Ketone bodies in Right Ventricular Failure: A Unique Therapeutic Opportunity

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Abstract

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 approaches that increase ketone concentrations exert beneficial effects in rodents and humans. However, the regulation of ketones in right ventricular failure (RVF) are unexplored. Here, we show in human pulmonary arterial hypertension (PAH), a compensatory ketosis is absent in patients with RVF. In the monocrotaline rat model of PAH-mediated RVF, a dietary-induced ketosis improves RV function, suppresses NLRP3 inflammasome activation, and combats RV fibrosis. These data suggest ketogenic therapies may particularly effective in RVF, and future studies evaluating the effects of ketones in RVF are warranted.

***To the Editor*,**

Right ventricular failure(RVF) is the leading cause of death in pulmonary arterial hypertension (PAH), but there are no therapies that effectively combat RVF(1). This is in juxtaposition to left ventricular failure(LVF) treatment as several medications can improve LV function and survival(2). Differences in ventricular development, morphology, load, and adaptability may partially explain why LVF therapies are largely ineffective for RVF(3). Additionally, there are differing degrees of end-organ dysfunction when comparing RVF and LVF. In particular, RVF impairs liver function to a greater extent, and it can even result in cirrhosis(4). This raises the possibility that a RV-liver axis exists, and it could be targeted to combat RVF.

Ketone bodies, metabolites that are primarily synthesized by the liver, regulate diverse biological functions ranging from energy homeostasis, inflammation, and epigenetic regulation(5). In LVF preclinical models and patients with LVF, ketone levels are elevated and correlate with HF severity(6). This response is widely believed to be compensatory as ketogenic interventions improve cardiac function in rodent LVF and ketone infusion acutely increases cardiac output in LVF patients(6). Additionally, the ketone body β-hydroxybutyrate (βOHB) suppresses nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3) inflammasome activity(7), a pathway recently implicated in PAH-mediated RVF(8). While there is abundant data evaluating ketones in LVF, the relationships among ketone body concentrations, RV inflammasome activation, and RV function in preclinical and human RVF are unexplored.

Here, 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 classified into two groups: a compensated (cardiac index>2.2 L/min/m^2 as defined by Thermodilution) or a decompensated (cardiac index ≤2.2 L/min/m^2) RV phenotype (Table 1). RV function was further characterized using both hemodynamic and echocardiographic parameters. In rodent studies, 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). 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. Picrosirius red staining of RV free wall sections quantified fibrosis. Immunoblots of RV extracts were probed with antibodies to NLRP3, Caspase-1, interleukin-1β, and apoptosis-associated speck-like protein containing a CARD (ASC) to biochemically characterize inflammasome activation. RV sections were stained with antibodies to galectin-3 and ASC to identify total macrophage and ASC-positive macrophage. Confocal micrographs were collected on a Zeiss LSM 900 Airyscan 2.0 microscope. RV fibrosis and macrophage analyses were blindly performed by MB. Serum ketones were measured using UPLC-MS/MS as described(9). All data were evaluated for normality using Sharpio-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. All statistical analyses were performed on GraphPad Prism. Rodent and human studies were approved by the University of Minnesota Institutional Animal Care and Use Committee and Institutional Review Board respectively.

We first validated divergent RV phenotypes from our PAH patient cohort. Both hemodynamic and echocardiographic analyses demonstrated more severe RV dysfunction in the decompensated group (**Figure 1A**). Surprisingly, unlike prior observations in patients with LVF, circulating concentrations of the two ketone bodies acetoacetate (AcAc) and βOHB were not different when the two groups were compared (**Figure 1B**). Moreover, AcAc and βOHB levels were not associated with cardiac index, right atrial pressure, RV global longitudinal strain, or tricuspid annular plane systolic excursion (TAPSE) (**Figure 1 C and D**). Thus, a compensatory ketosis was lacking in humans with PAH-mediated RVF.

Next, we determined how a dietary-induced ketosis modulated RVF in MCT rats (**Figure 2A**). In agreement with our human data, MCT-Standard rats were not ketotic (**Figure 2B**). However, the ketogenic diet increased serum concentrations of both AcAc and βOHB (**Figure 2B**). One of the MCT-Keto rats died, but compared to MCT-Standard rats, the remaining MCT-Keto rats showed higher TAPSE, percent RV free wall

thickening, and cardiac output despite no differences in pulmonary artery acceleration time (**Figure 2C**). Thus, a diet-induced ketosis was associated with augmented RV function in preclinical RVF.

Finally, we probed the effects of the ketogenic diet intervention on RV macrophage NLRP3 activation. Immunoblots showed the MCT-Keto animals had decreased RV levels of NLRP3, pro-caspase-1, pro and mature interleukin-1β, and ASC relative to the MCT-Standard group, but not all changes were statistically significant (**Figure 2D**). In concordance with our Western blots, both total macrophage and ASC+ macrophage abundances were normalized by the ketogenic diet (**Figure 2E**). Finally, RV fibrosis was blunted in MCT-Keto animals (**Figure 2F**).

In summary, circulating 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. The absence of ketosis in PAH-induced RVF agrees with other data showing this biochemical response is dysregulated by RVF. In particular, circulating βOHB concentrations in patients with arrhythmogenic right ventricular cardiomyopathy with isolated RV-involvement are not as high as those with biventricular involvement(10). Certainly, Future studies are required to gain a deeper understanding of our proposed RV-liver axis, and in particular, a detailed analysis of systemic ketone metabolism in RVF will be crucial. Interestingly, a clinical trial evaluating a ketogenic diet intervention in patients with pulmonary hypertension due to heart failure with preserved ejection fraction is ongoing (NCT04942548). Hopefully, this trial will shed more light on the RV-ketone relationship and determine if elevating ketones modulates RV function. In conclusion, the summation of our preclinical and clinical data suggests approaches to augment ketosis could combat RVF, and it is possible that patients with RVF are uniquely primed for ketogenic interventions.

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Figure 1: Circulating ketone body concentrations were not elevated in PAH-mediated RV failure. (A) Both hemodynamic and echocardiography revealed a greater degree of RV dysfunction in the decompensated group. (B) Ketone body concentrations were not elevated in the decompensated group. (C) Hemodynamic measures of RV function were not associated with serum ketone body concentrations. (D) Echocardiographic measures of RV function were not strongly associated with AcAc or βOHB. Red dots signify decompensated patients (*n*=25) and blue dots signify compensated patients (*n*=26).

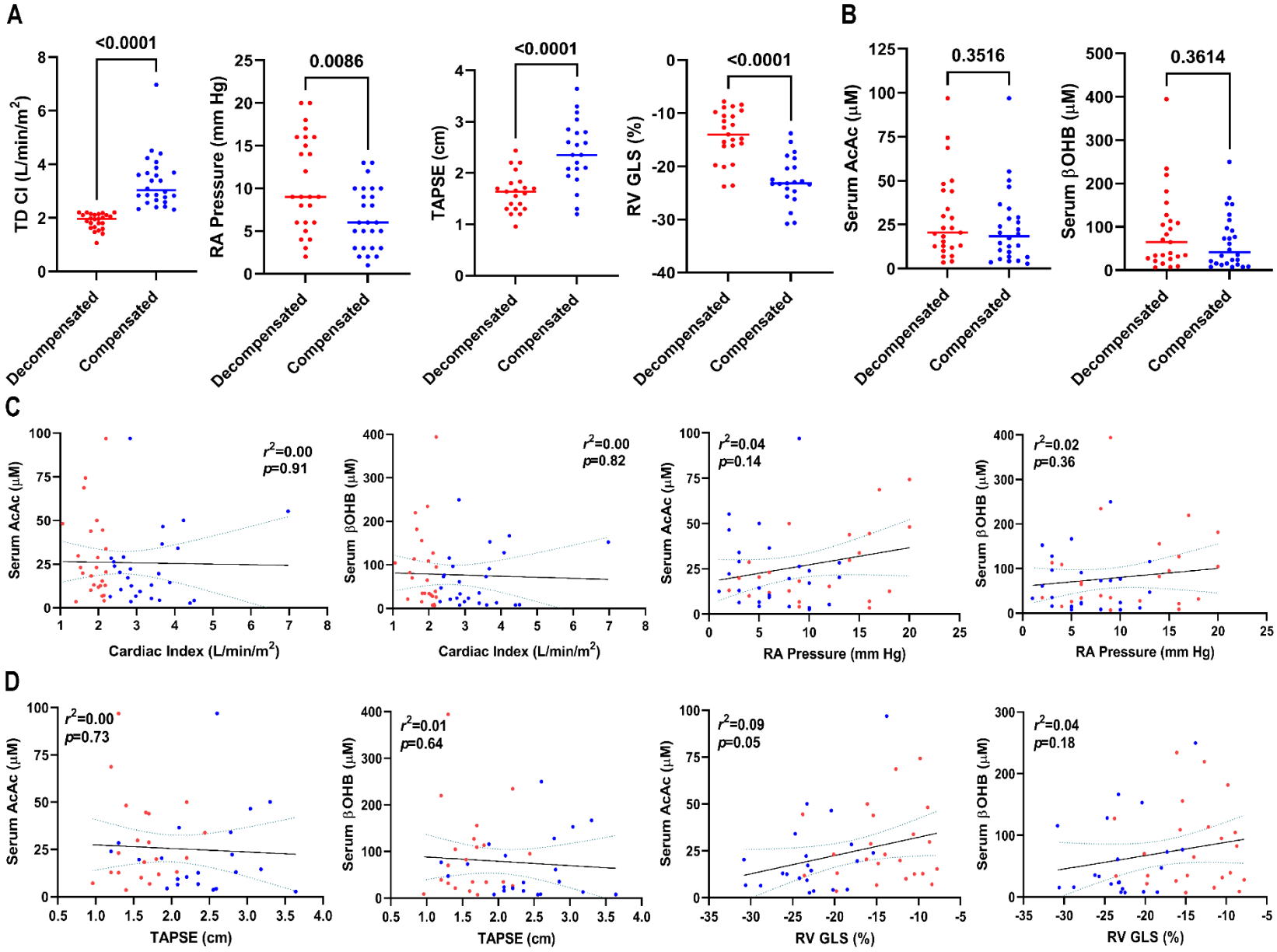
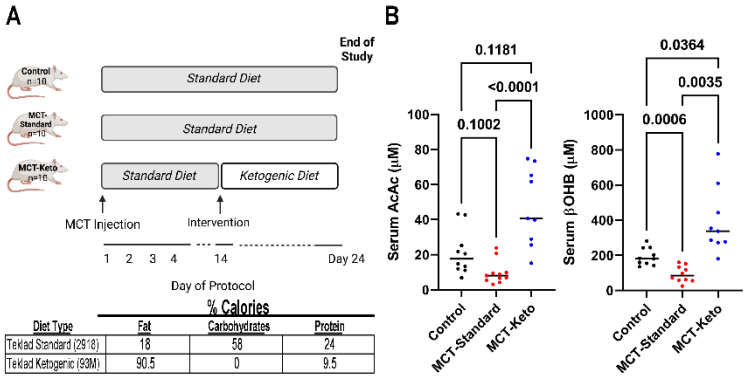
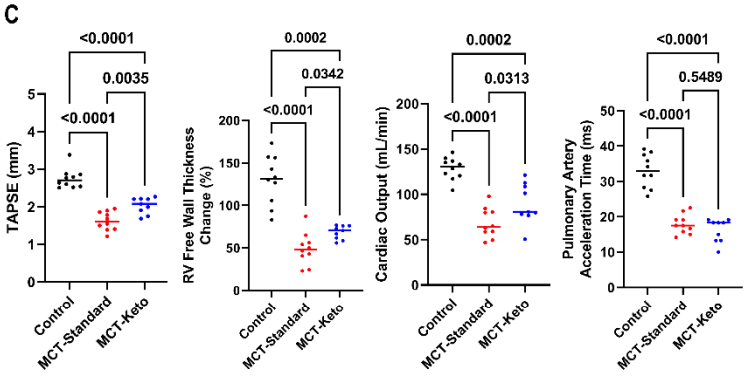
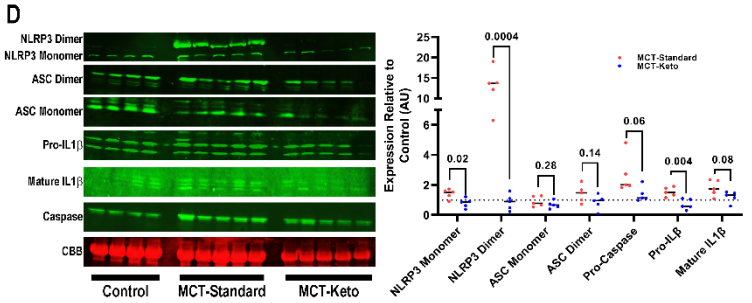


Figure 2: A ketogenic diet increased serum ketone body concentrations, augmented RV function, and suppressed macrophage NLRP3 activation in monocrotaline rats. (A) Diagram of experiment approach and caloric composition of diets. (B) A ketogenic diet increased serum concentrations of AcAc and βOHB in MCT-Keto rats. (C) Ketogenic diet improved RV function in MCT rats. (D) 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. (E) 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 20x objective from three distinct animals per experimental group. (F) Representative Picrosirius Red section with quantification of percent fibrosis on right. MCT-Keto rats had less RV fibrosis than MCT-Standard.







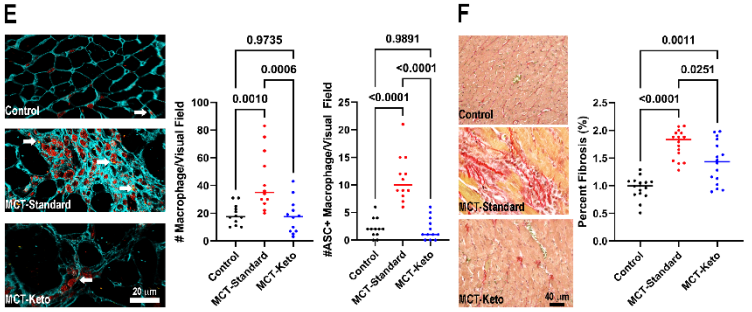


Table 1 Clinical, Echocardiographic, and Hemodynamic Characteristics of Study Cohort

Characteristics	Total Cohort (*n* = 51)	Preserved RV Function (*n* = 26)	Depressed RV Function (*n* = 25)	*p*-value
Age, years	58.0 ± 15	59.0 ± 12	57.0 ± 17	0.56
Female, *n* (%)	31 (60)	17 (65)	14 (56)	0.62
Body mass index, kg/m^2	28 ± 7	28 ± 7	28 ± 6	0.61
WHO Functional Class (*n* = 48)				
II	13 (25)	8 (31)	5 (20)	
III	32 (63)	13 (50)	19 (73)	
IV	1 (0)	0 (0)	1 (0)	
Comorbidities, *n* (%)				
Hypertension	23 (45)	12 (46)	11 (44)	0.76
Diabetes	12 (24)	6 (23)	6 (24)	0.85
Hyperlipidemia	16 (31)	9 (35)	7 (28)	0.69
Coronary artery disease	7 (14)	3 (12)	4 (16)	0.92
Atrial fibrillation	5 (10)	1 (4)	4 (16)	0.14
COPD	8 (16)	5 (19)	3 (12)	0.28
Medications, *n* (%)				
Dual oral therapy	15 (29)	12 (46)	3 (12)	< 0.01
Triple oral therapy	11 (22)	4 (15)	7 (28)	0.63
Parental Prostacyclin plus oral	15 (29)	5 (19)	10 (40)	0.09
Lab				
Serum hemoglobin, g/dl (*n=51*)	13 ± 2	13 ± 2	14 ± 2	0.34
Serum creatinine, mg/dl (*n=51*)	1 ± 0.40	1 ± 0.27	1 ± 0.43	0.36
Serum NT-proBNP (*n=50*)	1786 ± 2705	508 ± 1067	2988 ± 3220	< 0.001
Serum Bilirubin	0.67 ± 0.33	0.56 ± 0.23	0.76 ± 0.39	< 0.05
Serum ALT	26 ± 10	28 ± 12	24 ± 8	0.30
Serum AST	21 ± 9	20 ± 8	23 ± 9	0.21
Echocardiography				
Right ventricular FAC (*n=44*)	15 ± 15	2 ± 9	26 ± 8	< 0.001
RVEDA	26 ± 8	24 ± 7	29 ± 9	< 0.05
RVESA	18 ± 8	15 ± 6	22 ± 9	< 0.01
TAPSE	2 ± 0.63	2 ± 0.64	2 ± 0.38	< 0.001

S’	11 ± 4	13 ± 4	9 ± 3	< 0.01
Tricuspid regurgitation severity	3 ± 1	2 ± 1	3 ± 1	< 0.05
Hemodynamics				
Heart rate, beats/min (*n=51*)	74 ± 19	71 ± 12	77 ± 24	0.25
Mean right atrial, mm Hg (*n =51*)	7 ± 5	7 ± 4	11 ± 6	< 0.005
Mean PAP, mm Hg (*n=51*)	43 ± 12	36 ± 8	49 ± 14	< 0.001
Cardiac output, liters/min (*n=51*)	5 ± 2	6 ± 2	4 ± 1	< 0.001
Cardiac index, liters/min/m^2 (*n=51*)	3 ± 1	3 ± 1	2 ± 0.3	< 0.001
PVR, Wood units (*n=50*)	8 ± 5	5 ± 2	11 ± 6	< 0.001