SUPPLEMENTAL MATERIAL

Cardiovascular Effects of Prolonged Oral Ketone Ester Treatment in Patients with Heart Failure with Reduced Ejection Fraction. A Randomized, Controlled, Double-Blind Trial

Kristoffer Berg-Hansen, MD,[a,b] Nigopan Gopalasingam, MD,[a,b] Kristian Hylleberg Christensen, MD,[a,b] Bertil Ladefoged, MD,[a,b] Mads Jønsson Andersen,[a] Steen Hvitfeldt Poulsen,[a,b] Barry A. Borlaug, MD, Prof,[c] Roni Nielsen, MD, PhD,[a] Niels Møller, MD, DMSc, Prof,[b,d] Henrik Wiggers, MD, DMSc, Prof[a,b]

a: Department of Cardiology, Aarhus University Hospital, Aarhus, Denmark
b: Department of Clinical Medicine, Faculty of Health, Aarhus University, Aarhus, Denmark
c: Department of Cardiovascular Medicine, Mayo Clinic Hospital, Rochester, Minnesota, USA
d: Department of Endocrinology and Metabolism, Aarhus University Hospital, Aarhus, Denmark

Address for correspondence:
Kristoffer Berg-Hansen
Department of Cardiology, Aarhus University Hospital
Palle Juul-Jensens Boulevard 99, DK-8200 Aarhus N, Denmark
E-mail: krisbe@rm.dk
Twitter: KrisBergHansen

S1 – Eligibility Criteria

Inclusion Criteria

Participants qualify for randomization in the study only when they meet all inclusion criteria and none of the exclusion criteria.:

1. Left ventricular ejection fraction ≤40%
2. Experience of dyspnea symptoms (NYHA II-III) without a non-cardiac or ischemic explanation.
3. Age ≥18 years old
4. The participant must be on optimal, stable guideline-recommended treatment for heart failure with reduced ejection fraction, as per the local Danish heart failure guidelines aligned with the European Guidelines. This regimen should include an angiotensin-converting enzyme inhibitor (ACE-I), an angiotensin II receptor blocker (ARB), or an angiotensin receptor-neprilysin inhibitor (ARNI), a beta-blocker, and a mineralocorticoid receptor antagonist (MRA) before randomization. If indicated, a device such as an implantable cardioverter defibrillator (ICD) should be implanted 30 days before randomization, and cardiac resynchronization therapy (CRT) should be implanted 90 days before randomization, in accordance with National Guidelines.

Exclusion Criteria

1. A history of type I or type II diabetes
2. Glycated hemoglobin (HbA1c) ≥48 mmol/mol (≥6.5%)
3. Severe stable angina pectoris
4. Recent hospitalization (<30 days)
5. Significant valvular heart disease (>moderate stenosis, >moderate regurgitation)
6. Dyspnea due to primary lung disease or myocardial ischemia in the opinion of the investigator
7. Severe kidney disease (estimated GFR<20 and/or current dialysis) or liver disease
8. Women of childbearing potential unwilling to use a medically accepted method of contraception or those currently pregnant (confirmed with a positive pregnancy test) or breastfeeding are not eligible.
9. Subjects with severe hepatic impairment (Child-Pugh class C)
10. Dementia
11. Immobilization and inability to perform an exercise test
12. Pregnancy
13. Subjects on current ketogenic diet

S2 – Supplemental Equations

Calculation of the left ventricular end-diastolic pressure-volume relationship was based on the following equations:

$$\textbf{Eq. 1: } V_0 = \text{LVEDV} \cdot (0.6 - 0.006 \cdot \text{LVEDP})$$

$$\textbf{Eq. 2: } V_{30} = V_0 + \frac{(\text{LVEDV} - V_0)}{\left(\frac{\text{LVEDP}}{A_n}\right)^{B_n}}$$

$$\textbf{Eq. 3: } \beta = \frac{\log\left(\frac{\text{LVEDP}}{30}\right)}{\log\left(\frac{\text{LVEDV}}{V_{30}}\right)}$$

$$\textbf{Eq. 4: } \alpha = \frac{30}{(V_{30})^{\beta}}$$

$$\textbf{Eq. 5: } \text{LVEDP} = \alpha \cdot \text{LVEDV}^{\beta}$$

Constants A_n (28.2 mmHg) and B_n (2.79) are derived from a previous study describing and validating the method in detail.[1] LVEDP indicates left ventricular end-diastolic pressure (derived from pulmonary capillary wedge pressure) and LVEDV indicates left ventricular end-diastolic volume.

[1] Klotz S, Hay I, Dickstein ML, Yi GH, Wang J, Maurer MS, Kass DA, Burkhoff D. Single-beat estimation of end-diastolic pressure-volume relationship: A novel method with potential for noninvasive application. *Am J Physiol - Hear Circ Physiol* 2006;291:403–412. doi:10.1152/ajpheart.01240.2005.

S3 – Supplemental Tables

Table S1: Composition of the Ketone Ester and Isocaloric Comparator

	Ketone ester		Isocaloric comparator	
	Per serving	Per day	Per serving	Per day
Carbohydrate, g (kcal)	-	-	10.3 (40)	41.2 (160)
Fat, g (kcal)	-	-	8.9 (80)	35.6 (320)
Ketone ester, g (kcal)	25 (120)	100 (480)	-	-
Sodium, mg	30	120	1.3	5.2
Potassium, mg	83	332	-	-
Total fluid volume, mL	53	212	53	212
Energy, kcal	120	480	120	480

The Ketone Ester was taste-matched to the isocaloric comparator by adding a bitterness additive (denatonium benzoate, Mentholatum Go' Negl, Mentholatum, Denmark); both interventions were added equal amounts of stevia drops (Easis, Denmark).

Table S2: Interaction between Treatment Effect on Hemodynamic Parameters and Treatment Sequence or Study Period at Trough Level and After Dosing

	Trough levels		**After dosing**	
	Interaction (Treatment sequence)	Interaction (Visit 1 vs. 2)	Interaction (Treatment sequence)	Interaction (Visit 1 vs. 2)
Cardiac Output, L/min	0.319	0.459	0.923	0.586
Stroke Volume, mL	0.410	0.253	0.814	0.325
Heart rate, min^{-1}	0.541	0.192	0.963	0.231
Systolic BP, mmHg	0.698	0.951	0.643	0.733
Diastolic BP, mmHg	0.554	0.842	0.832	0.458
SaO_2, %	0.354	0.982	0.544	0.959
SvO_2, %	0.699	0.771	0.131	0.960
AVO_2-difference, mL/dL	0.321	0.220	0.574	0.369
PCWP, mmHg	0.976	0.753	0.558	0.932
mPAP, mmHg	0.085	0.372	0.403	0.354
RAP, mmHg	0.697	0.473	0.466	0.579
PCWP/CO	0.939	0.374	0.896	0.544
SVR, dyn·s/cm^5	0.733	0.250	0.584	0.210
PVR, dyn·s/cm^5	0.060	0.118	0.886	**0.028**
Ea, mmHg/mL	0.493	0.133	0.858	0.154
PRSW, g/cm^2	0.314	0.768	0.995	0.985
LVSW/EDV, g/mL	0.355	0.180	0.625	0.199
Ees, mmHg/mL	0.493	**0.041**	0.891	0.069
Ea/Ees	0.725	0.334	0.815	0.266
V_{15}, mL	0.466	**0.028**	0.852	0.053
V_{30}, mL	0.049	**0.028**	0.934	0.053
LV stiffness ß	0.647	0.757	0.471	0.795

Values are associated interaction *P*-values from a mixed model which incorporated repeated measurements for after dosing analysis. Bold values indicate $P < 0.05$.

AVO_2-difference, Arterio-venous oxygen difference; BP, blood pressure; CO, cardiac output; Ea, systemic effective arterial elastance; Ea/Ees, vascular-ventricular coupling, EDV, end-diastolic volume; Ees, end-systolic elastance; LV, left ventricle; LVSW, left ventricular stroke work; MAP, mean arterial pressure; mPAP, mean pulmonary arterial pressure; PA, pulmonary arterial; PCWP, pulmonary capillary wedge pressure; PRSW, preload recruitable stroke work; PVR, pulmonary vascular resistance; RAP, right atrial pressure; SaO_2, arterial oxygen saturation; SvO_2, mixed venous saturation; SVR, systemic vascular resistance; V15, left ventricular (LV) end-diastolic volume (LVEDV) at a common LV end-diastolic pressure of 15 mmHg; V30, LVEDV at a common LV end-diastolic pressure of 30 mmHg.

Table S3: Correlation between Change in 3-OHB and Change in Endpoints

Δ Trough 3-OHB levels (KE – IC)		
Δ KE - IC	**R**	***P*-value**
Δ Cardiac output	0.013	0.930
Δ PCWP	0.1	0.490
Δ LVEF	0.23	0.140
Δ NT-proBNP	0.03	0.850
Δ KCCQ-12-CSS	-0.18	0.270
Δ Peak 3-OHB levels after KE dosing		
Δ Time 60 min	**R**	***P*-value**
Δ Cardiac output	-0.062	0.780
Δ PCWP	-0.43	**0.047**
Δ Systolic BP	-0.11	0.630
Δ LVEF	-0.059	0.80

3-OHB, 3-hydroxybutyrate; IC, isocaloric comparator; KCCQ-12-CSS, Kansas City Cardiomyopathy Questionnaire (12-item) Clinical Summary Score; KE, ketone ester; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro brain-natriuretic-peptide; PCWP, pulmonary capillary wedge pressure. Bold values indicate $P < 0.05$.

Table S4: Additional Resting Echocardiographic Parameters at Plasma Trough and After Dosing

	Ketone ester	**Isocaloric comparator**	**Pairwise difference (95% CI)**	***P*-value**
TROUGH LEVEL				
3D-LVEF, %	37 ± 5	34 ± 6	3 (1, 6)	**0.006**
3D-LVEDV, mL	196 ± 48	205 ± 52	-12 (-25, 2)	0.077
3D-LVESV, mL	125 ± 37	136 ± 40	-15 (-24, -5)	**0.006**
Mitral inflow E velocity, cm/sec	61 ± 16	65 ± 15	-3 (-8, 1)	0.103
Mitral inflow A velocity, cm/sec	73 ± 20	69 ± 18	3 (-1, 8)	0.123
Lateral e´ velocity, cm/sec	-3.3 ± 1.5	-3.6 ± 1.9	-0.4 (-0.8, 0.1)	0.127
Lateral a´ velocity, cm/sec	-5.8 ± 1.3	-5.5 ± 1.3	-0.3 (-0.6, -0.1)	**0.024**
AFTER DOSING				
3D-LVEF, %	37 ± 5	33 ± 5	4 (3, 5)	**<0.001**
3D-LVEDV, mL	206 ± 49	221 ± 53	-14 (-23, -5)	**0.002**
3D-LVESV, mL	132 ± 38	150 ± 42	-19 (-25, -12)	**<0.001**
Mitral inflow E velocity, cm/sec	59 ± 16	62 ± 16	-3 (-5, 0)	**0.031**
Mitral inflow A velocity, cm/sec	72 ± 18	70 ± 19	3 (1, 5)	**0.005**
Lateral e´ velocity, cm/sec	-3.7 ± 1.6	-3.2 ± 1.4	-0.6 (0.3, 0.9)	**<0.001**
Lateral a´ velocity, cm/sec	-5.9 ± 1.3	-5.5 ± 1.3	-0.5 (-0.6, -0.3)	**<0.001**

Values are mean±SD or geometric mean (95% CI) for each treatment and between-treatment pairwise comparison (95% CI) and associated *P*-values from a mixed model which incorporated repeated measurements for after dosing analysis. Bold values indicate $P < 0.05$.

3D, three-dimensional, e' and a', early and late diastolic mitral plane tissue velocities, respectively; LVEDV and LVESV, left ventricular end-diastolic and end-systolic volumes, respectively; LVEF, left ventricular ejection fraction. 3D-echocardiography was available in 16/24 (67%) participants.

Table S5: Interaction between Treatment Effect on Biomarkers, Quality of Life, and Echocardiographic Parameters and Treatment Sequence or Study Period

	Interaction (Treatment sequence)	Interaction (Visit 1 vs. 2)
3-OHB, µmol/L	0.763	0.865
NT-proBNP, ng/L	0.885	0.388
ALT, IU/L	**0.026**	0.994
Cystatin C, mg/L	0.185	0.777
eGFR, ml/min/1,73m²	0.406	0.260
Hemoglobin, g/dL	**0.001**	0.392
Hematocrit, %	**0.020**	0.242
Platelets, 10^9/L	0.224	0.137
Hs-cTnI, ng/L	0.390	0.175
P-3-Methoxyadrenalin, nmol/L	0.565	0.705
P-3-Methoxynoradrenalin, nmol/L	0.096	0.651
Weight, kg	0.853	**0.018**
Plasma volume, mL	0.110	**0.041**
KCCQ-12 Clinical Summary	0.932	0.768
KCCQ-12 Physical Limitation	0.701	0.983
LVEF, %	0.663	0.428
LVEDV, mL	0.241	**0.032**
LVESV, mL	0.179	**0.030**
GLS, %	0.923	0.531
S'max, cm/s	0.143	0.688
LA maximal volume, mL	0.352	0.302
LV mass, g	0.857	0.015
E/A	0.213	0.660
E/e´	0.811	0.323
TAPSE, mm	0.653	0.918

Values are associated interaction *P*-values from a mixed model which incorporated repeated measurements for after dosing analysis. Bold values indicate $P < 0.05$.

3-OHB, 3-hydroxybutyrate; ALT, Alanine transaminase; E/e', ratio of E and early diastolic mitral plane tissue velocity; eGFR, estimated glomerular filtration rate (estimated from cystatin c); GLS, global longitudinal strain; Hs-cTnI, high-sensitive cardiac troponin I; KCCQ-12, 12-item version of Kansas City Cardiomyopathy Questionnaire; LA, left atrial; LV, left ventricular; LVEDV and LVESV, LV end-diastolic and end-systolic volume, respectively; LVEF, LV ejection fraction; NT-proBNP, N-terminal pro brain-natriuretic-peptide; S'max, systolic mitral plane peak excursion velocity (6-point average); TAPSE, tricuspid annular peak systolic excursion.

Table S6: Resting Electrolytes at Trough Level and After Dosing

	TROUGH LEVEL				AFTER DOSING			
	Ketone ester	Isocaloric comparator	Pairwise difference (95% CI)	*P*-value	Ketone ester	Isocaloric comparator	Pairwise difference (95% CI)	*P*-value
pH	7.40 ± 0.03	7.39 ± 0.03	0.02 (0.00, 0.03)	**0.018**	7.39 ± 0.03	7.39 ± 0.03	-0.01 (-0.02, 0.00)	0.192
HCO_3^-, mmol/L	25.1 ± 2.0	24.3 ± 1.8	0.7 (0.2, 1.2)	**0.005**	23.8 ± 2.4	24.4 ± 1.6	-0.4 (-0.9, 0.1)	0.088
Sodium, mmol/L	139 ± 2	140 ± 2	-1 (-2, 0)	0.095	138 ± 2	138 ± 2	-1 (-1, 0)	0.083
Chloride, mmol/L	106.5 ± 2.9	107.9 ± 2.4	-1.3 (-2.5, 0.0)	**0.045**	106 ± 3	108 ± 2	-1 (-3, 0)	**0.024**
Potassium, mmol/L	4.0 ± 0.3	4.0 ± 0.3	0.1 (0.0, 0.1)	0.100	4.0 ± 0.3	3.9 ± 0.3	0.1 (0.0, 0.2)	**0.011**
Lactate, mmol/L	1.5 ± 0.6	1.5± 0.4	0.0 (-0.2, 0.2)	0.879	1.2 ± 0.5	1.1 ± 0.5	0.1 (0.0, 0.2)	**0.032**
Hematocrit, %	43.3 ± 3.3	42.6 ± 4.1	0.4 (-0.5, 1.3)	0.380	-	-	-	-
Platelets, 10^9/L	215 ± 54	219 ± 56	-3 (-11, 4)	0.373	-	-	-	-

Values are mean±SD or geometric mean (95% CI) for each treatment and between-treatment pairwise comparison (95% CI) and associated *P*-values from a mixed model which incorporated repeated measurements for after dosing analysis. Bold values indicate $P < 0.05$.

3-OHB, 3-hydroxybutyrate.

Table S7: Endpoint Parameters at Submaximal Exercise (75% of Maximal Exercise Capacity)

	Ketone ester	**Isocaloric comparator**	**Pairwise difference (95% CI)**	***P*-value**
INVASIVE HEMODYNAMICS				
Cardiac Output, L/min	10.2 ± 2.7	9.6 ± 2.7	0.4 (-0.5, 1.3)	0.409
Stroke volume, mL	104 ± 29	108 ± 57	-4 (-22, 13)	0.620
PCWP, mmHg	24 ± 9	26 ± 8	-2 (-5, 1)	0.171
mPAP, mmHg	31 ± 8	33 ± 7	-2 (-5, 1)	0.228
RAP, mmHg	5.8 ± 4.5	7.3 ± 4.8	-1 (-3, 1)	0.194
PCWP/CO	2.5 ± 1.2	3.0 ± 1.5	-0.4 (-0.8, -0.1)	**0.021**
SvO_2, %	36 ± 10	34 ± 9	3 (-2, 7)	0.222
AVO_2-difference, mL/dL	11.9 ± 1.6	12.0 ± 1.8	-0.2 (-1.2, 0.7)	0.591
EXERCISE PARAMETERS				
VO_2, mL/kg/min	13.7 ± 4.2	13.6 ± 4.3	0.1 (-1.2, 1.4)	0.886
Heart rate, min^{-1}	100 ± 17	97 ± 19	3 (-5, 11)	0.499
MAP, mmHg	105 ± 34	99 ± 22	4 (-9, 17)	0.533
METABOLIC PARAMETERS				
Lactate, mmol/L	2.75 ± 1.25	2.72 ± 1.45	0.1 (-1.0, 1.1)	0.890

Values are mean±SD. Bold values indicate $P < 0.05$.

AVO_2-difference, Arterio-venous oxygen difference; CO, cardiac output; MAP, mean arterial pressure; mPAP, mean pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; RAP, right atrial pressure; SvO_2, mixed venous saturation; VO_2, oxygen uptake during inspiration.

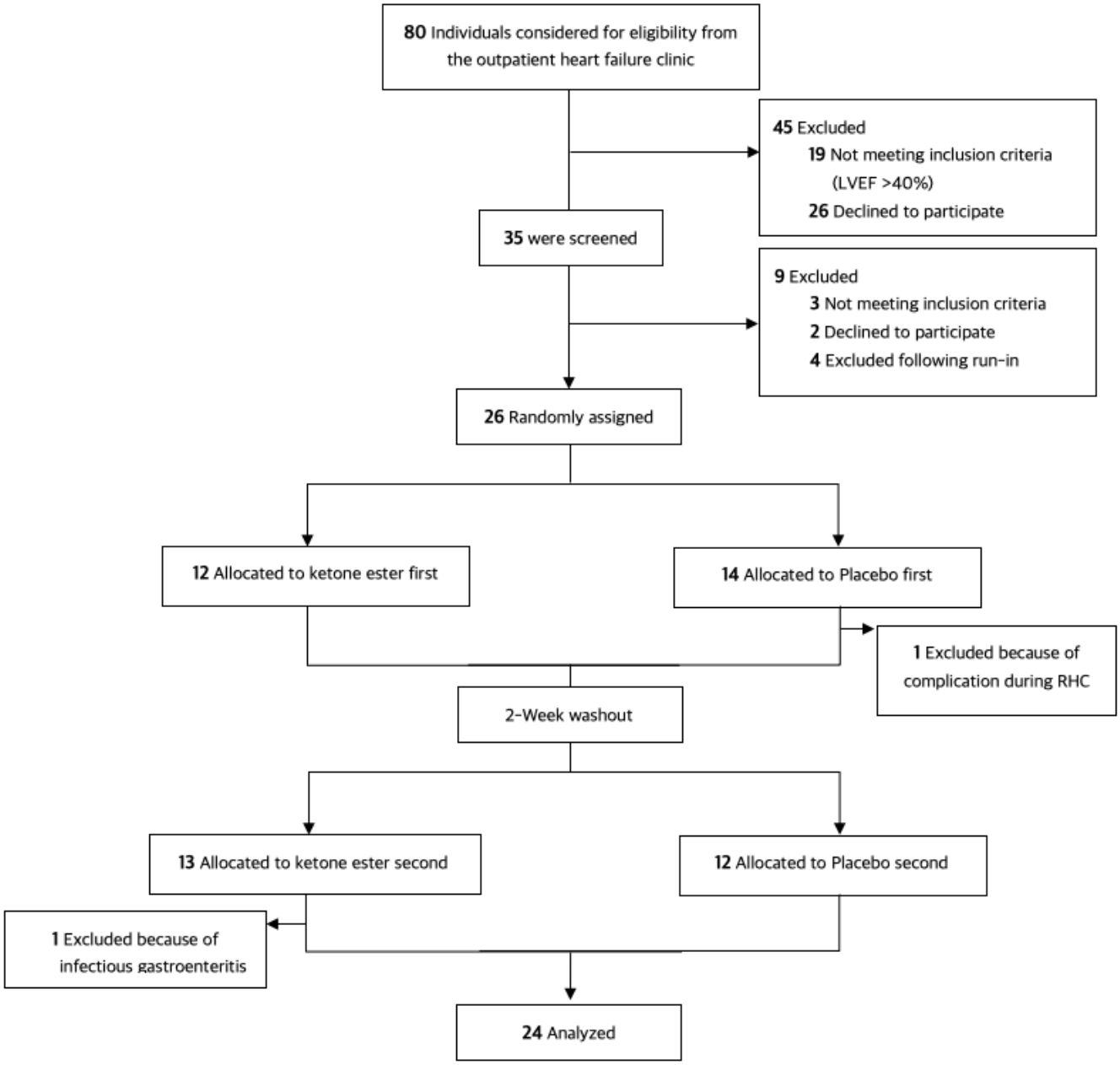
Table S8: Compliance and Adverse Events

	Ketone ester	Isocaloric comparator
COMPLIANCE		
Compliance (>80%)	24 (100%)	24 (100%)
Missed drinks	0.3±0.5	0.4±0.7
ADVERSE EVENTS		
Reflux	1 (2.1%)	0 (0%)
Mild fatigue	4 (8.3%)	0 (0%)
Headache	3 (6.2%)	0 (0%)
Angina	3 (6.2%)	2 (4.1%)
Abdominal pain	2 (4.2%)	0 (0%)
Insomnia	1 (2.1%)	0 (0%)
Reduced appetite	2 (4.2%)	0 (0%)
Flatulence	2 (4.2%)	1 (2.0%)
Palpitations	2 (4.2%)	1 (2.0%)
Cough	0 (0%)	2 (4.1%)
Diarrhea	2 (4.2%)	4 (8.2%)
Dizziness	1 (2.1%)	0 (0%)
Cold	1 (2.1%)	0 (0%)
Serious adverse events	0 (0%)	1 (2.0%)

Distribution of compliance and adverse events. The values are presented as absolute numbers and percentages.

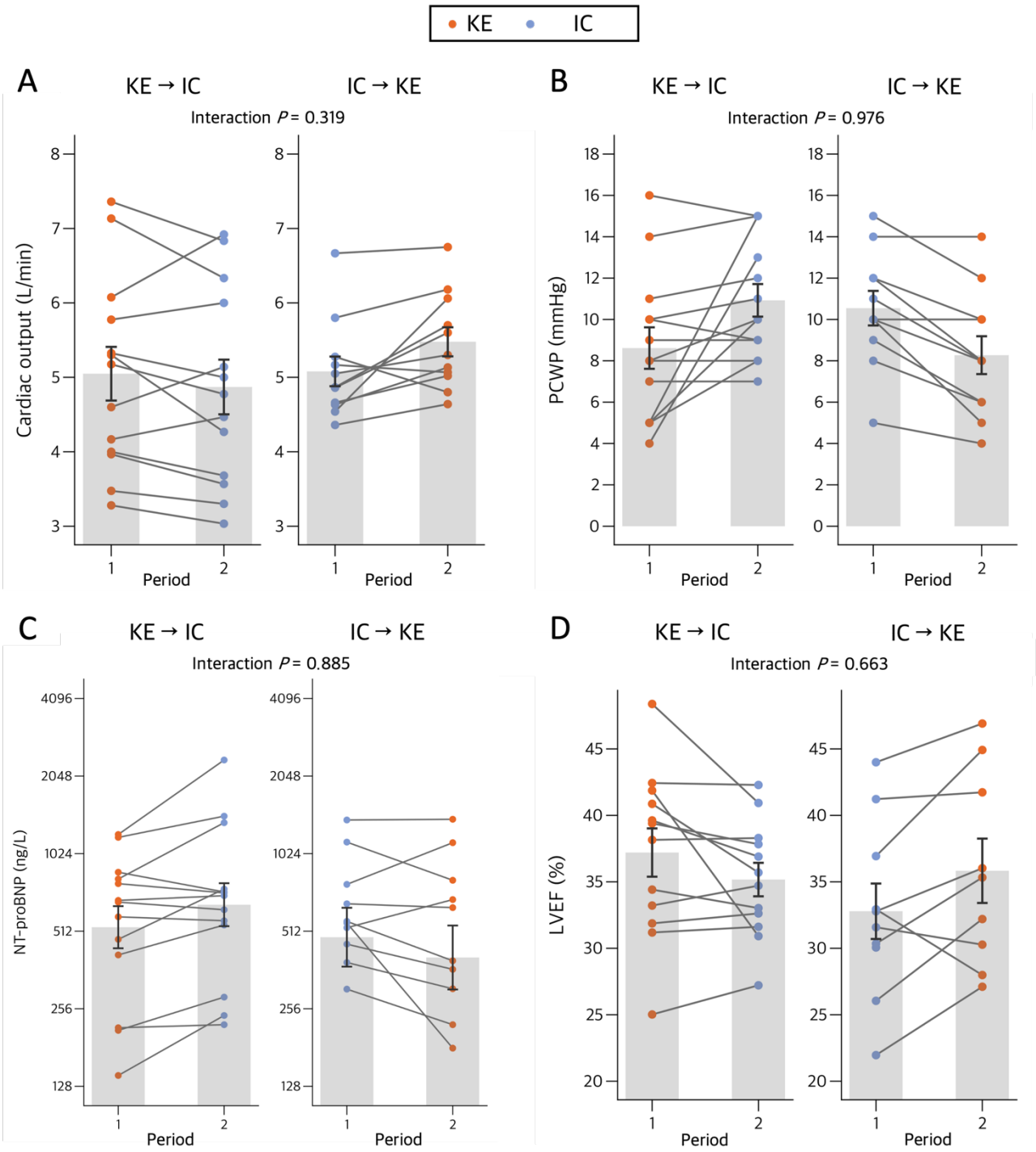
S4 – Supplemental Figures

Figure S1: Consort Diagram



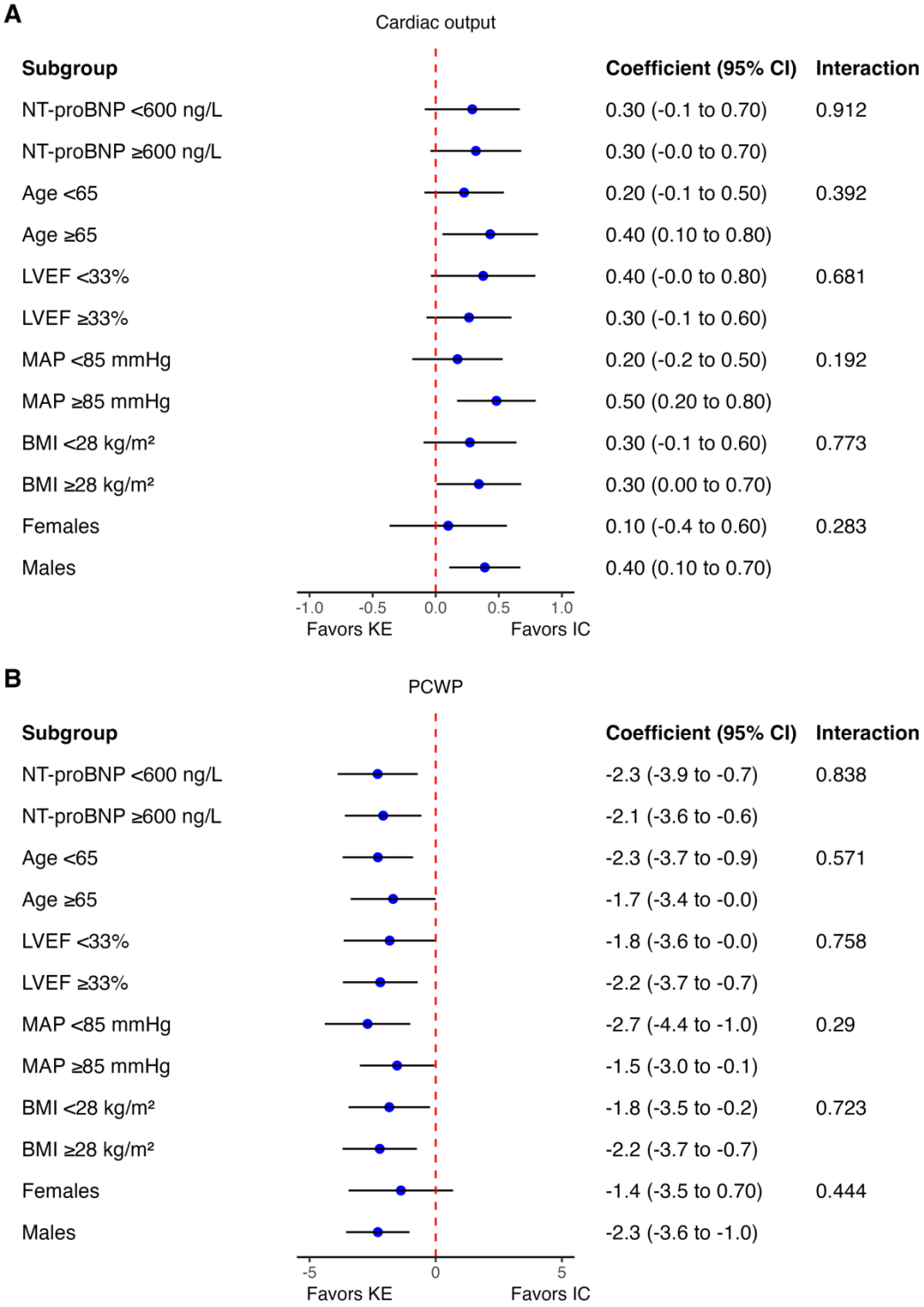
CONSORT, Consolidated Standards of Reporting Trial; RHC, right heart catheterization.

Figure S2: Effects of 14-day Treatment with Ketone Ester versus Isocaloric Comparator on Primary and Secondary Endpoints Stratified by Treatment Sequence.



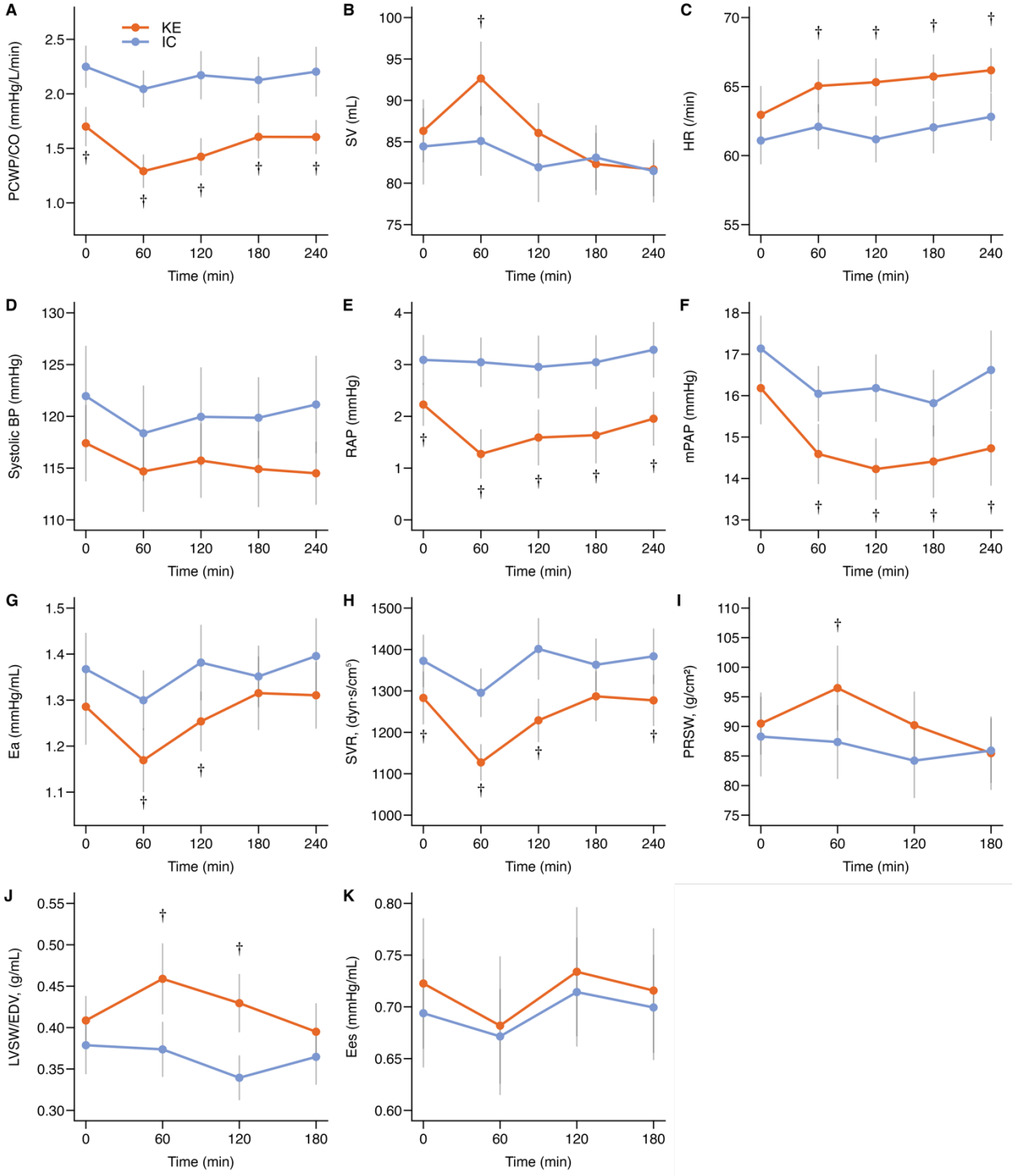
Mean or geometric mean at period 1 and 2 with bars indicating standard error. Cardiac output (**A**), pulmonary capillary wedge pressure (PCWP; **B**), N-terminal pro-B-type natriuretic peptide (NT-proBNP; **C**), and left ventricular ejection fraction (LVEF; **D**) changed following 14-day ketone ester (KE) treatment compared with isocaloric comparator (IC) with no significant treatment sequence interaction.

Figure S3: The Impact of KE Treatment on Cardiac Output and PCWP Across Exploratory Subgroups



Forest plots display the between-treatment pairwise comparisons (coefficients) and 95% CI for cardiac output (**A**) and pulmonary capillary wedge pressure (PCWP; **B**) in each subgroup, while *P*-values indicate subgroup comparisons for interaction testing. There were no significant treatment-by-subgroup differences observed. BMI, body mass index; IC, isocaloric comparator; KE, ketone ester; LVEF, left ventricular ejection fraction; MAP, mean arterial blood pressure; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SGLT2-inh, sodium-glucose cotransporter 2 inhibitor.

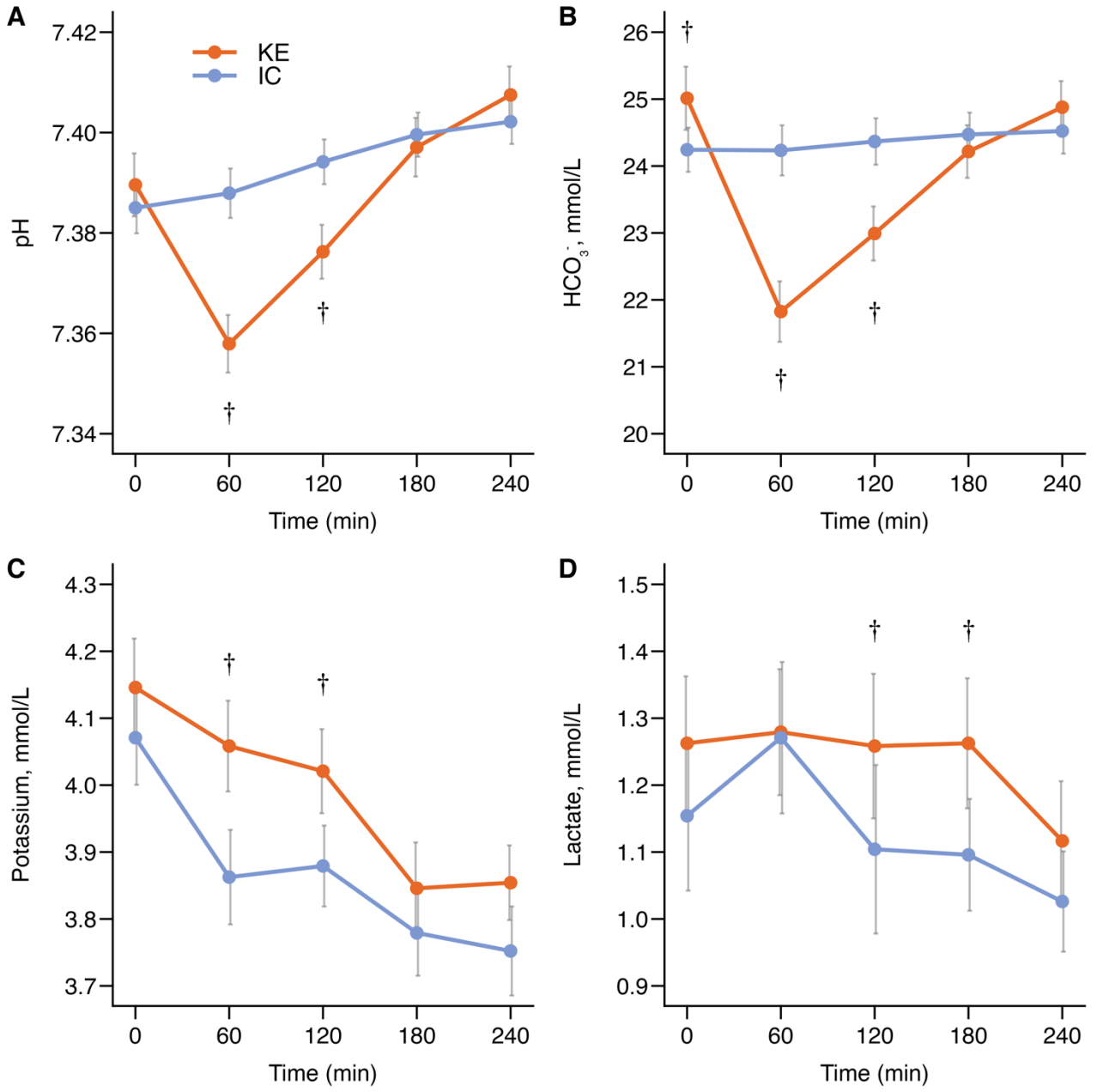
Figure S4: Temporal Changes in Other Hemodynamic Parameters After Intervention Dosing



Mean with bars indicating standard error of the mean. **A**, pulmonary capillary wedge pressure to cardiac output ratio (PCWP/CO); **B**, stroke volume (SV); **C**, heart rate (HR); **D**, systolic blood pressure (BP); **E**, right atrial pressure (RAP); **F**, mean pulmonary arterial pressure (mPAP); **G**, arterial elastance (Ea); **H**, systemic vascular resistance (SVR); **I**, preload recruitable stroke work (PRSW); **J**, left ventricular stroke work normalized to end-diastolic volume (LVSW/EDV); **K**, end-systolic elastance (Ees). IC, isocaloric comparator; KE, ketone ester.

† $P<0.05$ vs. IC

Figure S5: Temporal Changes in Blood Gasses after Intervention Dosing



Mean with bars indicating standard error of the mean. **A**, pH; **B**, HCO_3^-; **C**, Potassium; **D**, Lactate. IC, isocaloric comparator; KE, ketone ester.

Statistical Analysis Plan

Modulation of circulating levels of the ketone body 3-hydroxybutyrate in patients with chronic heart failure: Cardiovascular effects (KETO-CHF)

Trial registrations:
ClinicalTrials.gov NCT05161650, Registered on 03 December 2021
Dietary intervention approved by the Danish Food Agency and confirmed by the Danish Medical Agency (LMST journal number: 2018110888)

Protocol version
Protocol version 7, 9 September 2021
SAP revision history: None

Collaborators
Kristoffer Berg-Hansen, MD,[a,b]
Nigopan Gopalasingam, MD,[a,b]
Kristian Hylleberg Christensen, MD,[a,b]
Roni Nielsen, MD, PhD,[a]
Niels Møller, MD, DMSc, Prof,[b,c]
Henrik Wiggers, MD, DMSc, Prof[a,b]

[a]Department of Cardiology, Aarhus University Hospital, Aarhus, Denmark
[b]Department of Clinical Medicine, Faculty of Health, Aarhus University, Aarhus, Denmark
[c]Department of Endocrinology and Metabolism, Aarhus University Hospital, Aarhus, Denmark

Principal investigator
Kristoffer Berg-Hansen, MD[1]
Department of Cardiology, Aarhus University Hospital,
Palle Juul-Jensens Boulevard 99, DK-8200 Aarhus N, Denmark,
email: krisbe@rm.dk

Sponsor investigator
Henrik Wiggers, MD, DMSc, Prof[1]
Department of Cardiology, Aarhus University Hospital,
Palle Juul-Jensens Boulevard 99, DK-8200 Aarhus N, Denmark,
email: henrikwiggers@dadlnet.dk

Table of contents

1 Introduction

1.1 Background

Heart Failure (HF) is a major public health issue because the disease affects 1-2% of the Western population and the lifetime risk of HF is 20%.[1,2] HF is responsible for 1-2% of all healthcare expenditures and 5% of all hospital admissions.[3] The cornerstone in the medical treatment of chronic HF with reduced ejection fraction (HFrEF) is a combination of ACE-inhibitors/ATII-receptor antagonists, beta-blockers, and mineralocorticoid receptor antagonists. Despite major improvements in the management and care of patients with HF, the 1-year mortality in patients with HF is 13% and >50% of HF- patients is admitted during a 2.5-year period.[4,5] Furthermore, patients with HF have markedly decreased physical capacity and quality of life. Thus, there is a need for new treatment modalities in this group of patients.

Ketone bodies are produced in the liver and are of vital importance in the human body for energy generation in the heart and brain during fasting, exercise and severe illness.[6,7] However, ketosis can be safely obtained using dietary supplements and can increase exercise capacity in athletes.[8,9] The most important ketone bodies are 3-OHB and acetoacetate.[10] Recently, it was demonstrated that patients with severe HF have increased myocardial utilization of the ketone body 3-hydroxybutyrate (3-OHB).[11] It has been hypothesized that ketone bodies may act as "superfuel" for the failing heart.[6] In support of this, the glucose-lowering SGLT-2 inhibitor empagliflozin reduces the risk of hospitalizations and cardiovascular death in diabetic patients with HF and also increases circulating levels of 3-OHB.[12,13]

We have shown, using positron emission tomography, that ketone body infusion reduces myocardial glucose uptake and increases myocardial blood flow in healthy subjects.[14] Data from another study conducted by our group show a significant increase in cardiac output (CO) and left ventricular ejection fraction (LVEF) during infusion of 3-OHB.[15] Presently there are no data on the clinical cardiovascular and metabolic effects of long- or short-term oral ketone-supplementation in patients with chronic HF.

1.2 Hypothesis

We hypothesize that the acute beneficial hemodynamic effects of increasing circulating 3-OHB can persist with prolonged treatment with a ketone ester (KE). Thus, the primary hypothesis is that 14-day treatment with KE increases CO at rest compared with placebo treatment in patients with stable HFrEF (NYHA II/III). The secondary hypotheses are that 14-day treatment with KE compared with placebo: a) reduces cardiac filling pressures at rest and during exercise, b) increases resting LVEF, c) decreases NT-proBNP levels, d) increases exercise capacity.

1.3 Objectives

To explore the impact of a 14-day modulation of circulating 3-OHB levels through a KE on hemodynamic and cardiac function, both at rest and during exercise, as well as on exercise capacity in stable patients with heart failure with reduced ejection fraction (HFrEF).

2 Study design

2.1 Trial design

This is a single-center, randomized, double-blind, placebo-controlled crossover study of the hemodynamic effects of 14-day treatment with oral ketone ester in stable chronic HFrEF patients.

2.2 Randomization

Patients with stable chronic HFrEF are randomized 1:1 to receive either oral KE (25 gram; Ketone Aid Inc., Falls Church, Virginia, USA) x 4 daily or isocaloric placebo drink x 4 daily. Each study period lasts 14 days, separated by a 14-day washout period.

2.3 Endpoints

Primary: The primary endpoint is the between-treatment difference in CO at rest following an overnight fast (i.e. during trough levels of circulating 3-OHB).

Secondary: Between-treatment difference in:

1. other hemodynamic parameters (including right atrial pressure, pulmonary artery and wedge pressures, non-invasive blood pressure, and heart rate) at rest during trough levels of circulating 3-OHB
2. Exercise changes in invasive hemodynamic indices
3. Metabolic equivalents (METs)

Exploratory:

1. LVEF at rest during trough levels of circulating 3-OHB
2. NT-proBNP
3. Peak oxygen consumption (VO_2) during exercise
4. 12-item Kansas City Cardiomyopathy Questionnaire (KCCQ-12)

2.3 Timing of endpoints

The primary and secondary endpoints are measured at each study visit following the 14-day treatment period.

2.4 Data integrity

The clinical trial data will be collected and stored in a REDCap database in line with the local patient data integrity administration policy (administered by Aarhus University). Study data within the database will be analyzed through a pipeline from the REDCap database by an application programming interface unique key.

3 Cohort size

For the primary outcome of CO at rest, we anticipate a 4% coefficient of variation based on previous data. Thus, by enrolling 24 patients in the final analysis, we aim to detect a relative difference of 9% in the primary endpoint (with a standard deviation of 1 liter/min), a power of 80%, and a two-tailed significance level of 5%. Such change in the primary endpoint is expected to be related to clinical outcome.[16]

4 Patient recruitment

4.1 Eligibility criteria

Inclusion and exclusion criteria are specified in the protocol.

4.2 Study dropout

The occurrence of any condition necessitating withdrawal from the study, whether due to safety concerns, disease progression, subject choice, or non-adherence to protocol requirements, may serve as a reason for study withdrawal. Participants who withdraw will be replaced to reach the target enrollment as specified in the power analysis. The CONSORT flow diagram will present the timing, numbers, and reasons for withdrawals.

5 Statistical principles

5.1 Statistical thresholds

Confidence intervals (95%) and *P* values are two-tailed. A *P* value of <0.05 is deemed statistically significant for all conducted analyses.

5.2 Analysis population

All collected data from each participant will be analyzed using a linear mixed effects model as specified in *6.2 Statistical methods*. As this study aims to investigate the cardiovascular effects of ketone ester treatment in patients with HFrEF, certain modifications to the primary analysis may become necessary in a "modified Intention-to-Treat" approach. For instance, if a participant is unable to complete both study periods for reasons justified in the *4.2 Study dropout* section their data will not be included in the final analysis.

5.3 Screening

For all screened patients the following will be presented: the total number of patients screened, the count of screened patients not recruited, the number of patients successfully recruited, and the rationale for non-recruitment. The count of screened patients are individuals excluded before the screening visit and those excluded between the screening visit and randomization; they will be presented in a detailed summary.

5.4 Baseline characteristics

Patient characteristics will be presented as an overall summary regardless of treatment sequence. Normally and non-normally distributed continuous variables will be summarized as mean ± standard deviation (SD) and median (interquartile range (IQR)). Categorical variables will be presented as numbers and percentages.

6 Statistical plan

6.1 Statistical methods

The mean between-treatment (±SD) change in the primary endpoint of resting CO at trough levels will be analyzed using a linear mixed model with treatment, period, and treatment sequence included as fixed effects and participants as random effects. The fixed parameters will be estimated using a restricted maximum likelihood (REML) procedure and compared by Kenward-Roger's method. Assumptions for the statistical analysis involve inspection of linearity and variance homogeneity of residuals, and normal distribution of

residuals through plots (residuals vs. fitted values, scale location, and normal Q-Q). If violation occurs, data will be log-transformed and reported accordingly. Between-treatment effect will be reported with a 95% CI and a *P* value as previously defined.

Secondary endpoints are independently analyzed and interpreted, irrespective of the primary endpoint results, and without adhering to a hierarchical testing sequence. The analysis methods for the secondary endpoints will mirror those outlined for the primary outcome. There will be no imputation for missing data.

Analysis of temporal effects during acute-on-chronic intervention and during incremental exercise will be conducted using a repeated measures linear mixed model. To explore between-treatment fixed effects on outcome variables, the model will incorporate the same elements as described above. Additionally, a treatment-by-time or a treatment-by-workload interaction will be included as fixed effect and period nested within participants will be added as random effect to the model. Pairwise difference between treatment effects was adjusted for multiple comparisons in the fitted model to establish mean between-treatment effects on study outcomes during a given time or incremental workloads. The fixed effects will be estimated as described previously. If the test is considered statistically significant, model-based means will be created to compare variables at each time point or each exercise level.

6.2 Log-transformation

For statistical analyses, log-transformation will be applied in cases of significant skewness without log-transformation. For the linear mixed model, log-transformation will be applied if residuals vs. fitted values are significantly skewed. Reporting of variables will be displayed on a logarithmic scale and between-treatment effects are derived from the relative change as demonstrated by the model outcome using log-transformed variables.

7 Quality of statistical programming

All statistical analyses will be performed using R version 4.1.1 or later. Packages and functions used in the analysis will be frozen to the project file using the prodigenr package manager. Git repositories will be used to archive study programming code and data to provide version control.

8 References

1. Savarese G, Lund LH. Global Public Health Burden of Heart Failure. *Card Fail Rev* 2017;3:7–11. doi:10.15420/cfr.2016:25:2.
2. Mosterd A, Hoes AW. Clinical epidemiology of heart failure. *Heart* 2007;93:1137–1146. doi:10.1136/hrt.2003.025270.
3. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, González-Juanatey JR, Harjola V-P, Jankowska EA, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J* 2016;37:2129–2200. doi:10.1093/eurheartj/ehw128.
4. Schou M, Gustafsson F, Videbaek L, Tuxen C, Keller N, Handberg J, Sejr Knudsen A, Espersen G, Markenvard J, Egstrup K, et al. Extended heart failure clinic follow-up in low-risk patients: a randomized clinical trial (NorthStar). *Eur Heart J* 2013;34:432–442. doi:10.1093/eurheartj/ehs235.
5. Nakano A, Johnsen SP, Frederiksen BL, Svendsen ML, Agger C, Schjødt I, Egstrup K. Trends in quality of care among patients with incident heart failure in Denmark 2003-2010: a nationwide cohort study. *BMC Health Serv Res* 2013;13:391. doi:10.1186/1472-6963-13-391.
6. Lopaschuk GD, Ussher JR. Evolving Concepts of Myocardial Energy Metabolism: More Than Just Fats and Carbohydrates. *Circ Res* 2016;119:1173–1176. doi:10.1161/CIRCRESAHA.116.310078.
7. Lopaschuk GD, Karwi QG, Tian R, Wende AR, Abel ED. Cardiac Energy Metabolism in Heart Failure. *Circ Res* 2021:1487–1513. doi:10.1161/CIRCRESAHA.121.318241.
8. Stubbs BJ, Cox PJ, Evans RD, Santer P, Miller JJ, Faull OK, Magor-Elliott S, Hiyama S, Stirling M, Clarke K. On the metabolism of exogenous ketones in humans. *Front Physiol* 2017;8:1–13. doi:10.3389/fphys.2017.00848.
9. Cox PJ, Kirk T, Ashmore T, Willerton K, Evans R, Smith A, Murray AJ, Stubbs B, West J, McLure SW, et al. Nutritional Ketosis Alters Fuel Preference and Thereby Endurance Performance in Athletes. *Cell Metab* 2016;24:256–268. doi:10.1016/j.cmet.2016.07.010.
10. Møller N. Ketone Body, 3-Hydroxybutyrate: Minor Metabolite - Major Medical Manifestations. *J Clin Endocrinol Metab* 2020;105:2884–2892. doi:10.1210/clinem/dgaa370.
11. Bedi KC, Snyder NW, Brandimarto J, Aziz M, Mesaros C, Worth AJ, Wang LL, Javaheri A, Blair IA, Margulies KB, et al. Evidence for Intramyocardial Disruption of Lipid Metabolism and Increased Myocardial Ketone Utilization in Advanced Human Heart Failure. *Circulation* 2016;133:706–716. doi:10.1161/CIRCULATIONAHA.115.017545.
12. Fitchett D, Zinman B, Wanner C, Lachin JM, Hantel S, Salsali A, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE. Heart failure outcomes with empagliflozin in patients with type 2 diabetes at high cardiovascular risk: results of the EMPA-REG OUTCOME® trial. *Eur Heart J* 2016;37:1526–1534. doi:10.1093/eurheartj/ehv728.
13. Ferrannini E, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A "Thrifty Substrate" Hypothesis. *Diabetes Care* 2016;39:1108–1114. doi:10.2337/dc16-0330.
14. Gormsen LC, Svart M, Thomsen HH, Søndergaard E, Vendelbo MH, Christensen N, Tolbod LP, Harms HJ, Nielsen R, Wiggers H, et al. Ketone body infusion with 3-hydroxybutyrate reduces myocardial glucose uptake and increases blood flow in humans: A positron emission tomography study. *J Am Heart Assoc* 2017;6:1–11. doi:10.1161/JAHA.116.005066.
15. Nielsen R, Møller N, Gormsen LC, Tolbod LP, Hansson NH, Sorensen J, Harms HJ, Frøkiær J, Eiskjaer H, Jespersen NR, et al. Cardiovascular Effects of Treatment With the Ketone Body 3-Hydroxybutyrate in Chronic Heart Failure Patients. *Circulation* 2019;139:2129–2141. doi:10.1161/CIRCULATIONAHA.118.036459.

16. Doughty RN, Whalley GA, Walsh HA, Gamble GD, López-Sendón J, Sharpe N. Effects of carvedilol on left ventricular remodeling after acute myocardial infarction: the CAPRICORN Echo Substudy. *Circulation* 2004;109:201–206. doi:10.1161/01.CIR.0000108928.25690.94.