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ORIGINAL RESEARCH

Stimulation of the Hydroxycarboxylic Acid Receptor 2 With the Ketone Body 3-Hydroxybutyrate and Niacin in Patients With Chronic Heart Failure: Hemodynamic and Metabolic Effects

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BACKGROUND: The ketone body 3-hydroxybutyrate (3-OHB) increases cardiac output (CO) in patients with heart failure through unknown mechanisms. 3-OHB activates the hydroxycarboxylic acid receptor 2 (HCA_2), which increases prostaglandins and suppresses circulating free fatty acids. We investigated whether the cardiovascular effects of 3-OHB involved HCA_2 activation and if the potent HCA_2-stimulator niacin may increase CO.

METHODS AND RESULTS: Twelve patients with heart failure with reduced ejection fraction were included in a randomized crossover study and examined by right heart catheterization, echocardiography, and blood sampling on 2 separate days. On study day 1, patients received aspirin to block the HCA_2 downstream cyclooxygenase enzyme, followed by 3-OHB and placebo infusions in random order. We compared the results with those of a previous study in which patients received no aspirin. On study day 2, patients received niacin and placebo. The primary end point was CO. 3-OHB increased CO (2.3 L/min, $P<0.01$), stroke volume (19 mL, $P<0.01$), heart rate (10 bpm, $P<0.01$), and mixed venous saturation (5%, $P<0.01$) with preceding aspirin. 3-OHB did not change prostaglandin levels, neither in the ketone/placebo group receiving aspirin nor the previous study cohort. Aspirin did not block 3-OHB-induced changes in CO ($P=0.43$). 3-OHB decreased free fatty acids by 58% ($P=0.01$). Niacin increased prostaglandin D_2 levels by 330% ($P<0.02$) and reduced free fatty acids by 75% ($P<0.01$) but did not affect CO.

CONCLUSIONS: The acute increase in CO during 3-OHB infusion was not modified by aspirin, and niacin had no hemodynamic effects. These findings show that HCA_2 receptor-mediated effects were not involved in the hemodynamic response to 3-OHB.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT04703361.

Key Words: HCA_2 receptor ■ heart failure ■ hemodynamics ■ ketone body ■ metabolic

The ketone body 3-hydroxybutyrate (3-OHB) is produced in the liver during ketogenic dieting, fasting, and stress[1] and is oxidized in vital organs such as brain, heart, and skeletal muscle.[2] We recently demonstrated that 3-OHB infusion increases myocardial blood flow by 75% in healthy participants[3] and cardiac output (CO) by 40% in patients with chronic heart failure with reduced ejection fraction (HFrEF).[4] Animal studies

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CLINICAL PERSPECTIVE

What Is New?

- In patients with heart failure with reduced ejection fraction, infusion of the ketone body 3-hydroxybutyrate (3-OHB) increased cardiac output by 2.3 L/min and reduced circulating free fatty acids by 58%.
- The increase in cardiac output during 3-OHB infusion was not blocked when the HCA_2 downstream enzyme cyclooxygenase was inhibited with aspirin, and circulating prostaglandin levels did not change during 3-OHB infusion compared with placebo.
- Stimulation of HCA_2 with the potent niacin (B_3 vitamin) suppressed free fatty acids to a similar extent as 3-OHB and increased circulating prostaglandins but did not change cardiac output.

What Are the Clinical Implications?

- 3-OHB may be a beneficial treatment for patients with heart failure with reduced ejection fraction because it increases cardiac output. The hemodynamic changes were not mediated through HCA_2 activation, prostaglandin release, or circulating free fatty acid suppression.

Nonstandard Abbreviations and Acronyms

3-OHB	3-hydroxybutyrate
CO	cardiac output
FFA	free fatty acid
HCA_2	hydroxycarboxylic acid receptor 2
HFrEF	heart failure with reduced ejection fraction
PAP	mean pulmonary artery pressure
PCWP	pulmonary capillary wedge pressure
PGD_2	prostaglandin D_2
PGE_2	prostaglandin E_2
PGI_2	prostaglandin I_2

have shown that 3-OHB has cardioprotective effects in heart failure[5]; however, the exact underlying mechanism is not fully understood. In patients with heart failure, myocardial genes coding for enzymes involved in 3-OHB oxidation are upregulated.[6] Experimental studies have reported that heart failure is accompanied by a cardiac metabolic remodeling process that shifts the substrate utilization from free fatty acids (FFAs) toward 3-OHB.[7,8] Enzymes involved in 3-OHB oxidation are upregulated in cardiomyocytes from patients with HFrEF,[6] and studies show that the failing heart increases utilization of 3-OHB[9] without compromising glucose or FFA consumption.[10,11] Thus, current data support that 3-OHB is an important adjunct fuel in the failing heart, potentially exerting its beneficial effects through enhanced ATP production.[12,13]

In addition to its metabolic effects, 3-OHB has pleiotropic effects. The metabolite 3-OHB is the only physiologically important endogenous ligand of the G-protein-coupled receptor hydroxycarboxylic acid receptor 2 (HCA_2).[14,15] Physiological 3-OHB concentrations activate HCA_2[16] on immune cells and adipocytes.[15] In macrophages, HCA_2 activation has downstream effects resulting in production and release of prostaglandin D_2 (PGD_2) and prostaglandin E_2 (PGE_2) through cyclooxygenase enzymes.[15,17] Aspirin blocks the cyclooxygenase enzymes and thereby prostaglandin production.[18] In adipocytes, HCA_2 activation by 3-OHB decreases FFA release by inhibition of the hormone-sensitive lipase and adipocyte triglyceride lipase.[15,19] This is known to constitute a negative feedback loop, whereby hepatic ketogenesis from circulating FFAs during fasting prevents excessive FFA release from adipose tissue[15] (Figure 1). Our previous study showed that circulating FFAs are suppressed during 3-OHB infusion.[4] Therefore, it is possible that the observed favorable hemodynamic effects of 3-OHB infusion were due to both reduced plasma FFA concentrations and altered myocardial substrate metabolism. However, it is not fully understood whether the hemodynamic effect of 3-OHB in patients with HFrEF involves a HCA_2-mediated decrease in circulating FFAs.

The hemodynamic effects of 3-OHB in patients with HFrEF[4] involve a marked reduction in systemic vascular resistance (SVR) that could play a major role in the increased CO observed. Therefore, in the present study, we addressed the potential effects of 3-OHB-mediated activation of the HCA_2 receptor and release of vasodilatory prostaglandins (PGD_2, PGE_2, and prostaglandin I_2). Prostaglandins stimulate the receptors DP1 and EP2/4 on blood vessels, which activate the process of cAMP-mediated vasodilation.[17] It is unclear whether the observed increase in CO during 3-OHB infusion is a consequence of increased left ventricular contractility, direct vasodilatory effect on vessels, or a combination thereof.

Niacin (vitamin B_3) is a well-described potent HCA_2 receptor stimulator.[20] Niacin stimulation of HCA_2 has an antilipolytic effect and has therefore been used in dyslipidemia treatment.[19,21] However, the hemodynamic effects of niacin have never been investigated.

The aims of this study were to investigate, first, if the hemodynamic effect of 3-OHB in patients with HFrEF involves HCA_2 activation; second, to explore the hemodynamic effects of the exogenous and even stronger HCA_2-agonist niacin; third, to investigate the release of prostaglandins by HCA_2 activation; and finally, to examine the interaction between HCA_2 stimulation,

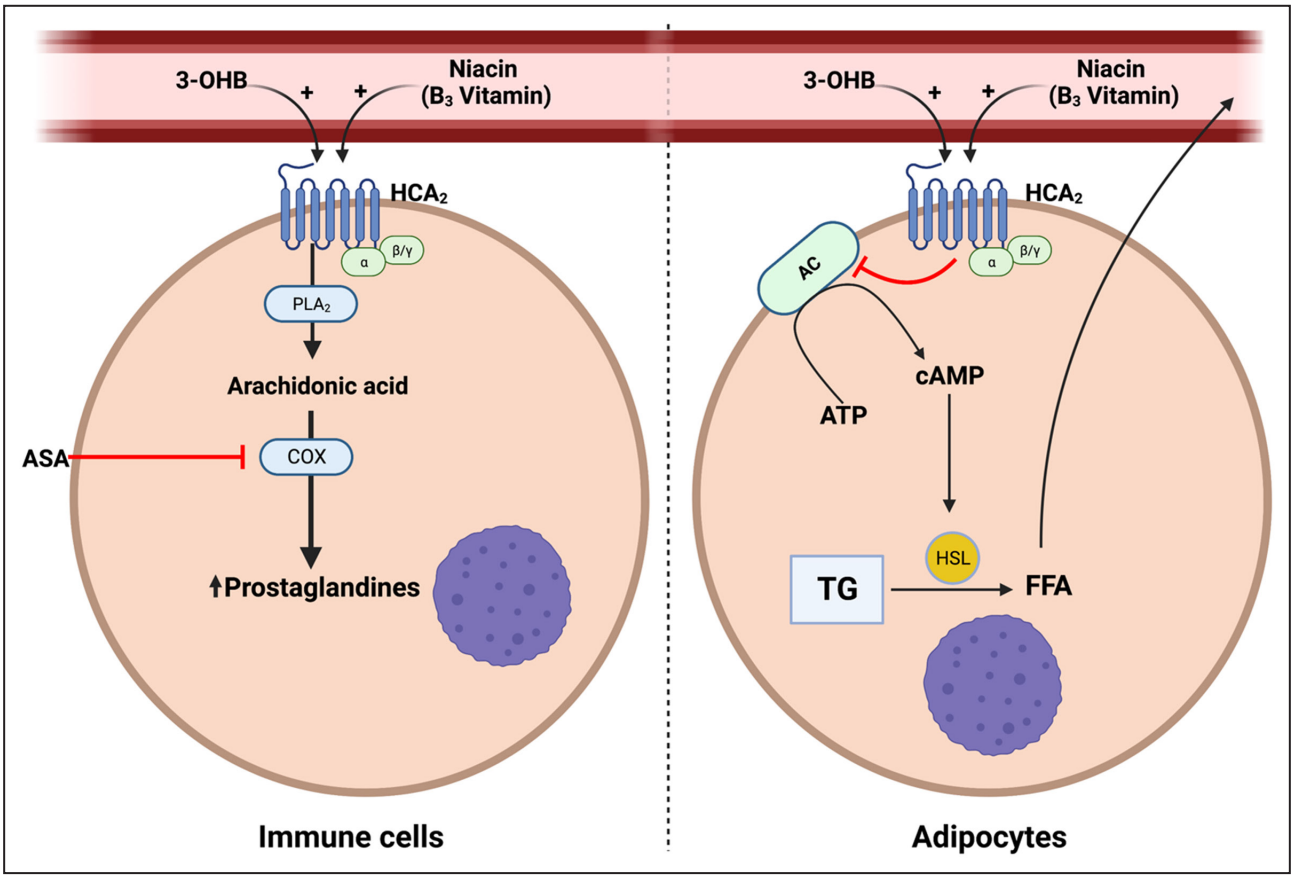

Figure 1. HCA_2.
Modulation of prostaglandin and free fatty acid production through HCA_2 receptor activation. 3-OHB indicates 3-hydroxybutyrate; AC, adenylyl cyclase; ASA, aspirin; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; COX, cyclooxygenase; FFA, free fatty acid; HCA_2, hydroxycarboxylic acid 2 receptor; HSL, hormone-sensitive lipase; PLA_2, phospholipase A2; and TG, triglyceride. Created with BioRender.com

suppressed FFA release from adipose tissue, and hemodynamic changes in patients with HFrEF.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Participants

We included patients with HFrEF with left ventricular ejection fraction ≤40% who were in New York Heart Association class II to III and able to give informed consent. Exclusion criteria were significant cardiac valve disease (symptomatic cardiac valve disease or disease requiring intervention according to guidelines), history of myocardial infarction within 1 month, insulin treatment, or significant liver disease. All patients were recruited from the Heart Failure Clinic, Department of Cardiology, Aarhus University Hospital, Denmark.

Design

This was a randomized, controlled, single-blinded crossover study (Figure 2). Twelve patients were studied on 2 days separated by a minimum 7-day washout period. All participants fasted overnight and avoided heavy exercise before each visit. On study days, a pulmonary artery catheter was placed, and a peripheral venous catheter was inserted in the cubital vein. Patients were then left to rest for at least 45 minutes before baseline measurements were made.

On study day 1 (Ketone/placebo with preceding aspirin), all participants received a 650 mg bolus of intravenous aspirin (Bayer, Germany), which is known to irreversibly block the cyclooxygenase enzyme.[18] Thirty minutes later, the patients received a continuous 3-OHB infusion (0.18 g/kg per hour) and isovolumetric placebo (isotonic saline) for 3 hours, administered in random order (Figure 2).

Sixteen control patients with HFrEF (ketone/placebo without aspirin) were derived from a previous study by our group.[4] These patients also received the infusions

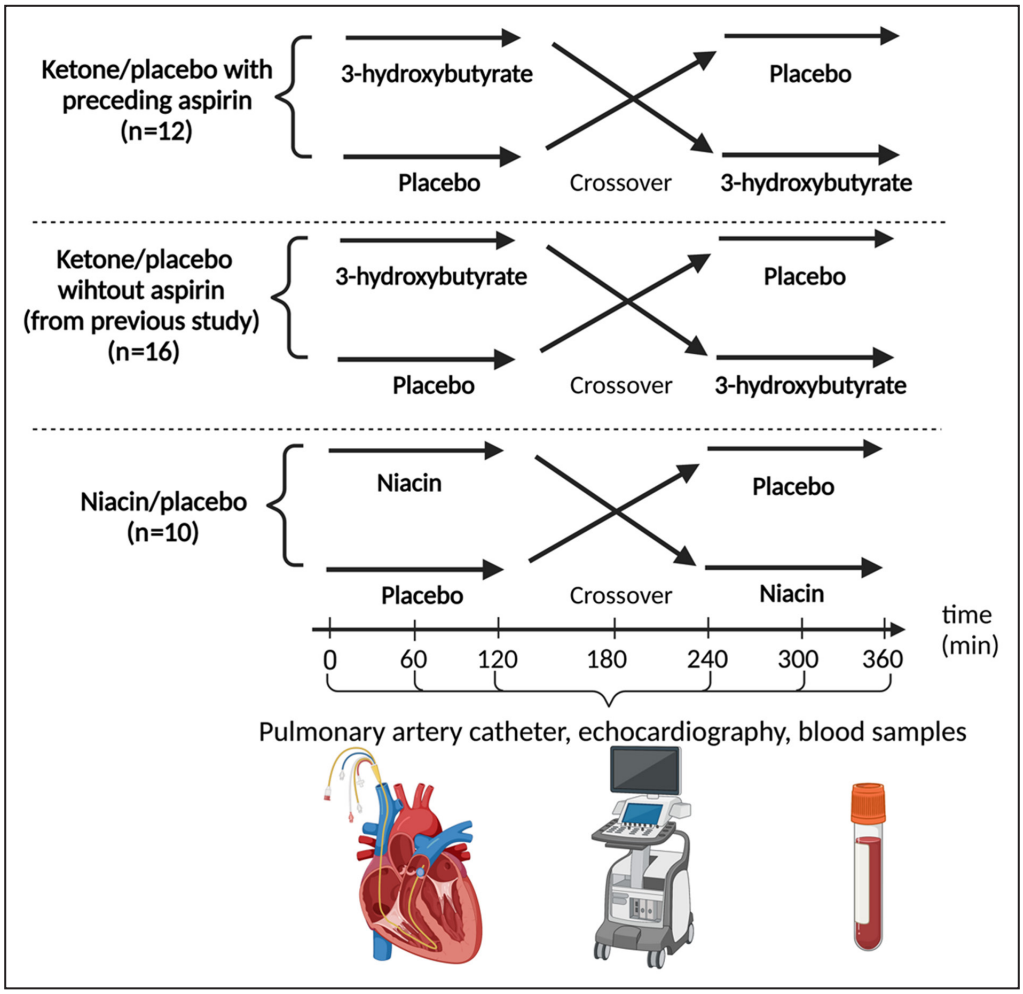

Figure 2. Study flowchart.
Overview of the study design for all 3 study groups: Ketone/placebo with preceding aspirin, Ketone/placebo without aspirin (from previous study), Niacin/placebo. The interventions were initiated after baseline measurements (0 minutes) and were crossed over to the other intervention at 180 minutes. Invasive measurements using pulmonary artery catheter, echocardiography, and blood samples were conducted hourly during the study period.

and investigations described above for study day 1 but received no intravenous aspirin (Figure 2).

On study day 2 (Niacin/placebo), all participants received tablets of 1000 mg niacin orally (Natur Drogeriet, Denmark) and placebo tablets (lactose monohydrate, the Hospital Pharmacy Aarhus University Hospital, Aarhus, Denmark) in random order (Figure 2). The purpose of utilizing niacin in this study was to serve as a positive control for the HCA_2 receptor, as it is a well-known strong agonist of this receptor. Measurements were made for 3 hours after intake of each study drug. The niacin dose was reduced to 500 mg halfway through the study because 4 patients experienced flushing and discomfort.

Solutions and Infusions

The 3-OHB solution was prepared as a 7.5% concentration of 3-OHB by the local pharmacy. Potassium chloride (60 mmol/L) was added to the 3-OHB solution. Infusion was initiated following baseline measurements. Infusion was changed at crossover after 3 hours. The patients received an oral bolus of 60 mmol KCl when randomized to initiate with 3-OHB and 20 mmol when randomized to initiate with placebo. To maintain euglycemia and minimize endogenous ketogenesis, all patients received a low-dose insulinemic euglycemic clamp (0.3 mU insulin/kg per min) with glucose (20% solution, 60 mmol/L KCl) throughout the 6-hour study period.

End Points and Measurements

All patients were monitored with pulmonary artery catheter measurements, echocardiography, and blood samples at baseline and hourly for a 6-hour period on each study day. The primary end point

was difference between CO at the end of each infusion period. Secondary end points were mixed venous saturation, pulmonary capillary wedge pressure, mean pulmonary artery pressure (PAP), pulmonary vascular resistance, systemic vascular resistance (SVR), left ventricular ejection fraction, left ventricular global longitudinal strain, FFAs, and circulating prostaglandin levels.

Noninvasive Hemodynamic Measurements

Blood pressure and heart rate were measured with Phillips IntelliVue X3 (Phillips, Netherlands) monitoring system. Mean arterial pressure was calculated as one-third systolic blood pressure + two-thirds diastolic blood pressure.

Right Heart Catheterization

The pulmonary artery catheter was placed with fluoroscopy guidance under sterile conditions through the internal jugular vein. Right arterial pressure, PAP, PCWP, mixed venous saturation, and CO were measured at baseline and hourly after the intervention was initiated. CO was measured by the thermodilution technique, and the mean of 3 measurements was used. SVR was calculated as (mean arterial pressure–right arterial pressure)/CO. Pulmonary vascular resistance was calculated as (PAP–PCWP)/CO.

Echocardiography

All echocardiographic acquisitions were made using GE Vivid E95 or GE Vivid E9 (GE Healthcare, USA). EchoPAC software (GE-Vingmed Ultrasound, Norway) was used for analysis.

Blood Samples

Blood samples were drawn from the cubital vein via a peripheral venous catheter. pH, potassium, sodium, calcium, and lactate were analyzed immediately after sampling using YSI STAT 2100 (YSI Inc., Netherlands). Glucose was analyzed using Contour Xt Glucometer (Bayer, Germany). D-3-OHB was measured with FreeStyle Precision Neo (Abbott, USA). All other samples were analyzed as a batch at the end of the study. Total 3-OHB was measured with hydrophilic interaction liquid chromatography tandem mass spectrometry. Prostaglandin I_2 (PGI_2), PGE_2, and PGD_2 were analyzed with the 6-keto Prostaglandin F1alpha ELISA kit (Cayman Chemical, USA), Prostaglandin E Metabolite ELISA kit (Cayman Chemical, USA), and the Prostaglandin D2-MOX Express ELISA Kit (Cayman Chemical, USA), respectively.

Statistical Analysis

The SD of CO (primary end point) is 0.8 L/min.[4] By enrolling 12 patients, a relative difference in CO of 20% can be detected with a power of 90% and a 2-sided significance level of 5%. For the niacin part of the study, 10 patients completed the study. Post-hoc power for this part of the study was 88.5%, based on the given parameters of 10 participants, a 20% difference in CO, and an SD of 0.8 L/min. Randomization was made in STATA 17 (StataCorp, USA) by the investigator. Participants were blinded to the randomization order. Data were inspected for normal distribution as required. Baseline data (Table 1) are presented as mean±SD if normally distributed or median (interquartile range) if not normally distributed. Paired 2-tailed *t* test was used to compare the effect of the interventions with that of placebo. A 2-sample *t* test was used to compare the means of continuous variables between the 2 groups: ketone/placebo with the preceding aspirin and ketone/placebo without aspirin, whereas Fisher exact test was utilized to compare the categorical variables between the 2 groups. Differences are presented as mean with 95% CI. A *P*-value <0.05 was considered statistically significant. Data analyses were performed with STATA 17 (StataCorp, USA). All figures were created with Prism 8 (Graphpad Software, LLC).

Ethics

The local Ethical Committee of the Central Denmark Region approved the study. All patients provided written informed consent for participation. The study was registered with the Danish Data Protection Agency and clinicaltrials.gov (NCT04703361). NG had full access to all study data and takes responsibility for its integrity and data analysis.

RESULTS

Patients

Sixteen patients were eligible for inclusion. Three patients declined to participate, and 1 patient did not meet the inclusion criteria. Thus, 12 patients were included in the study. They all completed study day 1 (ketone/placebo with preceding aspirin), but 2 did not complete study day 2 (the niacin/placebo day). One declined to participate; the other had a vasovagal syncope on the day of examination. All patients received treatment in the intended randomly assigned order. The patient studies were conducted from February 2021 to September 2021, and blood sample analyses were finalized in September 2022. The baseline characteristics are shown in Table 1. Patient characteristics for patients from our previous study[4] (ketone/placebo without aspirin) are also presented in Table 1.

Table 1. Baseline Characteristics

	Ketone/placebo with preceding aspirin and niacin/placebo (n=12)	Ketone/ placebo without aspirin (n=16)	*P* value
Sex (male/female)	11/1	14/2	1.00
Age, y	64±11	60±12	0.38
BMI, kg/m^2	30±5	26±5	0.07
IHD, n (%)	6 (50%)	12 (75%)	0.17
AFIB, n (%)	3 (25%)	1 (6%)	0.28
Heart rate, beats/ min	72 (67–84)	67 (58–72)	0.07
Systolic BP, mm Hg	120±14	130±18	0.15
Diastolic BP, mm Hg	76±13	77±10	0.78
NT-proBNP, ng/L	360 (206–1113)	617 (418–1306)	0.15
HbA1c, mmol/mol	41±4	39±4	0.24
eGFR, mL	85 (71–90)	90 (66–90)	0.82
NYHA class	2 (2–3)	2 (2–2)	0.06
LVEF, (%)	33±5	36±4	0.16
GLS, (%)	−9.3±4	−10±3	0.60
ACE-I/ARB, n (%)	8 (67%)	15 (94%)	0.13
Platelet inhibitors, n (%)	4 (33%)	12 (75%)	0.05
β-blockers, n (%)	12 (100%)	16 (100%)	1.00
Diuretics, n (%)	7 (58%)	8 (50%)	0.66
MRA, n (%)	11 (92%)	10 (63%)	0.08
Statin, n (%)	8 (67%)	14 (88%)	0.35
SGLT2 inhibitor	3 (25%)	0 (0%)	0.07
ICD/CRT/CRT-D, n (%)	7 (58%)	12 (75%)	0.43

Data are mean±SD or median (interquartile range). ACE-I indicates angiotensin-converting enzyme inhibitors; AFIB, atrial fibrillation; ARB, angiotensin-2 receptor blockers; BMI, body mass index; BP, blood pressure; CRT, cardiac resynchronization therapy; CRT-D, implantable cardioverter defibrillators with cardiac resynchronization capabilities; eGFR, estimated glomerular filtration rate; GLS, global longitudinal strain; HbA1c, hemoglobin A1c; ICD, implantable cardioverter-defibrillator; IHD, ischemic heart disease; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonists; NT-proBNP, N-terminal pro brain-natriuretic-peptide; NYHA, New York Heart Association; and SGLT2, sodium-glucose cotransporter-2.

Hemodynamic Effects of 3-OHB With Preceding Aspirin

Intravenous aspirin was administrated 42±1 minutes before interventions were initiated. Circulating 3-OHB levels increased by 4.1 mmol/L (95% CI, 3.5–4.6 mmol/L) during the ketone infusion period compared with the placebo period. CO increased by 2.3 L/min (95% CI, 1.6–2.9 L/min) after 3 hours of 3-OHB infusion compared with 3 hours of placebo infusion (*P*<0.01). The increase in CO was driven by a 19 mL increase in stroke volume (SV) (95% CI, 14–24 mL) and an increase in heart rate of 10 beats per minute (95% CI, 7–13 beats per minute). Mixed venous saturation increased by 5 percentage points (95% CI, from 2%–8%) after 3-OHB infusion compared with placebo (*P*<0.01). SVR was reduced by 5 Wood units compared with placebo (95% CI, −6 to −4 Wood units). PCWP, PAP, and pulmonary vascular resistance did not change significantly (Table 2).

Echocardiographic systolic parameters including global longitudinal strain, left ventricular ejection fraction, and systolic myocardial velocity at mitral annulus (s') all increased during 3-OHB infusion compared with placebo (Table 2).

Hemodynamic Effects of 3-OHB With and Without Preceding Aspirin Treatment

Hemodynamic data for ketone/placebo without aspirin treatment have previously been published by our group,[4] and the results are included in Table 2. These data showed an increase in CO of 2.0 L/min (95% CI, 1.6–2.4 L/min) after 3 hours of 3-OHB infusion as compared with placebo.

In the present study, where aspirin treatment preceded infusions, we observed an increase in CO of 2.3 L/min (95% CI, 1.6–2.9 L/min) after 3 hours of 3-OHB infusion as compared with placebo. Thus, no significant difference was observed between the increase in CO between patients with HFrEF with and without preceding aspirin treatment (0.3 L/min; 95% CI, −0.4 to 0.9 L/min). Likewise, no differences in other hemodynamic parameters were observed between patients with HFrEF with and without preceding aspirin treatment (Table 2).

Hemodynamic Effects of Niacin

Plasma niacin levels increased by 8.1 mg/mL (95% CI, 3.6–12.6 mg/mL) after niacin administration compared with placebo. CO did not change (0 L/min; 95% CI −0.4 to 0.4 L/min) after niacin administration compared with placebo. Likewise, no differences were observed in mixed venous saturation, SVR, PCWP, PAP, and pulmonary vascular resistance (Table 3). No changes were observed in echocardiographic parameters (Table 3).

Effects of 3-OHB and Niacin on Prostaglandin Release

PGI_2 and PGE_2 levels in plasma did not differ during 3-OHB infusion with or without preceding aspirin treatment when compared with placebo (Table 2). No differences were observed in PGI_2 levels (*P*=0.84) or PGE_2 levels (*P*=0.98) during 3-OHB infusion between patients with HFrEF with and without preceding aspirin-induced cyclooxygenase inhibition (Table 2). Niacin treatment significantly increased PGD_2 levels by 58% (55.3 pg/mL [95% CI, 0.9–109.6 pg/mL]) compared with placebo (Table 3, Figure 3). No significant

Table 2. Ketone/Placebo With Preceding Aspirin and Without Aspirin

	Ketone/placebo with preceding aspirin				Ketone/placebo without aspirin				Ketone/placebo with preceding aspirin vs without aspirin	
	Placebo (saline) (n=12)	3-OHB (n=12)	Difference	*P* value	Placebo (saline) (n=16)	3-OHB (n=16)	Difference	*P* value	Difference of difference	*P* value
Hemodynamic parameters										
CO, L/min	5.1±1.5	7.4±2.0	2.3 [1.6, 2.9]	<0.01*	4.8±0.6	6.8±1.0	2.0 [1.6–2.4]	<0.01*	0.3 [–0.4, 0.9]	0.43
SV, mL	80±15	100±16	19 [14– 24]	<0.01*	75±16	95±18	20 [15–24]	<0.01*	0 [7–6]	0.90
RAP, mmHg	5±2	5±2	0	0.83	4.4±2.7	3.7±3.0	–0.7 [–1.4, –0.02]	0.05	1 [0–1.6]	0.23
PAP, mmHg	19±6	19±5	0 [–2, 2]	0.91	19.8±10.6	19.6±8.7	–0.2 [–2.0, 1.7]	0.80	0 [–2, 3]	0.82
PCWP, mmHg	9±2	8±2	–1 [0–1]	0.11	10±5	9±5	–1 [–2, 0]	0.03*	1 [–1, 2]	0.37
MAP, mmHg	81±16	77±12	–4 [–9, 0.8]	0.09	92±13	91±10	–1 [–5, 2]	0.48	–3 [–9, 3]	0.32
SVR, WU	15±3	10±3	–5 [–6, –4]	<0.01*	19±3	13±2	–5 [–4, –2]	<0.01*	0 [–1, 2]	0.60
Heart rate, bpm	63±9	73±11	10 [7–13]	<0.01*	66± 16	73±14	7 [4–11]	<0.01*	3 [–2, 7]	0.21
SVO_2, %	70±7	75±5	5 [2–8]	<0.01*	72±3	79±4	6 [5–8]	<0.01*	–2 [– 4, 1]	0.28
Echocardiography										
LVOT VTI, cm	22.4±3.8	23.3±3.9	0.9 [–1.7, 3.5]	0.46	18.1±3.5	22.1±4.3	4.0 [2.8–5.2]	<0.01*	–3.1 [–5.6, –0.6]	<0.02*
GLS, %	10.3±3.9	12.3±4.2	2.1 [1.2–3.0]	<0.01*	9.7±3.2	11.7±3.6	2.0 [1.3–2.7]	<0.01*	0.1 [–0.9, 1–1]	0.83
S′, cm/s	4.4±1.1	5.6±1.9	1.2 [0.5– 1.8]	<0.01*	3.7±0.8	4.5±1.3	0.8 [0.3–1.3]	<0.01*	0.4 [–0.4, 1–1]	0.32
LVEF, %	40±8.6	45±7	6 [3–9]	<0.01*	35±7	43±9	8 [5–11]	<0.01*	–2 [–6, 1]	0.21
LVEDV, mL	143±41	127±40	–16 [–29, 3]	0.02*	174±48	165±47	–9 [–20, 2]	0.09	–7 [–23, 9]	0.38
LVESV, mL	97±46	72±30	–25 [–44, –6]	0.02*	115±40	97±41	–17 [–24, 10]	<0.01*	–8 [–25, 9]	0.35
TAPSE, cm	1.8±0.4	2.0±0.5	0.2 [0.0, 0.4]	0.07	1.9±0.5	2.1±0.5	0.2 [0.1–0.3]	<0.01*	0.0 [–0.2, 0.2]	0.96
Substrates and hormones										
3-OHB, mM	0.4±0.4	4.4±0.9	4.1 [3.5–4.6]	<0.01*	0.3±0.3	3.3±0.4	2.9 [2.7–3.1]	<0.01*	1.1 [0.6–1.6]	<0.01*
D-3-OHB, mM	0.1±0.1	1.7±0.6	1.6 [1.2, 1.9]	<0.01*	NA	NA	NA	NA	–	–
Lactate, mmol/L	0.9±0.2	1.3±0.3	0.4 [0.2–0.60.0]	<0.01*	1.1±0.5	1.5±0.7	0.4 [0.3–0.6]	<0.01*	0.0 [–0.2, 0.2]	0.84
Glucose, mmol/L	7.6±0.6	7.7±0.8	0.0 [–0.2, 0.3]	0.72	5.7±0.7	5.7±0.6	0.0 [–0.4, 0.4]	0.99	–0.1 [–0.8, 0.3]	0.30
FFA, mmol/L	0.17±0.13	0.07±0.08	–0.10 [–0.17, – 0.03]	0.01*	0.17±0.18	0.09±0.12	- 0.08 [–0.14, –0.01]	0.02*	–0.02 [–0.12, 0.07]	0.60
Insulin, pM	225.4±95.0	236.6±135.4	11.2 [–33.7, 56.0]	0.59	175.3±72.1	183.4±48.4	8.2 [–20.4, 36.8]	0.55	53.16 [–21.8, 128.1]	0.16
Prostaglandins										
PGE_2, pg/mL	126.1±137.2	78.0±27.2	–48.0 [–125.6, 29.5]	0.20	157.3±87.2	108.3±50.8	- 49.0 [–107.9, 9.8]	0.10	1.0 [–89.6, 91.7]	0.98

(*Continued*)

Table 2. Continued

	Ketone/placebo with preceding aspirin				Ketone/placebo without aspirin				Ketone/placebo with preceding aspirin vs without aspirin	
	Placebo (saline) (n=12)	3-OHB (n=12)	Difference	*P* value	Placebo (saline) (n=16)	3-OHB (n=16)	Difference	*P* value	Difference of difference	*P* value
PGI_2, pg/mL	669.3±526.9	788.7±560.9	119.4 [−299.9, 538.7]	0.54	554.6±1236.7	581.4±660	26.8 [−815.4, 869.0]	0.95	92.6 [−851.5, 1036.7]	0.84
PGD_2, pg/mL	60.2±30.1	57.3±25.6	−2.8 [−10.0, 4.3]	0.40	117.7±44.9	109.4±40.1	−8.3 [−21.2, 4.6]	0.19	5.5 [−10.1, 21.0]	0.48

The data are shown as mean±SD. The measures were performed at the end of each intervention period. *P* values refer to paired *t* tests. bpm indicates beats per minute; 3-OHB, 3-hydroxybutyrate; CO, cardiac output; D-3-OHB, D-3-hydroxybutyrate; FFA, free fatty acids; GLS, numeric global longitudinal strain; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; LVOT VTI, left ventricular outflow tract velocity time integral; MAP, mean arterial pressure; NA, not available; PAP, mean pulmonal artery pressure; PCWP, pulmonary capillary wedge pressure; PGD_2, prostaglandin D_2; PGE_2, prostaglandin E_2; PGI_2, prostaglandin I_2; RAP, right arterial pressure; S′, systolic mitral plane peak excursion velocity; SV, stroke volume; SVO_2, mixed venous saturation; SVR, systemic vascular resistance;TAPSE, tricuspid annular peak systolic excursion; and WU, Wood units.

*$P<0.05$.

change was observed in PGI_2 and PGE_2 levels after niacin treatment compared with placebo.

FFA and Glucose

FFA levels were suppressed by 58% (−0.10 mmol/L; 95% CI, −0.17 to −0.03 mmol/L) during 3-OHB infusion compared with placebo in patients with HFrEF who received preceding aspirin (Table 2). In patients without aspirin administration, FFA levels were suppressed by 47% (−0.08 mmol/L; 95% CI, −0.14 to −0.01) during 3-OHB infusion compared with placebo (Table 2, Figure 4). No difference was recorded between the decrease in FFA levels during 3-OHB infusion between patients with HFrEF with and without preceding aspirin treatment (*P*=0.60). FFA levels were suppressed by 75% during niacin treatment compared with placebo (*P*<0.01). Glucose levels were stable in all study arms (Tables 2 and 3).

Safety

No serious adverse events were observed during the ketone study day. However, 1 patient experienced facial heat sensation during ketone infusion. Flushing and facial heat sensations were experienced by 9 of 10 (90%) of the patients during the niacin study day. One patient experienced vasovagal syncope after niacin administration, and the study day was discontinued for this patient. Four patients experienced nausea and vomiting after niacin intake. To avoid this, the niacin dose was then reduced to 500 mg.

DISCUSSION

The present study investigated the acute effects of circulating 3-OHB on prostaglandin release, circulating FFAs, and hemodynamics. We also tested the effects of the even stronger HCA_2 stimulator niacin. The main findings of the present study were as follows: First, CO increased by 2.3 L/min and left ventricular ejection fraction by 6 percentage points during 3-OHB infusion as compared with placebo, similar to our previous findings.[4] Second, the CO increase during 3-OHB infusion was not affected by aspirin-induced inhibition of the HCA_2 downstream cyclooxygenase enzyme. Third, PGI_2, PGE_2, and PGD_2 plasma levels did not change during 3-OHB infusion compared with placebo. Fourth, 3-OHB reduced circulating FFAs by 58%, consistent with our previous data.[4] Fifth, niacin, a high-affinity HCA_2 agonist, did not affect CO compared with placebo even though it increased prostaglandin levels. Last, niacin reduced FFA levels to the same extent as during 3-OHB infusion but did not change CO. In summary, our findings support that acute hemodynamic changes during 3-OHB infusion are not mediated through HCA_2 stimulation, prostaglandin release, or HCA_2-induced FFA reduction.

Table 3. Study Day 2 (Niacin/Placebo Day)

	Placebo (n=10)	Niacin (n=10)	Difference	*P* value
Hemodynamic parameters				
CO, L/min	4.5±0.9	4.5±0.6	0 [−0.4, 0.4]	0.98
SV, mL	72±12	70±11	- 3 [−9, 3]	0.35
RAP, mm Hg	5±1	6±1	1 [0.1–1.7]	0.03†
PAP, mm Hg	18±8	18±7	0 [−2, 3]	0.76
PCWP, mm Hg	10±6	9±6	−1 [−2, 0]	0.06
TPG, mm Hg	8.9±4.3	9.3±3.7	0.4 [−2.6–3.36]	0.77
PVR, WU	2.0±1.0	2.0±0.8	0.0 [−0.7–1.0]	0.91
MAP, mm Hg	86±13	84±14	- 2 [−12, 8]	0.72
SVR, WU	18.3±2.6	17.5±2.3	−0.8 [−3.6, 2.0]	0.55
Heart rate, bpm	62±8	64±59	3 [−1, 6]	0.18
SVO_2, %	65±7	63±6	−2 [−6, 2]	0.33
Echocardiography				
LVOT VTI, cm	18.3±3.6	17.2±2.6	−1.0 [−3.0, 0.9]	0.25
GLS, %	9.1±2.4	10.3±6.9	1.1 [−0.4, 2.6]	0.12
S′, cm/s	4.1±1.4	4.2±1.6	0.1 [−0.7, 0.9]	0.75
LVEF, %	38.8±7.5	38.4±6.8	−0.4 [−2.3, 1.6]	0.66
LVEDV, mL	135.4±57.9	134±59.1	−1.4 [−30.5, 27.7]	0.91
LVESV, mL	84.3±42.6	84.4±45.4	0.1 [−18.7, 19.0]	0.99
TAPSE, cm	16.3±3.5	17.6±4.6	1.2 [−2.5, 4.9]	0.47
Blood samples				
Lactate, mmol/L	1.0±0.2	1.1±0.2	0.1 [−0.1, 0.3]	0.25
Glucose, mmol/L	6.0±0.8	5.5±0.8	−0.5 [−1.3, 0.3]	0.18
FFA*, mmol/L	0.3±0.1	0.2±0.1	−0.2 [−0.3, −0.7]	<0.01†
pH	7.4±0.0	7.4±0.0	0.0 [0.0–0.0]	0.22
Niacin, µg/mL	1.6±1.0	9.7±7.2	8.1 [3.6–12.6]	<0.01†
Prostaglandins				
PGE_2, pg/mL	138.8±97.7	125.6±69.5	−13.2 [−102.9, 76.5]	0.74
PGI_2, pg/mL	634.1±352.8	1308.4±1658	674.2 [−543.2, 1891.7]	0.24
PGD_2, pg/mL	95.6±54.3	150.8±97.7	55.3 [0.9–109.6]	0.047†

The data are shown as mean±SD. The measures were performed at the end of each intervention period. *P* values refer to paired *t* test. bpm indicates beats per minute; CO, cardiac output; FFA, free fatty acids, GLS, numeric global longitudinal strain; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; LVOT VTI, left ventricular outflow tract velocity time integral; MAP, mean arterial pressure; PAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; PGD_2, prostaglandin D_2; PGE_2, prostaglandin E_2; PGI_2, prostaglandin I_2; PVR, pulmonary vascular resistance; RAP, right arterial pressure; S′, systolic mitral plane peak excursion velocity; SV, stroke volume; SVO_2, mixed venous saturation; SVR, systemic vascular resistance; TAPSE, tricuspid annular peak systolic excursion; TPG, transpulmonary pressure gradient; and WU, Wood units.

*Calculated from the area under the curve during the 3-hour intervention.

†$P<0.05$.

HCA_2 Stimulation by 3-OHB, Prostaglandin Release, and Hemodynamic Effects

PGE_2 levels are elevated in patients with heart failure,[22] and PGI_2 treatment of patients with HFrEF increases cardiac index.[23] We hypothesized that 3-OHB is involved in prostaglandin release and that prostaglandins mediate a beneficial hemodynamic response. HCA_2 activation is known to promote production and release of PGD_2 and PGE_2 from macrophages,[17] and macrophages are involved in the HCA_2-mediated PGD_2 and PGE_2 production and cutaneous flushing during niacin treatment.[24] Activation of the G-protein-coupled receptor HCA_2 initiates a cascade of downstream effects in the macrophages. These effects include activation of the cytosolic phospholipase A_2 and release of arachidonic acid, which are converted into prostaglandins by cyclooxygenase enzymes.[17] An animal study has shown that 3-OHB activates HCA_2 and is tissue-protective through release of prostaglandin from monocytes and macrophages.[25]

3-OHB activates HCA_2 with an EC_{50} of ≈750 µmol/L.[26] In the present study, we reached levels of 3-OHB that

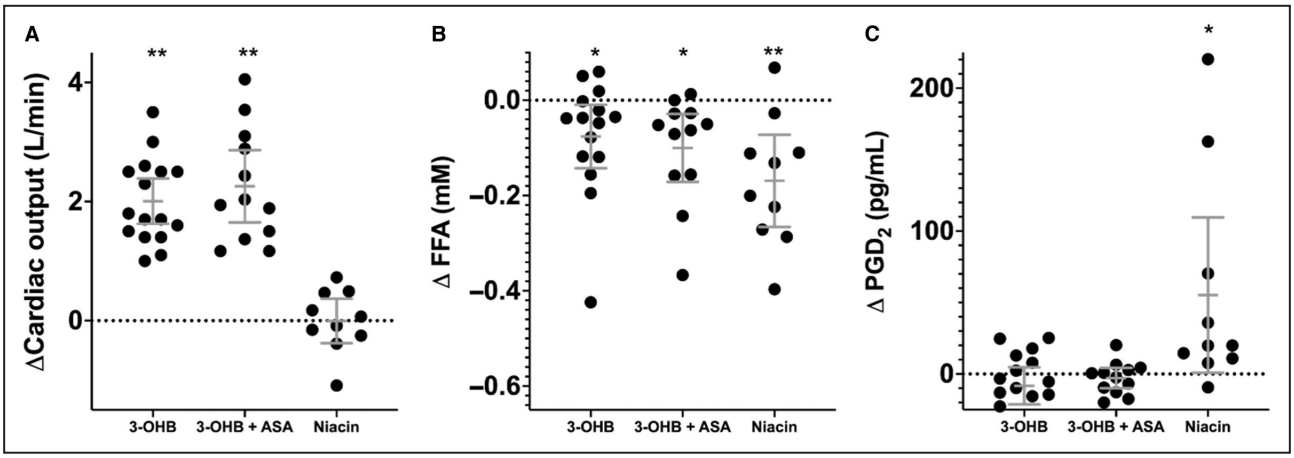

Figure 3. Changes in cardiac output, free fatty acids, and prostaglandin D_2.
ΔCardiac output (**A**), ΔFFA (**B**), and ΔPGD_2 (**C**) recorded during the intervention period as compared with the placebo period. 3-OHB indicates 3-hydroxybutyrate; ASA, aspirin; FFA, free fratty acid; and PGD_2, prostaglandin D_2. **P*<0.05 ***P*<0.01. 3-OHB: HCA_2 receptors are activated; 3-OHB+ASA: HCA_2 receptors are activated, whereas the downstream cyclooxygenase enzyme is inhibited with ASA; Niacin: HCA_2 receptors are activated by an even stronger HCA_2 agonist than 3-OHB.

were 4 times higher. We therefore assume that the HCA_2 receptor was fully stimulated. Intravenous aspirin is documented to fully suppress prostaglandin levels after 5 minutes and until 48 hours after administration.[18] In the present study, 3-OHB infusion did not affect circulating prostaglandin levels (PGE_2, PGI_2, PGD_2) compared with placebo (Table 2). Thus, this study showed that 3-OHB infusion did not change HCA_2-mediated prostaglandin release and that PGI_2, PGE_2, and PGD_2 were not involved in the acute hemodynamic effects of 3-OHB.

Niacin Does Not Affect Hemodynamics

Niacin is an even stronger HCA_2 agonist than 3-OHB with an EC_{50} of 0.1 μmol/L.[26] Therefore, niacin was chosen as a positive control in the present study. We reached niacin plasma levels of 9.7 mg/mL (≈79 430 μmol/L) after niacin treatment. Niacin stimulates prostaglandin release,[27] resulting in vasodilation and cutaneous flushing.[28,29] The present study confirmed the increase in prostaglandin release (PGD_2) during niacin treatment in patients with HFrEF. However, no hemodynamic changes were observed during niacin treatment when compared with placebo. The vasodilatory effect of niacin is predominantly described in the cutaneous vessels.[17] The lack of significant change in SVR following niacin administration may therefore be explained by isolated cutaneous vasodilation and thus is only a minor effect on SVR. Other potential explanations of no change of SVR include the possibility of a rapid and transient effect of oral niacin,[30] compensatory hemodynamic reflexes to counteract the vasodilatory effect and that most patients were on angiotensin-converting enzyme inhibitors, and β-blocker treatment. However, no increase in CO corroborates that prostaglandin

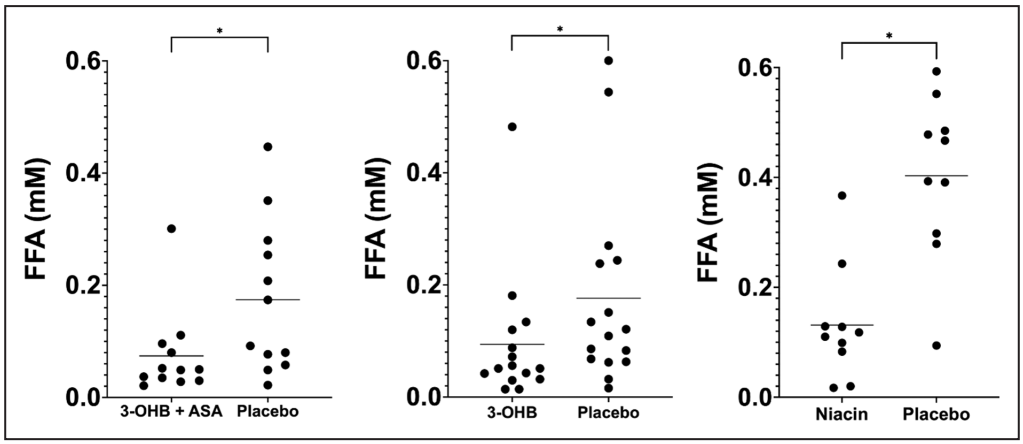

Figure 4. FFA concentrations.
FFA levels in all 3 study groups compared with placebo. 3-OHB indicates 3-hydroxybutyrate; ASA, aspirin; and FFA, free fatty acid. **P*<0.05.

downstream release of HCA_2 stimulation does not induce any major hemodynamic changes.

HCA_2-Mediated Metabolic Effects

It is well known that HCA_2 stimulation on adipocytes decreases FFA levels[19,20] (Figure 1). In the adipocyte, HCA_2 stimulation inhibits adenylyl cyclase and decreases cAMP production. This results in decreased conversion of triglyceride to FFA via the hormone-sensitive lipase and adipocyte triglyceride lipase, which decrease the level of FFA release from the adipocytes.[15] This downstream regulation is not cyclooxygenase enzyme dependent or related to prostaglandin release.[16] In the present study, 3-OHB infusion decreased FFA levels by 58% as compared with placebo. Aspirin administration did not affect the decrease in FFA levels (Table 2 and Figure 3). The present study further confirmed that niacin decreased FFA levels (Table 3 and Figure 3). These results show that suppression of circulating FFA levels through HCA_2 stimulation by 3-OHB involves a cyclooxygenase-independent mechanism.

In an experimental study, 3-OHB infusion increased cardiac FFA uptake.[5] The beneficial hemodynamic response during 3-OHB infusion could therefore be mediated through reduced plasma FFA levels and an altered myocardial substrate metabolism.[3,10] However, during niacin treatment, FFA levels were reduced to similar levels as during 3-OHB infusion without significant hemodynamic changes. We did not measure myocardial FFA uptake, but it is well documented that plasma FFA levels correlate with myocardial FFA uptake.[31,32] Our findings indicate that the increase in CO during 3-OHB infusion was not mediated through changes in circulating FFAs. This is further supported by other studies reporting no change in myocardial FFA uptake during 3-OHB infusion[3] and no impact on left ventricular ejection fraction.[33]

Mechanism Behind Hemodynamic Effect of 3-OHB

3-OHB infusion increased CO through an increase in stroke volume and heart rate and a decrease in SVR (Table 2). Further studies are needed to investigate whether the CO increase involves afterload reduction or increased contractility. Whether the changes in hemodynamic response during 3-OHB infusion are mediated through a direct metabolic effect involving increased myocardial 3-OHB uptake and metabolization remains unknown and warrants further studies.

Study Limitations

This study investigated the acute effects of 3-OHB and niacin on hemodynamics and metabolism. Long-term 3-OHB treatment may have different effects due to HCA_2 activation and prostaglandin release. Furthermore, the present study did not inhibit the HCA_2 receptor directly. However, HCA_2 antagonists are unavailable for human use. The infusion of 3-OHB consisted of 50% L-enantiomer and 50% D-enantiomer. Only the D-enantiomer is endogenously produced. However, HCA_2 has affinity for both enanatiomers.[20]

The data pertaining to patients who received ketone and placebo without aspirin were obtained from a previous study.[4] Although the study design utilized a retrospective cohort, the participants were sourced from the same medical center and were investigated by the same research team. Furthermore, all measurements were performed using the same equipment as in the current study, leading to the assumption that the 2 groups can be compared.

Circulating prostaglandin levels did not change significantly during 3-OHB infusion compared with placebo. However, circulating prostaglandins are very unstable, so the lack of change in circulating prostaglandins during 3-OHB infusion should be interpreted cautiously. We argue that prostaglandins were not involved in ketone-related increase in CO, since aspirin in the given dose is documented to block prostaglandin release and production.[18]

The ketone body Na-3-OHB dissociates into 3-OHB^- and Na^+. 3-OHB^- is a weak base, which is why pH increases during 3-OHB infusion.Alkalosis may mediate vasodilation and afterload reduction and thus increase CO. However, in most vascular beds, alkalosis either has no effect on vascular tone or causes vasoconstriction,[34] and it is unlikely that alkalosis is the only mediator of the CO increase. First, the increase in pH during 3-OHB infusion was minimal (from 7.38 to 7.46). Second, pH did not decrease during the placebo period in the group that started with 3-OHB, whereas CO decreased during the placebo period.

There was a statistically significant increase in the peak 3-OHB levels in the aspirin-receiving group when compared with the nonaspirin group. Nonetheless, we deem it improbable that the observed difference in peak circulating 3-OHB levels can be attributed to the effect of aspirin treatment, because no effect of aspirin on ketone metabolism is known to exist. Rather, it is plausible that these fluctuations could be attributed to variations in physiological parameters or a type 1 error.

Despite active attempts to recruit female patients, we only managed to include 1. The hemodynamic findings for this 1 female participant were similar to those of the male participants.

CONCLUSIONS

In conclusion, our data demonstrated that the increase in CO during 3-OHB infusion in patients with HFrEF is

not mediated through HCA_2 stimulation, prostaglandin release, or suppression of circulating FFAs.

ARTICLE INFORMATION

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Disclosures

Dr Wiggers has been the principal or a subinvestigator in studies involving the following pharmaceutical companies: MSD, Bayer, Daiichi-Sankyo, Novartis, Novo Nordisk, Sanofi-Aventis, and Pfizer. The remaining authors have no disclosures to report.

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