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BRIEF COMMUNICATION

Intermittent Fasting Enhances Right Ventricular Function in Preclinical Pulmonary Arterial Hypertension

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BACKGROUND: Intermittent fasting (IF) confers pleiotropic cardiovascular benefits including restructuring of the gut microbiome and augmentation of cellular metabolism. Pulmonary arterial hypertension (PAH) is a rare and lethal disease characterized by right ventricular (RV) mitochondrial dysfunction and resultant lipotoxicity and microbiome dysbiosis. However, the effects of IF on RV function in PAH are unexplored. Therefore, we investigated how IF altered gut microbiota composition, RV function, and survival in the monocrotaline model of PAH.

METHODS AND RESULTS: Male Sprague Dawley rats were randomly allocated into 3 groups: control, monocrotaline-ad libitum feeding, and monocrotaline-IF (every other day feeding). Echocardiography and invasive hemodynamics showed IF improved RV systolic and diastolic function despite no significant change in PAH severity. IF prevented premature mortality (30% mortality rate in monocrotaline-ad libitum versus 0% in monocrotaline-IF rats, P=0.04). IF decreased RV cardiomyocyte hypertrophy and reduced RV fibrosis. IF prevented RV lipid accrual on Oil Red O staining and ceramide accumulation as determined by metabolomics. IF mitigated the reduction in jejunum villi length and goblet cell abundance when compared with monocrotaline-ad libitum. The 16S ribosomal RNA gene sequencing demonstrated IF changed the gut microbiome. In particular, there was increased abundance of *Lactobacillus* in monocrotaline-IF rats. Metabolomics profiling revealed IF decreased RV levels of microbiome metabolites including bile acids, aromatic amino acid metabolites, and gamma-glutamylated amino acids.

CONCLUSIONS: IF directly enhanced RV function and restructured the gut microbiome. These results suggest IF may be a non-pharmacological approach to combat RV dysfunction, a currently untreatable and lethal consequence of PAH.

Key Words: gut microbiome ■ intermittent fasting ■ *Lactobacillus* ■ lipotoxicity ■ metabolism ■ metabolomics ■ pulmonary arterial hypertension ■ right ventricular function

Intermittent fasting (IF) is an emerging non-pharmacological intervention in multiple chronic diseases because of its ability to extend lifespan in a wide array of species ranging from single-cell organisms to non-human primates.[1] The benefits of IF are pleiotropic, but proposed mechanisms include enhanced cellular metabolism and restructuring of the gut microbiome.[1] Microbiota produce metabolites such as short-chain fatty acids, amino acids, and bile acids that modulate host metabolism, immune response, and intestinal homeostasis.[2] Regulation of microbiota metabolites is crucial for normal physiology as microbiota dysbiosis is implicated in multiple diseases.[1]

Pulmonary arterial hypertension (PAH) is a rare, but lethal disease characterized by metabolic defects[3] and microbiome dysbiosis.[4] Right ventricular (RV) failure

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is the leading cause of death in PAH.[3] Disrupted RV metabolism including impaired mitochondrial fatty acid oxidation leads to lipotoxicity and ceramide accumulation.[3] RV lipotoxicity is proposed to be a significant contributor to RV dysfunction as it is present in both preclinical and clinical PAH.[3]

Because IF corrects metabolic defects and combats gut dysbiosis, it may be a non-pharmacological approach to augment RV function in PAH. To investigate this hypothesis, we analyzed the effects of IF on gut microbiota composition, weight gain, microbiota metabolite abundance in the RV, RV lipotoxicity, RV function, and survival in the monocrotaline rat model of PAH.

METHODS

The primary data supporting our findings are available from the corresponding author upon reasonable request.

Male Sprague Dawley rats (200–250 g; 7–8 weeks old) purchased from Charles River Laboratories were randomized into 3 experimental groups: phosphate buffered saline injected (control), monocrotaline-ad libitum, and monocrotaline-IF (Figure 1A). The monocrotaline-ad libitum and monocrotaline-IF rats received a single subcutaneous injection of monocrotaline (60 mg/kg) (Sigma-Aldrich, St. Louis, MO). Rats were fed regular chow ad libitum until they received the monocrotaline injection. The monocrotaline-IF group began every other day fasting the day following monocrotaline injection (Figure 1A). Because of the experimental design, researchers were unable to be masked to the experimental group. All animal studies were approved by the University of Minnesota Institutional Animal Care and Use Committee.

Echocardiography was completed with a Vevo2100 ultrasound system as previously described.[5] Invasive closed-chest RV pressure-volume loops were performed using a high-fidelity Scisense 1.9F catheter (Transonic Systems, Ithaca, NY) with values continuously recorded with a Transonic AV500 Pressure-Volume Measurement System (Figure S1). Results were analyzed on LabScribe version 4 (iWorx Systems, Dover, NH).

For histological analysis, jejunum was fixed in 10% formalin, embedded in paraffin, sectioned at 10-µm, and stained with hematoxylin and eosin by the University of

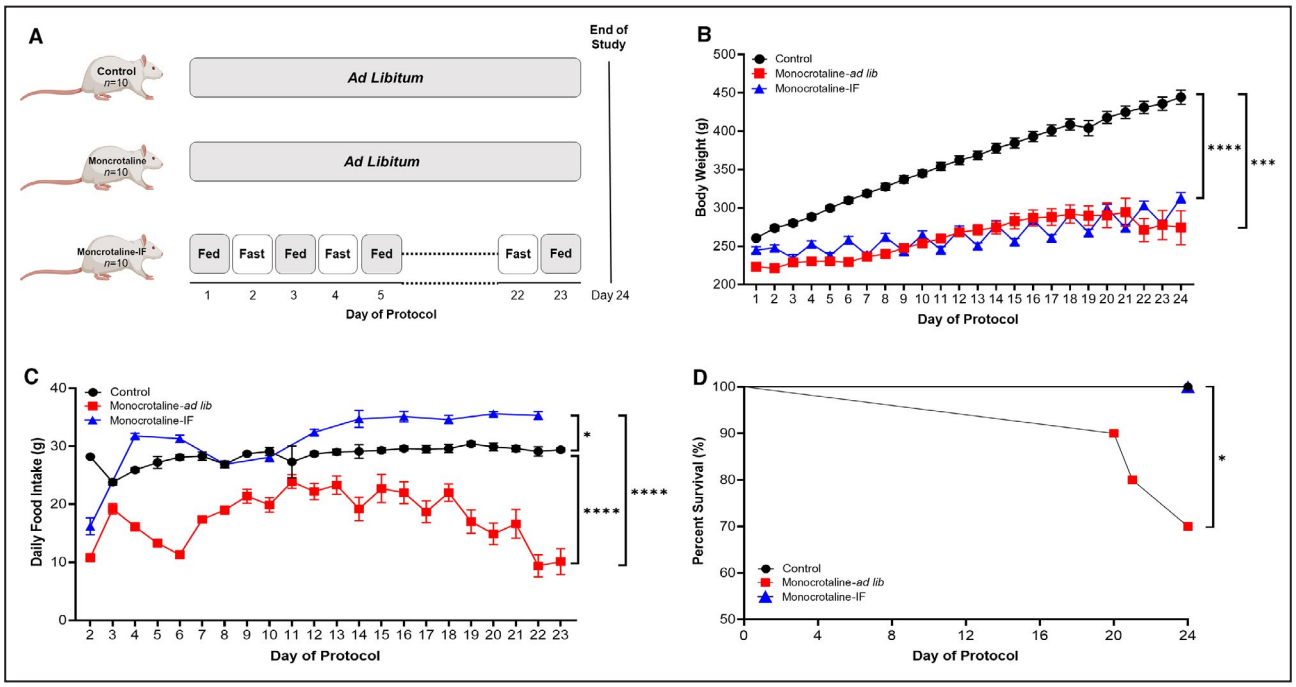

Figure 1. Intermittent fasting monocrotaline rats maintain their body weight, consume more food on feeding days, and have improved survival.

A, Experimental approach. Rats were fed regular chow ad libitum until 7 to 8 weeks of age. Then, they were randomized to receive a 1-time subcutaneous phosphate buffered saline or monocrotaline injection. The monocrotaline-intermittent fasting (IF) rats began every other day fasting the day after monocrotaline injection. Each group started with 10 rats. **B**, Monocrotaline-ad libitum and monocrotaline-IF rats were smaller than controls. Monocrotaline-IF rats maintained their body weight at the end of the study while monocrotaline-ad libitum rats began to lose weight (last measured weight for control: 444±29, monocrotaline-ad libitum: 274±49, monocrotaline-IF: 313±21 g, P=0.049 between monocrotaline-ad libitum and monocrotaline-IF by 1-way ANOVA with Tukey post hoc analysis). Plot showing mean±SEM. **C**, Monocrotaline-IF rats ate more food on feeding days as compared with control and monocrotaline-ad libitum. Data shown as mean±SEM. **D**, There was no premature mortality in the monocrotaline-IF group as compared with monocrotaline-ad libitum (30% mortality rate at end of study in monocrotaline-ad libitum vs 0% mortality rate in control and monocrotaline-IF, P=0.04, n=10 per group at the start of the study). *P<0.05, ***P<0.001, ****P<0.0001 with 2-way repeated measures ANOVA with Tukey multiple comparisons test for (**B** and **C**) and log-rank test for (**D**). IF indicates intermittent fasting.

Minnesota Histology and Research Laboratory in the Clinical and Translational Science Institute. RV free wall sections were fixed in 10% formalin, embedded in paraffin, sectioned at 10-μm, and stained with hematoxylin and eosin or cryosectioned at 10-μm and stained with trichrome (Abcam, Cambridge, MA). Lungs were fixed in 10% formalin, embedded in paraffin, sectioned at 4-μm, and stained with hematoxylin and eosin.

Stool was collected the last day of the study. Fecal samples were immediately frozen after collection. DNA was extracted from thawed stool samples using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany). PCR amplification of the V4 hypervariable region of the 16S ribosomal RNA gene was completed using the 515F/806R primer set. Dual-index, paired-end sequencing was performed on an Illumina MiSeq platform (Illumina Inc., San Diego, CA) at a read length of 300 nucleotides at the University of Minnesota Genomics Center. Sequence data were processed using mothur version 1.41.1 with a previously published pipeline.[6]

Global metabolomics profiling of frozen RV free wall specimens was performed by Metabolon Inc. (Durham, NC) on the Waters ACQUITY ultra-performance liquid chromatography and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer.

Daily body weight and food consumption data were analyzed with 2-way repeated measures ANOVA with Tukey multiple comparisons test. Survival was compared by the log-rank test. One-way ANOVA with Tukey post hoc analysis was completed if there was equal variance as determined by the Brown–Forsythe test and the data were normally distributed by the Shapiro–Wilk test. If the data were normally distributed but there was unequal variance, Brown–Forsythe, and Welch ANOVA with Dunnett post hoc analysis was performed. If the data were not normally distributed, Kruskal–Wallis ANOVA with Dunn post hoc test was completed. Statistical significance was defined as $P<0.05$. Sparse partial least squares discriminant analysis, random forest classification of 16S ribosomal RNA sequencing of stool samples, and hierarchical cluster analyses of RV metabolomics data were performed using MetaboAnalyst[7] software. All other statistical analysis and graphing were completed with GraphPad Prism version 9. Data are presented as mean±SD in the text.

RESULTS

IF Rats Maintained Body Weight, Ate More Food on Feeding Days, and Had Improved Survival Rates

We first examined the effects of IF on body mass, food consumption, and survival. Control rats displayed a consistent weight gain throughout the observation period, but both monocrotaline-ad libitum and monocrotaline-IF were smaller than control rats and exhibited a blunted growth pattern (Figure 1B). However, monocrotaline-IF had less weight loss over the last week of experimentation compared with monocrotaline-ad libitum rats (Figure 1B). When food consumption was evaluated, monocrotaline-IF ate more than both controls and monocrotaline-ad libitum on feeding days (Figure 1C). Finally, survival analysis showed IF prevented premature mortality (Figure 1D).

IF Augmented RV Function and Decreased RV Hypertrophy, Fibrosis, and Lipotoxicity

Then, we characterized the effects of IF on RV function. Echocardiographic analysis showed monocrotaline-IF rats had higher tricuspid annular plane systolic excursion (Figure 2A) and percent RV free wall thickening (Figure 2B) than monocrotaline-ad libitum. Pressure-volume loop analysis (Figure 2C) identified a reduction in RV ejection fraction (Figure 2D) and depressed RV-pulmonary arterial coupling (Figure 2E) in monocrotaline-ad libitum rats. However, IF improved both RV ejection fraction (Figure 2D) and RV-pulmonary arterial coupling (Figure 2E) when compared with monocrotaline-ad libitum. monocrotaline-ad libitum rats had impaired RV diastolic function as defined by RV tau, which IF normalized (Figure 2F).

Monocrotaline-ad libitum rats had significant RV hypertrophy as the Fulton index (Figure 2G), RV mass relative to body mass (Figure 2H), and cardiomyocyte cross-sectional area were increased compared with controls (Figure 2I and 2J). However, IF lessened RV hypertrophy at the whole organ and cardiomyocyte level (Figure 2G through 2J). Monocrotaline-ad libitum RVs had more fibrosis than controls, which IF ameliorated (Figure 2K and 2L). In summation, IF combated RV hypertrophy/fibrosis and augmented both RV systolic and diastolic function.

Subsequently, we determined the impact of IF on RV lipotoxicity. Monocrotaline-ad libitum RVs exhibited ectopic lipid accumulation in Oil Red O staining (Figure 2M and 2N). Metabolomics profiling revealed increased ceramide, dihydroceramide, lactosylceramide, and hexosylceramide levels, which IF mitigated (Figure 2O). In total, IF curtailed RV lipid accumulation (Figure 2M and 2N) and normalized levels of multiple ceramide species in the RV (Figure 2O).

IF Had Minimal Effect on Pulmonary Vascular Remodeling

Finally, we evaluated how IF modulated pulmonary vascular disease severity. There were no significant

differences between monocrotaline-ad libitum and monocrotaline-IF in pulmonary artery acceleration time (Figure 2P), RV systolic pressure (Figure 2Q), and effective arterial elastance (Figure 2R). However, there was a slight but significant decline in the histological pulmonary vascular remodeling (Figure 2S and 2T).

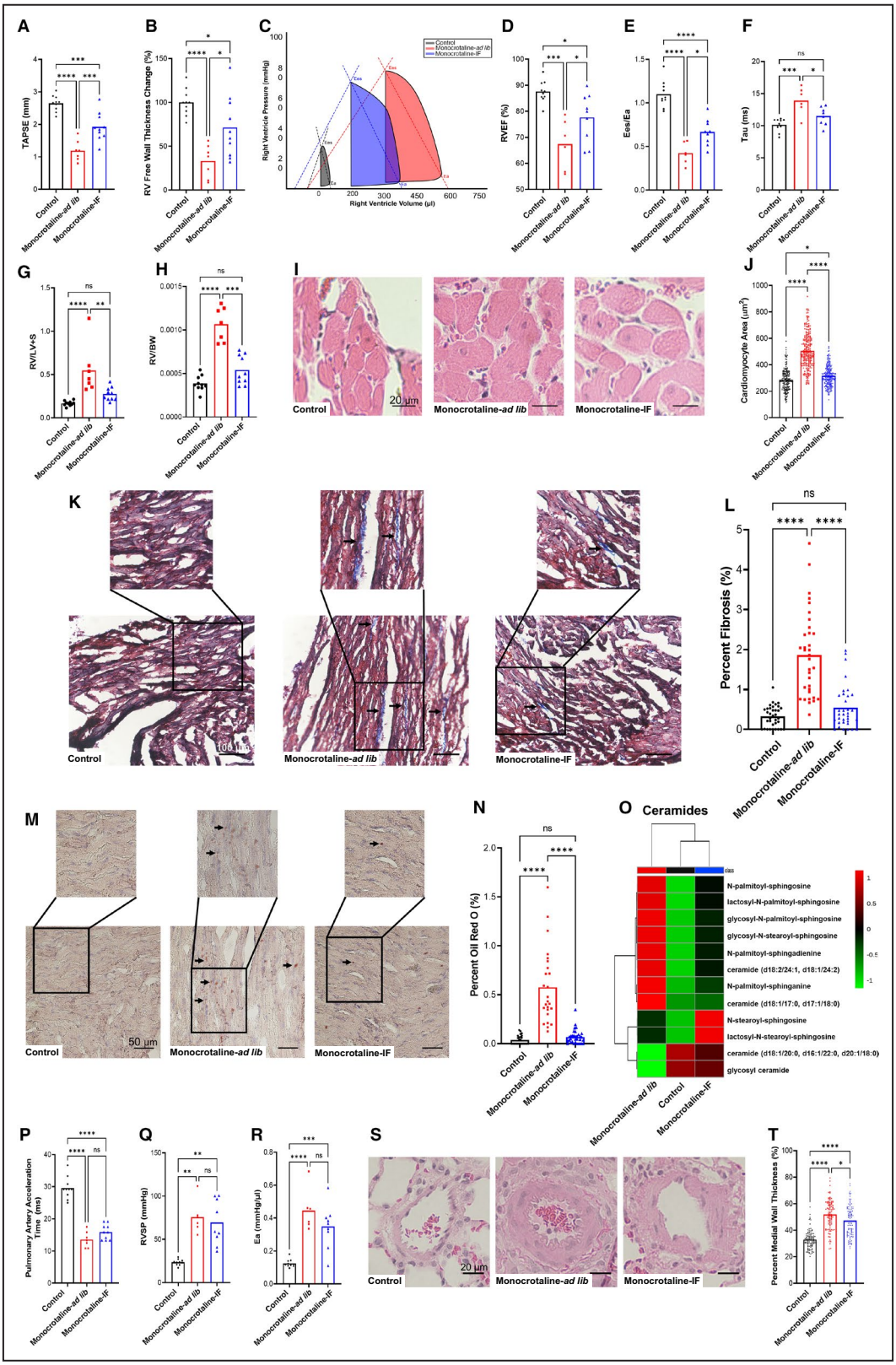

Figure 2. Intermittent fasting augments right ventricular (RV) systolic and diastolic function and decreases RV hypertrophy, RV fibrosis, and RV lipotoxicity.

Echocardiographic analysis demonstrated (**A**) greater tricuspid annular plane systolic excursion (control: 2.6±0.2, monocrotaline-ad libitum: 1.2±0.3, monocrotaline-intermittent fasting [IF]: 1.9±0.4 mm, *P*=0.0006 between monocrotaline-ad libitum and monocrotaline-IF, n=10 control, 7 monocrotaline-ad libitum, and 10 monocrotaline-IF) and (**B**) RV free wall thickening (control: 100±15%, monocrotaline-ad libitum: 33±19%, monocrotaline-IF: 71±34%, *P*=0.01 between monocrotaline-ad libitum and monocrotaline-IF, n=10 control, 7 monocrotaline-ad libitum, and 10 monocrotaline-IF) in monocrotaline-IF as compared with monocrotaline-ad libitum. **C**, Schematic of representative pressure-volume loops in all 3 groups. Pressure-volume loop analysis revealed (**D**) higher RV ejection fraction (control: 88±4%, monocrotaline-ad libitum: 67±10%, monocrotaline-IF: 78±9%, *P*=0.047 between monocrotaline-ad libitum and monocrotaline-IF, n=10 control, 6 monocrotaline-ad libitum, and 9 monocrotaline-IF), (**E**) enhanced RV-pulmonary arterial coupling (end-systolic elastance/effective arterial elastance) (control: 1.1±0.2, monocrotaline-ad libitum: 0.4±0.1, monocrotaline-IF: 0.7±0.2, *P*=0.01 between monocrotaline-ad libitum and monocrotaline-IF, n=10 control, 6 monocrotaline-ad libitum, and 9 monocrotaline-IF), and (**F**) improved diastolic function (tau) (control: 10±1, monocrotaline-ad libitum: 14±2, monocrotaline-IF: 12±1 ms, *P*=0.02 between monocrotaline-ad libitum and monocrotaline-IF, n=10 control, 6 monocrotaline-ad libitum, and 8 monocrotaline-IF) in monocrotaline-IF rats as compared with monocrotaline-ad libitum. IF diminished RV hypertrophy at the organ level as assessed by (**G**) the Fulton index (control: 0.17±0.03, monocrotaline-ad libitum: 0.55±0.28, monocrotaline-IF: 0.28±0.08, *P*=0.003 between monocrotaline-ad libitum and monocrotaline-IF, n=10 control, 7 monocrotaline-ad libitum, and 10 monocrotaline-IF) and (**H**) RV normalized to body weight (control: 0.0004±0.00009, monocrotaline-ad libitum: 0.001±0.0002, monocrotaline-IF: 0.0005±0.0002, *P*=0.0004 between monocrotaline-ad libitum and monocrotaline-IF, n=10 control, 7 monocrotaline-ad libitum, and 10 monocrotaline-IF), and at the cardiomyocyte level (control: 287±85, monocrotaline-ad libitum: 505±131, monocrotaline-IF: 317±77 μm^2, *P*<0.0001 between monocrotaline-ad libitum and monocrotaline-IF, n=190–257 cardiomyocytes measured from 4 RV tissues per group) with representative images in (**I**) and quantification in (**J**). **K**, IF also mitigated RV fibrosis (control: 0.3±0.3%, monocrotaline-ad libitum: 1.9±1.1%, monocrotaline-IF: 0.5±0.5%, *P*<0.0001 between monocrotaline-ad libitum and monocrotaline-IF, n=7 RV per group with 4–5 areas measured per RV) (arrows) as quantified in (**L**). **M**, IF mitigated RV lipotoxicity as quantified by Oil Red O staining (control: 0.04±0.05%, monocrotaline-ad libitum: 0.6±0.4%, monocrotaline-IF 0.07±0.07%, *P*<0.0001 between monocrotaline-ad libitum and monocrotaline-IF, n=3–7 RV areas assessed from 6 control, 5 monocrotaline-ad libitum, 7 monocrotaline-IF rats) (arrows) (**N** and **O**) a reduction in RV ceramide, dihydroceramide, lactosylceramide, and hexosylceramide concentrations as compared with monocrotaline-ad libitum. When assessing pulmonary arterial hypertension severity, there was no difference in (**P**) pulmonary artery acceleration time (control: 30±3, monocrotaline-ad libitum: 14±2, monocrotaline-IF: 16±2 ms, *P*=0.23 between monocrotaline-ad libitum and monocrotaline-IF, n=10 control, 7 monocrotaline-ad libitum, and 10 monocrotaline-IF) assessed by echocardiography or (**Q**) RV systolic pressure (control: 24±4, monocrotaline-ad libitum: 76±19, monocrotaline-IF: 69±26 mm Hg, *P*=0.93 between monocrotaline-ad libitum and monocrotaline-IF, n=10 control, 6 monocrotaline-ad libitum, and 9 monocrotaline-IF) and (**R**) effective arterial elastance (control: 0.1±0.02, monocrotaline-ad libitum: 0.4±0.1, monocrotaline-IF: 0.3±0.1 mm Hg/μL, *P*=0.18 between monocrotaline-ad libitum and monocrotaline-IF, n=10 control, 6 monocrotaline-ad libitum, and 9 monocrotaline-IF) measured from invasive hemodynamics. **S**, There was a subtle decrease in small arteriole remodeling in monocrotaline-IF lungs (control: 33±7%, monocrotaline-ad libitum: 52±11%, monocrotaline-IF: 47±12% medial wall thickness, *P*=0.04 between monocrotaline-ad libitum and monocrotaline-IF, n=90–101 small arterioles assessed from 4 lungs per group) as quantified in (**T**). **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001, and (ns) not significant with one-way ANOVA with Tukey post hoc test in (**A**, **B**, **D** through **G**, **P**, and **R**), Brown−Forsythe and Welch ANOVA with Dunnett multiple comparison test in (**H** and **Q**), and Kruskal–Wallis test with Dunn multiple comparisons test in (**J**, **L**, **N**, and **T**). BW indicates body weight; Ea, effective arterial elastance; Ees, end-systolic elastance; IF, intermittent fasting; LV+S, left ventricle+septum weight; RV, right ventricle; RVEF, right ventricular ejection fraction; RVSP, right ventricular systolic pressure; and TAPSE, tricuspid annular plane systolic excursion.

IF Combated Intestinal Epithelial Pathology, Altered the Gut Microbiome, and Normalized Microbiome Metabolite Levels in the RV

Next, we determined how IF modified intestinal pathology and gut microbiome composition. As compared with controls, monocrotaline-ad libitum had shortened villi length and less goblet cells per villi in the jejunum (Figure 3A through 3C). However, IF normalized both villi length and goblet cell abundance (Figure 3A through 3C). Sparse partial least squares discriminant analysis (Figure 3D), Shannon diversity index (Figure 3E), and inverse Simpson index (Figure 3F) analysis of 16S ribosomal RNA sequencing of stool samples all revealed IF modulated the microbiome. Random forest classification identified the 15 most divergent bacterial genera between the 3 groups (Figure 3G). Interestingly, the relative abundance of *Lactobacillus* was higher in monocrotaline-IF as compared with control and monocrotaline-ad libitum (Figure 3H). In summary, IF altered microbiome ecology with increased abundance of *Lactobacillus*.

To further delineate the consequences of gut microbiome modulation by IF, we quantified levels of bile acids, tryptophan, tyrosine, and phenylalanine metabolite intermediates and gamma-glutamylated amino acids (all microbiome metabolites) in RV specimens. Multiple bile acids, aromatic amino acid metabolites, and gamma-glutamylated amino acids were elevated in monocrotaline-ad libitum as compared with controls (Figure 3I through 3M). However, IF normalized the concentrations of nearly all of these microbiome metabolites (Figure 3I through 3M). Thus, IF combated dysregulation of multiple microbiome metabolites in the RV.

DISCUSSION AND CONCLUSIONS

In summary, we show IF significantly enhances RV systolic and diastolic function and reduces RV

hypertrophy and fibrosis, which likely explains the survival benefit in monocrotaline rats. Importantly, the RV changes are observed in the absence of significant alteration of PAH severity, demonstrating an RV-specific effect of IF. In the RV, lipotoxicity is blunted as demonstrated by a normalization in Oil Red O staining and RV ceramide levels with IF. IF corrects jejunal pathology, restructures the microbiome, and increases abundance of *Lactobacillus*. IF also combats the systemic consequences of microbiome dysbiosis as there is a restoration of bile acids, aromatic amino acid metabolites, and gamma-glutamylated amino acids levels in the RV. In total, these data provide multiple levels of support of the advantageous effects of IF for RV function in PAH.

The ability of IF to augment RV without significantly altering PAH severity has important implications for PAH. First, it suggests that microbiome dysbiosis in

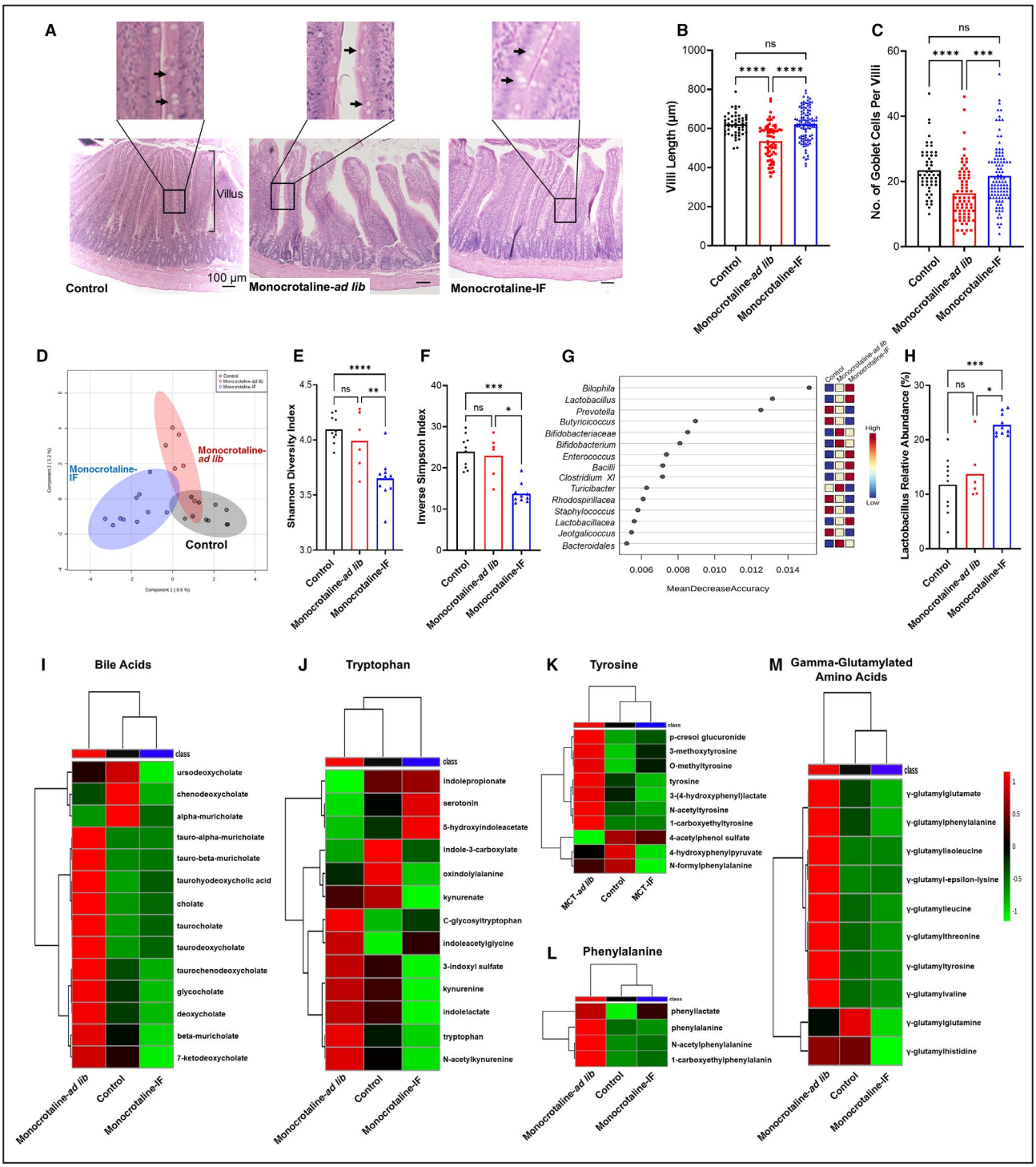

Figure 3. Intermittent fasting combats intestinal epithelial pathology, modulates the microbiome, and normalizes microbiome metabolite levels in the right ventricle (RV).
A, In histological analysis of the jejunum, intermittent fasting (IF) normalized (**B**) villi length (control: 622±55, monocrotaline-ad libitum: 534±91, monocrotaline-IF: 621±84 μm, $P<0.0001$ between monocrotaline-ad libitum and monocrotaline-IF, n=52 total villi from 4 controls, 71 villi from 8 monocrotaline-ad libitum, and 106 villi from 8 monocrotaline-IF rats measured) and (**C**) goblet cell abundance (control: 23±7, monocrotaline-ad libitum: 16±8, monocrotaline-IF: 22±9 goblet cells/villi, $P=0.0002$ between monocrotaline-ad libitum and monocrotaline-IF, n=52 villi from 4 controls, 70 villi from 8 monocrotaline-ad libitum, and 106 villi from 8 monocrotaline-IF rats assessed) (arrows). **D**, Sparse partial least squares discriminant analysis of 16S RNA sequencing of stool samples showed distinct microbiome compositions between control, monocrotaline-ad libitum, and monocrotaline-IF. IF restructured the microbiome as assessed by the (**E**) Shannon diversity index and (**F**) inverse Simpson index. **G**, Random forest classification identified the 15 most divergent bacterial genera. **H**, IF increased *Lactobacillus* abundance as compared with control and monocrotaline-ad libitum. Hierarchical cluster analysis of (**I**) bile acids, (**J**) tryptophan, (**K**) tyrosine, (**L**) phenylalanine pathway intermediates, and (**M**) gamma-glutamylated amino acids highlighted the restoration of microbiota metabolites in the RV with IF. *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$, and not significant with Brown–Forsythe and Welch ANOVA with Dunnett post hoc analysis in (**B**), Kruskal–Wallis test with Dunn multiple comparisons test in (**C**, **F**, and **H**), and 1-way ANOVA with Tukey post hoc test in (**E**). IF indicates intermittent fasting, and ns, not significant.

PAH[4] may be related to RV dysfunction and not solely pulmonary vascular remodeling. Second, the ability of IF to improve RV function while also combating lipotoxicity further validates this metabolic derangement as a significant contributor to impaired RV contractility in PAH. Finally, the fact that IF is able to extend lifespan without mitigating PAH severity further validates the RV as a viable therapeutic target in PAH.[8] Thus, IF is a potential non-pharmacologic approach to directly enhance RV function, which may be additive to proposed pharmaceutical approaches.[8]

The microbiome impacts multiple organ systems because of its influence on metabolic activity and systemic inflammation. In particular, there are 3 well-defined microbial metabolites (short-chain fatty acids, amino acids, and bile acid metabolites) that may directly regulate the immune system.[2] Immune dysregulation is hypothesized to modulate RV function in PAH,[8] and thus the RV-enhancing effects of IF may be attributable to anti-inflammatory properties of a restructured microbiome. Interestingly, excess bile acids are associated with decreased fatty acid metabolism and cardiac dysfunction.[9] These results are consistent with our study as monocrotaline-ad libitum RVs had increased levels of almost all bile acids and evidence of impaired fatty acid oxidation. Unfortunately, our metabolomics study did not quantify short-chain fatty acids, but the consistent normalization of amino acid and bile acid metabolites with IF supports the fact that IF combats the systemic effects of a pathologically altered gut microbiome.

Our observation of increased abundance of *Lactobacillus* with IF is consistent with other preclinical[10] and human studies[11,12] evaluating the effects of IF on the microbiome. *Lactobacilli* enhance the intestinal epithelial cell health and decrease intestinal permeability via their ability to stimulate goblet cell mucus production and replication.[13] Thus, our finding that IF normalized jejunal epithelial morphology (both villi length and goblet cell abundance) may be partially because of higher levels of *Lactobacillus*. However, several bacterial genera are proposed to regulate intestinal epithelial morphology and function,[13] and thus it is likely that changes in the abundance of multiple bacteria underlie this beneficial effect. In addition to modulation of intestinal epithelium, there is evidence that *Lactobacilli* have direct cardioprotective effects. *Lactobacillus* supplementation following myocardial infarction attenuates the development of left ventricular systolic function in rats.[14] In particular, *Lactobacillus* treatment restores the leptin to adiponectin ratio and corrects left ventricle myocardial metabolism.[14] Our finding that IF fasting combats RV lipotoxicity are congruent with these previously described metabolic effects. However, future studies are needed to dissect the effects of *Lactobacillus* supplementation on RV metabolism and function in PAH.

A next logical question is could IF negate microbiome dysbiosis and disrupted metabolism to combat RV dysfunction in patients with PAH. Currently, IF is under active investigation in multiple clinical trials in a diverse array of disease states ranging from obesity, diabetes, stroke, to multiple types of cancer. Query of ClinicalTrials.gov shows there are 67 ongoing studies investigating IF, suggesting IF can be safely performed in the proper clinical scenario, and thus a clinical trial of IF in PAH would be a feasible next approach. Importantly, a recent proof-of-principle clinical trial showed IF effectively modifies the microbiome and increases *Lactobacillus* abundance in patients with multiple sclerosis.[12] Thus, a clinical trial evaluating the effects of IF on RV dysfunction in PAH, a currently untreatable and ultimately fatal consequence of PAH,[8] could be considered in the future.

Our study has important limitations that we must acknowledge. It is known that IF has pleiotropic benefits, and thus our findings are likely not solely attributable to combating microbiome dysbiosis. Other potential mediators of the favorable effects of IF include but are not limited to decreasing inflammation, enhancing mitochondrial stress response and biogenesis, augmenting

autophagy, and improving DNA damage repair.[15] Interestingly, many of these molecular signatures are hypothesized to promote RV dysfunction in PAH.[8] Although our study links IF to enhanced RV function, further studies are required to investigate the precise mechanism by which the microbiota and/or specific microbial metabolites modulate RV function in PAH. While IF improved RV function without substantially altering pulmonary vascular disease severity, it is unknown whether initiating IF during a later time period after monocrotaline injection would still be beneficial because we initiated our protocol the day after monocrotaline injection (prevention as opposed to reversal). Finally, we only studied male rats as they develop more severe RV failure than females[8] and potential sex differences in response to IF were not the aim of this study.

ARTICLE INFORMATION

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Disclosures

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Supplementary Material

Figure S1

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Supplemental Material

Figure S1. Representative pressure-volume loops with end-systolic elastance (Ees) line.