Supplemental Material

Data S1. Supplemental Methods.

Animal Facility

The pigs were obtained from a specific pathogen-free facility and were acclimatized (12 hours light: 12 hours dark; 20–22 °C; 50–55 % humidity; restricted pellet diet (Svin Foder VAK, Danish Agro, Denmark)) for one to two weeks before the study day. All pigs were fasted for at least 16 hours but had free access to tap water while fasting.

High resolution respirometry (HRR)

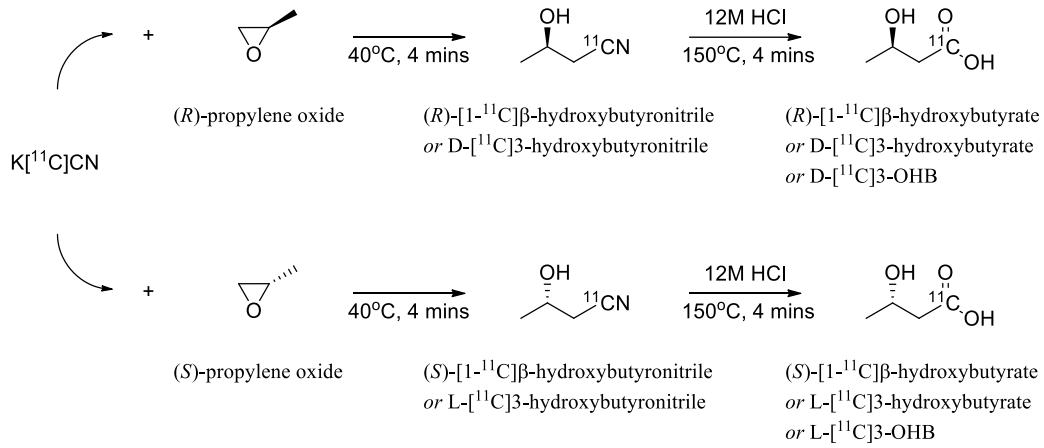
Myocardial biopsies were immediately transferred to a biopsy preserving solution (BIOPS: 10Ca-EGTA buffer 10^{-4} free Ca^{2+}, 20 imidazole, 20 taurine, 50 K-MES, 0.5 dithiothretiol, 6.56 $MgCl_2$, 5.77 ATP, and 15 phosphocreatine, pH 7.1). Each biopsy was manually dissected to form fibre bundles (~1.5mm) and permeabilized in a cold BIOPS solution mixed with Saponin (50 μg/mL) by gentle agitation for 30 min. Permeabilized fibers were rinsed twice in cold mitochondrial respiration medium (MiR05: in mmol/L: 110 sucrose, 60 K-lactobionate, 0.5 EGTA, 0.1% BSA, 3 $MgCl_2$, 20 taurine, 10 KH_2PO_4 and 20 Hepes; pH 7.4). Fiber bundles were added to the chambers of the Oxygraph-2k, containing 2mL MiR05 at 37°C.

Mitochondrial respiratory capacity was evaluated by substrate-uncoupler-inhibition-titration (SUIT) protocols. Glucose linked respiration was evaluated by the following SUIT protocol: Glutamate (10mmol/L) and Malate (10mmol/L) stimulated LEAK respiration. The addition of ADP (5mmol/L) was used to evaluate complex I coupled respiration. Subsequent addition of succinate (10mmol/L) was added to stimulated complex I+II respiration. Oligomycin (complex V inhibitor) (2μg/mL) was added to evaluate uncoupling the inner mitochondrial membrane. Finally, rotenone (complex I inhibitor) (0.5 μmol/L) and antimycin A (complex III inhibitor) (2.5mmol/L) was added to measure residual oxygen consumption. 3-OHB linked respiration was evaluated by the following SUIT protocol: Glutamate (10mmol/L) and 3-OHB (1.65mmol/L and further titrated until no subsequent rise in O_2 consumption) stimulated LEAK respiration. ADP (5mmol/L) was added to stimulate coupled respiration and subsequent addition of succinate stimulated fully coupled respiration.

Cytochrome c (10 μmol/L) was added to test the integrity of the inner mitochondrial membrane and an increase of >10% compared to complex I coupled respiration led to exclusion.

PET

The synthesis of D-[^{11}C]3-hydroxybutyrate acid was based on the work of Thorrell et al[53] Briefly, [^{11}C]CN was generated from [^{11}C]CO_2 and delivered to the synthesis module as NH_4[^{11}C]CN. [^{11}C]CN was then trapped as K[^{11}C]CN on the KCN trap[54] in the initial step of the synthesis, and subsequently reacted with the precursor *(R)*-propylene oxide and generated the intermediate D-[^{11}C]3-hydroxybutyronitrile. The end product D-[^{11}C]3-hydroxybutyrate (D-[^{11}C]3-OHB) was finally produced by acidic hydrolysis. The production of L-3-hydroxybutyrate (L-[^{11}C]3-OHB) employed *(S)*-propylene oxide as the precursor, which, due to the stereospecificity of the initial reaction step with K[^{11}C]CN, in turn, generated the L-enantiomer. Radio chemical purities was > 95% and enantiomeric purities was > 97%.



PET/CT scans were performed on a Siemens Biograph Vision 600 PET/CT (Siemens, Erlangen, Germany). Injections of radiotracers were done in a catheter placed in a femoral vein and used solely for infusions. The pigs were placed with the heart and liver in the field of view and a low-dose CT scan was obtained for attenuation and anatomic localization purposes. All [^{11}C]3-OHB injections were given as a bolus (256 MBq ± 131 MBq) followed by a 60-min list mode scan (frame structure 12 x 5 s, 4 x 10 s, 4 x 20 s, 4 x 30 s, 5 x 60 s, 6 x 300 s, 2 x 600 s). Image data were reconstructed using PSF + TOF with 4 iterations, 5 subsets, 128 x 128 matrix, and a 5 mm Gaussian postfilter. All dynamic PET data were decay corrected to scan start. Voxel size for the Vision scans was 3x3x3 mm.

Myograph

Physiological saline solution (PSS) consisting of (in mM): 119 NaCl, 22 $NaHCO_3$, 10 HEPES, 1.2 $MgSO_4$, 2.82 KCl, 5.5 glucose, 1.18 KH_2PO_4, 0.03 EDTA, 1.6 $CaCl_2$. The experimental bath was heated to 37°C and continuously bubbled with 5% CO_2/balance air. Following a 30-minute stabilization period, vessels were normalized to 90% of the internal diameter corresponding to a transmural pressure of 100 mmHg[55]; this internal diameter of the investigated arteries was on average (±SEM) 240±51 µm. The arteries were initially activated by two 2-minute long contractions elicited by increasing the extracellular K^+ concentration to 60 mM followed by one 5-minute long contraction elicited by 3 µM of the thromboxane analogue U46619. The 60 mM K^+-solution was prepared through equimolar substitution of K^+ for Na^+. Vascular responses to Na-D/L-3-OHB or NaCl were tested in arteries contracted with U46619 to a stable active tension equivalent to ~75% of the contraction achieved with 3 µM U46619.

These image-derived input functions were individually corrected for metabolites [^{11}C]CO_2 (described in detail in[56]) drawn every 10 minutes (Supplemental Figure 1) to yield the parent fraction. Tissue TACs were drawn on summed images (4-15 minutes) using the iso contouring VOI in PMOD.

Table S1. Dobutamine effect on hemodynamics.

	Before Dobutamine (n=24)[1]	After Dobutamine (n=24)[1]	Effect size (95 % CI)	*P*-value
Pulmonary artery catheter				
CO, L/min	5.8 ± 1.4	6.9 ± 1.6	1.2 (0.9, 1.5)	<0.001
HR, bpm	89 ± 24	101 ± 20	12 (7, 17)	<0.001
SVR, WU	15.2 ± 4.7	13.2 ± 4.1	-2 (-3, -1)	<0.001
mPAP, mmHg	23 ± 7	22 ± 7	-2 (-3, -1)	0.003
PVR, WU	3.2 ± 1.7	2.3 ± 1.3	-1 (-1, -1)	<0.001
Pressure volume loops				
Ees, mmHg/mL	0.71 ± 0.40	0.95 ± 0.57	0.24 (0.10, 0.38)	0.002
Ea, mmHg/mL	1.66 (1.22, 2.28)	1.52 (1.08, 2.00)	-0.13 (-0.40, 0.13)	0.3
EF, %	34 ± 12	43 ± 13	9 (6, 11)	<0.001

[1] Mean ± SD

The effect size is shown as mean (95 % confidence interval). The effect size indicates the variation in change observed for each outcome during dobutamine infusion compared with the intervention infusion alone.

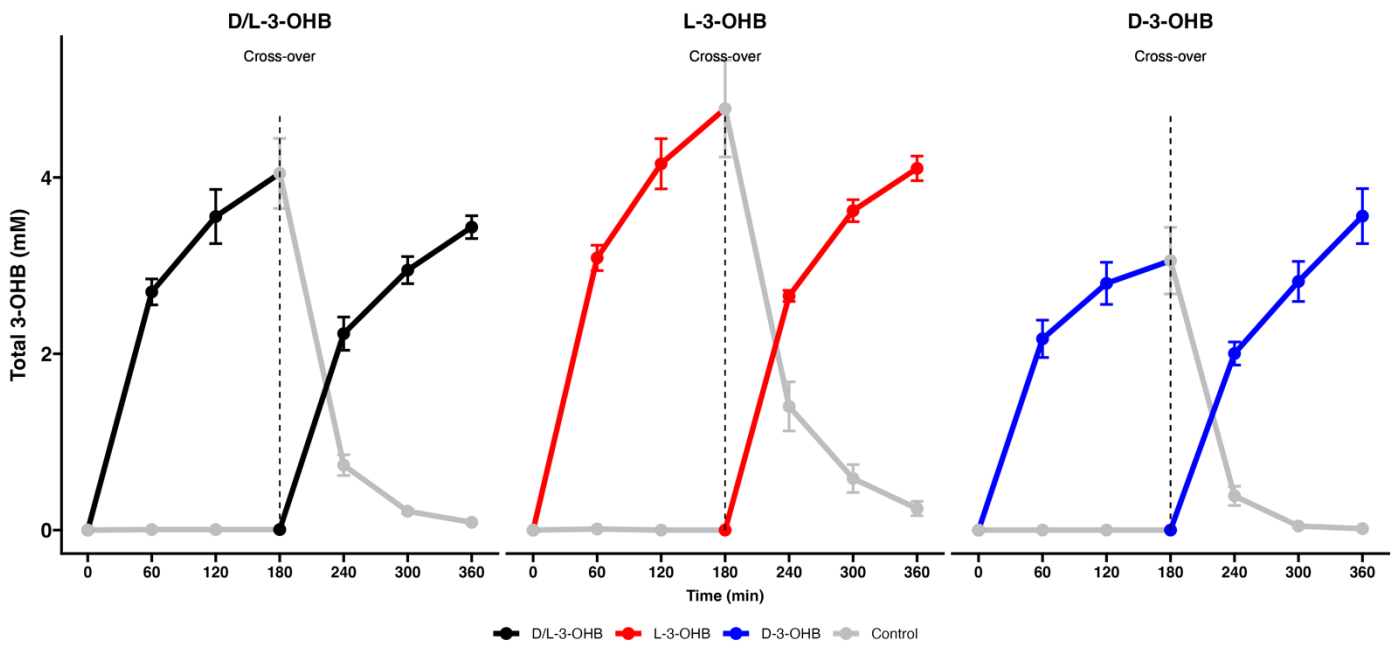
CO: Cardiac output; HR: Heart rate; SVR: systemic vascular resistance, mPAP: Mean pulmonary arterial pressure; PVR: Pulmonary vascular resistance; Ees: End-systolic elastance; Ea: Arterial elastance; EF: Ejection fraction.

Table S2. Myocardial D-[^{11}C]3-OHB kinetics during low and elevated ketones.

	Low circulating ketone levels (n=2)		High circulating ketone levels (n=2)	
	1-tissue	**2-tissue**	**1-tissue**	**2-tissue**
Vb	0.11 ± 0.02	0.11 ± 0.02	0.21 ±0.04	0.17 ± 0.10
K1, mL/min/mL	0.62 ± 0.02	0.62 ± 0.02	0.64 ±0.14	0.76 ± 0.04
k2, min^{-1}	0.14 ± 0.00	0.14 ± 0.01	0.20 ± 0.07	0.93 ± 0.80
k3, min^{-1}		0.25 ± 0.36		2.45 ± 1.22
k4, min^{-1}		7.33 ± 0.32		4.12 ± 5.45
Vt	4.57 ± 0.21	4.58 ± 0.19	3.20 ± 0.49	3.25 ± 0.55
AIC	19.06	24.12	33.28	26.65

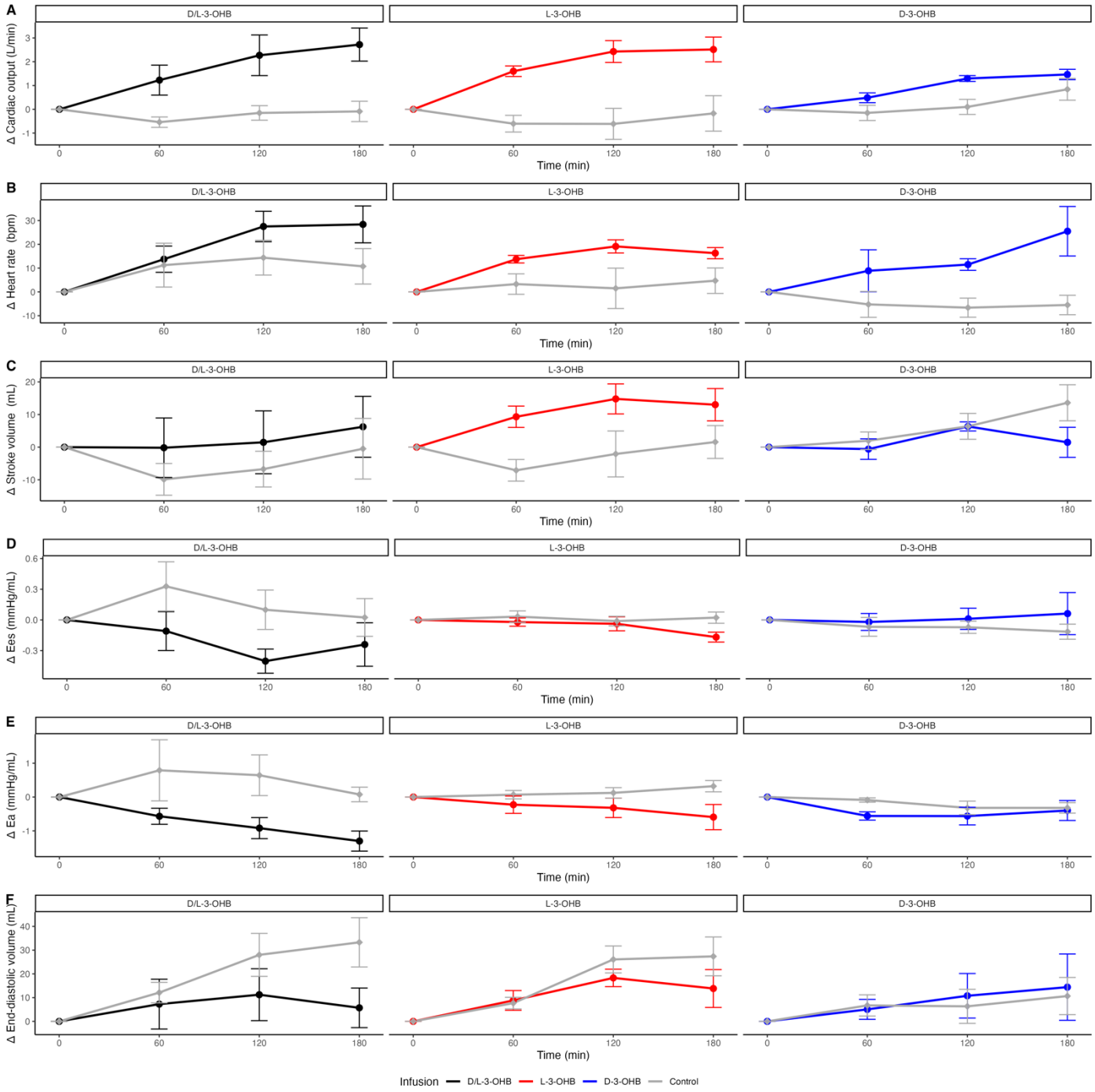
One-tissue and two-tissue models fit for heart tissue during low and high ketonemia. Data are presented as mean ± standard deviation. Vb: Blood volume; K1: Rate constant for the transfer of a radiotracer from plasma to tissue; k2: Rate constant for the transfer of a radiotracer from tissue back to plasma; k3: Rate constant for the reversible binding of a radiotracer to a specific receptor or enzyme; k4: Rate constant for the irreversible binding of a radiotracer to a specific receptor or enzyme; Vt: Tissue distribution volume; AIC: Akaike information criterion, which is a statistical measure used to compare the goodness of fit of different models in data analysis. A lower AIC value indicates a better fit of the model to the data.

Figure S1. Temporal changes in plasma concentrations of total 3-OHB.



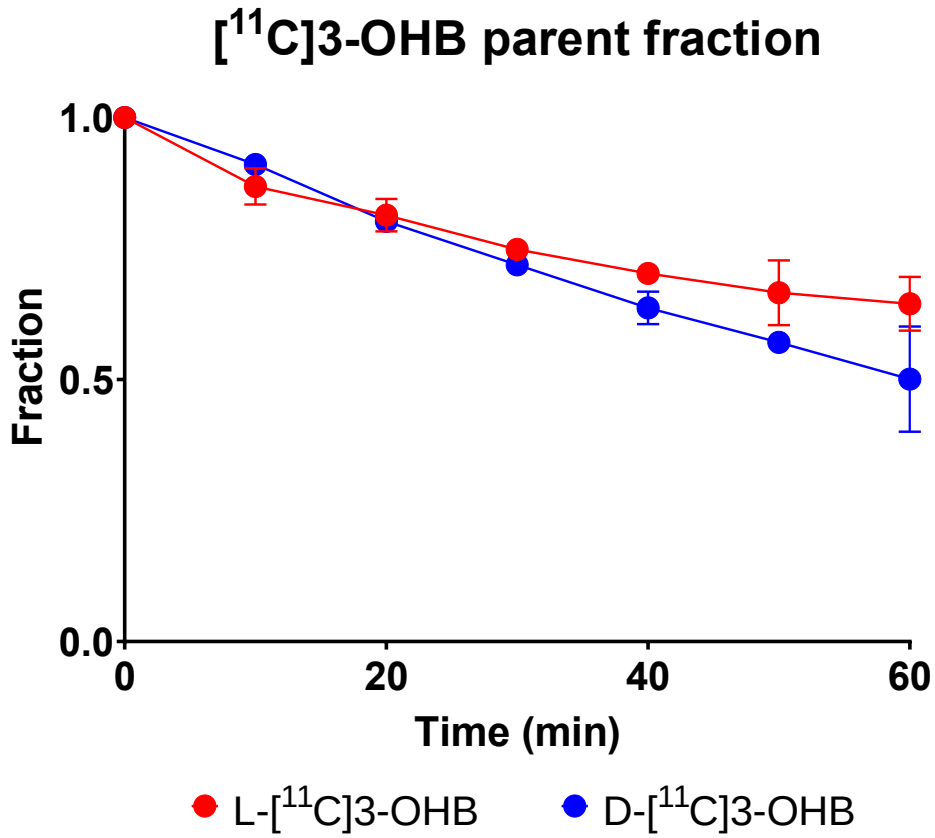
Changes in total 3-OHB plasma concentration during each 3-OHB infusion (D/L-3-OHB, L-3-OHB, D-3-OHB) and their respective control infusion

Figure S2. Relative temporal changes in hemodynamic measurements during each 3-OHB infusion and their respective control infusions.



Relative temporal changes during each 3-OHB infusion (D/L-3-OHB, L-3-OHB, D-3-OHB) and their respective control infusion in (A) Cardiac Output, (B) Heart rate, (C) Stroke Volume, (D) End-systolic pressure-volume relationship (ESPVR), (E) Arterial elastance (Ea) and (F) End-diastolic volume. Bars indicate the standard error of the mean.

Figure S3. [^{11}C]3-OHB fraction.



Unmetabolized [^{11}C]3-OHB tracer. Both tracers were metabolized to [^{11}C]CO_2 over the course of the study.