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Ketone bodies in right ventricular failure: A unique therapeutic opportunity

Madelyn Blake [a], Patrycja Puchalska [b], Felipe Kazmirczak [a], Jeffrey Blake [a], Ryan Moon [a], Thenappan Thenappan [a], Peter A. Crawford [b,c], Kurt W. Prins [a,*]

[a] *Lillehei Heart Institute, Cardiovascular Division, University of Minnesota, United States*
[b] *Division of Molecular Medicine, Department of Medicine, University of Minnesota, United States*
[c] *Department of Biochemistry, Molecular Biology, and Biophysics, United States*

A B S T R A C T

Background: Ketone bodies are pleotropic metabolites that play important roles in multiple biological processes ranging from bioenergetics to inflammation regulation via suppression of the NLRP3 inflammasome, and epigenetic modifications. Ketone bodies are elevated in left ventricular failure (LVF) and multiple approaches that increase ketone concentrations exert advantageous cardiac effects in rodents and humans. However, the relationships between ketone bodies and right ventricular failure (RVF) are relatively unexplored.

Methods: 51 PAH patients were dichotomized into preserved or impaired RV function based on a cardiac index of 2.2 L/min/m^2. Impaired RV function patients were further segmented into intermediate or severe RV dysfunction based on a right atrial pressure of 8 mm Hg. Serum ketone bodies acetoacetate (AcAc) and beta-hydroxybutyrate (βOHB) were quantified using ultra performance liquid chromatography and mass spectrometry. In rodent studies, male Sprague Dawley rats were assigned to three groups: control (saline injection), monocrotaline (MCT) standard chow diet (MCT-Standard), and MCT ketogenic diet (MCT-Keto). Immunoblots and confocal microscopy probed macrophage NLRP3 activation in RV extracts and sections. RV fibrosis was determined by Picrosirus Red. Echocardiography evaluated RV function. Pulmonary arteriole remodeling was assessed from histological specimens.

Results: Human RVF patients lacked a compensatory ketosis as serum AcAc and βOHB levels were not associated with hemodynamic, echocardiographic, or biochemical measures of RV dysfunction. In rodent studies, AcAc and βOHB levels were also not elevated in MCT-mediated RVF, but the ketogenic diet significantly increased AcAc and βOHB levels. MCT-Keto exhibited suppressed NLRP3 activation with a reduction in NLRP3, ASC (apoptosis-associated speck-like protein), pro-caspase-1, and interleukin-1 beta on immunoblots. Moreover, the number of ASC-positive macrophage in RV sections was reduced, RV fibrosis was blunted, and RV function was augmented in MCT-Keto rats.

Conclusion: The ketogenic response is blunted in pulmonary arterial hypertension (PAH) patients with RVF. In the MCT rat model of PAH-mediated RVF, a dietary-induced ketosis improves RV function, suppresses NLRP3 inflammasome activation, and combats RV fibrosis. The summation of these data suggest ketogenic therapies may be particularly efficacious in RVF, and therefore future studies evaluating ketogenic interventions in human RVF are warranted.

Abbreviations: LVF, Left ventricular failure; RVF, Right ventricular failure; AcAc, Acetoacetate; βOHB, Beta-hydroxybutyrate; MCT, Monocrotaline; PAH, Pulmonary arterial hypertension; SGLT2, Sodium-glucose co-transporter-2; NLRP3, Nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3; RVFAC, RV fractional area change; RVEDA, RV end diastolic area; RVESA, RV end systolic area; TAPSE, Tricuspid annular plane systolic excursion; UPLC-MS/MS, Ultra performance liquid chromatography and mass spectrometry; ASC, Apoptosis-associated speck-like protein containing a CARD; mPAP, Mean pulmonary arterial pressure; PVR, Pulmonary vascular resistance; CTEPH, Chronic thromboembolic pulmonary hypertension; OXCT1, 3-oxoacid CoA-transferase 1; BDH1, 3-hydroxybutyrate dehydrogenase.

* Corresponding author. Medicine Director of Translational Research in Pulmonary Hypertension University of Minnesota Minneapolis, MN, United States.

E-mail address: Prin0088@umn.edu (K.W. Prins).

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1. Introduction

RVF is the leading cause of death in pulmonary arterial hypertension (PAH), but at present there are no therapies that effectively combat RVF [[1]]. This is in juxtaposition to LVF treatment as several medications can both improve LV function and long-term survival [[2]]. Additionally, there are differing degrees of end-organ dysfunction when comparing RVF and LVF. In particular, RVF is associated with more severe liver impairments, and in many cases RVF can ultimately result in cirrhosis [[3]]. At present, the impact of RVF on earlier phenotypes of liver function are not well described. In particular, the consequences of RVF on hepatic metabolic function are unknown, and it is plausible that impairment of liver metabolism could be another detrimental effect of RVF. This raises the possibility that a RV-liver axis exists, and it could serve as a novel therapeutic target for this uniformly lethal consequence of PAH.

An important metabolic function of the liver is the synthesis of ketone bodies, metabolites that regulate diverse biological functions ranging from energy homeostasis, inflammation, and epigenetic regulation [[4]]. Both preclinical models and patients with LVF have elevated ketone levels, and ketone concentrations are inversely associated with echocardiographic and biochemical measures of LV function [[5]]. Multiple interventions to induce ketogenic states including intermittent fasting, ketogenic diets, ketone body

Table 1
Clinical, echocardiographic, and hemodynamic characteristics of study cohort.

Characteristics	Total Cohort	TD CI > 2.2	TD CI < 2.2, RAP <8	TD CI < 2.2, RAP >8	*p*-value
	(*n* = 51)	(*n* = 26)	(*n* = 10)	(n = 15)	
Age, years	58 ± 15	59 ± 12	55 ± 16	59 ± 18	0.70
Female, *n* (%)	31 (60)	17 (65)	6 (60)	8 (53)	0.75
Body Mass Index, kg/mˆ2	28 ± 7	28 ± 7	29 ± 8	27 ± 4	0.77
WHO Functional Class (*n* = 48)					
I	2 (4)	2 (9)	0 (0)	0 (0)	
II	13 (27)	8 (35)	4 (40)	1 (7)	
III	32 (67)	13 (57)	6 (60)	13 (87)	
IV	1 (2)	0 (0)	0 (0)	1 (7)	
Comorbidities, *n* (%)					
Hypertension	23 (45)	12 (46)	4 (40)	7 (47)	0.94
Diabetes	12 (24)	6 (23)	0 (0)	6 (40)	0.07
Hyperlipidemia	16 (31)	9 (35)	3 (30)	4 (27)	0.87
Coronary Artery Disease	7 (14)	3 (12)	1 (10)	3 (20)	0.70
Atrial Fibrillation	5 (10)	1 (4)	0 (0)	4 (27)	0.03
COPD	8 (16)	5 (19)	1 (10)	2 (13)	0.76
Medications, *n* (%) (*n* = 49)					
Monotherapy	7 (14)	4 (15)	1 (10)	2 (13)	0.92
Dual Oral Therapy	15 (31)	12 (46)	1 (10)	2 (13)	0.03
Triple Oral Therapy	12 (24)	5 (19)	2 (20)	5 (33)	0.57
Parental Prostacyclin Plus Oral	15 (31)	5 (19)	4 (40)	6 (40)	0.27
Lab					
Serum Hemoglobin, g/dl (*n* = *51*)	13 ± 2	13 ± 2	14 ± 3	13 ± 2	0.63
Serum Creatinine, mg/dl (*n* = *51*)	1 ± 0.35	1 ± 0.27	1 ± 0.37	1.1 ± 0.5	0.89
Serum NT-proBNP (*n* = *50*)	1786 ± 2705	508 ± 1067	1402 ± 1190	4330 ± 3799	<0.0001
Serum Sodium	139 ± 3	139 ± 2	140 ± 2	138 ± 3	0.40
Serum Bilirubin	0.7 ± 0.3	0.6 ± 0.2	1.1 ± 0.2	1.1 ± 0.1	0.09
Serum ALT	26 ± 10	28 ± 12	23 ± 6	25 ± 10	0.74
Serum AST	21 ± 9	20 ± 8	23 ± 7	23 ± 11	0.46
Serum INR	1.5 ± 0.6	1.4 ± 0.6	1.4 ± 0.4	1.5 ± 0.7	0.71
MELD Score	10.8 ± 5.0	10.2 ± 4.1	11.1 ± 3.3	12.5 ± 6.1	0.48
MELD NA Score	10.4 ± 5.5	8.8 ± 4.9	11.4 ± 3.9	12.8 ± 6.0	0.13
MELD-XI Score	10.4 ± 2.5	10.4 ± 1.7	11.2 ± 2.2	11.8 ± 3.2	0.39
Serum Acetoacetate	26 ± 23	23 ± 21	21 ± 12	32 ± 29	0.63
Serum β-Hydroxybutyrate	77 ± 79	66 ± 63	74 ± 66	92 ± 109	0.69
Echocardiography					
Right Ventricular FAC (*n* = *44*)	33 ± 10	39 ± 7	29 ± 7	23 ± 8	<0.0001
RVEDA	26 ± 8	24 ± 7	28 ± 8	29 ± 10	0.12
RVESA	18 ± 8	15 ± 6	20 ± 7	23 ± 10	0.01
TAPSE	2 ± 0.6	2.4 ± 0.6	1.7 ± 0.4	1.6 ± 0.4	<0.0001
S'	11 ± 4	13 ± 4	10 ± 4	9 ± 2	<0.001
Tricuspid Regurgitation Severity	3 ± 1	2 ± 1	3 ± 1	4 ± 1	<0.01
RV Global Longitudinal Strain	-18 ± 6	-23 ± 4	-16 ± 4	-13 ± 5	<0.0001
Hemodynamics					
Heart Rate, beats/min (*n* = *51*)	74 ± 19	71 ± 12	69 ± 14	84 ± 28	0.20
Mean Right Atrial, mm Hg (*n* = *51*)	7 ± 5	7 ± 4	5 ± 2	14 ± 4	<0.0001
Mean PAP, mm Hg (*n* = *51*)	43 ± 12	36 ± 8	42 ± 16	51 ± 13	<0.0001
Cardiac Output, liters/min (*n* = *51*)	4.9 ± 1.8	6.3 ± 1.9	3.5 ± 0.8	3.7 ± 0.8	<0.0001
Cardiac Index, liters/min/m^2 (*n* = *51*)	2.6 ± 1.0	3.3 ± 1.0	1.9 ± 0.2	1.9 ± 0.4	<0.0001
PVR, Wood units (*n* = *50*)	8 ± 5	5 ± 2	8 ± 5	13 ± 6	<0.0001

injections, sodium-glucose co-transporter-2 (SGLT2) inhibitor use, and genetic manipulation of ketone metabolism impart cardio-protective changes in both preclinical and human LVF [[6,7]]. At present, most of the studies evaluating the advantageous effects of ketone bodies in cardiac dysfunction have focused on the favorable metabolic properties that ketone bodies possess [[6,7]]. However, the ketone body β-hydroxybutyrate (βOHB) suppresses nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3) inflammasome activity, which suggests ketogenic interventions may have important anti-inflammatory ramifications that could also combat cardiac dysfunction. We recently showed the NLRP3 inflammasome pathway promotes PAH-mediated RVF, but the interplay between RV inflammation/NLRP3 activation, RV function, and circulating ketones has not been studied in-depth [[8,9]]. Ketones exert therapeutic effects through suppression of the NLRP3 inflammasome and exhibit favorable metabolic properties in LVF. However, the relationships among serum ketone bodies, RV inflammasome activation, and RV function in preclinical and human RVF are unexplored.

To address these important knowledge gaps, we performed a translational study that first evaluated relationships between RV function and serum ketone body levels in 51 patients with PAH. Then, we probed the effects of a ketogenic diet on RV function, NLRP3 inflammasome activation, and RV fibrosis in preclinical RVF. We showed human RVF patients lack a compensatory ketosis as serum ketone body concentrations were not associated with hemodynamic, biochemical, or echocardiographic measures of RV function. In rodent studies, a ketogenic diet increased circulating ketones, suppressed NLRP3 inflammasome activation, blunted RV fibrosis, and augmented RV function. In total, our preclinical and clinical data suggest a therapeutic ketosis could combat RVF. Moreover, patients with RVF may be uniquely primed for ketogenic interventions as they appear to exhibit an insufficient ketogenic response.

2. Materials and methods

2.1. Human patients

We examined the relationship between RV function and serum ketone bodies in 51 PAH-patients from the University of Minnesota Pulmonary Hypertension Program. PAH patients were sorted into three groups: a compensated (cardiac index>2.2 L/min/m^2 as defined by Thermodilution), intermediate decompensated (cardiac index ≤2.2 L/min/m^2, right atrial pressure <8 mm Hg), or severe decompensated (cardiac index ≤2.2 L/min/m^2, right atrial pressure >8 mm Hg) RV phenotype. Patients were stratified into compensated or decompensated RV phenotype cohorts based upon the threshold cardiac index value of 2.2 as previously defined by Boucherat et al. (Table 1) [[10]]. g. Patients with known hepatic conditions were excluded. RV function was characterized using hemodynamic and comprehensive echocardiographic analysis (RV strain, RV fractional area change (RVFAC), RV end diastolic area (RVEDA), RV end systolic area (RVESA), tricuspid annular plane systolic excursion (TAPSE), S', tricuspid regurgitation severity) (Table 1). Echocardiography images were analyzed offline and blindly by FK. Finally, RV-pulmonary artery coupling was estimated using TAPSE/mean pulmonary arterial pressure. Human studies were approved by the University of Minnesota Institutional Review Board.

2.2. Serum ketone measurements

Fasting blood samples were drawn from each patient prior to a planned hemodynamic assessment. Circulating serum ketone bodies acetoacetate (AcAc) and beta-hydroxybutyrate (βOHB) were quantified using ultra performance liquid chromatography and mass spectrometry (UPLC-MS/MS) [[11]].

2.3. Rodent ketogenic intervention

Male Sprague Dawley rats (Charles River Laboratories) were randomized into three experimental groups: phosphate buffered saline injected (control), monocrotaline (MCT, 60 mg/kg subcutaneous injection) rats fed standard (MCT-Standard) chow (Teklad:2918), and monocrotaline rats fed ketogenic (MCT-Keto) chow (Teklad:93M). To ensure a translational approach, dietary intervention began two weeks after MCT injection. End point analyses were performed 24 days post-MCT exposure. Rodent RV function was examined with echocardiography using a Vevo2100 ultrasound system as previously described [[12]]. The University of Minnesota Institutional Animal Care and Use Committee approved rodent studies.

2.4. Serum ketone measurements

Animals were fasted overnight and then blood was drawn for analysis. Blood was allowed to coagulate for 30 min and then was spun at 1000*g* for 20 min; the serum fraction was aspirated, and immediately snap frozen in liquid nitrogen. Ketone bodies were measured using UPLC-MS/MS as described above.

2.5. RV fibrosis assessment

RV free wall sections were collected and fixed in 10 % formalin before being embedded in paraffin, sectioned at 10-μm, and stained with Picrosirius Red by the University of Minnesota Histology and Research Laboratory in the Clinical and Translational Science Institute.

2.6. Confocal microscopy

RV free wall sections were deparaffinized using Xylene and subsequent incubations with 100 % ethanol, 95% ethanol, and 70% ethanol. Slides were then placed in a water bath with 10 % decloaking solution, before being permeabilized with 1% Triton X-100 in PBS. Slides were blocked with 5 % goat serum before incubation with primary antibodies galectin-3 and ASC overnight at 4 °C. Following primary antibody incubation, sections were blocked with 5% goat serum before incubation with VectaFluor Amplifier Antibody. Secondary antibody incubation was performed for 30 min at 37 °C. Sections were washed with PBS and incubated with 0.1% Hoechst stain before treatment with a fluorescence quenching kit and mounted in anti-fade reagent. Confocal micrographs were collected on a Zeiss LSM 900 Airyscan 2.0 microscope. RV fibrosis and macrophage analyses were blindly performed by MB.

2.7. Lung histology

Lung tissue specimens underwent fixation in a 10 % formalin solution, followed by paraffin embedding. Subsequently, these specimens were sectioned at a thickness of 4-μm and subjected to hematoxylin and eosin (H&E) staining. The Histology and Research Laboratory within the Clinical and Translational Science Institute at the University of Minnesota performed these procedural steps. Visual documentation was facilitated by utilization of a Zeiss AxioCam IC system while quantitative analyses were conducted using the FIJI software by JB. Quantification of the percent medial thickness of diminutive pulmonary arterioles was achieved through the application of the following formula: 100 × (outer diameter - inner diameter)/outer diameter.

2.8. Immunoblots

RV free wall extracts were prepared and subjected to Western blotting protocol to quantify protein expression as previously described [[13]]. Antibodies to NLRP3, Caspase-1, interleukin-1β, and apoptosis-associated speck-like protein containing a CARD (ASC) were used to biochemically characterize inflammasome activation. A complete list of antibodies used for immunoblots is included in Supplemental Table 1.

2.9. Statistical analysis

All data were evaluated for normality using Shapiro-Wilk test. When analyzing differences between two groups, normally distributed variables were compared using unpaired *t*-test and Mann-Whitney *U* test was used for non-normally distributed variables. When comparing three groups with normal distributions, one-way ANOVA with Tukey post hoc analysis was performed if variance was equal as determined by Brown-Forsythe test. If variance was unequal, Brown-Forsythe and Welch ANOVA with Dunn post hoc analysis was completed. If the data were not normally distributed, the Kruskal-Wallis and Dunn's multiple-comparisons tests were employed. Linear regression analysis was used to determine the relationships among serum ketone concentrations, RV function, and PAH severity. All statistical analyses were performed on GraphPad Prism version 9.0. Statistical significance was defined by $p < 0.05$.

Ethics approval

All entities involved in the present study, clinical or preclinical, provided ethics approval for the experimental design and associated analysis.

3. Results

3.1. Circulating ketone bodies were not strongly associated with biochemical, echocardiographic, or hemodynamic measures of RV function in human PAH-mediated RVF

We first validated divergent RV phenotypes when we separated our cohort into three groups: patients with either compensated, intermediate decompensated, or severe decompensated RV function. Hemodynamic, echocardiographic, and biochemical markers of RV function were more most deranged in the severely decompensated group (Table 1 and Supplemental Fig. 1). When comparing clinical characteristics between the three populations, there were no significant differences in cardiovascular comorbidities, age, and sex distributions. However, laboratory assessments revealed higher serum NT pro-BNP and total bilirubin in the decompensated groups (Table 1). Moreover, there were trends for higher MELD scores in the patients with the most compromised cardiac function, suggesting a right heart liver axis may be present. Finally, the decompensated patients had more advanced PAH with higher mean pulmonary arterial pressure (mPAP) and pulmonary vascular resistance (PVR) (Table 1).

Unlike prior observations in patients with LVF, circulating concentrations of the two ketone bodies acetoacetate (AcAc) and beta-hydroxybutyrate (βOHB) were not different when the compensated and decompensated groups were compared (Supplemental Fig. 1) [[13]]. Furthermore, when comparing the intermediate and severe decompensated groups, there was still no significant difference between their serum AcAc and βOHB levels (Table 1). Surprisingly, AcAc and βOHB levels were not strongly associated with cardiac index, right atrial pressure, RV global longitudinal strain, tricuspid annular plane systolic excursion (TAPSE), serum NT pro-BNP, or TAPSE/mPAP (Fig. 1 A-F). Additionally, there were no significant relationships between mPAP and PVR and AcAc and βOHB (Supplemental Fig. 2).

3.2. A ketogenic diet increased serum ketone body concentrations and improved RV function in rodent RVF

Next, we determined how a dietary-induced ketosis, starting after PAH was allowed to develop, modulated RVF in MCT rats (Fig. 2A). In agreement with our human data, MCT-Standard rats were not ketotic, and in fact they had lower βOHB levels than controls (Fig. 2B). Importantly, the ketogenic diet (MCT-Keto) significantly increased serum AcAc and βOHB (Fig. 2B). Then, we quantified the RV-effects of the ketogenic diet using RV-focused echocardiographic analysis. As compared to MCT-Standard, MCT-Keto rats had higher TAPSE, percent RV free wall thickening, and cardiac output despite (Fig. 2C). The improvements in RV function occurred despite no differences in pulmonary artery acceleration time, a well-validated echocardiographic marker of pulmonary hypertension

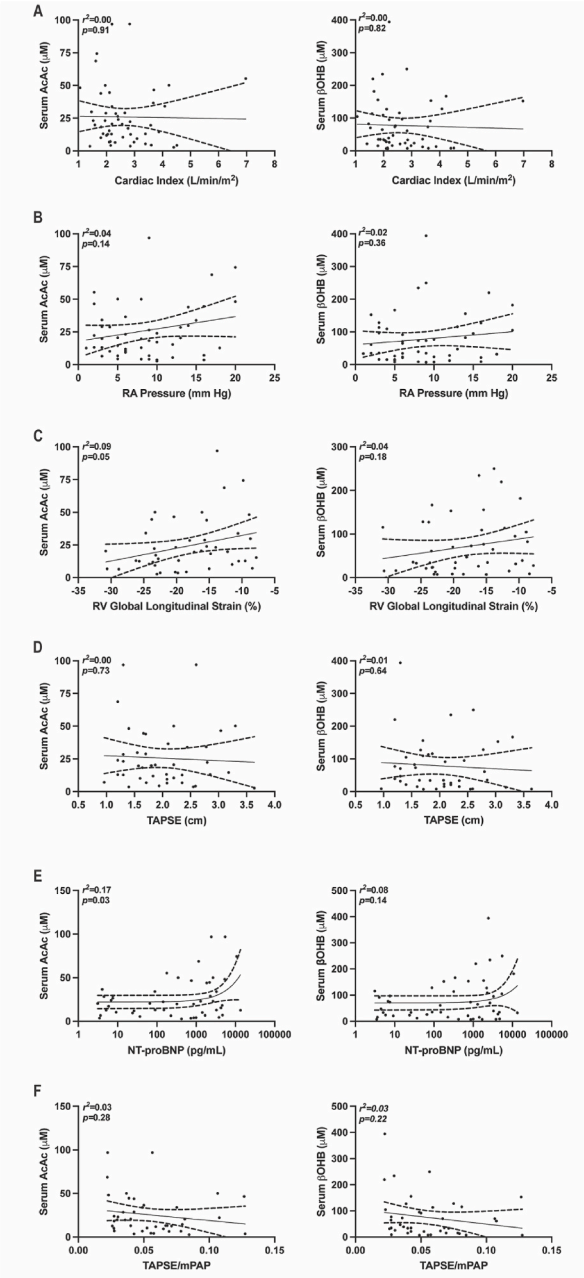

Fig. 1. Serum ketones were not strongly associated with echocardiographic, hemodynamic, or biochemical measures of RV function or RV-pulmonary artery coupling (A) Cardiac index was not associated with serum ketone body concentrations. (B) Right atrial pressure was not strongly associated with AcAc or βOHB. (C) RV global longitudinal strain was not associated with serum ketone body concentrations. (D) TAPSE was not significantly associated with AcAc or βOHB. (E) NT-proBNP was not strongly associated with serum ketone body concentrations. (F) TAPSE/mPAP was not meaningfully associated with serum ketone body concentrations.

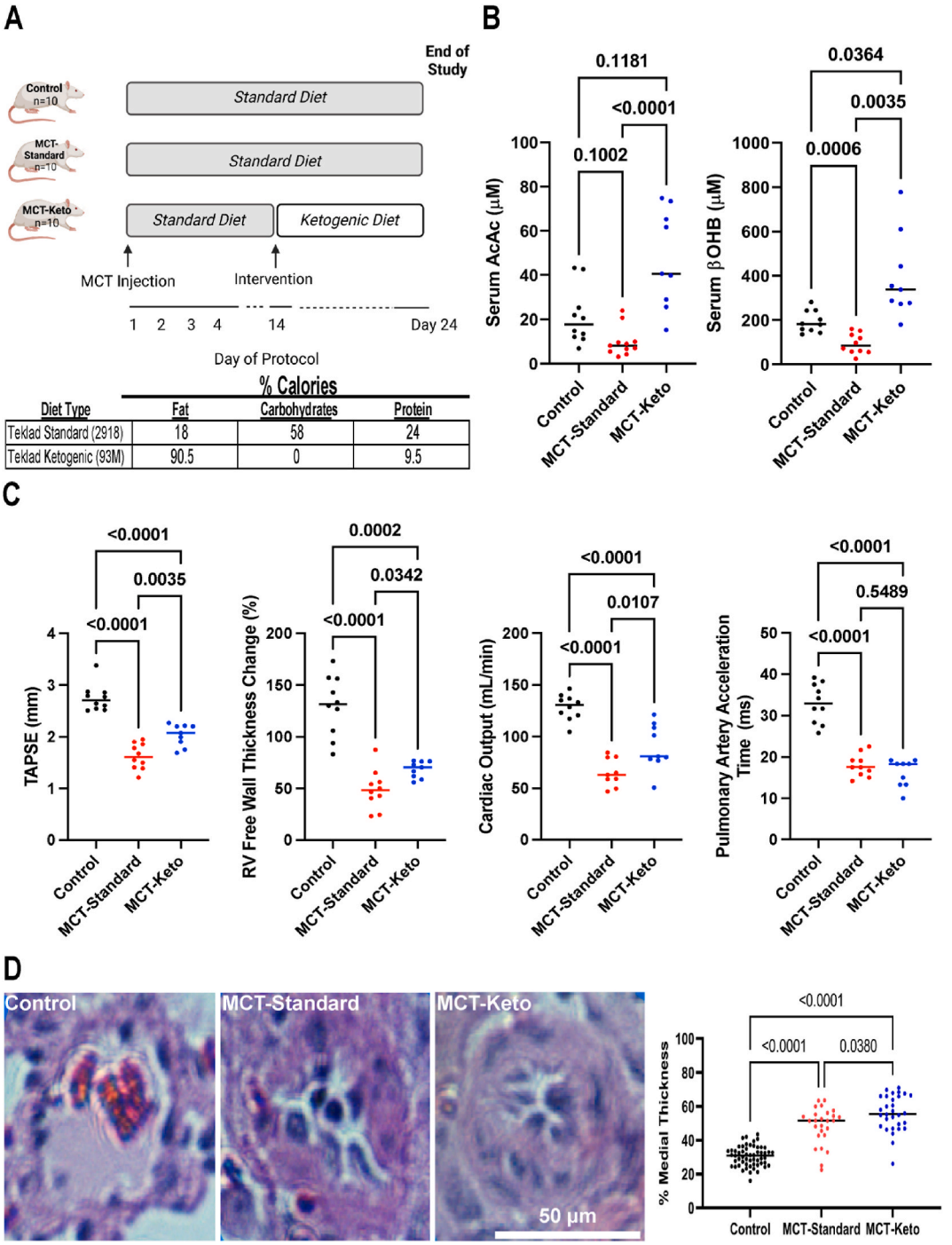

Fig. 2. A ketogenic diet increased serum ketone body concentrations and augmented RV function in monocrotaline rats (A) Diagram of experiment approach and caloric composition of diets. (B) A ketogenic diet increased serum concentrations of AcAc and βOHB in MCT-Keto rats. (C) Ketogenic diet significantly increased TAPSE (control: 2.7 ± 0.2 mm, MCT-standard: 1.6 ± 0.2 mm, MCT-keto: 2.0 ± 0.2 mm), RV free wall thickness change (control: 130 ± 29 %, MCT-standard: 49 ± 19 %, MCT-keto: 68 ± 8 %), and cardiac output (control: 129 ± 12 mL/min, MCT-standard: 65 ± 14 mL/min, MCT-keto: 90 ± 22 mL/min) without changing pulmonary artery acceleration time (control: 33 ± 5 ms, MCT-standard: 18 ± 3 ms, MCT-keto: 16 ± 3 ms). (D) Ketogenic diet intervention did not significantly combat histological severity of pulmonary vascular disease severity in MCT rats (control: 30 ± 6 %, MCT-standard: 49 ± 11 %, MCT-keto: 56 ± 11 %).

severity and an even slightly increased degree of histology pulmonary vascular remodeling (Fig. 2D). Thus, a diet-induced ketosis augmented RV function in preclinical RVF.

3.3. Ketogenic diet blunted NLRP3 inflammasome activation and significantly reduced macrophage accumulation in the RV

Finally, we probed how the ketogenic diet intervention modulated RV macrophage infiltration and NLRP3 inflammasome activation. Immunoblots showed MCT-Keto animals had decreased RV levels of NLRP3, pro-caspase-1, pro and mature interleukin-1β, and apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) relative to the MCT-Standard group, but not all changes were statistically significant (Fig. 3A). In accordance with our Western blots, confocal microscopy revealed that both total macrophage and ASC + macrophage abundances in the RV were normalized by the ketogenic diet (Fig. 3B). Additionally, RV fibrosis was blunted in MCT-Keto animals (Fig. 3C). In summary, our data showed a ketogenic diet suppressed pathological NLRP3 activation, macrophage infiltration, and RV fibrosis in MCT rats, and these results may explain the RV-enhancing effects the ketogenic diet afforded.

4. Discussion

In summary, we show circulating ketone body concentrations are not elevated in human PAH-mediated RVF and they do not change as the severity of RVF, as determined by three distinct but complementary approaches, increases. This is in direct opposition to what is observed in LVF as more severe LV dysfunction results in an even more pronounced ketosis [[14]]. In rodent studies, a ketogenic diet augments RV function and suppresses pathological macrophage NLRP3 inflammasome activation and RV fibrosis.

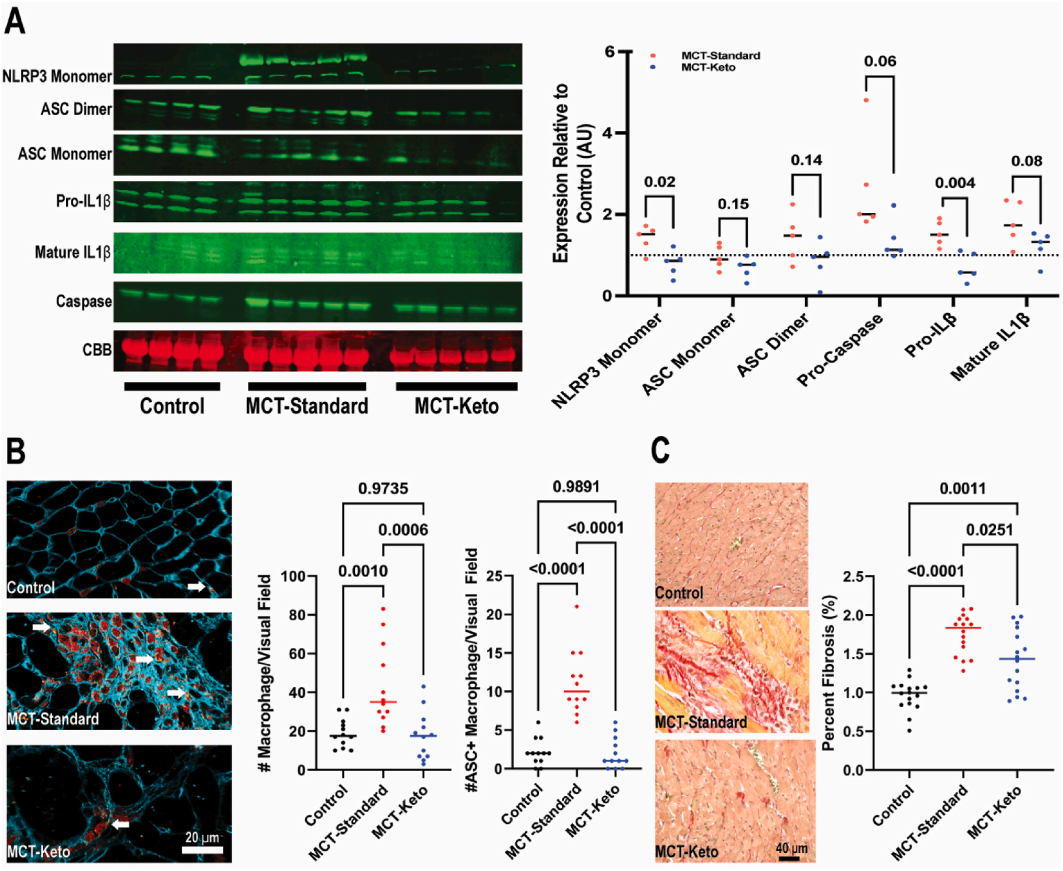

Fig. 3. A ketogenic diet suppressed macrophage NLRP3 activation and RV fibrosis in monocrotaline rats.
(A) Representative Western blots and subsequent quantification of protein abundance from $n = 4$ control, $n = 5$ MCT-Standard, and $n = 5$ MCT-Keto RV extracts. Signals from the four control animals were averaged to serve as an arbitrary standard of 1. MCT-Standard and MCT-Keto were then compared to each other. (B) Representative confocal micrographs stained with wheat germ agglutinin (Blue), galectin-3 (Red), and ASC (Yellow) to show total macrophage (galectin-3 positive) and ASC + macrophage in RV sections. Arrows highlight ASC + macrophage in each section. Quantification of total and ASC + macrophage in four randomly selected areas per RV section on a 20× objective from three distinct animals per experimental group. (C) Representative Picrosirius Red section with quantification of percent fibrosis on right. MCT-Keto rats had less RV fibrosis than MCT-Standard.

Importantly, the enhancement of RV function in MCT rats occurs without any overt changes in PAH severity as defined by histological and echocardiographic measures. Thus, our preclinical works shows one method to enhance ketosis improves RV function, potentially through an anti-inflammatory mechanism. Overall, our data suggests ketogenic interventions may have therapeutic relevance for a highly morbid and untreatable form of heart failure.

The absence of a robust ketosis in PAH-induced RVF agrees with other data showing this biochemical response could be blunted in isolated RVF. In particular, circulating βOHB concentrations in patients with arrhythmogenic right ventricular cardiomyopathy with isolated RV-involvement are lower than those in patients with biventricular involvement [[15]]. Consistent with the hypothesis that RVF patients have an impaired ketogenic response, Heresi et al., found no significant association between serum βOHB and cardiac index in a cohort of 33 chronic thromboembolic pulmonary hypertension (CTEPH) patients [[16]]. In addition, Nielsen et al., found patients with CTEPH ($n = 10$) and PAH ($n = 10$) have nearly identical serum βOHB levels, despite the fact that the CTEPH patients had significantly worse RV function as determined by strain echocardiography [[17]]. Thus, our data are congruent with findings from other groups, and the summation of these studies suggests patients with isolated RVF may have a diminished ketogenic response.

An additional explanation for the observed benefits afforded by the ketogenic diet in our rodent studies may be the favorable metabolic properties that ketone bodies possess. Ketone bodies are an energetically efficient fuel source, and thus can overcome mitochondrial dysfunction [[5]]. RV failure is characterized by mitochondrial deficits that culminates in the loss of the RV's ability to utilize its preferred energy source: fatty acids [[4]]. It is possible that ketone bodies help the dysfunctional RV overcome the metabolic inflexibility and thereby restore RV bioenergetics. These important metabolic changes could serve as an addition to the anti-inflammatory effects we observed. To gain more insight into this hypothesis, we queried previously published proteomics data, and found 3-hydroxybutyrate dehydrogenase (BDH1), the enzyme that converts βOHB to AcAc and thus serves as the initiating event in ketone oxidation, is actually downregulated in the MCT-RV (Supplemental Fig. 3). However, 3-oxoacid CoA-transferase 1 (OXCT1), the enzyme that governs ketone catabolism into the tricyclic acid cycle, is unaltered in MCT-RV (Supplemental Fig. 3) [[18]]. These data suggest that the anti-inflammatory properties of ketones may have been more important than the bioenergetic changes in our preclinical studies as MCT rats would be predicted to have suppressed ketolytic activity. In contrast, proteomics data from human RVF show BDH1 is significantly upregulated while OXCT1 levels are unchanged in the failing RV (Supplemental Fig. 3) [[10]]. The divergent species response in RV ketone metabolism suggests ketone bodies may have even greater therapeutic utility in human RVF via their synergistic anti-inflammatory and metabolic effects.

At present, approaches that induce ketosis are being investigated in pulmonary hypertension, and these studies are starting to shed light on the utility of ketogenic approaches in RVF. Interestingly, a recent study demonstrated an acute infusion of βOHB exerts beneficial hemodynamic changes in human PAH, and echocardiographic analysis reveals an augmentation of RV function with βOHB. The specific effects of βOHB on the RV versus the pulmonary vasculature are difficult to differentiate as βOHB appears to have pulmonary vasodilatory properties, which could underlie the echocardiographic changes in RV function [[17]]. Nonetheless, this acute study highlights the potential utility of ketosis in PAH, but chronic studies are still needed to evaluate both the long-term therapeutic efficacy and RV-remodeling capabilities. Additionally, a second clinical trial is evaluating how a ketogenic diet impacts symptoms and disease severity in pulmonary hypertension due to heart failure with preserved ejection fraction (NCT04942548). Echocardiography will be used as a secondary endpoint in this study, and hopefully this trial will provide additional insights in the role that ketone bodies play in RV function.

Certainly, future studies are required to gain a deeper understanding of our proposed RV-liver metabolic axis, and in particular, a detailed analysis of systemic ketone metabolism in RVF will be crucial. Additionally, it will be important to perform in-depth analyses of the molecular consequences of RVF on hepatic metabolic function. Moreover, there are clear clinical observations that chronic RVF can manifest as cirrhosis, but the mechanisms underlying this phenomenon are unknown, and therefore defining the molecular mediators of RVF-mediated hepatic dysfunction are sorely needed as these studies may illuminate therapeutic targets for this highly morbid complication of RVF [[4,19]].

5. Limitations

Our study has important limitations that we must acknowledge. First, while the ketogenic diet increases AcAc and βOHB in preclinical PAH, we cannot be rule out that the beneficial effects may be due to low carbohydrate or high fat content. Second, our rodent intervention only involved male rats because they develop more severe RV failure than females. Potential sex-specific differences in response to increased ketone levels were not the aim of this study, but they should be evaluated in the future. Third, there is a discrepancy between our preclinical rodent data where the MCT-standard animals had diminished ketone levels in the presence of RVF whereas our patient data exhibited no change in ketone levels with RVF severity. At present, we are not entirely confident in the exact reason for this difference, but it is likely due to a species differences between humans and rodents. Alternatively, it may also be the case that in MCT-mediated RVF, there are other tissues that have heightened ketone metabolism that consequently caused serum ketone levels to decrease. Fourth, we focused on the anti-inflammatory effects of the ketogenic diet for the purposes of this study due to previous proteomic data from the RV demonstrating that ketolytic enzymes are actually downregulated in rodent RV failure, as seen in Supplemental Fig. 3. With this in mind, we did not evaluate potential therapeutic benefit conferred through metabolic alterations in the RV, but it is possible that the ketogenic diet may have afforded advantageous bioenergetic effects as well.

6. Conclusion

In summary, circulating ketone body concentrations are not associated with hemodynamic, echocardiographic, or biochemical

assessments of RV function. Furthermore, ketone body concentrations are not elevated in both rodent and human PAH-mediated RVF. However, in rodents, a ketogenic diet augments RV function and suppresses pathological macrophage NLRP3 inflammasome activation and RV fibrosis. Collectively, these data suggest approaches to augment ketosis could combat RVF, and relative to rodents, it is possible that patients with RVF are uniquely primed for ketogenic interventions due to both the anti-inflammatory effects via NLRP3 suppression and beneficial bioenergetic effects via ketolysis.

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Data availability

All data referenced in the article is available either directly within the article or the supplemental material. Additional data not included in the article or supplement can be potentially requested from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Madelyn Blake: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Patrycja Puchalska:** Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Felipe Kazmirczak:** Data curation, Formal analysis, Methodology, Writing – review & editing. **Jeffrey Blake:** Data curation, Investigation. **Ryan Moon:** Data curation, Formal analysis. **Thenappan Thenappan:** Investigation, Methodology, Writing – review & editing. **Peter A. Crawford:** Conceptualization, Methodology, Writing – review & editing. **Kurt W. Prins:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Kurt Prins reports financial support was provided by National Heart Lung and Blood Institute. KWP Served as a consultant to Edwards. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22227.

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