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ORIGINAL RESEARCH

Hemodynamic Effects of Ketone Bodies in Patients With Pulmonary Hypertension

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BACKGROUND: Pulmonary arterial hypertension (PAH) or chronic thromboembolic pulmonary hypertension (CTEPH) are debilitating diseases with a high mortality. Despite emerging treatments, pulmonary vascular resistance frequently remains elevated. However, the ketone body 3-hydroxybutyrate (3-OHB) may reduce pulmonary vascular resistance in these patients. Hence, the aim was to assess the hemodynamic effects of 3-OHB in patients with PAH or CTEPH.

METHODS AND RESULTS: We enrolled patients with PAH (n=10) or CTEPH (n=10) and residual pulmonary hypertension. They received 3-OHB infusion and placebo (saline) for 2 hours in a randomized crossover study. Invasive hemodynamic and echocardiography measurements were performed. Furthermore, we investigated the effects of 3-OHB on the right ventricle of isolated hearts and isolated pulmonary arteries from Sprague–Dawley rats. Ketone body infusion increased circulating 3-OHB levels from 0.5±0.5 to 3.4±0.7 mmol/L (P<0.001). Cardiac output improved by 1.2±0.1 L/min (27±3%, P<0.001), and right ventricular annular systolic velocity increased by 1.4±0.4 cm/s (13±4%, P=0.002). Pulmonary vascular resistance decreased by 1.3±0.3 Wood units (18%±4%, P<0.001) with no significant difference in response between patients with PAH and CTEPH. In the rat studies, 3-OHB administration was associated with decreased pulmonary arterial tension compared with saline administration (maximal relative tension difference: 12±2%, P<0.001) and had no effect on right ventricular systolic pressures (P=0.63), whereas pressures rose at a slower pace (dP/dtmax, P=0.02).

CONCLUSIONS: In patients with PAH or CTEPH, ketone body infusion improves cardiac output and decreases pulmonary vascular resistance. Experimental rat studies support that ketone bodies relax pulmonary arteries. Long-term studies are warranted to assess the clinical role of hyperketonemia.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT04615754.

Key Words: echocardiography ■ invasive hemodynamics ■ ketone bodies ■ pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) or chronic thromboembolic pulmonary hypertension (CTEPH) are debilitating diseases that affect both the pulmonary vasculature and the heart. PAH is treated with medications that reduce pulmonary pressure and pulmonary vascular resistance (PVR), whereas in CTEPH, the main treatment is pulmonary endarterectomy. For patients with persistent pulmonary hypertension (PH) after pulmonary endarterectomy or considered inoperable, additional treatment options are balloon pulmonary angioplasty or specific PH medication.

Despite optimal treatment, patients with PAH and CTEPH are often left with a substantial degree of PH, which is associated with increased morbidity and mortality.[1,2]

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CLINICAL PERSPECTIVE

What Is New?

- In patients with pulmonary arterial hypertension or chronic thromboembolic pulmonary hypertension, infusion of the ketone body 3-hydroxybutyrate increased cardiac output by 1.2 L/min (27%) and decreased pulmonary vascular resistance by 18%.
- Experimental data demonstrated no effect on right ventricular pressure, whereas pulmonary arterial tension decreased in response to Na-3-hydroxybutyrate compared with NaCl.

What Are the Clinical Implications?

- This is the first human study to demonstrate that modulation of circulating ketone body levels improves cardiac output and lowers pulmonary vascular resistance in patients with pulmonary arterial hypertension or chronic thromboembolic pulmonary hypertension in addition to established treatment.

Nonstandard Abbreviations and Acronyms

3-OHB	3-hydroxybutyrate
CTEPH	chronic thromboembolic pulmonary hypertension
CO	cardiac output
HR	heart rate
mPAP	mean pulmonary arterial pressure
PAH	pulmonary arterial hypertension
PH	pulmonary hypertension
RAP	right atrial pressure
SV	stroke volume
WU	Wood units

Ketone bodies are produced in the liver and used for energy production in peripheral tissues in numerous physiological states such as fasting, exercise, and severe illness. The most important ketone bodies are 3-hydroxybutyrate (3-OHB) and acetoacetate. In patients with PH, a shift in energy substrates toward increased ketone availability has been demonstrated.[3] This led the authors to speculate that such a shift in metabolism is a beneficial adaptation compensating for the failing right heart. In addition, 3-OHB infusion has been shown to increase cardiac output (CO) and perfusion in brain, skeletal muscles, kidneys, and heart.[4–8] Intriguingly, 3-OHB was reported to have caused a decreased in PVR among patients with left-sided heart failure and reduced ejection fraction.[8] If similar effects of 3-OHB were to be present in patients with PH, this may pave the way for a novel treatment approach. However, the effect of increased circulating ketone body levels on pulmonary hemodynamics and cardiac function in patients with PH remains unsettled. We hypothesized that 3-OHB infusion would increase right ventricular (RV) output and decrease PVR in patients with PAH and CTEPH. To assess the direct effects of 3-OHB on RV function and pulmonary arteries, we performed additional studies in isolated rat hearts and pulmonary arteries.

METHODS

Upon request, anonymized data may be made available to other researchers following approval by the local ethics committee. Additional information about animal studies, statistics, and ethics is provided in Data S1.

Patients

We studied 20 patients with either CTEPH (n=10) or PAH (n=10), all with residual PH. The inclusion criteria were (1) precapillary PH (defined as mean pulmonary arterial pressure [mPAP] ≥25 mm Hg, PVR >3 Wood units, and pulmonary capillary wedge pressure (PCWP) <15 mm Hg); (2) tricuspid valvular regurgitated flow velocity >2.8 m/s; (3) preserved left ventricular (LV) ejection fraction >50%; and (4) ability to give informed consent. The exclusion criteria were significant pulmonary, mitral, or aortic valve disease. All patients were recruited from the outpatient PH clinic at Aarhus University Hospital, Denmark.

Design

The participants fasted overnight and avoided strenuous exercise for 48 hours before the investigations. At 8:00 AM, venous catheters were inserted into the participants' cubital veins. The local pharmacy prepared the dissolved Na-3-OHB at a 7.5% concentration. Placebo and 3-OHB alike were provided in red bags to ensure concealment. Potassium chloride (KCl) was added at a concentration of 60 mmol/L. A low-dose insulinemic euglycemic clamp (0.3 IE insulin/kg per hour) was administered to all participants to minimize endogenous 3-OHB production during prolonged fasting throughout the study. Glucose (20% solution, 60 mmol/L KCl) was infused to maintain euglycemia. All participants were investigated once for 5 hours and received either 3-OHB infusion (0.18 g/kg per hour) (Na-3-OHB, Sigma-Aldrich, St. Louis, MO [racemic mixture 50/50 D- and L-3OHB]) or placebo (isotonic saline) at an equivalent volume for 2 hours followed by 30 minutes of end point measurements in a randomized crossover design. The participants were blinded to the order of infusion and were continuously monitored with a Swan-Ganz catheter (Edward Lifesciences, Irvine, CA), systemic blood

pressure measurements, repeated blood sampling, and echocardiography. To avoid hypokalemia, which may occur during 3-OHB infusion,[7,8] they received an oral load of 60 mmol KCl when randomized to initiate with 3-OHB and with 20 mmol when randomized to initiate with placebo. All hemodynamic measurements were performed with the patient in the supine position following a 10-minute resting period.

Right Heart Catheterization

CO was assessed by thermodilution and averaged over 3 measurements. Right atrial pressure (RAP), mPAP, PCWP, and mixed venous oxygen saturation were measured. Heart rate (HR) and blood pressure were measured noninvasively. Stroke volume (SV) [SV=CO/HR], mean arterial pressure (MAP), and systemic [systemic vascular resistance=(MAP–RAP/CO)] and pulmonary [PVR=(mPAP–PCWP)/CO] vascular resistance were calculated.

Echocardiography

A GE Vivid E95 (GE Healthcare, Chicago, IL) was used for echocardiographic acquisition and EchoPAC 204 (GE-Vingmed Ultrasound, Norway) for analysis. The following variables were assessed in the 4-chamber view: RV free wall and global (RV-global longitudinal strain), RV systolic tricuspid annular velocity by pulsed doppler, tricuspid annular plane systolic excursion, RV area (systolic and diastolic), RV fractional area [(RV end-diastolic area–RV end-systolic area)/RV end-diastolic area×100%], right atrium area (before opening of the tricuspid valve). LV ejection fraction, global longitudinal strain (numeric values), and LV outflow tract velocity time integral were also measured. All measures were averaged over 3 heartbeats. The assessor was blinded to randomization.

Animal Studies

Healthy male Sprague–Dawley rats (10 weeks, 350–400 g, Taconic, Ry, Denmark; n=15) were used. Rat hearts were isolated and perfused ex vivo. Isolated hearts were randomized to receive either 2 mmol/L NaCl and 2 mmol/L Na-3-OHB (n=7) or 10 mmol/L NaCl and 10 mmol/L Na-3-OHB (n=8) in a crossover design.

Segments of fourth-order pulmonary arteries (approximately 2 mm long) were isolated from rats (n=7) and mounted on wire myographs for isometric evaluation. From each animal, 2 pulmonary arteries were investigated. One was subjected to NaCl (2 and 10 mmol/L—randomized order); the other, to Na-3-OHB (2 and 10 mmol/L—randomized order).

End Points

The primary end point was CO measured by thermodilution technique. End point measurements were compared at the end of each intervention period (ie, saline versus 3-OHB). Because medical treatment with PAH and CTEPH among other factors may potentially differ, we assessed the interaction between the underlying disease and end points. Consequently, 10 participants were enrolled with each disease. In the exploratory animal study, systolic RV pressures, dP/dtmax, and relative pulmonary arterial tension were assessed.

Statistical Analysis

Unpaired 2-tailed *t* tests were used for comparison between groups (baseline characteristics). Changes in data are presented as mean±SEM and between-group differences as mean±SD or median with interquartile range (25%; 75%) if not stated otherwise. For additional statistics refer to Data S1.

Ethics

All patients gave informed consent and the local Ethics Committee of the Central Denmark Region and the Danish Data Protection Agency approved the study. The study is registered at clinicaltrials.gov (NCT04615754), and all animal experiments were conducted in accordance with the institutional and Animal Research: Reporting of In Vivo Experiments guidelines.

RESULTS

Patients

Twenty patients were enrolled (PAH: n=10, hereof idiopathic PAH: n=6; hereditary PAH: n=2; associated with scleroderma: n=1; corrected congenital heart disease: n=1; and CTEPH: n=10). All enrolled patients completed the protocol (Figure S1, Consolidated Standards of Reporting Trials diagram). Due to technical issues, the euglycemic clamp could not be performed in 1 patient with CTEPH.

Patient characteristics are given in Table 1. At inclusion, all patients were stable and on individualized guideline-recommended PH-specific treatment with no change in therapy for at least 6 months and with more than 6 months since pulmonary endarterectomy or balloon pulmonary angioplasty. The baseline hemodynamic parameters, echocardiographic measures and PH specific treatment did not differ significantly between randomization groups (3-OHB→saline versus saline→3-OHB) ($P>0.05$, for all measurements).

Blood Samples

Circulating 3-OHB levels increased from 0.5±0.5 to 3.4±0.7 mmol/L. We observed an increase in lactate, pH, and sodium ($P<0.001$, for all) and a decrease in

Table 1. Baseline Characteristics of Patients

Demographics	PAH (n=10)	CTEPH (n=10)	*P* value	All (n=20)
Age, y	47±16	70±9	0.001	59±17
Sex, n, male/female	1/9	4/6	0.30	5/15
World Health Organization class (1/2/3/4)	2/7/1/0	1/7/2/0	1.00	3/14/3/0
6-minute walk distance, m	497±86	399±117	0.06	445±112
Pulmonary hypertension duration, y	6.5±4	7.3±5	0.77	6.9±4.7
Body mass index, kg/m^2	26±6	26±5	0.97	26±5
Estimated glomerular filtration rate, mL/min per 1.73 m^2	92±31	65±16	0.03	80±28
N-terminal pro-B-type natriuretic peptide, pg/mL	186 (117, 466)	533 (484, 847)	0.01	466 (132, 635)
Hemodynamics				
Cardiac output, L/min	4.7±1.0	4.5±1.5	0.85	4.6±1.2
Stroke volume, mL	61±13	54±7	0.18	58±11
Right arterial pressure, mm Hg	4±2	5±4	0.70	4±3
Mean pulmonary arterial pressure, mm Hg	42±11	36±8	0.20	39±9
Pulmonary capillary wedge pressure, mm Hg	10±3	10±4	0.95	10±3
Transpulmonary gradient, mm Hg	32±9	26±7	0.16	29±9
Pulmonary vascular resistance, WU	7.0±2.6	6.4±2.7	0.59	6.7±2.6
Mean arterial pressure, mm Hg	91±16	97±13	0.47	94±14
Systemic vascular resistance, WU	20±4	21±4	0.50	20±4
Heart rate, bpm	76±11	84±18	0.27	80±15
Mixed venous oxygen saturation, %	70±5	67±11	0.37	69±8
Echocardiographic parameters				
RV-free wall strain, %	18.7±4.8	14.4±3.4	0.04	16.5±4.6
RV-global strain, %	17.2±3.3	14.6±3	0.09	15.9±3.4
RV systolic tricuspid annular velocity, cm/sec	12.7±3.0	10.7±3.4	0.24	11.7±3.5
Tricuspid annular plane systolic excursion, mm	18.4±3.5	14.2±3.3	0.01	16.3±4.0
RV-diastolic area, cm^2	22.1±4.1	23.6±7.1	0.58	22.8±5.7
RV-systolic area, cm^2	14.8±3.5	17.1±4.4	0.21	15.9±4.1
RV-fractional area, %	33.3±9.9	25.8±12.1	0.15	29.5±11.4
Right atrium area, cm^2	15.8±4	24.0±8.8	0.02	19.7±7.8
LV-global longitudinal strain, %	19.3±2.3	17.6±3.4	0.23	18.4±3
LV-ejection fraction, %	60±6	56±8	0.23	58±7
LV outflow tract velocity time integral, cm	21.7±4.1	19.8±4.5	0.33	20.8±4.3
Treatment				
Phosphordiesterase-5 inhibitors, n	10	7	0.21	17 (85%)
Soluble guanylate cyclase activator, n	0	2	0.09	2 (10%)
Parental prostaglandin analogue, n	3	0	0.58	3 (15%)
Endothelin receptor antagonists, n	9	1	0.001	10 (50%)
Calcium channel blockers, n	1	0	1.00.30	1 (5%)
Loop diuretics, n	3	6	0.37	9 (45%)
Anticoagulation, n	3	10	0.003	13 (65%)
Balloon pulmonary angioplasty, n	0	7	0.003	7
Pulmonary endarterectomy, n	0	8	0.001	8

CTEPH indicates chronic thromboembolic pulmonary hypertension; LV, left ventricular; PAH, pulmonary arterial hypertension; RV, right ventricular; and WU, Wood units.

magnesium levels (*P*=0.03) (Table 2). For both pH and sodium, the interaction of infusion sequence was significant (*P*<0.001). Hence, in those who were randomized to receive 3-OHB infusion in the first period, pH and sodium levels remained elevated throughout the placebo period (Figure S2). Insulin levels were approximately 15% higher during 3-OHB administration (*P*=0.03).

Effects of Ketone Body Infusion on Hemodynamics

Ketone body infusion was associated with a 27% increase in CO (1.2±0.1 L/min; Table 3; Figures 1A and Figure 2; Figure S3 [individualized data]). This was due to a combination of a 13% increase in SV (8±1 mL) and a 13% increase in HR (9±1 beats per minute). Only a minor increase in mPAP of 4% (P=0.03) was observed as a consequence of increased systolic PAP (3±1 mm Hg, P=0.05), whereas diastolic PAP was unchanged (0±0.7 mm Hg, P=1.0). PCWP remained unchanged (P=0.91), and therefore, PVR decreased by 18% (1.3±0.3 Wood units; P<0.001). A small decrease in RAP was observed (P=0.02) in the PAH group only (test for interaction: P=0.02). Consequently, systolic pulmonary artery pulsatile index increased for the entire study population (2.1 [0; 11.2], P=0.02), whereas the changes did not reach statistical significance for each study group separately (PAH: P=0.11, CTEPH: P=0.17). Changes in pulmonary vascular capacitance were not significant (–0.04±0.02 mm Hg/mL, P=0.08) during ketone body infusion compared with saline. Mixed venous oxygen saturation improved in patients with PAH and CTEPH (P<0.001). Three-way ANOVA analysis showed an interaction of infusion order (3-OHB→saline versus saline→3-OHB) with CO (P=0.01) and HR (P=0.004; Table 3). Hence, we performed additional analysis of CO data acquired only during the first infusion period (ie, after the first 2 hours of infusion). We observed an increase in CO of 1.2±0.2 (P=0.001, unpaired t test) from baseline for those who received 3-OHB (n=10) compared with those who received placebo (n=10) in the first infusion period.

Echocardiographic Measurement

Tricuspid annular plane systolic excursion was lower and the right atrium area was larger in patients with CTEPH than in patients with PAH (P=0.01 and P=0.02, respectively; Table 1). Ketone body infusion was associated with an increase in contractile function measured by right ventricular systolic tricuspid annular velocity (P=0.002), whereas no significant changes in strain (RV-free wall, RV-global longitudinal strain), tricuspid annular plane systolic excursion, and RV-fractional area were detected (Table 3). This was in contrast with measurement of LV function in which both LV-global longitudinal strain, LV ejection fraction, and forward SV measured as LV outflow tract velocity time integral increased during ketone body infusion (P<0.001, P=0.01, and P<0.001, respectively; Table 3).

Animal Results

During increasing RV diastolic pressure 3-OHB compared with NaCl at high levels (10 mmol/L) did not affect RV systolic pressure (P=0.63, Figure 3). However, the rate of rise in RV systolic pressure was prolonged; thus, dP/dtmax (P=0.02) was lower during increasing RV diastolic pressure. At low levels of 3-OHB compared with NaCl (2 mmol/L), no differences were observed in either RV systolic pressure (P=0.42) or dP/dtmax (P=0.19). Compared with NaCl, 3-OHB had no effect on HR (2 mmol/L: P=0.15; 10 mmol/L: P=0.35).

No effect on vascular tension was observed at low concentrations (2 mmol/L) of 3-OHB (P=0.73), whereas both high NaCl and 3-OHB levels (10 mmol/L) increased vascular tension (P<0.001; Figure 3). However, pulmonary vascular tension was relatively reduced by 12%±2% (P<0.001) during high 3-OHB levels as compared with NaCl (Figure 4).

DISCUSSION

The present study is the first to assess hemodynamic changes during 3-OHB infusion in patients with PAH or CTEPH. First, we observed an increase in CO and a decrease in PVR during 3-OHB infusion as compared with placebo. Second, we observed no significant differences in response to 3-OHB infusion in patients with PAH or CTEPH despite ongoing PH medication or previous pulmonary endarterectomy or balloon pulmonary angioplasty. Third, 3-OHB had no significant effect on RV systolic pressure in an isolated perfused rat heart model, whereas tension development in isolated pulmonary arterial segments decreased compared with NaCl, suggesting that 3-OHB may have a pulmonary vasodilating effect.

Ketone Body 3-OHB Improves Cardiac Function in Patients With PAH or CTEPH

We observed increments in both SV and HR with a resulting increase in CO and a decrease in PVR during 3-OHB infusion. It is unlikely that the CO increase was due to an increase in preload as RAP decreased only minimally (0.6 mm Hg, P=0.02) during 3-OHB infusion compared with saline; and PCWP did not differ (P=0.91) between 3-OHB and saline infusion. Furthermore, in patients with CTEPH, in whom RAP did not differ significantly during saline and 3-OHB administration, the hemodynamic response to 3-OHB was equivalent to that observed in patients with PAH. The echocardiographic findings may indicate an increase in RV contractile function, but these results are equivocal as only right ventricular systolic tricuspid annular velocity increased significantly. However, right ventricular systolic tricuspid annular velocity is a more sensitive marker of changes in RV contractile function than tricuspid annular plane systolic excursion.[9] Hence, the results support increased RV contractile function.

Table 2. Blood Sample Measurements and Quantities of Infused Volumes

Blood sample parameter	PAH (n=10)		CTEPH (n=10)		Overall change (n=20)	*P* value (3-way repeat ANOVA)	Interaction (group)	Interaction (inf. seq.)
	Saline	3-hydroxybutyrate	Saline	3-hydroxybutyrate				
3-hydroxybutyrate, mmol/L	0.4±0.4	3.3±0.8‡	0.5±0.5	3.5±0.7‡	2.9±0.2	<0.001	0.78	0.61
Lactate, mmol/L	0.8±0.3	1.3±0.5†	0.9±0.2	1.2±0.3†	0.4±0.06	<0.001	0.31	0.45
Glucose, mmol/L	5.0±0.6	5.1±0.7	4.7±0.5	5.1±0.8	0.3±0.2	0.08	0.38	0.48
pH	7.42±0.03	7.45±0.02*	7.43±0.05	7.47±0.02*	0.04±0.01	<0.001	0.72	<0.001
Potassium, mmol/L	3.8±0.2	3.7±0.3	3.8±0.2	3.7±0.3	−0.1±0.1	0.07	0.65	0.19
Sodium, mmol/L	142±1	144±2‡	143±2	145±2†	2.4±0.4	<0.001	0.86	<0.001
Magnesium, mmol/L	0.72±0.06	0.70±0.07	0.77±0.07	0.76±0.09	−0.02±0.01	0.03	0.53	<0.001
Calcium (ionized), mmol/L	1.18±0.06	1.16±0.06	1.19±0.07	1.21±0.08	−0.01±0.01	0.14	0.01	<0.001
Hematocrit, %	39±5	38±5	42±3	42±3	−0.1±0.1	0.33	0.32	0.38
Free fatty acids, mmol/L	0.09±0.07	0.07±0.08	0.16±0.2	0.05±0.03	−0.07±0.03	0.07	0.21	0.83
Insulin, pmol/L	122±40	134±43	135±41	157±41	17±7	0.03	0.42	0.83
Brain natriuretic peptide, pg/mL	780 (458, 1151)	708 (400, 1197)	1175 (601, 3201)	1141 (594, 3200)	0 (−97, 38)	0.65	0.18	0.35
Infused volumes								
Glucose vol., mL	140±74	189±87	105±60	153±93	49±24	0.01	0.72	<0.001
Ketone/saline vol., mL	368±65	380±72	421±83	417±86	4±11	0.70	0.48	0.30

Blood sample measures and infused volumes during the study. Data are mean±SD, median (interquartile range) or mean±SEM (overall change). The measures were performed at the end of each intervention period, and the difference between 3-hydroxybutyrate and placebo was calculated for the entire study cohort (overall change). *P* value refers to 3-way repeated measures ANOVA test for effect of intervention, interaction of group (idiopathic PAH vs CTEPH), and interaction between infusion sequence (inf. seq: [saline→3-hydroxybutyrate versus 3-hydroxybutyrate→saline]). BNP levels were analyzed using log-transformed data. CTEPH indicates chronic thromboembolic pulmonary hypertension; and PAH, pulmonary arterial hypertension.

*$P<0.05$ vs saline.

†$P<0.01$ vs saline.

‡$P<0.001$ vs saline (pairwise comparisons).

Table 3. Hemodynamic Measures and Echocardiographic Findings

Hemodynamic parameter	PAH (n=10)		CTEPH (n=10)		Overall change (n=20)	*P* value (3-way repeat. ANOVA)	Interaction (group)	Interaction (inf. seq.)
	Saline	3-OHB	Saline	3-OHB				
Cardiac output, L/min	4.5±0.6	5.8±0.3‡	4.0±0.9	5.0±1.2†	1.2±0.1	<0.001	0.27	0.01
Stroke volume, mL	68±7	75±9†	55±8	63±8†	8±1	<0.001	0.96	0.73
Right atrial pressure, mm Hg	3.5±2.0	2.4±2.1†	5.9±4.2	5.9±4.4	−0.6±0.2	0.02	0.02	0.48
Mean pulmonary arterial pressure, mm Hg	43±11	45±12*	39±10	40±9	1.7±0.8	0.03	0.29	0.24
Pulmonary capillary wedge pressure, mm Hg	9.0±4.5	9.3±3.3	12±4	11±4	0.1±0.4	0.91	0.59	0.34
Transpulmonary gradient, mm Hg	34±10	36±10	27±9	28±9	1.6±0.9	0.07	0.55	0.13
Pulmonary vascular resistance, WU	7.5±2.4	6.2±1.8†	7.3±3.4	6.0±1.4*	−1.3±0.3	<0.001	0.98	0.99
Mean arterial pressure, mm Hg	90±13	84±11*	94±11	92±13	−4.2±1.6	0.02	0.26	0.84
Systemic vascular resistance, WU	20±5	15±4‡	23±5	17±3†	−5.2±0.7	<0.001	0.81	0.17
Heart rate, bpm	67±5	77±3‡	72±11	80±12†	8.9±1.4	<0.001	0.22	0.004
Transcutaneous oxygen tension, %	95±2	96±2	93±3	93±3	0.8±0.5	0.12	0.91	0.25
Mixed venous oxygen saturation, %	68±4	73±3‡	65±7	70±6‡	5.2±0.6	<0.001	0.87	0.42
Echocardiographic findings								
RV-free wall strain, %	17.8±3.4	18.3±4.4	14.2±4.9	15.1±5.3	0.9±0.9	0.39	0.73	0.67
RV-global strain, %	17.0±2.8	17.8±3.5	14.5±3.3	15.8±3.9*	1.0±0.6	0.11	0.70	0.67
RV systolic tricuspid annular velocity, cm/s	11.8±1.3	13.5±2.1†	10.3±1.8	11.4±3.1	1.4±0.4	0.002	0.45	0.80
Tricuspid annular plane systolic excursion, mm	17.6±3.3	20.1±5.0	14.7±4.4	14.1±3.6	0.9±0.9	0.31	0.10	0.38
RV-diastolic area, cm^2	23.5±4.2	25.0±4.5	24.3±7.8	24.3±6.9	0.2±0.7	0.75	0.72	0.38
RV-systolic area, cm^2	16.1±3.8	15.5±5.6	17.4±6.0	16.8±5.1	−0.7±0.5	0.28	0.97	0.97
RV-fractional area, %	31.7±9.2	35.4±12	28.3±6.7	30.3±10.4	2.8±2.3	0.26	0.74	0.38
Right atrial area, cm^2	17.3±4.5	16.4±3.7	26.5±6.2	24.2±6.7	−1.6±0.8	0.06	0.40	0.31
LV-global longitudinal strain, %	19.2±1.8	21.2±2.5‡	18.6±1.7	19.5±1.9†	1.5±0.3	<0.001	0.04	0.98
LV ejection fraction, %	61±6	66±4*	59±8	61±8	4±2	0.01	0.38	0.02
LV outflow tract velocity time integral, cm	21.1±3.4	26.3±3.1‡	19.6±5.2	21.3±5.4	3.5±0.7	<0.001	0.01	0.07

Hemodynamic measures in each study group. Data are mean±SD or mean±SEM (overall change). The measures were performed at the end of each intervention period, and the difference between 3-OHB and saline was calculated for the entire study cohort (overall change). *P* value refers to 3-way repeated measures ANOVA test for effect of intervention, interaction of group (idiopathic PAH vs CTEPH) and interaction between infusion sequence (inf. seq: [saline→3-OHB versus 3-OHB→saline]). 3-OHB indicates 3-hydroxybutyrate 4; CTEPH, chronic thromboembolic pulmonary hypertension; LV, left ventricular; PAH, pulmonary arterial hypertension; RV, right ventricular; and WU, Wood units.

*$P<0.05$ vs saline.

†$P<0.01$ vs saline.

‡$P<0.001$ vs saline (pairwise comparisons).

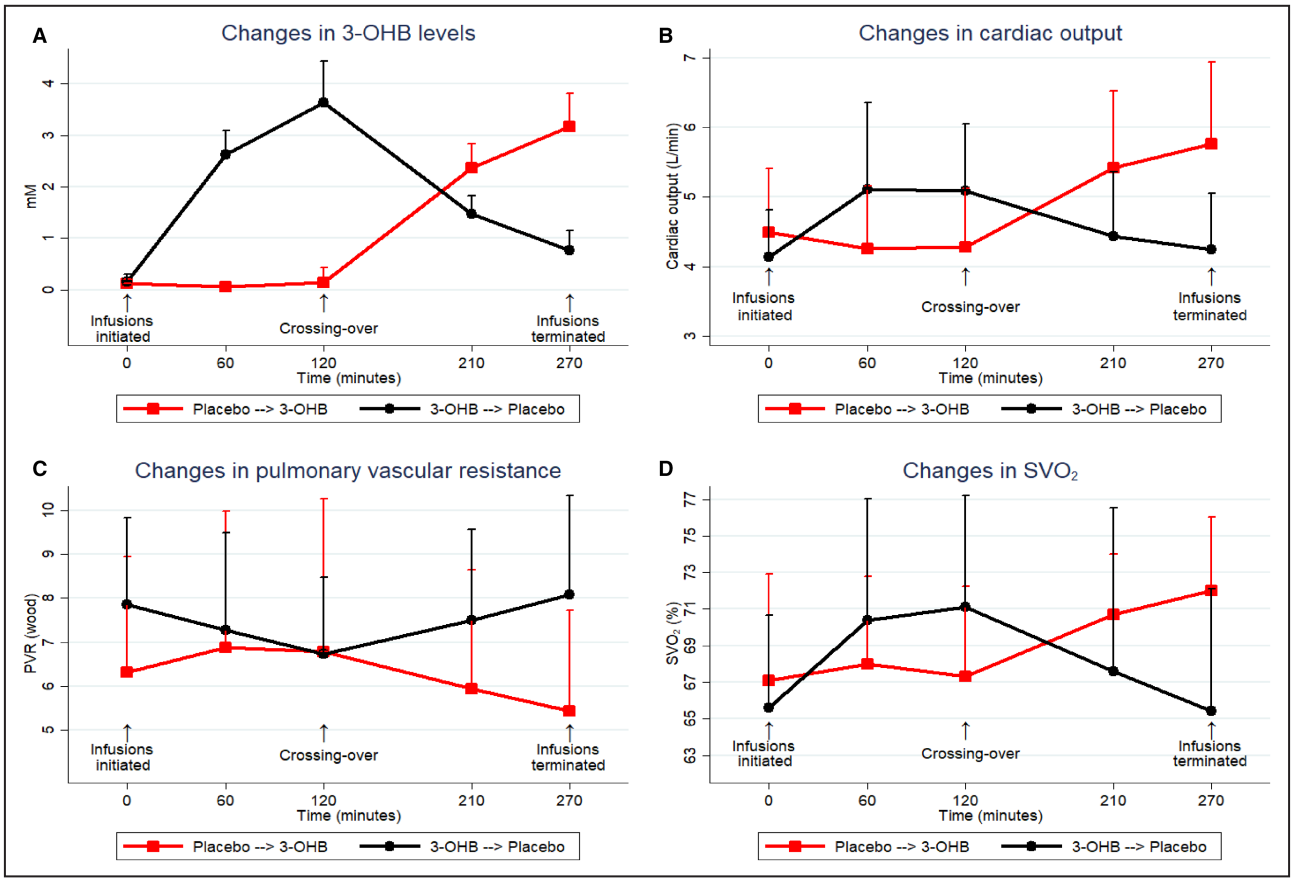

Figure 1. Changes in plasma 3-OHB levels, cardiac output, pulmonary vascular resistance, and SVO_2 during the study. **A**, 3-OHB levels were low until 3-OHB infusion was initiated and decreased after 3-OHB was substituted with placebo. **B**, Cardiac output increased from placebo to 3-OHB infusion and decreased when 3-OHB infusion was terminated. Pulmonary vascular resistance decreased (**C**) whereas SVO_2 increased (**D**) during 3-OHB infusion. Mean with bars indicating SEM. Crossover was performed following the measurements at 120 minutes (ie, 120–150 minutes). 3-OHB indicates 3-hydroxybutyrate; PVR, pulmonary vascular resistance; and SVO_2, mixed venous saturation.

In the isolated heart model, low 3-OHB concentrations compared with NaCl (2 mmol/L) had no significant effect on either peak RV systolic pressure or the rate of rise in RV systolic pressure (dP/dtmax). However, at a high concentration of 3-OHB compared with NaCl (10 mmol/L), the rate of rise in RV pressure was prolonged and, hence, lower despite similar RV systolic pressures, suggesting a negative inotropic effect on the RV. This runs contrary to previously reported data from the LV of isolated hearts.[10–12] It remains undetermined whether the present observations are due to a different impact of 3-OHB on the RV and LV or whether they are explained by differences between an conditioned RV in PAH and CTEPH high-pressure LV.

PVR Is Lowered by 3-OHB Irrespective of the Cause of Pulmonal Hypertension

Despite the decrease in PVR, we observed a small but significant increase in mPAP of 1 to 2 mm Hg (P=0.03). First, such observations should suggest caution in future trials in patients with PH, even though the mPAP increase was minor and no significant changes in transpulmonary gradient were observed. Second, in combination with the profound increase in CO, these findings may suggest that the decrease in PVR is not predominantly caused by vasodilatation but more likely due to recruitment of pulmonary vessels as a consequence of increased CO.[13] Hence, the observed hemodynamic effects of 3-OHB (ie, increased CO, decreased PVR, and a moderate, significant increase in mPAP) are similar to previously reported hemodynamic changes during exercise, which were mainly due to recruitment of pulmonary vessels rather than distension of the vasculature.[14] Future studies are needed to define the dose of 3-OHB that yields the optimal balance between the increase in CO, the drop in PVR, and the resultant effect on mPAP.

Like PVR, systemic vascular resistance decreased during 3-OHB infusion compared with placebo (Table 3). In addition, the increase in CO and the associated decrease in systemic vascular resistance

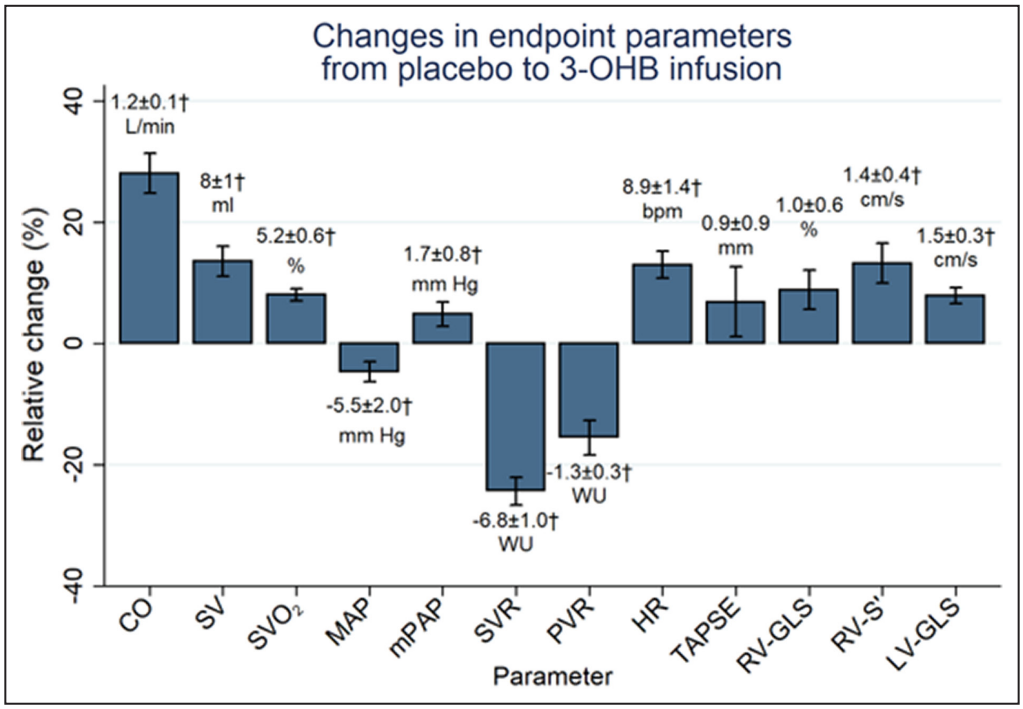

Figure 2. Mean relative hemodynamic change with SEM and the corresponding mean absolute change±SEM listed above or below each bar.

†Denotes relative changes with a *P* value <0.05. 3-OHB indicates 3-hydroxybutyrate; CO, cardiac output; HR, heart rate; LV-GLS, left ventricular global longitudinal strain; MAP, mean arterial pressure; mPAP, mean pulmonary pressure; PVR, pulmonary vascular resistance; RV-GLS, right ventricular global longitudinal strain; RV-S′, right ventricular systolic tricuspid annular velocity; SV, stroke volume; SVO_2, mixed venous saturation measured in the pulmonary artery; SVR, systemic vascular resistance; TAPSE, tricuspid annular plane systolic excursion; and WU, Wood units.

may initiate a feedback mechanism in the autonomic nervous system with a resultant vasorelaxation of the pulmonary vessels that contributes to the decrease in PVR. However, no changes in catecholamine levels have been observed previously during 3-OHB infusion, which argues against changes in sympathetic activity.[8]

Overall, there were no significant differences between patients with PAH and CTEPH regarding hemodynamic response to 3-OHB infusion. It should be noted that most of the enrolled patients had moderate symptoms (World Health Organization-class 2), their 6-minute walking distance was rather good (mean 445 m), and their NT-proBNP (N-terminal pro-B-type natriuretic peptide) levels were only slightly elevated (466 pg/mL; Table 1). Hence, it is unknown whether differences in hemodynamics between the groups during ketone infusion will be present in patients with more advanced PH.

In isolated pulmonary arteries, at lower 3-OHB concentrations (2 mmol/L), we observed no effect on pulmonary vascular tension. At concentrations of 10 mmol/L, 3-OHB produced a relative reduction in pulmonary arterial tension compared with NaCl. However, both 3-OHB and NaCl caused vasocontraction (Figure 4). This association between elevated osmolality and pulmonary arterial tension has previously been demonstrated in experimental studies.[15,16] As compared with NaCl at 10 mmol/L, the increase in pulmonary arterial tension was followed by a decrease in tension during 3-OHB at 10 mmol/L. Thus, 3-OHB produced a relative reduction in pulmonary arterial tension compared with NaCl. However, it is important to highlight that the largest change in pulmonary pressures resides in the capillaries and to a lesser extent in the small arteries as here assessed.[17] Despite this, the capillaries are devoid of vasomotor control, whereas the arteries, as investigated here, are the primary sites of vasoactive mediators.[18]

In Patients With PAH and CTEPH 3-OHB Is an Inodilator

The present results may have clinical implications as management of acute hemodynamic worsening is challenging in patients with PAH and CTEPH. Dobutamine may be used to increase CO and decrease PVR like the observed findings during 3-OHB infusion.[19] This effect of dobutamine is considered to be due to recruitment of pulmonary vessels, but, as opposed to 3-OHB, dobutamine simultaneously increases transpulmonary gradient.[19] When dobutamine

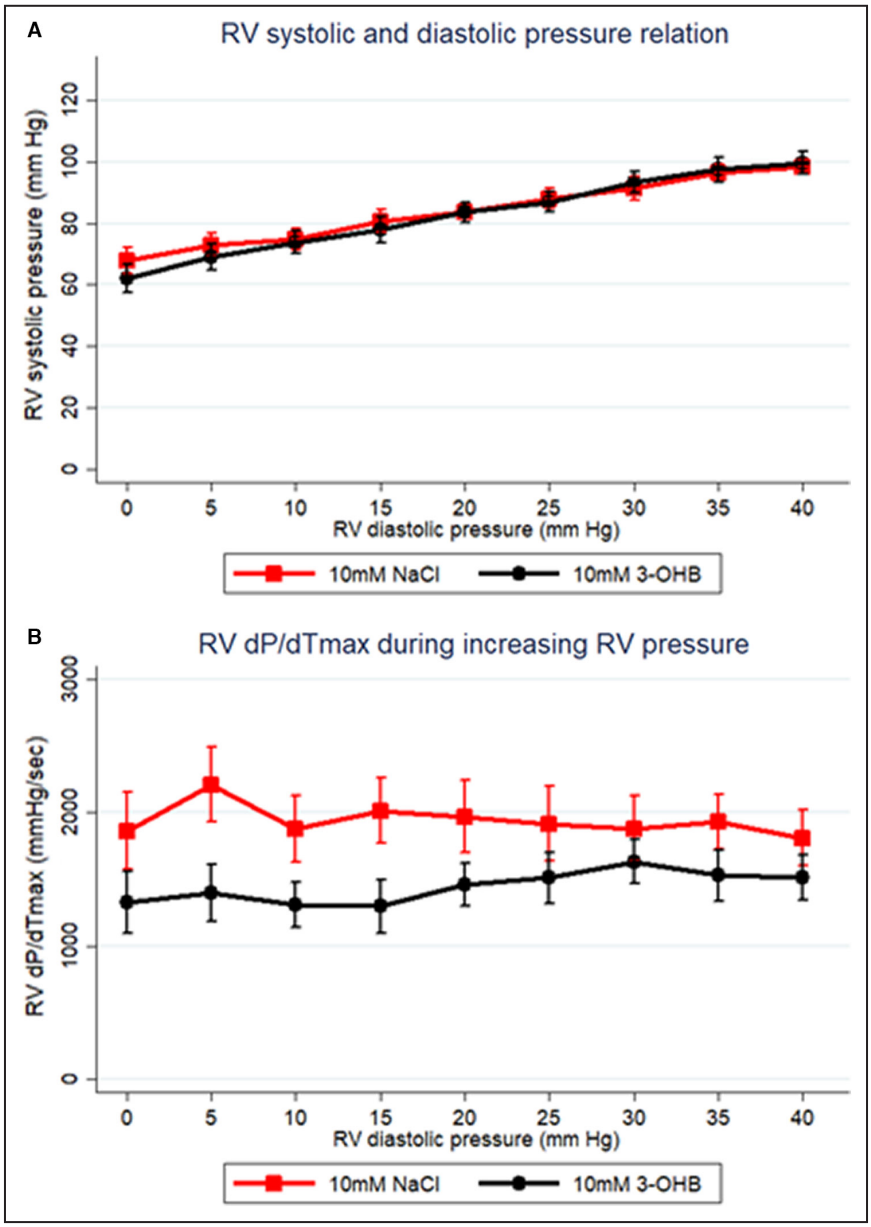

Figure 3. Right ventricular (RV) pressure (A) and dP/dtmax in the isolated rat heart (B) during increased diastolic pressure at 10 mmol/L NaCl (red line) vs 10 mmol/L Na-3-OHB (black line).
No difference was observed in RV systolic pressure (P=0.63), but RV dP/dtmax (P=0.02) was lower in the group receiving 3-OHB. Mean with bars indicating standard error of mean. 3-OHB indicates sodium-3-hydroxybutyrate; NaCl, sodium chloride; and RV, right ventricle.

is combined with a direct vasodilator, such as inhaled nitric oxide, an increase in transpulmonary gradient is not observed.[19] Consequently, 3-OHB has effects similar to those observed with a combination of dobutamine and inhaled nitric oxide. In addition, the present experimental data support that 3-OHB may elicit a direct pulmonary vasodilation beyond recruitment of pulmonary vessels. Hence, the present study forms the basis for future studies in patients with PAH and CTEPH with acute hemodynamic worsening.

It has been speculated that the cardiac effects of 3-OHB are related to a more favorable energy profile in terms of adenosine triphosphate generation.[10,20,21] However, 3-OHB administration has no effect on myocardial external energy efficiency despite a rapid shift in energy substrate metabolism during short-term intravenous infusion of 3-OHB.[7,8] Hence, we find it unlikely that 3-OHB administration exerts the present observed hemodynamic effects due to changes in cardiac energy metabolism.

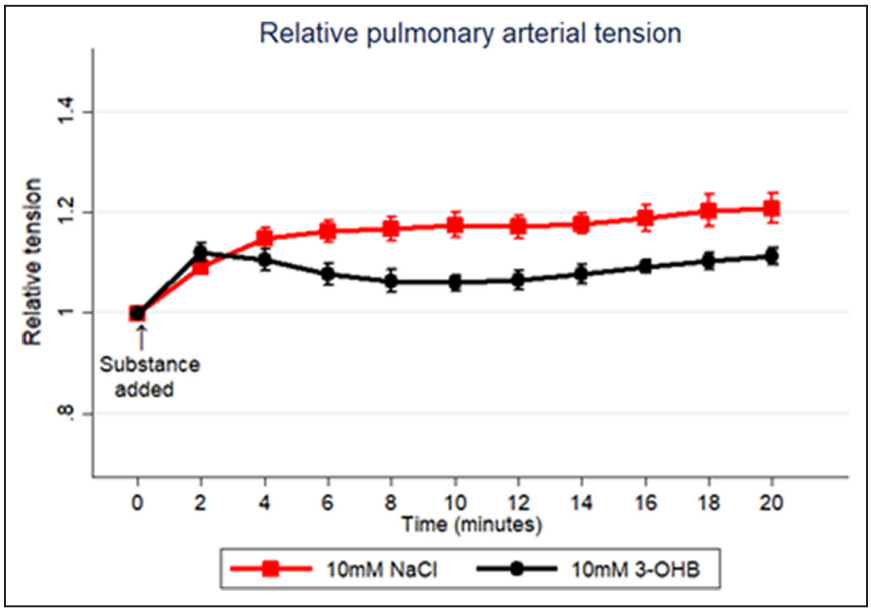

Figure 4. Relative changes in pulmonary arterial tension (isolated rat pulmonary arteries).
Tension increased in response to both solutions but was lower with Na-3-OHB (P<0.001). Mean with bars indicating SEM. 3-OHB indicates sodium-3-hydroxybutyrate; and NaCl, sodium chloride.

Study Limitations

CO and HR increased less in patients who received 3-OHB in the first infusion period, which may suggest a carryover effect from the 3-OHB infusion into the placebo period. Despite this, on both variables, the effect of 3-OHB infusion remained significant compared with saline.

Future studies should address the impact on contractility by use of pressure-volume catheters in a whole-body setting. However, irrespective of the mechanism, 3-OHB had beneficial hemodynamic effects.

Administration of 3-OHB was associated with increases in lactate levels. This association has been demonstrated previously[7,8] and is conceivably due to increased lactate production from pyruvate[22–24] and is not believed to affect CO.[25]

Na-3-OHB dissociates into 3-OHB^- and Na^+ 3-OHB^- acts as a weak base and increases pH.[26] However, it is improbable that extracellular pH changes caused the observed hemodynamic effects because the changes in pH were marginal with an overall mean increase from 7.43 to 7.46 (Table 3, Figure S2A). Furthermore, CO decreased during placebo infusion in patients randomized to 3-OHB followed by placebo (Figure 1B) despite persistent pH elevation during the placebo phase in these patients (Figure S2A).

The minor increase of 15% in circulating insulin levels during 3-OHB administration is unlikely to increase CO.[27–29]

It remains unknown whether a PH animal model would have produced different results. We decided to assess the effects in a healthy model as it has previously been shown that the hemodynamic effects of ketones are also present in both healthy test subjects and patients with cardiovascular disease.[8] Likewise, it remains undetermined whether sex or age can have an impact on the effects of 3-OHB administration.

Finally, we cannot infer whether long-term treatment or repetitive infusion with ketone bodies would produce different effects. Likewise, it is unknown whether similar hemodynamic effects would be achieved with a ketogenic diet and in patients with de novo PAH or CTEPH or whether background PH therapy might have an impact on the effects of 3-OHB.

CONCLUSIONS

In chronic and stable patients with PAH or CTEPH and residual PH, 3-OHB administration increased CO and reduced PVR. In an experimental setup, we observed that 3-OHB reduced pulmonary vascular tension, compared with NaCl.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Data S1
Figures S1–S3
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Supplemental Material

Data S1.

Supplemental Methods

Design

The participants were randomized to receive 3-OHB or saline or opposite in 1:1 order. The randomization sequence was coded in STATA version 14 (StataCorp, USA) and imported into the electronic case report forms (eCRF).

The participants were enrolled and investigated from August 2020 to Marts 2021.

Blood samples

Blood samples were drawn from the study participants before and during the examinations. Glucose, electrolytes, lactate, and pH were analyzed using a YSI STAT 2100 (YSI Inc., Netherlands) immediately after sampling. All other samples were stored at –20^{o}C and analyzed in batches. Plasma (P) 3-OHB was measured using hydrophilic interaction liquid chromatography tandem mass spectrometry (HILIC-MS/MS). Insulin was measured with ELISA (Mercodia, Sweden) and free fatty acids (FFA) quantified using an *in vitro* enzymatic colorimetric method assay (Trichem, Denmark).

Animal studies

Healthy male Sprague-Dawley rats (10 weeks, 350-400 g, Taconic, Ry, Denmark) (n=15) were used. The rat hearts were isolated and perfused ex vivo. Briefly, rats were anesthetized with a mixture of Dormicum (midazolam (0.5mg kg-1 body weight); Matrix Pharmaceuticals, Herlev, Denmark) and Hypnorm (fentanyl citrate (0.158 mg kg-1 body weight) and fluanisone (0.5mg Kg-1 body weight)) administered subcutaneously. Following anesthesia, the rats were tracheotomized and

coupled to a mechanical ventilator with atmospheric air (Ugo Basile 7025 rodent ventilator, Comerio, Varese, Italy) whereupon a thoracotomy and laparotomy were performed. Subsequently, a bolus of 500 IU heparin (Leo Pharma, Ballerup, Denmark) was administered intravenously and the ascending aorta was cannulated and retrogradely perfused at constant pressure of 80 mmHg with an oxygenated (95% O2 and 5% CO2) KH buffer. The heart was excised and transferred under perfusion to an isolated perfused heart system (IH-SR type844/1; HSE, March-Huhstettem, Germany) with a constant temperature of 37 °C. Following isolation and perfusion ex vivo, an interventricular balloon (size 7, HSE, March-Hugstetten, Germany) was inserted into the right ventricle through the right atrial appendage, and the volume was adjusted to a RV end-diastolic pressure of 0 mmHg. After 40 minutes of stabilization, systolic pressures and the rate of rise in systolic pressures (dP/dtmax) of the right ventricle were obtained during gradual 5-mmHg increments in end-diastolic pressure until a final pressure of 40 mmHg was reached, as previously described.[30] The isolated hearts were randomized to receive either 2 mM NaCl and 2 mM Na-3-OHB (n=7), or 10 mM NaCl and 10 mM Na-3-OHB (n=8) in a cross-over design.

Segments of fourth order pulmonary arteries (approximately 2 mm long) were isolated from rats (n=7) and mounted on wire myographs for isometric evaluation, as previously described.[31] Briefly, pulmonary arteries were mounted in PSS-filled wire myographs (DMT, Aarhus, Denmark) aerated with 5% CO2/balance air using 40 µm wires.[32] Vessels were normalized to the internal diameter corresponding to a transmural pressure of 3,9 kPa as previously described.[33] Following normalization all vessels were exposed to a warm-up procedure consisting of five contractions of 1 minute duration, obtained by increasing extracellular K+ concentration to 60 mM followed by a maximal contraction obtained with 120 mM K+ and 0.1 µM U46619 (thromboxane A2 analogue). Vasorelaxations were tested in arteries developing a stable level of pre-constriction (corresponding to 50% of initial maximal contraction) using U46619. All force data is shown relative to this pre-constriction.

From each animal, two pulmonary arteries were investigated. One was subjected to NaCl (2 mM and 10 mM – randomized order) and the other to Na-3-OHB (2 mM and 10 mM – randomized order). For all animal measures, the assessors were blinded for treatment.

Power calculation and statistics

The standard deviation of the differences in CO measurement is 4%.[34] Hence, using a paired design and comparing changes in CO from placebo to 3-OHB infusion at the end of each infusion periods, we expected to be able to detect relative differences of 3% (N=20) in CO at a power of 80% and an alpha of 5%.

Data were investigated for normal distribution by qq-plots and transformed when appropriate. Unpaired two-tailed t-test, Kruskal-Wallis or Fisher's exact test were used for comparison between groups (baseline characteristics). Changes in data are presented as mean ± standard error of mean (SEM) and between-group differences as mean ± standard deviation (SD) if not stated otherwise. Three-way repeated measures ANOVA tests were applied with the following independent variables: intervention, group (CTEPH versus PAH) and intervention sequence (placebo→ 3-OHB versus 3-OHB → placebo). The dependent variables were blood sample measurements, infused volumes, hemodynamic and echocardiographic measurements with each dependent variables assessed separately. Pairwise comparisons were made when the overall ANOVA analysis demonstrated a significant difference. The animal results were analyzed using mixed effects models with the following independent variables: Intervention (placebo versus 3-OHB),RV diastolic pressure (isolated RV) and time (isolated pulmonary arteries). Time as an independent variable was applied for both 2 mM and 10 mM concentrations and no comparisons between 2 mM and 10 mM was performed. The dependent variables were RV systolic pressure and dP/dTmax (isolated RV) and for the isolated pulmonary arteries it was relative vasorelaxation. P-values <0.05 were considered

statistically significant. All data analyzes were performed using STATA version 14 (StataCorp, USA) or Prism 9 (GraphPad, USA).

Ethics

Study data were collected into the eCRF and managed using REDCap electronic data capture tools hosted at Aarhus University hospital.

All animal handling was performed in accordance with Danish guidelines and legislation (Act No. 1306 of 23/11/2007) and following Guide for the Care and Use of Laboratory Animals. The experimental setup was approved by The Danish Animal Experiments Inspectorate, Ministry of Environment and Food of Denmark with license number: 2018-15-0201-01475. All animal experiments were conducted in accordance with the ARRIVE guidelines.

The full protocol will be available upon request.

Figure S1: CONSORT diagram

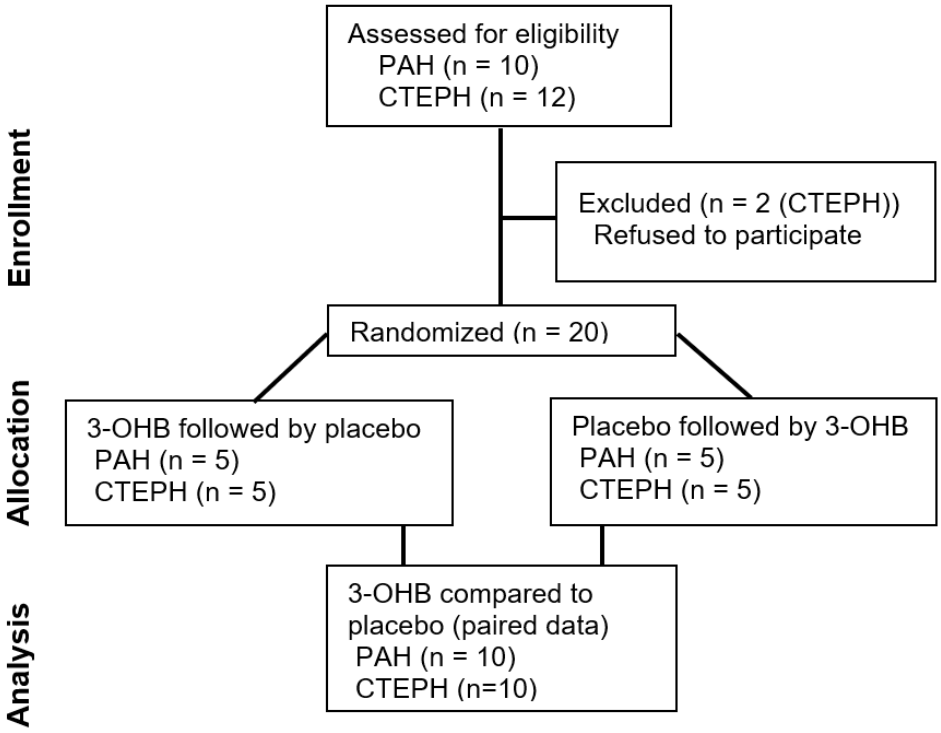
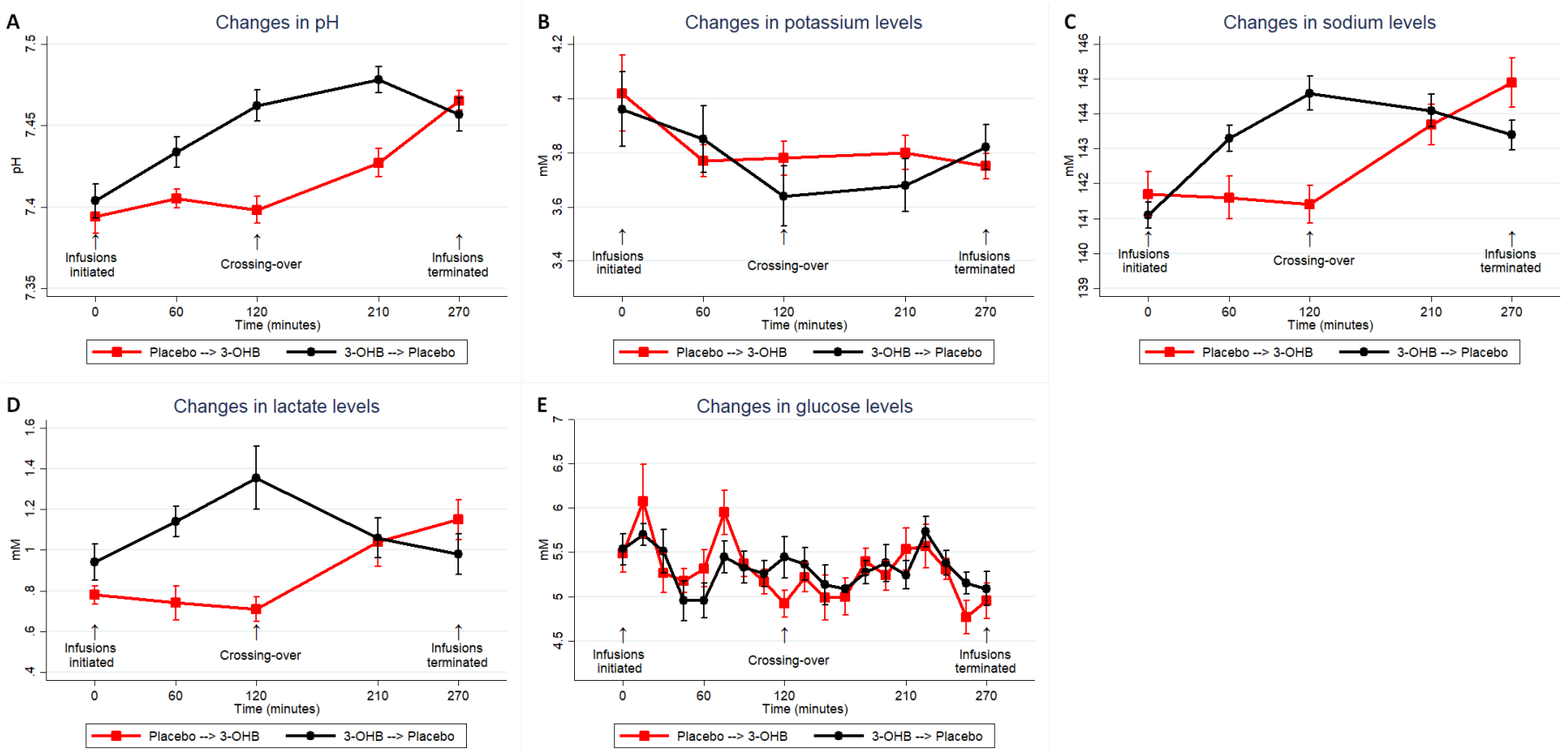


Figure S1: 3-OHB: 3-hydroxybutyrate, CTEPH: chronic thromboembolic pulmonary hypertension, PAH: pulmonary arterial hypertension.

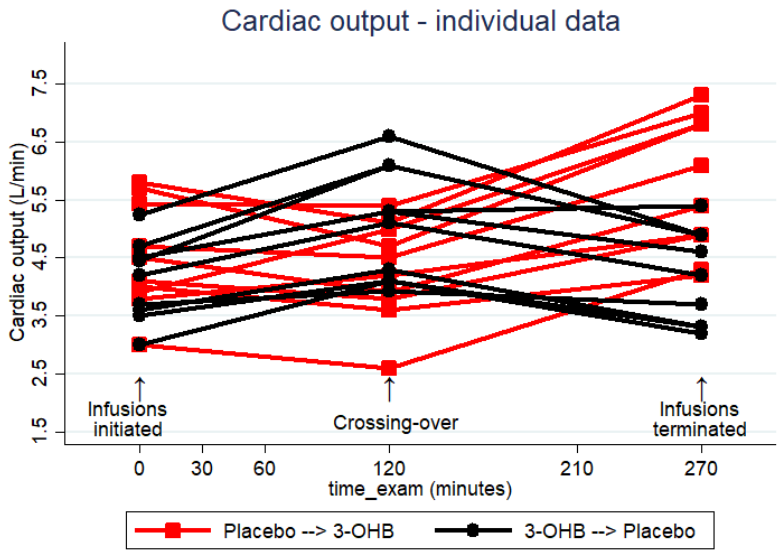
Figure S2: The figure demonstrates changes in (A) pH, (B) potassium, (C) sodium, (D) lactate, and (E) glucose levels during the study.



Cross-over was performed following the measurements at 120 minutes (i.e. 120-150 minutes).

Grey lines with squares depict patients initiated on placebo followed by 3-OHB and black lines with dots depict patients initiated on 3-OHB followed by placebo. Mean with bars indicating standard error of mean. 3-OHB: 3-hydroxybutyrate.

Figure S3: Changes in cardiac output in each study subject.



Cross-over was performed following the measurements at 120 minutes (i.e. 120-150 minutes).

Black lines with squares depict patients initiated on placebo followed by 3-OHB and black lines with dots depict patients initiated on 3-OHB followed by placebo.

3-OHB: 3-hydroxybutyrate.