

Supplementary Material for

**Ketone body 3-hydroxybutyrate elevates cardiac output through peripheral vasorelaxation and enhanced cardiac contractility**

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<b>Baseline characteristics, echocardiography</b>			
	NaCl (before treatment)	Na-3-OHB (before treatment)	<i>P</i> -value
Weight (g)	326±3	317±5	0.21
Cardiac output (mL/min)	144±7	125±11	0.15
Ejection fraction (%)	62.4±1.8	60.7±2.6	0.88
Heart rate (min <sup>-1</sup> )	364±10	350±13	0.40
Stroke volume (μL)	397±19	358±28	0.26
End-diastolic volume (μL)	639±32	585±27	0.22
End-systolic volume (μL)	242±20	227±14	0.57
<b>Baseline characteristics, blood pressure measurements</b>			
	NaCl (before treatment)	Na-3-OHB (before treatment)	<i>P</i> -value
Weight (g)	322±4	311±7	0.17

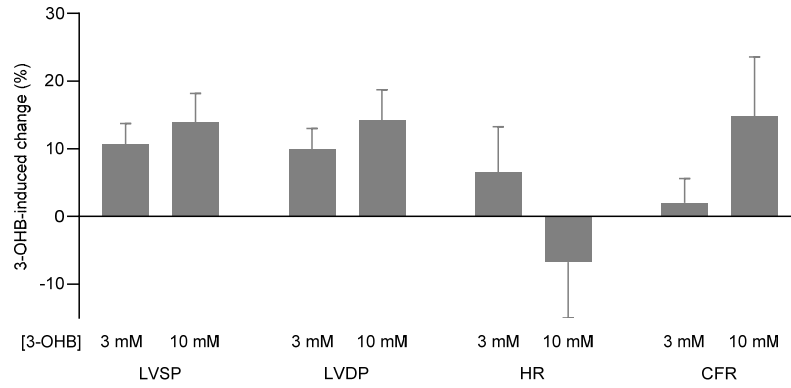
**Supplementary Table S1.** Baseline values from the *in vivo* echocardiography (n=8) and blood pressure (n=6-7) measurements. The data are mean±SEM and were compared using unpaired two-tailed Student's *t*-tests.

<b>Baseline characteristics, isolated perfused hearts</b>			
	NaCl (before treatment)	Na-3-OHB (before treatment)	<i>P</i> -value
Left ventricular systolic pressure (mmHg)	156.9±3.4	156.6±3.1	0.95
Left ventricular developed pressure (mmHg)	150.2±3.4	150.5±3.1	0.94
Heart rate (min <sup>-1</sup> )	233±10	238±10	0.76
Coronary flow rate (mL/min)	16.2±1.5	15.3±1.0	0.61

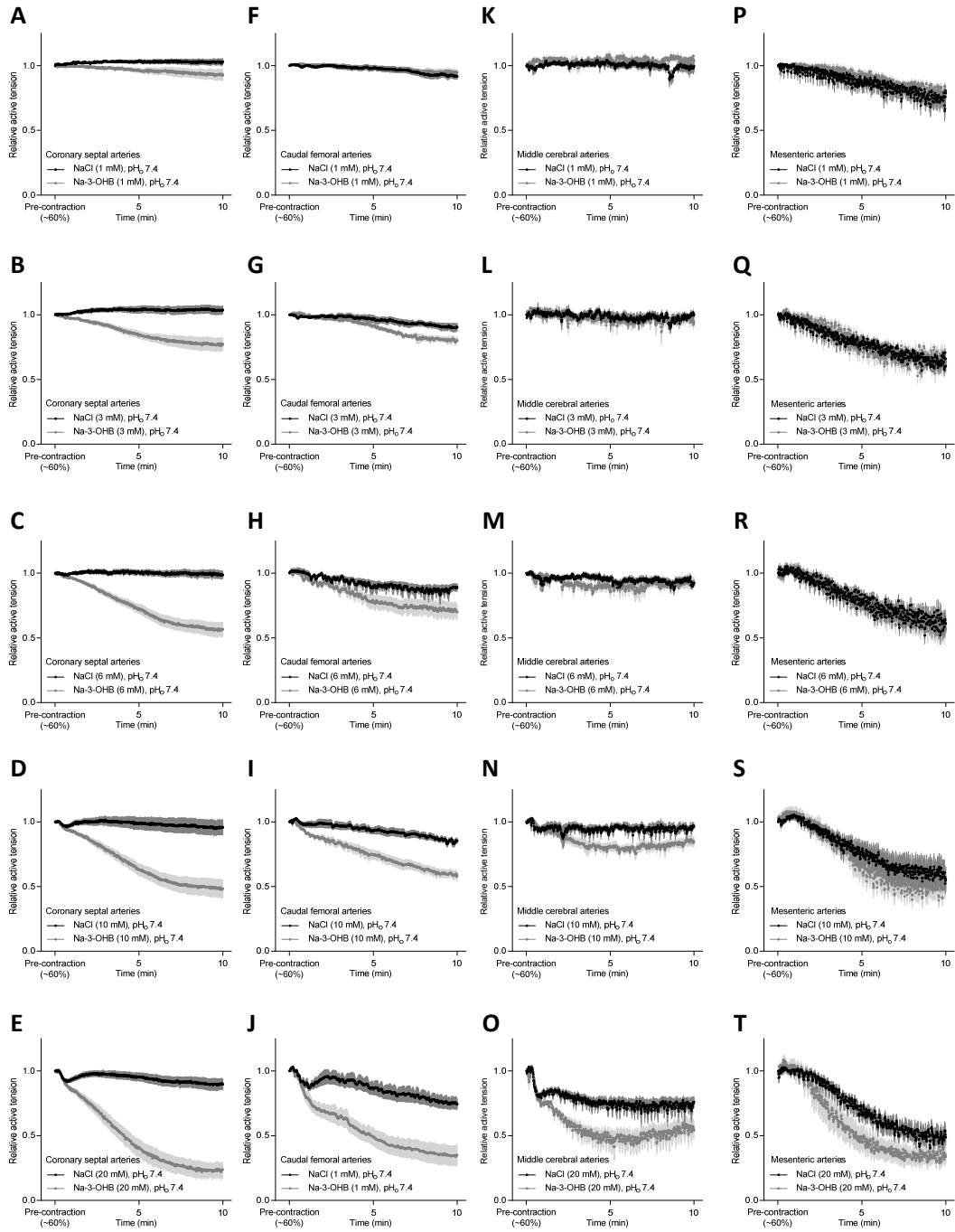
**Supplementary Table S2.** Baseline values recorded at the end of the stabilization period from *ex vivo* experiments on isolated hearts. The table summarizes 21 hearts tested with 3 or 10 mM Na-3-OHB and 19 hearts tested with matching concentrations of NaCl. The data are mean±SEM and were compared using unpaired two-tailed Student's *t*-tests.

Isolated blood vessel characteristics		
	Lumen diameter ( $\mu\text{m}$ )	Initial maximal active tension (N/m)
Arteries		
Coronary septal	309 $\pm$ 6	3.23 $\pm$ 0.13
Caudal femoral	199 $\pm$ 7	4.09 $\pm$ 0.26
Middle cerebral	228 $\pm$ 8	2.70 $\pm$ 0.21
Mesenteric	240 $\pm$ 12	7.44 $\pm$ 0.30
Renal interlobar	344 $\pm$ 18	4.46 $\pm$ 0.50
Veins		
Caudal femoral	367 $\pm$ 19	1.49 $\pm$ 0.10
Profound brachial	616 $\pm$ 60	2.44 $\pm$ 0.40
Mesenteric	341 $\pm$ 39	1.74 $\pm$ 0.19

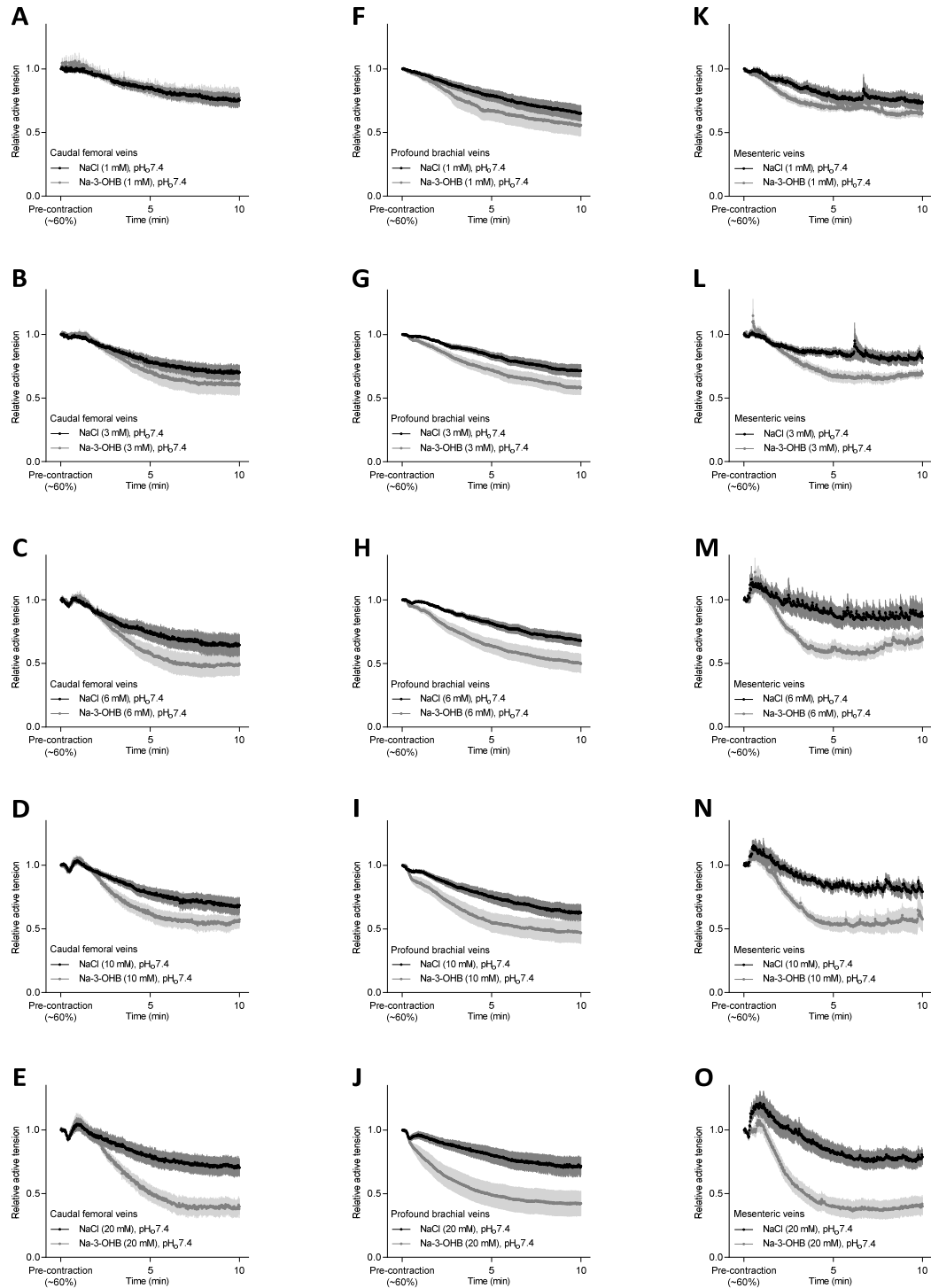
**Supplementary Table S3.** Characteristics of isolated blood vessels from the *ex vivo* experiments. The arteries and veins were evaluated based on a paired design where each blood vessel was exposed to multiple interventions (e.g., multiple concentrations of Na-3-OHB and NaCl) in alternating order between experiments. Alternatively, two blood vessels from the same animal were tested in parallel (e.g., Na-3-OHB vs. NaCl in presence of pharmacological inhibitors). The data are mean $\pm$ SEM, and n equals number of blood vessels: coronary septal arteries (n=66), caudal femoral arteries (n=20), middle cerebral arteries (n=15), mesenteric arteries (n=20), renal interlobar arteries (n=12), caudal femoral veins (n=23), profound brachial veins (n=10), and mesenteric veins (n=7).



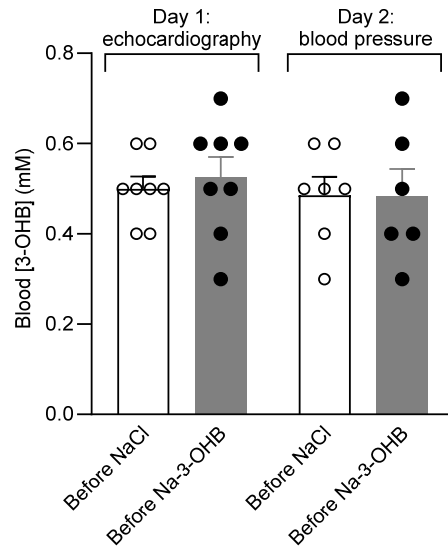
**Supplementary Figure S1.** 3-OHB-induced changes in cardiac parameters measured from isolated perfused hearts *ex vivo* (n=9-12) 20 minutes after buffer change. Values after 10 minutes are reported in Figure 2. Bars represent mean±SEM. Abbreviations: CFR, coronary flow rate; HR, heart rate; LVDP, left ventricular developed pressure; LVSP, left ventricular systolic pressure.



**Supplementary Figure S2.** Concentration-dependent arterial responses to 3-OHB, illustrated relative to a stable U46619-induced pre-contraction. **A-E**, coronary septal arteries ( $n=7$ , summarized data are shown in Figure 3B and 4B). **F-J**, caudal femoral arteries ( $n=8$ , summarized data are shown in Figure 4D). **K-O**, middle cerebral arteries ( $n=5$ , summarized data are shown in Figure 4F). **P-T**, mesenteric arteries ( $n=7$ , summarized data are shown in Figure 4H). The data are presented as mean $\pm$ SEM.



**Supplementary Figure S3.** Concentration-dependent venous responses to 3-OHB, illustrated relative to a stable U46619-induced pre-contraction. **A-E**, caudal femoral veins (n=10, summarized data are shown in Figure 5B). **F-J**, profound brachial veins (n=10, summarized data are shown in Figure 5C). **K-O**, mesenteric veins (n=8, summarized data are shown in Figure 5D). The data are presented as mean±SEM.



**Supplementary Figure S4.** Baseline blood concentrations of 3-OHB measured with a point-of-care device prior to administration of exogenous 3-OHB on day 1 (echocardiography) and day 2 (blood pressure) of the study. The bars represent mean±SEM.