyzed for gasses, glucose and lactate.

After preparations, the pigs were placed in prone position. The burr hole was drilled using a manual drill, exposing the dura over the central sulcus. Using a 30-gauge needle and a microinjector pump, the ET-1 dissolved in saline was injected over 10 minutes. Then, the pigs underwent MRI as described below. After the experimental protocol was complete, the pigs were euthanized with an IV overdose of pentobarbital.

MRI protocol

In both pigs and volunteers, the proton and carbon images were obtained with separate coils. In the volunteers, proton images were obtained with a commercial 32-channel head coil (NOVA Medical, USA). The proton images of the pigs were obtained with a flexible 16-channel coil (GE Healthcare) placed above the skull of the pig. For the ^{13}C hyperpolarized MRI, we used a single-tuned quadrature birdcage coil as a transceiver for healthy volunteers and a single-tuned custom-made 14-channal phased-array receive coil combined with a commercial volume transmit coil of clamshell design for the preclinical experiments (RAPID Biomedical, Germany). The custom coil was designed as a single ring of elements (80 mm diameter) fixed to a rigid cylinder (250 mm diameter). For further details, please refer to coil #6 in Sánchez-Heredia et al[1]

The proton protocol used the following imaging parameters. Pseudo-Continuous Arterial Spin Labeling was a 3D sequence, with 3.6×3.6×3.6 mm^3 resolution and 2025 ms label-delay. Diffusion weighted imaging was performed using an echo-planner imaging sequence with b = 0 and 1000 and 1.9×1.9×5 mm^3 resolution. Spectroscopy was performed as 2×2×2 cm^3 single-voxel PROBE with 8 averages and TR/TE = 2500/135 ms. T1 maps were obtained with inversion recovery prepped scans (7 TIs, 100 to 1500 ms). T2 and T2* maps were acquired using fast spin echo and gradient echo sequences with multiple echo times (12 TEs from 5 to 80 ms and 16 TEs from 2 to 50 ms, respectively). DSC (gradient echo echo-planner imaging, 1.9×1.9×4 mm^3, flip angle = 30°, TR = 800 ms) was performed during gadolinium injection (10 ml Dotarem flushed with 20 ml saline, ~5 ml/s, GE Healthcare). In addition, ^{23}Na images were acquired with a radial readout 3D sequence (10×10×10 mm^3, TR = 6 ms, flip angle = 20°) using a 20 cm diameter Helmholtz loop-coil (PulseTeq Limited, UK).

For hyperpolarized MRI, hyperpolarized [1-^{13}C]pyruvate was injected IV (in the jugular vein for pigs and in the antecubital vein for volunteers) and flushed with 20 ml saline. Then, imaging was started using constant flip angle, single-band spectral-spatial excitation of the pyruvate, lactate and bicarbonate resonances (Δf = 0, 392 and 322). For the preclinical studies, we used a fully-sampled sequence with a stack-of-spirals readout for encoding. It covered a volume of 30×30×6 cm^3 (TR = 120 ms for 4 excitations per full volume). Reconstructed resolution was 1×1×1.5 cm^3. For the human studies, we used fully-sampled, multi-slice 2D imaging with an echo planar imaging readout.[2] In both preclinical and human exams, transmit gain and center frequency was calibrated using a ^{13}C phantom placed with the subject in the coil (urea for volunteers, bicarbonate for pigs). A spectrum was obtained after imaging to ensure correct prescription of the center frequency. An anatomical reference proton image was obtained with the built-in body coil of the scanner.

Hyperpolarization

Dynamic nuclear polarization of [1-^{13}C]pyruvic acid (Sigma-Aldrich) was performed with AH111501 (15 mM) as the radical. The SPINLab operates at 5T and 0.8K, while the SpinAligner operates at 6.7T and 1.3K. Dissolution was performed with superheated water and the product was buffered with NaOH in water. For the human studies, the radical was filtered out through column filtration, and the hyperpolarized pyruvate injection was passed through a 0.2 µm sterilization filter. Further, the solution underwent automated quality control of pH, temperature, pyruvate and radical concentration, volume and polarization before injection. The quality control was performed by the SPINLab quality control module.

Image reconstruction and postprocessing

Apparent diffusion coefficient (ADC), CBF, T2 and R2* maps were calculated on the scanner. T1 maps were fitted in Fiji.[3] Spectroscopy was processed in LCModel.[4] Perfusion-weighted imaging was analyzed using the DSC MRI Toolbox for Matlab available on Github.

Raw signals from the human and preclinical hyperpolarized MRI data were reconstructed using either the Orchestra or Fidall Matlab toolboxes (GE Healthcare), respectively. The preclinical data underwent non-Cartesian Fourier transform, a Gaussian filter (15 Hz line broadening) and zerofilling.[5] Pyruvate reference sensitivity maps were used for optimal coil combination.[6,7] The first order apparent rate constant of exchange from pyruvate to lactate (k_{PL}) was modeled using the Hyperpolarized MRI Matlab toolbox from University of California, San Francisco available on Github. Only the forward reaction from pyruvate to lactate was considered, and the starting value for k_{PL} was set to 0.02 s^{-1}. The global relaxation rates of pyruvate and lactate were fixed at 1/30 s^{-1} and 1/25 s^{-1}, respectively.[8]

Biochemical analyses

A number of biological samples were obtained from the pigs. Lactate, glucose and blood gasses in blood from the femoral artery and jugular vein were analyzed using a blood gas analyzer (ABL90 FLEX PLUS, Radiometer Copenhagen, Denmark). This was used for determining a global arterial input function and the arterial to venous differences of metabolites and gasses. Biopsies obtained at sacrifice was frozen in liquid nitrogen and stored at -80° Celsius until analyses. The following assays were used for analyses: Sigma references MAK064, MAK183 and MAK066.

References

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Supplementary Figures and Tables

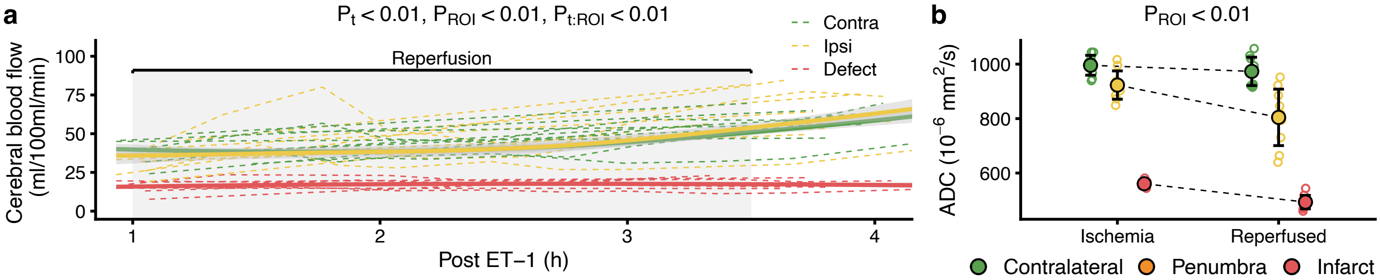

Figure S1: Changes over time in cerebral blood flow (CBF) and apparent diffusion coefficient after endothelin 1 (ET-1) injection. Quantified with arterial spin labeling, CBF increased with time after reperfusion, except in the remaining, shrinking perfusion defect (a). ADC values across regions of interest decreased throughout the experiment (b).

Data are shown as individual observations (n = 10) with mean ± SD. In, a locally weighted smoothing function was added for visualization (thick line). Tested with linear mixed-effect models. ROI is region of interest, t is time, t:ROI denotes their interaction.

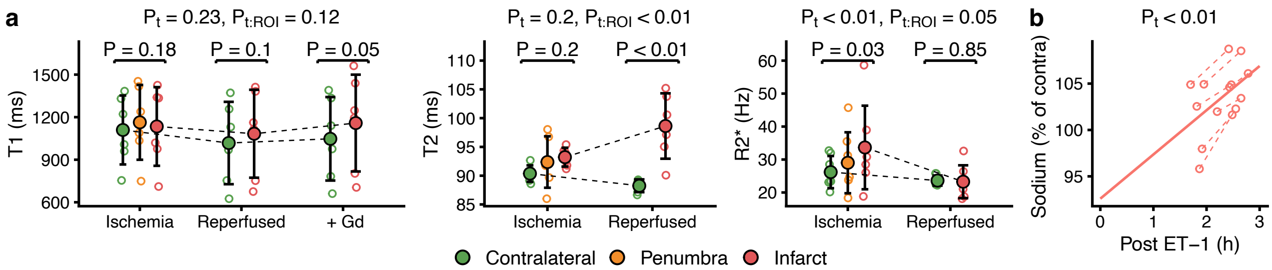

Figure S2: Characterization of ischemic stroke in pigs using parametric proton and sodium MRI. Assessed with parametric ^{1}H MRI (a), T1 relaxation time did not change with ischemia, reperfusion or gadolinium (Gd). T2 was increased in the infarct after reperfusion. R2* was increased in ischemic regions. The ^{23}Na MRI signal from the infarct normalized to contralateral increased 0.09 % points per minute (95% CI, 0.06 to 0.1) after stroke (b). The intercept with time = 0 was 90.59 %.

Data (n= 6-7) are shown as individual observations with mean ± SD (a) or a linear model-fit (b). Tested with linear mixed-effect models (a). ROI is region of interest, t is time, the colon (:) marks an interaction.

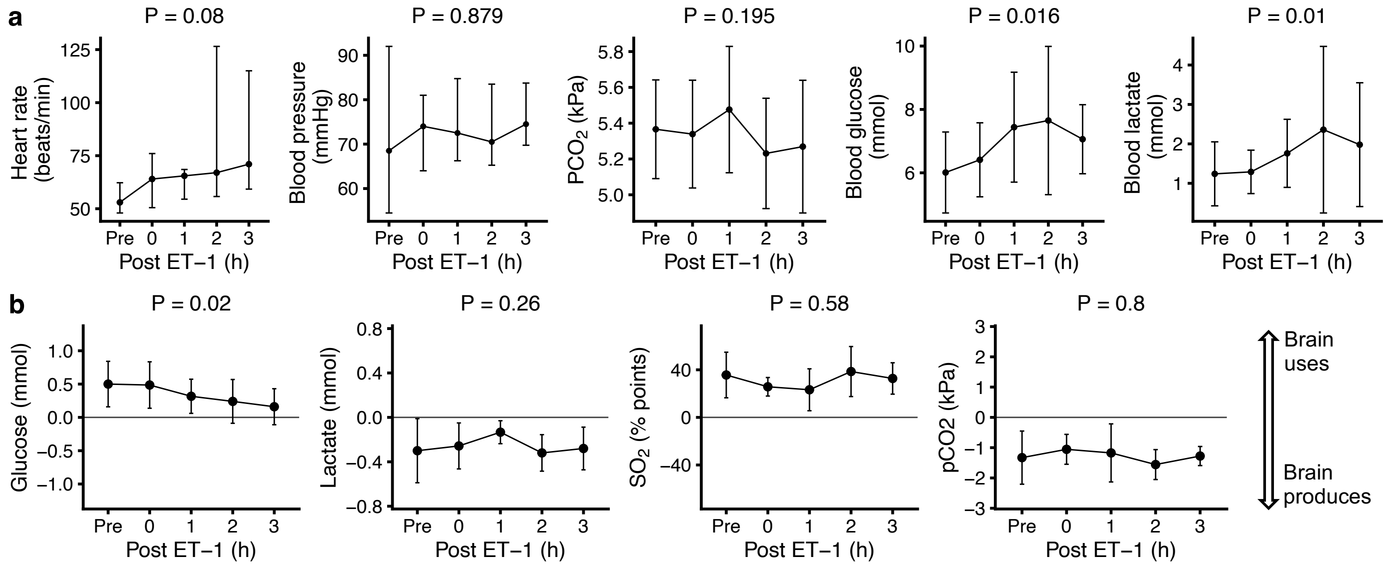

Figure S3: Physiological changes after endothelin 1 (ET-1) injection. No significant changes were observed in heart rate, mean arterial blood pressure or arterial partial pressure of carbon dioxide (pCO_2) during the experiment. Arterial glucose and lactate increased slightly as a function of time (a). The brain arterial-to-venous difference of glucose, lactate, oxygen saturation (SO_2) and pCO_2 were obtained repeatedly throughout the protocol (b). Only glucose changed with time.

Data (n = 5-10) are shown as medians ± intra-quartile range (heart rate and blood glucose) or mean ± SD (remaining panels). Tested with linear mixed-effect models or Friedman's test.

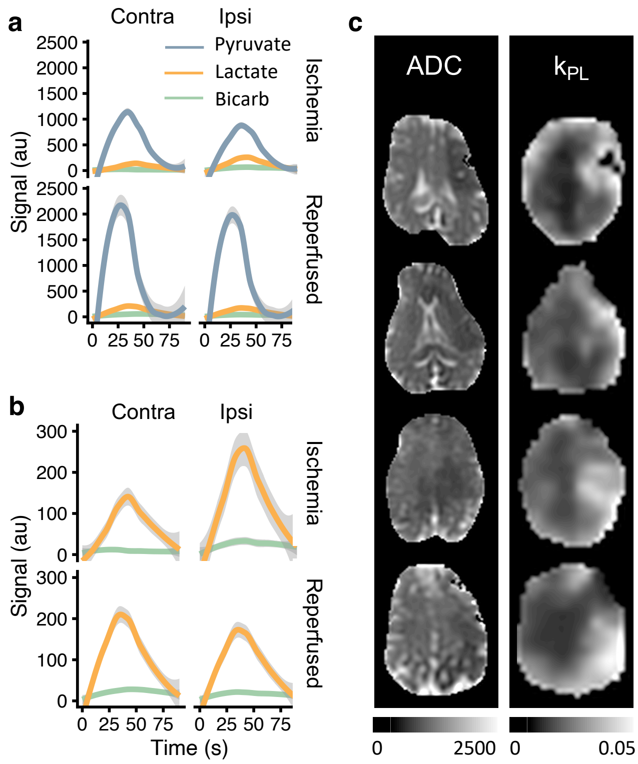

Figure S4: Signal over time after injection of hyperpolarized [1-^{13}C]pyruvate. Mean signal of lactate, bicarbonate and pyruvate (a) or metabolites shown without pyruvate (b). The lactate and pyruvate signals were used for fitting of k_{PL}. Here, the four pigs analyzed before reperfusion are shown with corresponding maps of the apparent diffusion coefficient (c).

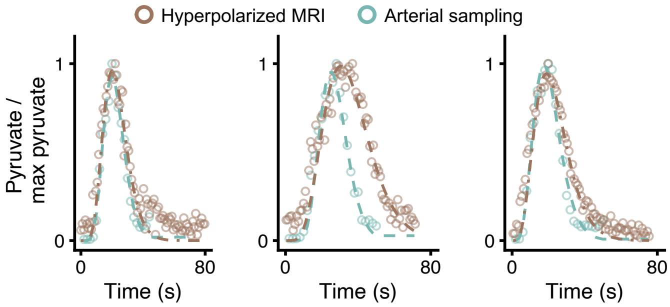

Figure S5: Comparisons of arterial input functions (AIF) measured with hyperpolarized pyruvate MRI and by sampling from the femoral artery in three pigs. The AIF from hyperpolarized pyruvate represents signal change in the automatically selected arteries used for perfusion weighted imaging. The AIF in blood was sampled during injection of hyperpolarized [1-^{13}C]pyruvate and subsequently analyzed with colorimetric assays. They show good agreement, however with a deviation in one pig. The curves were fitted with gamma variate fits.

Table S1: Overview of missing preclinical data.

Method	Missing data points	Reason	Final N
Hyperpolarized MRI	3 at 1-hour time point 0 at 3.5-hour time point	Technical error of the polarizer (dissolution failure) -	4 7
Magnetic resonance spectroscopy	2 at 1-hour time point 2 at 3.5-hour time point	Poor data quality or spectral contamination Poor data quality or spectral contamination	8 8
Arterial-venous differences	2 pigs from 1 hours onwards	Jugular vein catheter malfunction from moving the animal to the scanner	5
Tissue biopsies	1 pig	An insufficient biopsy was obtained	6

Table S2: All mixed-effect models performed.

Dependent variable	Effects	Coefficient	95% confidence limits		Random effect SD
			Lower	Upper	
Figure 1					
Perfusion defect (ml)	Time in min (fixed)	-0.05	-0.06	-0.03	-
	Pig (random)	-	-	-	16.07
Infarct size (ml)	Timepoint (fixed)				
	Reperfusion = ref.	-	-	-	-
	Ischemia	-6.93	-10.82	-3.04	-
	Pig (random)	-	-	-	1.6
Figure 2					
Naa/Cr in infarct	Timepoint (fixed)				
	Reperfusion = ref.	-	-	-	-
	Ischemia	0.39	0.24	0.54	-
	Pig (random)	-	-	-	0.19
Lactate/Cr in infarct	Timepoint (fixed)				
	Reperfusion = ref.	-	-	-	-
	Ischemia	-0.89	-1.81	0.06	-
	Pig (random)	-	-	-	0.65
Lactate, tissue (ng/ul)	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Infarct	102.56	42	163.12	-
	Pig (random)	-	-	-	54
LDH activity, tissue, not normalized (units)	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Infarct	-2.343	-4.395	-0.29	-
	Pig (random)	-	-	-	0.00000002
LDH activity, tissue, normalized to protein (units/mg)	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Infarct	0.068	-0.299	0.435	-
	Pig (random)		-	-	0.356
PDH activity, tissue, not normalized (units)	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Infarct	-2.96	-5.9	-0.02	-
	Pig (random)	-	-	-	0.0000001
PDH activity, tissue, normalized to protein (units/mg)	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Infarct	-0.48	-1.16	0.2	-
	Pig (random)	-	-	-	0.2
Figure 3					
k_{PL} (s^{-1})	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Penumbra	0.0077	0.0051	0.0102	-
	Infarct	-0.0021	-0.0038	-0.0003	-
	Timepoint (fixed)				
	Reperfusion = ref.	-	-	-	-

	Ischemia	-0.0018	-0.0043	0.0005	-
	Infarct:ischemia (interact.)	0.0145	0.0114	0.0176	-
	Pig (random)	-	-	-	0.0016
k_{PL} (s^{-1}), only ischemia timepoint	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Penumbra	0.0077	0.0052	0.0101	-
	Infarct	0.0125	0.01	0.0149	-
	Pig (random)	-	-		0
k_{PL} (s^{-1}), only reperfusion timepoint	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Infarct	-0.0021	-0.0037	-0.0004	-
	Pig (random)	-	-	-	0.0021
Lactate/pyruvate	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Penumbra	0.11	0.01	0.21	-
	Infarct	-0.06	-0.13	0.01	-
	Timepoint (fixed)				
	Reperfusion = ref.	-	-	-	-
	Ischemia	-0.16	-0.21	-0.03	-
	Infarct:ischemia (interact.)	0.26	0.14	0.38	-
	Pig (random)	-	-	-	0.16
Lactate/pyruvate, only ischemia timepoint	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Penumbra	0.11	0.05	0.17	-
	Infarct	0.2	0.14	0.26	-
	Pig (random)	-	-	-	0.15
Lactate/pyruvate, only reperfusion timepoint	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Infarct	-0.06	-0.1	-0.02	-
	Pig (random)	-	-	-	0.11
Bicarbonate/pyruvate	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Penumbra	0.03	0.0006	0.066	-
	Infarct	-0.01	-0.0332	0.013	-
	Timepoint (fixed)				
	Reperfusion = ref.	-	-	-	-
	Ischemia	-0.02	-0.0537	0.0062	-
	Infarct:ischemia (interact.)	0.07	0.0306	0.1107	-
	Pig (random)	-	-	-	0.02
Bicarbonate/pyruvate, only ischemia timepoint	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-

	Penumbra	0.03	0.001	0.065	-
	Infarct	0.06	0.029	0.092	-
	Pig (random)	-	-	-	0.025
Bicarbonate/pyruvate, only reperfusion timepoint	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Infarct	-0.01	-0.03	0.01	-
	Pig (random)	-	-	-	0.03
Lactate/bicarbonate	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Penumbra	0.82	-0.495	2.135	-
	Infarct	-0.058	-0.988	0.871	-
	Timepoint (fixed)				
	Reperfusion = ref.	-	-	-	-
	Ischemia	-0.543	-1.754	0.672	-
	Infarct:ischemia (interact.)	0.227	-1.383	1.837	-
	Pig (random)	-	-	-	1.567
Lactate/bicarbonate, only ischemia timepoint	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Penumbra	0.82	-0.654	2.294	-
	Infarct	0.169	-1.305	1.642	-
	Pig (random)	-	-	-	1.836
Lactate/bicarbonate, only reperfusion timepoint	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Infarct	-0.058	-0.667	0.551	-
	Pig (random)	-	-	-	0.586
Figure 4					
No tests but a regression analysis was performed for these data					
Figure 5					
Cerebral blood flow (ml/100ml/min)	Region of interest (fixed)				
	No progression = ref.	-	-	-	-
	Progressed to infarct	-2.3	-6.1	1.5	-
	Pig (random)	-	-	-	4.2
ADC (10^{-6} mm^2/s)	Region of interest (fixed)				
	No progression = ref.	-	-	-	-
	Progressed to infarct	-33.5	-71.1	4.2	-
	Pig (random)	-	-	-	0
k_{PL} (s^{-1})	Region of interest (fixed)				
	No progression = ref.	-	-	-	-
	Progressed to infarct	0.0085	0.004	0.013	-

	Pig (random)	-	-	-	0.0051
Lactate/pyruvate	Region of interest (fixed)				
	No progression = ref.	-	-	-	-
	Progressed to infarct	0.17	0.08	0.27	-
	Pig (random)	-	-	-	0.06
Pyruvate rCBF	Region of interest (fixed)				
	No progression = ref.	-	.	-	-
	Progressed to infarct	-0.54	-0.98	-0.1	-
	Pig (random)	.	-	-	0.5
Pyruvate MTT (s)	Region of interest (fixed)				
	No progression = ref.	-	-	-	-
	Progressed to infarct	5.06	2.21	7.91	-
	Pig (random)	-	-	-	2.09
Figure 6					
No tests but a regression analysis was performed for these data					
Figure S1					
Cerebral blood flow (ml/100ml/min)	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Ipsilateral	-5.24	-14.15	3.66	-
	Infarct	-15.46	-24.57	-6.31	-
	Time in min (fixed)	0.11	0.07	0.15	-
	Time:ipsilateral (interact.)	0.04	-0.01	0.09	-
	Time:infarct (interact.)	-0.08	-0.13	-0.02	-
	Pig (random)	-	-	-	6.68
ADC (10^{-6} mm^2/s)	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Ipsilateral	-168.96	-208.2	-129.7	-
	Infarct	-480.07	-519.3	-440.8	-
	Timepoint (fixed)				
	Reperfusion = ref.	-	-	-	-
	Ischemia	22.44	-16.8	61.68	-
	Ischemia:ipsilateral (interact.)	96.26	40.76	151.8	-
	Ischemia:infarct (interact.)	45.02	-10.48	100.52	-
	Pig (random)	-	-	-	29.91
Figure S2					
T1 (ms)	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Penumbra	53.86	-55.08	162.8	-
	Infarct	65.47	-43.48	174.4	-
	Timepoint (fixed)				

	Reperfusion = ref.	-	-	-	-
	Ischemia	91.92	-17.02	200.9	-
	Reperfusion + gad	29.73	-79.22	138.7	-
	Infarct:ischemia (interact.)	-40.69	-194.8	113.4	-
	Infarct:gad (interact.)	45.46	-108.6	199.5	-
	Pig (random)	-	-	-	271.3
T1 (ms), only ischemia timepoint	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Penumbra	53.86	2.5	105.22	-
	Infarct	24.78	-26.58	76.14	-
	Pig (random)	-	-	-	257.7
T1 (ms), only reperfusion timepoint	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Infarct	65.47	-3.28	134.2	-
	Pig (random)	-	-	-	294.98
T1 (ms), only reperfusion plus gadolinium timepoint	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Infarct	110.93	17.01	204.8	-
	Pig (random)	-	-	-	309.27
T2 (ms)	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Penumbra	2	-1.26	5.27	-
	Infarct	10.38	7.11	13.65	-
	Timepoint (fixed)				
	Reperfusion = ref.	-	-	-	-
	Ischemia	2.12	-1.14	5.38	-
	Infarct:ischemia (interact.)	-7.53	-12.15	-2.91	-
	Pig (random)	-	-	-	1.56
T2 (ms), only ischemia timepoint	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Penumbra	2	-0.89	4.9	-
	Infarct	2.85	-0.04	5.74	-
	Pig (random)	-	-		1.25
T2 (ms), only reperfusion timepoint	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Infarct	10.38	5.8	14.97	-
	Pig (random)	-	-	-	0.76
R2* (Hz)	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Penumbra	2.83	-2.23	7.89	-
	Infarct	-0.36	-5.41	4.71	-

	Timepoint (fixed)				
	Reperfusion = ref.	-	-	.	-
	Ischemia	2.57	-2.5	7.63	-
	Infarct:ischemia (interact.)	7.82	0.67	14.98	-
	Pig (random)	-	-	-	5.83
R2* (Hz), only ischemia timepoint	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Penumbra	2.83	-1.98	7.64	-
	Infarct	7.47	2.66	12.28	-
	Pig (random)	-	-	-	8.29
R2* (Hz), only reperfusion timepoint	Region of interest (fixed)				
	Contralateral = ref.	-	-	-	-
	Infarct	-0.35	-4.02	3.31	-
	Pig (random)	-	-	-	1.65
Sodium MRI ipsi/contra	Time in min (fixed)	0.0009	0.0006	0.001	-
	Pig (random)	-	-	-	0.031
Figure S3					
Heart rate (beats/min)	Time in min*	-	-	-	-
Blood pressure (mmHg)	Time in min*	-	-	-	-
Arterial pCO_2 (kPa)	Time in min (fixed)	-0.0007	-0.00163	0.000328	0.2107
Arterial glucose (mmol)	Time in min (fixed)	0.006	0.001	0.011	1.114
Arterial lactate (mmol)	Time in min (fixed)	0.005	0.001	0.009	0.929
Glucose AV-difference (mmol)	Time in min (fixed)	-0.002	-0.003	-0.0001	-
	Pig (random)	-	-	-	0.18
Lactate AV-difference (mmol)	Time in min (fixed)	-0.0004	-0.001	0.0003	-
	Pig (random)	-	-	-	0.21
SO_2 AV-difference (% points)	Time in min (fixed)	0.025	-0.06	0.11	-
	Pig (random)	-	-	-	0
pCO_2 AV-difference (kPa)	Time in min (fixed)	-0.0004	-0.003	0.002	-
	Pig (random)	-	-	-	0.5

* Were tested with Freidman's test due to non-normality. Therefore, no effect estimates are provided.
Ref. denotes reference, interact. denotes interaction.