Metabolic Profiling Revealed Dysregulated Arachidonic Acid Metabolism and Tryptophan Metabolism in the Lungs from Canines with Pacing-induced Heart Failure

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Abstract

Background: Lung has critical pathophysiological connections to heart and lung congestion presents one of the hallmark features of heart failure (HF). This study aimed to explore the metabolic signatures and disturbances in lungs under HF condition and provide insights on the pathophysiology of the lungs under HF condition from the perspective of metabolism.

Methods: In this study, we established a rapid pacing induced HF canine model and applied a comprehensive untargeted metabolomics method to comparatively assessed the metabolomics profiles in the lung tissues from HF group and sham group.

Results: Distinct metabolic signatures were identified in the lungs between beagles in HF group and sham group. 81 dysregulated metabolites were identified as differential metabolites (adjusted $P < 0.05$, $FC \geq 2$ or ≤ 0.5) in positive ion mode and 80 dysregulated metabolites in negative ion mode, indicating a profound metabolic alteration in the lungs under HF condition. In pathway analysis, arachidonic acid metabolism and tryptophan metabolism were identified as the most significant dysregulated metabolic pathways in the lungs from HF beagles.

Conclusions: In this study, we identified profound metabolic variation and dysregulated metabolic pathways, which may deepen our understanding on the pathophysiology of the lungs under HF condition from the perspective of metabolism and open new avenues in lung congestion management in HF.

Introduction

Heart failure (HF) is a complex clinical syndrome characterized by impaired structural or functional ventricular filling or blood ejection(1). And it is a rapidly growing public health issue with an estimated prevalence of > 37.7 million individuals globally, causing enormous societal burden worldwide(2). There are critical pathophysiological connections between lung and heart. Lung congestion presents one of the hallmark features of HF, which not only impairs gas exchange capacity and pulmonary function, but also drives poor prognosis by promoting pulmonary vascular dysfunction and right ventricular failure(3, 4).The pathophysiology of lung congestion has been considered to result from passive fluid extravasation from lung capillaries towards the alveoli and interstitium(5). Briefly, the deterioration of cardiac pump function leads to the increase in cardiac filling pressure and accelerates the regular fluid filtration rate to the interstitium, and then pulmonary congestion and oedema. These changes further cause inflammation-triggered increase in permeability of the pulmonary vasculature and exacerbate the pathological process (6, 7). Therefore, conventional therapeutic strategies aiming at resolving lung congestion mainly focus on the reduction of capillary hydrostatic pressure, either through decreasing total intravascular volume with diuretics or through reducing afterload with vasodilators(5, 8). While clinical congestion may rapidly resolve with treatment, pulmonary congestion may persist for longer, and its resolution during hospitalization may be delayed or incomplete despite aggressive diuretics or vasodilators use(9, 10). In addition, side effects including diuretic resistance, electrolyte disturbances,

worsening of renal function, and adverse neurohormonal activation limited their use in clinical practice(11). Therefore, there is an unmet need to investigate the pathophysiology of lung under HF condition and find novel therapeutic strategies to tackle lung congestion.

Metabolites are the most proximal reflection of all the dynamic changes in response to transcriptional and translational regulation as well as posttranslational modifications(12). Metabolism dysregulation plays an increasing important role in cardiovascular disease. Metabolomics, a newly developed high-throughput technology, enables quantitation of thousands of small molecules metabolites simultaneously and depicts unbiased and comprehensive metabolic signatures (13–15). To our knowledge, few studies have demonstrated the pathophysiology of lung under HF condition from the view of metabolism.

In this study, a pacing-induced canine HF model was conducted. We applied the untargeted metabolomics technology to the lung samples harvested from HF dogs and control dogs and comparatively assessed the metabolomics profiles in HF lungs versus control lungs. By using a comprehensive untargeted metabolomics technology, we aimed to explore the metabolic signatures and disturbances in the lungs under HF condition and shed light on potential therapy target in lung congestion management.

Materials And Methods

Animal and study protocol

Eleven male beagles (12 months; 11–13 kg, Animal Center of Fuwai Hospital) were randomly assigned to control group (Sham, N=5) or HF group (HF, N=6). One week after settling in, all beagles underwent echocardiography and electrocardiography (ECG) test. Beagles in HF group underwent cardiac pacemaker implantation, while the control group underwent a sham operation. The animals were anesthetized with intravenous pentobarbital (3%, 30 mg/kg). Under fluoroscopic guidance, the tip of the pacing electrode (7231, Lepu Medical Equipment Company, Shanghai, China) was introduced to the right ventricular apex through the right external jugular vein. The pacing mode was set to AOO, and the pacing frequency was set to 250 beats/min. The output was 3.6 V, and the pulse width was 0.4 ms. The end of the electrode was connected to the pacemaker, which was placed on the right thoracic wall subcutaneously.

Beagles were feed on a high-protein diet after surgery and 80 U intramuscular penicillin was injected twice a day for 7 days to prevent infection. Two weeks after the operation, the pacemakers were turned on simultaneously, and the pacing went on for 6 weeks. Echocardiography and the ECG test were recorded again at the end of 6 weeks, and the peripheral blood samples and left ventricle and lung tissues samples were harvested. Tissue samples were collected and washed with PBS to remove the blood. The samples were quickly snap-frozen in liquid nitrogen and stored in –80°C until use. All animal experiments complied with the ARRIVE guidelines and were carried out in accordance with the National Institutes of

Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). The protocol was approved by the ethics committee of Fuwai hospital (approval number: 0077-3-40-G (X)).

Echocardiography and ECG

The echocardiogram (at baseline and endpoint) was performed by an experienced physician who was blinded to the group information. Left ventricle end-diastolic volume (LVEDV), left ventricle end-systolic volume (LVESV), left ventricle fractional shortening (LVFS), left ventricle ejection fraction (LVEF), and the peak flow rate ratio E/A of the mitral valve in early and late diastole were measured at sinus rhythm (IE33, Philips Company, Netherlands). ECG was recorded simultaneously. (ECG monitor, Mindray, China).

Metabolomics analysis

The samples were processed according to the procedure previously described (16). In brief, 400 μ l extraction solvent (methanol/water, 4/1, v/v) was added to 20 mg lung tissue sample. Samples were grinded in a ball mill at 60 Hz for 2 minutes. The homogenate was ultrasound-extracted for 10 minutes, and then stored at -20°C for 30 minutes. Then the extraction was centrifuged at 13000 rpm, 4°C for 10 minutes. The supernatant was transferred and collected for analysis in positive and negative electrospray ionization mode respectively.

A Dionex Ultimate 3000 RS UHPLC system (Thermo Fisher Scientific, Waltham, MA, USA) was equipped with ACQUITY UPLC BEH C18 column ($1.7\mu\text{m}$, $2.1\times 100\text{mm}$, Waters Corp, Milford, USA) for positive mode and UPLC HSS T3 column ($2.1\text{mm}\times 100\text{mm}$, $1.8\mu\text{m}$, Waters Corp, Milford, USA) for negative mode. In the positive mode analysis, the binary gradient elution system consisted of (A) water (containing 0.1% formic acid, v/v) (B) acetonitrile (containing 0.1% formic acid, v/v). The separation was accomplished through the following gradient: B start at 5%, 1-24 min to reach 100%, the composition was held at 100% B, then 27.5–27.6 min, 100% to 5% B, and 27.6–30 min holding at 5% B. In the negative mode analysis, the mobile phase A was 6.5mM ammonium acetate in water and mobile phase B was 6.5mM ammonium acetate in 95% methanol. The separation was accomplished through the following gradient: B start at 5%, 1-18 min to reach 100%, the composition was held at 100% B 18.1-22 min, then 22–22.1 min, 100% to 5% B, and 22.1–25 min holding at 5% B. The flow rate and column temperature were the same as in the positive mode.

The injection volume was 5 μ l. LC–MS/MS assessment was performed on a Q-Exactive quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with heated electrospray ionization (ESI) source in both positive and negative ion modes. Data acquisition was performed in full scan mode combined with Data Dependent Acquisition (DDA) mode. The mass spectrometer parameters were set as following: spray voltage, 3800V (positive) and 3000V (negative); capillary temperature, 320°C ; Aux gas heater temperature: 350°C ; sheath gas flow rate, 35 arbitrary units; auxiliary gas flow rate, 8 arbitrary units; S-lens RF level: 50. The mass range was from m/z 70 to 1000. The resolution was set at 70,000 for the full MS scans and 17500 for MS/MS scans. The collision energy was set at 20, and 40eV in NCE mode.

Data processing and metabolite identification

Metabolomics data was acquired using the XCMS software (1.50.1). The preprocessing procedure generated a data matrix that consisted of the retention time (RT), mass-to-charge ratio (m/z) values, and peak intensity. Variables with missing value >15% were removed from the data set. OSI-SMMS (version 1.0, Dalian Chem Data Solution Information Technology Co. Ltd.) was used for peak annotation after XCMS data processing with an in-house MS/MS database.

RT-qPCR

Total RNA was extracted from left ventricle tissues by RNeasy pure Tissue Kit (Qiagen, DP431) and was reverse transcribed to cDNA by SuperScript™ III First-Strand Synthesis SuperMix (Invitrogen, 11752050). Primers sequence was listed as following: BNP (forward primer 5'-CCA CTT CTC TCC AGC GAC AT-3' and reverse primer 5'-CAG CTC TGA AAC TGC GTC CT-3'); GAPDH (forward primer 5'-GGG TGA TGC TGG TGC TGA GTA T-3' and reverse primer 5'-TTG CTG ACA ATCTTG AGG GAG TT-3'). Quantitative RT-PCR (RT-qPCR) was performed using SYBR™ Select Master Mix (Applied Biosystems, 4472908). The Ct values of the target genes were subtracted from the mean of the Ct values of GAPDH (ΔCt). All samples were examined in duplicate, and the mean value of ΔCt was calculated. The transcription levels of the target genes in each sample were represented as an n-fold difference to GAPDH.

Statistical analysis

To compare the differences between the two groups, student's t-test was used for variables in normal distribution and wilcoxon test for variables in non-normal distribution. The *P* values were adjusted by a false discovery rate (FDR) method to account for multiple testing. Metabolomics data was auto-scale normalized for multivariate analysis. Principle component analysis (PCA) was used to discriminate among different groups in an unsupervised mode. The pathway analysis with the pathway topology analysis was used to identify the most relevant metabolic pathways. The statistical analysis was performed by R x64 3.6.2, SPSS Statistics 22.0 (IBM, New York, USA), SIMCA-P software 14.1, (Umetrics, Umea, Sweden) and MetaboAnalyst 4.0 online website(17).

Results

Establishment of a canine model of pacing-induced heart failure

All experimental animals had good pacing tests during the operation. The incision healed well after operation, and there were no infectious secretions in the capsular bag (Supplementary Fig. 1). Pre-operative ECG for all dogs were normal, neither bradycardia nor conduction block was seen before the operation. ECG and pacemaker's programming control after operation indicated that the pacemaker worked properly, and the pacing heart rate reached the assumed value (Supplementary Fig. 2).

The beagles in the HF group developed clinical symptoms including poor appetite, tachypnoea, and reduced physical activity, while no abnormal symptom was observed in the beagles in the sham group. Then the cardiac function was evaluated by echocardiographic measurements. All echocardiographic measurements at baseline showed no significant difference between the two groups. After 6 weeks of rapid-pacing, dogs in HF group showed significant deterioration in cardiac function (LVESV: 8.4 ± 2.0 ml vs. 23.2 ± 4.7 ml, $P = 0.0001$; LVEDV: 22.4 ± 6.4 ml vs. 38.2 ± 6.3 ml, $P = 0.0027$; LVFS: $36.6 \pm 5.0\%$ vs $22.1 \pm 3.3\%$, $P = 0.0003$; LVEF: $61.0 \pm 5.0\%$ vs $36.8 \pm 2.3\%$, $P < 0.0001$ Fig. 1a-e). In addition, the mRNA level of BNP in left ventricles significantly increased in the HF group than the sham group (Fig. 1f). There were also significantly increased interstitial collagen in left ventricles in the HF group than the sham group (Supplementary Fig. 3). The heart weight relative to body weight showed significant increase (Supplementary Fig. 4A). The lung weight relative to corresponding body weight also showed significant elevation, indicating a fluid retention in the lungs under HF (Supplementary Fig. 4B).

Metabolic profile of canine lungs in heart failure

4876 ion peaks were acquired in ESI+ mode, and 4870 ion peaks were acquired in ESI- mode. 77% of features in QC samples possessed a relative standard deviation (RSD) less than 30%. In addition, PCA plot showed that QC samples clustered together, which displayed good reproducibility and stability of the metabolomics method (Supplementary Fig.5).

The significant differential metabolites between the HF group and the sham group were analyzed in ESI+ and ESI- mode respectively. Volcano plots in fig.2a-b exhibited the differential ion peaks ($\text{adj-}P < 0.05$, $\text{FC} > 1.5$) generated through univariate analysis. Differential endogenous metabolites were then identified (Supplementary Table1, Supplementary Fig.6). PCA, an unsupervised multivariate algorithm, was applied for further analysis to discriminate different groups. The cumulative variance explained by the PCA analysis were 80.4% in positive ion mode and 88.6% in negative ion mode (Supplementary Fig.7). As shown in score plots in Fig. 2c-d, blue and red dots gathered at different cluster, indicating there was a distinct classification between these two groups. A heatmap consisting of the most significant dysregulated metabolites was demonstrated in supplementary Fig.8, which also suggested a profound metabolic alteration between the lungs from the HF group and the sham group.

Pathway analysis of the dysregulated metabolites between HF and sham group

The results of pathway analysis were illustrated in supplementary Fig.9. In total, 36 pathways were involved in the analysis. The arachidonic acid metabolism was ranked on top as the most significant pathway followed by tryptophan metabolism pathway.

In the arachidonic acid metabolism pathway, arachidonic acid was primarily metabolized by three types of critical enzymes including cyclooxygenase (COX) enzymes, lipoxygenase (LOX) enzymes and cytochrome P-450 (CYP) enzymes, which were all involved in the dysregulation observed in the lungs responded to HF (Fig.3a). 10 metabolites in this pathway were upregulated in the lungs in the HF group compared to the sham group (Fig.3b). The CYP-mediated metabolites were 5,6-EET (111249 ± 38897 vs.

558840±92274, $P=0.0025$), 11,12-EET (576169±282430 vs. 4883341±1367680, $P=0.0204$), 14,15-DHET (22098±14224 vs. 101198±25071, $P=0.0292$), 20-HETE (10269±3693 vs. 51124±12469, $P=0.0182$). The COX-mediated metabolites were prostaglandin G2 (8346±3567 vs. 33177±3507, $P=0.0008$), prostaglandin I2 (1113447±754800 vs. 3792112±682874, $P=0.0272$), and prostaglandin E2 (22516±6599 vs. 199541±55900, $P=0.0190$). The LOX mediated metabolites were 15(S)-HPETE (179933±85779 vs. 1121179±369445, $P=0.0498$), leukotriene C4 (64362±36154 vs. 463769±79382, $P=0.0021$), and 5(S)-HPETE (49755±17893 vs. 193588±55598, $P=0.0499$). These significant changes indicated a profound dysregulation in the arachidonic acid metabolism pathway, which was closely related to lung pathophysiology under HF condition.

In the tryptophan metabolism pathway, 6 metabolites significantly elevated in the HF lungs compared to those of the sham group (Fig.4a). The level of L-tryptophan was higher in lungs from HF group (115973±34697 vs. 205440±19888, $P=0.0442$). 2 out of 3 major metabolic pathways of L-tryptophan were altered including indoleamine 2,3-dioxygenase/tryptophan 2,3-dioxygenase (IDO/TDO) mediated pathway and tryptophan hydroxylase (TPH) mediated pathway. The downstream metabolites of IDO/TDO mediated pathway were L-kynurenine (328297±186723 vs. 1054659±148466, $P=0.0130$), and formylanthranilate (115860±65109 vs. 321741±51349, $P=0.0328$). TPH mediated metabolites contained 5-hydroxy-L-tryptophan (2738±526 vs. 4909±718.6, $P=0.0437$), 5-hydroxyindoleacetate (12754±2433 vs. 40615±7388, $P=0.0093$), 6-hydroxymelatonin (3390±1675 vs. 24371±3960, $P=0.0014$), all of which were elevated in the HF lungs (Fig.4b).

Discussion

In this study, we established a rapid pacing induced HF canine model and comparatively assessed the metabolomics profiles in the lung tissues from HF group and sham group through untargeted metabolomics approach. Distinct metabolic signatures were identified in lungs between beagles in the HF group and the sham group, indicating a profound metabolic alteration in the lungs under HF condition. Additionally, we found dysregulated arachidonic acid metabolism and tryptophan metabolism in the lungs from the beagles of HF, which may shed light on the pathophysiology of the lungs under HF condition from the perspective of metabolism.

Arachidonic acid (AA) and its derivatives link nutrient metabolism to immunity and inflammation, thus holding a key role in the emergence and progression of diseases such as cardiovascular disease, obesity, and diabetes (18). Three major enzymatic pathways process free AA, resulting in the generation of a plethora of AA derivatives with numerous functions. Prostaglandin G/H synthases, also known as cyclooxygenase 1 (COX1) and 2 (COX2), foster the production of thromboxane A2 (TXA2), prostacyclin (PGI2) and several prostaglandins (PGs), which plays an important role in microvascular endothelial cell function, vessel tone regulation, and platelet aggregation (19, 20). Lipoxygenases (LOX) processed AA to 15-hydroperoxyicosatetraenoic acid (15-HPETE) and 5-hydroxyicosatetraenoic acid (5-HETE), as well as various leukotrienes (LTs). These metabolites affect neutrophil recruitment, epithelial barrier function, vascular permeability and bronchoconstriction, which plays a vital role in lung congestion formation in

HF (21, 22). Moreover, the cytochrome P450 (CYP450) mediated epoxy-eicosatrienoic acids (EETs) and hydroxy-eicosatetraenoic acids (HETEs) also participate in regulating vessel constriction and dilation (23, 24).

Tryptophan, an essential amino acid in mammals, is involved in several physiological processes, including neuronal function, immunity, and gut homeostasis (25, 26). In humans, tryptophan is mainly metabolized via the kynurenine and serotonin pathways, leading to the generation of biologically active compounds, such as serotonin and melatonin. Only a small portion of tryptophan is metabolized into neuromodulators such as tryptamine (27). In this study, we identified increased metabolism of tryptophan in IDO mediated metabolism and TPH mediated metabolism in the lungs under HF. IDO is induced by proinflammatory stimuli and T-helper-cell cytokines, such as tumor necrosis factor- α , interleukin-6 (28, 29). Kynurenines pathway activation, by up-regulating NAD(P)H-derived superoxide, accelerates endothelial dysfunction and vascular inflammation, which further increases the vascular permeability and contribute to fluid filtration in lung congestion (30). TPH mediated serotonin synthesis plays an important role in pulmonary vasoconstriction and bronchoconstriction (31). In addition, serotonin and its membrane transporter also played an essential role in the pathogenesis of pulmonary artery smooth muscle cell proliferation in experimental and human pulmonary hypertension (32, 33). As persistent lung congestion promotes the pulmonary vascular dysfunction and ultimately right ventricular failure, the dysregulated tryptophan metabolism warrants concern as a therapeutic target in lung congestion management.

In this study, we identified profound metabolic variation in the lungs under HF condition. We also found dysregulated arachidonic acid metabolism and tryptophan metabolism pathway, which may deepen our understanding on the pathophysiology of the lungs under HF condition from the perspective of metabolism and open new avenues in lung congestion management in HF.

List Of Abbreviations

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JHZ, JW, SWY performed the animal experiment. RJ and XL collected the tissues samples. JHZ and SWY analyzed and interpreted the metabolomics data. SWY performed the histological examination. MG and CC contributed to the figure production. JHZ, and JW contributed to manuscript writing. WH designed the experiment and modified the manuscript. All authors read and approved the final manuscript.

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References

1. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Jr., Drazner MH, et al. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Journal of the American College of Cardiology*. 2013;62(16):e147-239.
2. Ziaeian B, Fonarow GC. Epidemiology and aetiology of heart failure. *Nature reviews Cardiology*. 2016;13(6):368-78.
3. Platz E, Merz AA, Jhund PS, Vazir A, Campbell R, McMurray JJ. Dynamic changes and prognostic value of pulmonary congestion by lung ultrasound in acute and chronic heart failure: a systematic review. *Eur J Heart Fail*. 2017;19(9):1154-63.
4. Metra M, Davison B, Bettari L, Sun H, Edwards C, Lazzarini V, et al. Is worsening renal function an ominous prognostic sign in patients with acute heart failure? The role of congestion and its interaction with renal function. *Circ Heart Fail*. 2012;5(1):54-62.

5. Bistola V, Polyzogopoulou E, Parissis J. A novel strategy for the management of lung congestion: targeting TRPV4 channel, the 'gate keeper' of pulmonary capillary permeability. *Eur J Heart Fail*. 2020.
6. Azarbar S, Dupuis J. Lung capillary injury and repair in left heart disease: a new target for therapy? *Clinical science (London, England : 1979)*. 2014;127(2):65-76.
7. Bursi F, McNallan SM, Redfield MM, Nkomo VT, Lam CS, Weston SA, et al. Pulmonary pressures and death in heart failure: a community study. *Journal of the American College of Cardiology*. 2012;59(3):222-31.
8. Kingsbury MP, Huang W, Donnelly JL, Jackson E, Needham E, Turner MA, et al. Structural remodelling of lungs in chronic heart failure. *Basic research in cardiology*. 2003;98(5):295-303.
9. Coiro S, Rossignol P, Ambrosio G, Carluccio E, Alunni G, Murrone A, et al. Prognostic value of residual pulmonary congestion at discharge assessed by lung ultrasound imaging in heart failure. *Eur J Heart Fail*. 2015;17(11):1172-81.
10. Melenovsky V, Andersen MJ, Andress K, Reddy YN, Borlaug BA. Lung congestion in chronic heart failure: haemodynamic, clinical, and prognostic implications. *Eur J Heart Fail*. 2015;17(11):1161-71.
11. Mullens W, Damman K, Harjola VP, Mebazaa A, Brunner-La Rocca HP, Martens P, et al. The use of diuretics in heart failure with congestion - a position statement from the Heart Failure Association of the European Society of Cardiology. *Eur J Heart Fail*. 2019;21(2):137-55.
12. Wishart DS. Emerging applications of metabolomics in drug discovery and precision medicine. *Nature reviews Drug discovery*. 2016;15(7):473-84.
13. Shah SH, Kraus WE, Newgard CB. Metabolomic profiling for the identification of novel biomarkers and mechanisms related to common cardiovascular diseases: form and function. *Circulation*. 2012;126(9):1110-20.
14. Johnson CH, Ivanisevic J, Siuzdak G. Metabolomics: beyond biomarkers and towards mechanisms. *Nature reviews Molecular cell biology*. 2016;17(7):451-9.
15. McGarrah RW, Crown SB, Zhang GF, Shah SH, Newgard CB. Cardiovascular Metabolomics. *Circulation research*. 2018;122(9):1238-58.
16. Yang XY, Li XZ, Zhang SN. Metabolomics analysis of oral mucosa reveals profile perturbation in reticular oral lichen planus. *Clinica chimica acta; international journal of clinical chemistry*. 2018;487:28-32.
17. J C, DS W, J X. Using MetaboAnalyst 4.0 for Comprehensive and Integrative Metabolomics Data Analysis. *Current protocols in bioinformatics*. 2019;68(1):e86.
18. Sonnweber T, Pizzini A, Nairz M, Weiss G, Tancevski I. Arachidonic Acid Metabolites in Cardiovascular and Metabolic Diseases. *International journal of molecular sciences*. 2018;19(11).
19. Cogolludo A, Moreno L, Bosca L, Tamargo J, Perez-Vizcaino F. Thromboxane A2-induced inhibition of voltage-gated K⁺ channels and pulmonary vasoconstriction: role of protein kinase C ζ . *Circulation research*. 2003;93(7):656-63.

20. Cheng K, Wu TJ, Wu KK, Sturino C, Metters K, Gottesdiener K, et al. Antagonism of the prostaglandin D2 receptor 1 suppresses nicotinic acid-induced vasodilation in mice and humans. *Proc Natl Acad Sci U S A*. 2006;103(17):6682-7.
21. Bray MA, Cunningham FM, Ford-Hutchinson AW, Smith MJ. Leukotriene B4: a mediator of vascular permeability. *British journal of pharmacology*. 1981;72(3):483-6.
22. Moos MP, Mewburn JD, Kan FW, Ishii S, Abe M, Sakimura K, et al. Cysteinyl leukotriene 2 receptor-mediated vascular permeability via transendothelial vesicle transport. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2008;22(12):4352-62.
23. Liu Y, Zhang Y, Schmelzer K, Lee TS, Fang X, Zhu Y, et al. The antiinflammatory effect of laminar flow: the role of PPARgamma, epoxyeicosatrienoic acids, and soluble epoxide hydrolase. *Proc Natl Acad Sci U S A*. 2005;102(46):16747-52.
24. Inceoglu B, Schmelzer KR, Morisseau C, Jinks SL, Hammock BD. Soluble epoxide hydrolase inhibition reveals novel biological functions of epoxyeicosatrienoic acids (EETs). *Prostaglandins & other lipid mediators*. 2007;82(1-4):42-9.
25. Platten M, Nollen EAA, Röhrig UF, Fallarino F, Opitz CA. Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond. *Nature reviews Drug discovery*. 2019;18(5):379-401.
26. van der Goot AT, Nollen EA. Tryptophan metabolism: entering the field of aging and age-related pathologies. *Trends in molecular medicine*. 2013;19(6):336-44.
27. Schwarcz R, Stone TW. The kynurenine pathway and the brain: Challenges, controversies and promises. *Neuropharmacology*. 2017;112(Pt B):237-47.
28. King NJ, Thomas SR. Molecules in focus: indoleamine 2,3-dioxygenase. *The international journal of biochemistry & cell biology*. 2007;39(12):2167-72.
29. O'Connor JC, André C, Wang Y, Lawson MA, Szegedi SS, Lestage J, et al. Interferon-gamma and tumor necrosis factor-alpha mediate the upregulation of indoleamine 2,3-dioxygenase and the induction of depressive-like behavior in mice in response to bacillus Calmette-Guerin. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2009;29(13):4200-9.
30. Wang Q, Zhang M, Ding Y, Wang Q, Zhang W, Song P, et al. Activation of NAD(P)H oxidase by tryptophan-derived 3-hydroxykynurenine accelerates endothelial apoptosis and dysfunction in vivo. *Circulation research*. 2014;114(3):480-92.
31. Naz S, Bhat M, Ståhl S, Forsslund H, Sköld CM, Wheelock Å M, et al. Dysregulation of the Tryptophan Pathway Evidences Gender Differences in COPD. *Metabolites*. 2019;9(10).
32. Guignabert C, Izikki M, Tu LI, Li Z, Zadigue P, Barlier-Mur AM, et al. Transgenic mice overexpressing the 5-hydroxytryptamine transporter gene in smooth muscle develop pulmonary hypertension. *Circulation research*. 2006;98(10):1323-30.
33. de Caestecker M. Serotonin signaling in pulmonary hypertension. *Circulation research*. 2006;98(10):1229-31.

Figures

Figure 1

Establishment of pacing-induced heart failure model. Representative M-mode echocardiograms obtained after sham surgery or 6 weeks pacing (a). Echocardiographic measurements at baseline and after 6 weeks of rapid ventricular pacing in beagles (b-e). The mRNA level of BNP in left ventricles after 6 weeks of rapid ventricular pacing (f). Student's t-test was used for variables in normal distribution and wilcoxon test for non-normal distribution variables. *P < 0.05, **P < 0.01, ***P < 0.001. Data were showed as mean ± SD.

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Figure 2

Metabolomics profiling in positive and negative ion modes. Volcano plots for dysregulated endogenous metabolites in lungs between sham group and HF group in positive ion mode (a) and negative ion mode (b). Each plot represents a metabolite. The differentiating metabolites (adj-P value < 0.05, FC > 1.5 or < 0.67) were highlighted in color. In the three-dimensional score plots, each plot represents a sample. They showed a distinct classification in sham (blue) and HF (red) in positive ion mode (c) and negative ion mode (d).

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Metabolomics profiling in positive and negative ion modes. Volcano plots for dysregulated endogenous metabolites in lungs between sham group and HF group in positive ion mode (a) and negative ion mode (b). Each plot represents a metabolite. The differentiating metabolites (adj-P value < 0.05, FC > 1.5 or < 0.67) were highlighted in color. In the three-dimensional score plots, each plot represents a sample. They showed a distinct classification in sham (blue) and HF (red) in positive ion mode (c) and negative ion mode (d).

Figure 3

Dysregulated arachidonic acid metabolism pathway observed in lungs between HF and sham group. Dysregulated arachidonic acid metabolism pathway in lung under HF condition (a). Significantly changed metabolites in arachidonic acid metabolism pathway (b). Student's t-test was used for variables in normal distribution and wilcoxon test for non-normal distribution variables. *P < 0.05, **P < 0.01, ***P < 0.001 Data were showed as mean ± SD. 15-LOX, arachidonate 15-lipoxygenase; CYP450, cytochrome P450; EPHX2, epoxide hydrolase 2; LTC4S, leukotriene C4 synthase; PTGES3, prostaglandin E synthase 3; PTGIS, prostaglandin I2 synthase; PTGS, prostaglandin-endoperoxide synthase.

Figure 3

Dysregulated arachidonic acid metabolism pathway observed in lungs between HF and sham group. Dysregulated arachidonic acid metabolism pathway in lung under HF condition (a). Significantly changed metabolites in arachidonic acid metabolism pathway (b). Student's t-test was used for variables in normal distribution and wilcoxon test for non-normal distribution variables. *P < 0.05, **P < 0.01, ***P < 0.001 Data were showed as mean ± SD. 15-LOX, arachidonate 15-lipoxygenase; CYP450, cytochrome P450; EPHX2, epoxide hydrolase 2; LTC4S, leukotriene C4 synthase; PTGES3, prostaglandin E synthase 3; PTGIS, prostaglandin I2 synthase; PTGS, prostaglandin-endoperoxide synthase.

Figure 4

Dysregulated tryptophan metabolism pathway observed in lungs between HF and sham group. Dysregulated tryptophan metabolism pathway in lung under HF condition (a). Significantly changed metabolites in tryptophan metabolism pathway (b). Student's t-test was used for variables in normal distribution and wilcoxon test for non-normal distribution variables. *P < 0.05, **P < 0.01, ***P < 0.001. Data were showed as mean ± SD. AADC, aromatic-l-amino acid decarboxylase; AFMID, kynurenine

formamidase; IDO, indoleamine-2,3-dioxygenase; TDO, tryptophan-2,3-dioxygenase; TPH, tryptophan hydroxylase.

Figure 4

Dysregulated tryptophan metabolism pathway observed in lungs between HF and sham group. Dysregulated tryptophan metabolism pathway in lung under HF condition (a). Significantly changed metabolites in tryptophan metabolism pathway (b). Student's t-test was used for variables in normal distribution and wilcoxon test for non-normal distribution variables. *P < 0.05, **P < 0.01, ***P < 0.001.

Data were showed as mean $\pm$ SD. AADC, aromatic-l-amino acid decarboxylase; AFMID, kynurenine formamidase; IDO, indoleamine-2,3-dioxygenase; TDO, tryptophan-2,3-dioxygenase; TPH, tryptophan hydroxylase.

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