DOI: 10.1002/pul2.12202

RESEARCH ARTICLE

Pulmonary Circulation Open Access | PVRI Pulmonary Vascular Research Institute | WILEY

# Metallomics in pulmonary arterial hypertension patients

**Karim El-Kersh[1] | C. Danielle Hopkins[2] | Xiaoyong Wu[3] | Shesh N. Rai[3] | Matthew C. Cave[4,5,6,7,8] | M. Ryan Smith[9] | Young-Mi Go[9] | Dean P. Jones[9] | Lu Cai[5,6,10,11] | Jiapeng Huang[2,5,6,8,12,13]**

[1]Division of Pulmonary Critical Care and Sleep Medicine, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, Nebraska, USA

[2]Department of Anesthesiology and Perioperative Medicine, University of Louisville School of Medicine, Louisville, Kentucky, USA

[3]Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio, USA

[4]Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine, University of Louisville School of Medicine, Louisville, Kentucky, USA

[5]The Center for Integrative Environmental Health Sciences, University of Louisville, Louisville, Kentucky, USA

[6]Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, Kentucky

[7]Department of Biochemistry and Molecular Genetics, University of Louisville School of Medicine, Louisville, Kentucky, USA

[8]The Transplant Program at UofL Health — Jewish Hospital Trager Transplant Center, Louisville, Kentucky, USA

[9]Division of Pulmonary, Allergy and Critical Care Medicine, Emory University, Atlanta, Georgia, USA

[10]Department of Pediatrics, Pediatric Research Institute, University of Louisville School of Medicine, Louisville, Kentucky, USA

[11]Department of Radiation Oncology, University of Louisville School of Medicine, Louisville, Kentucky, USA

[12]Department of Cardiovascular and Thoracic Surgery, Cardiovascular Innovation Institute, University of Louisville School of Medicine, Louisville, Kentucky, USA

[13]Division of Infectious Diseases, Department of Medicine, Center of Excellence for Research in infectious Diseases, University of Louisville, Louisville, Kentucky, USA

**Correspondence**
Lu Cai, Department of Pediatrics, Pediatric Research Institute, University of Louisville School of Medicine, Louisville, KY, USA.
Email: lu.cai@louisville.edu

Jiapeng Huang, Department of Anesthesiology and Perioperative Medicine, University of Louisville, 530 South Jackson Street, Louisville, KY, USA.
Email: j0huan03@louisville.edu

**Funding information**
National Institute of Environmental Health Sciences, Grant/Award Number: P30ES030283; National Center for Advancing Translational Sciences, Grant/Award Number: 1U18TR003787-01

## Abstract

Pulmonary arterial hypertension (PAH) prevalence is increasing worldwide, and the prognosis is poor with 5-year survival < 50% in high risk patients. The relationship between metal exposure/essential metal dyshomeostasis and PAH/right ventricular dysfunction is less investigated. The aim of this study is to investigate vegetable consumptions and metal levels between PAH patients and controls. This was a prospective, single center pilot study. Questionnaires were completed by all study subjects (20 PAH patients and 10 healthy controls) on smoking, metal exposure risks, metal supplements, and vegetable consumptions. Blood and urine samples were collected to measure 25 metal levels in blood, plasma, and urine using an X Series II quadrupole inductively coupled plasma mass spectrometry. Statistical analysis was conducted using SAS 9.5 and results with $p$ value $< 0.05$ were considered significant.

Guarantor: Jiapeng Huang is the guarantor for this manuscript.

Vegetables consumptions (broccoli risk ratio [RR] = 0.4, CI = (0.2, 0.9)], cabbage [RR = 0.2, CI = (0.1, 0.8)], and brussel sprouts [RR = 0.2, CI = (0.1, 0.5)]) are associated with less risks of PAH. In the plasma samples, silver ($p < 0.001$), and copper ($p = 0.002$) levels were significantly higher in PAH patients. There was significant positive correlation between cardiac output and cardiac index with plasma levels of silver ($r = 0.665$, $p = 0.001$ and $r = 0.678$ $p = 0.001$), respectively. There was significant correlation between mixed venous saturation, 6-min walk distance, and last BNP with plasma levels of chromium ($r = -0.520$, $p = 0.022$; $r = -0.55$, $p = 0.014$; $r = 0.463$, $p = 0.039$), respectively. In conclusion, there are significant differences between PAH and control groups in terms of vegetable consumptions and metal concentrations. Silver and chromium levels are correlated with clinical indicators of PAH severities.

**KEYWORDS**
chromium, copper, metal, pulmonary hypertension, silver

## INTRODUCTION

Pulmonary hypertension is a syndrome characterized by marked remodeling of the pulmonary vasculature and a progressive rise in the pulmonary vascular load, leading to hypertrophy and remodeling of the right ventricle.[1] It is estimated that 1% of the world population and up to 10% of persons older than 65 years of age have pulmona hypertension.[1] Pulmonary arterial hypertension (PAH, World Symposium on Pulmonary Hypertension classification Group 1) affects 25 persons (most of whom are women) per 1 million population in Western countries, with an annual incidence of 2–5 cases per million.[2] Death results from right ventricular failure if pulmonary hypertension is left untreated. Despite these therapeutic interventions, the prognosis is generally poor, necessitating further investigation into the pathogenesis of PAH to improve novel therapeutic strategies.[3]

In vitro and in vivo experimental models and clinical data showed that oxidative stress, an imbalance between the generation of reactive oxygen species (ROS) and the biological system's ability to detoxify these ROSs or to repair the resulting damages, appears as a significant mediator and predictor of worse outcomes in PAH.[3] Noteworthy, environmental factors are considered key determinants of cardiovascular disease (CVDs). Although lifestyle choices such as smoking, diet, and exercise are viewed as major environmental influences, the contribution of environmental pollutants, including nonessential metals, to CVDs is important and needs more attention. Exposures to nonessential metals, such as arsenic (As), lead (Pb), and cadmium,[4] have been linked to increased incidence of CVDs.[5,6] For instance, urinary alterations of nonessential metals, such as As and Cd, and essential metals, such as copper (Cu) and zinc (Zn), were associated with increased CVD risk.[7] A recent meta-analysis of 350,000 individuals from 37 countries showed that exposure to As, Pb, Cd, and Cu was directly associated with increased CVD incidence and mortality in a dose-response manner.[8,9] For peripheral vascular disease (PVD), Pb and Cd appear to be significant mediators of smoking-induced PVD.[7] Cd has been shown to increase atherosclerosis in animal models, and environmental exposure to Cd damages the heart and blood vessels by increasing ROS generation. However, current studies suffer from multiple drawbacks with most studies being cross sectional in design and the relationship between metal exposure/essential metal dyshomeostasis and PAH/RV dysfunction is less investigated.[10] A small study found an association between isolated RV failure and self-reported exposure to occupational dusts.[11] Biomass fuel exposure not only cause obstructive and/or restrictive lung disease but also leads to systolic and diastolic right ventricular dysfunction.[12] In another study, each 11.62-μg/m$^3$ increase in same-day mean PM2.5 concentration was associated with small but significant increases in estimated PA diastolic pressure [0.19 mmHg; 95% confidence interval (CI): 0.05–0.33] and RV diastolic pressure (0.23 mmHg; 95% CI: 0.11–0.34).[13]

Understanding the molecular mechanisms of nonessential metal toxicities and essential metal dyshomeostasis in PAH/RV dysfunction would have a significant impact on exploring potential therapeutic options. For

instance, metal toxicity by either generating ROS via Fenton reaction, or by competing/replacing essential metals from essential metal-containing proteins, transcription factors and enzymes to alter their functions, could be a central mechanism behind metal toxicity and essential metal imbalance-associated PAH/RV dysfunction.[14] In fact, the association between altered iron (Fe) homeostasis and PAH have attracted attention recently, because treatment of Fe deficiency in PAH improved patients' exercise tolerance, quality of life, hospitalization rates and mortality.[15] Furthermore, intracellular Fe deficiency in mouse pulmonary artery smooth muscles directly led to PAH and RV failure, demonstrating a causal relationship.[16]

We recently found that antimony, a nonessential metal, blood and plasma levels were significantly higher in PAH patients when compared to controls. In addition, there was a significant correlation between plasma antimony level and prognostic hemodynamic parameters of PAH including mean right atrial pressure (mRAP), cardiac output (CO), cardiac index (CI), pulmonary vascular resistance (PVR), and mixed venous oxygen saturation (SvO2).[17] We aim to explore the potential links between other essential and nonessential metals and PAH in the current study. We hypothesize that metal levels are different between PAH and controls and are correlated with clinical parameters of PAH.

## METHODS

This was a prospective, single center pilot study that recruited patients from the University of Louisville Health Pulmonary Hypertension center over a period of 3 months. Patients with PAH who were ≥18 years old were eligible to participate. The recruited PAH patients did not have any known prior history of occupational heavy metal exposure.

IRB approval (University of Louisville IRB# 20.0947) was obtained, and all the included patients signed an informed consent before recruitment. All participants completed a questionnaire on environmental exposures and vegetable consumptions (frequency, amounts, cooking methods and whether cooking water was consumed). Blood and urine samples were collected from 20 PAH patients and from 10 volunteer apparently healthy controls to measure 25 metal levels in whole blood, plasma, and urine using an X Series II quadrupole inductively coupled plasma mass spectrometry (ICP-MS, Thermo Fisher Scientific). The ICP-MS trace metal assay protocol included several steps with all procedures avoiding metal contamination. The sample digestion tubes (Eppendorf or metal free tubes) were treated with concentrated acid/nitric acid overnight and washed/rinsed by deionized (DI) water 5 times then tubes were dried. A total of 100 µl blood samples were transferred to digestion tubes. A total of 700 µl 70% HNO3 (cat# A509P500, trace metal grade; Fisher Scientific) were added into sample tubes, and digestion tubes were placed in 65°C incubation shaker for 4 h till sample solutions became clear. All digested solution was carefully transferred into 12 mL DI water (~4% HNO3 concentration required by ICPMS machine). The solution was mixed well and filtered by 45 µm cell strainer. Sample tubes were standard 15 mL (VWR metal free tube 89049–172). Five tubes 35 mL 4% HNO3/DI water solutions were made for assay blank and making standards (each tube 33 mL DI water +2 mL 70% HNO3 and mixed well, VWR 89049–176). At that point samples are ready for assay.

We collected available demographics, hemodynamics, 6-min walk distance (6MWD), brain natriuretic peptide (BNP) level, and REVEAL Lite 2 risk score of the recruited PAH patients. Due to the small size of this pilot study, normality of the data is difficult to check. The logarithmic transformation can produce approximately normally distributed metal levels.[18] The blood, plasma and urine metal levels were logarithmically transformed and then compared among groups using a two-sample *t*-test (or analysis of variance [ANOVA] when comparing more than two factors) for continuous variables and a $\chi^2$-test (or Fisher's exact test when the expected frequency within any cell was less than 5 in a 2×2 table) for categorical variables. The association of exposure to vegetables with PAH was also evaluated. The relative risk was used to evaluate the effect of exposure to vegetables on PAH. The individual variations among all PAH patients were examined in terms of the blood, plasma urine metal levels. In addition, the Pearson's correlation coefficient and p value were used to evaluate the relationship between heavy metals and clinical biomarkers. Statistical analysis was conducted using SAS 9.5 and results were declared significant at $\alpha = 0.05$.

## RESULTS

### Vegetable consumptions and PAH

A total of 30 subjects (20 PAH patients and 10 controls) were included in this study. Of the 20 PAH patients included, 80% were female and the mean age was 57.6 years. There were significant associations between PAH and more smoking ($p = 0.017$), older age ($p = <0.001$), less broccoli sprouts ($p = 0.020$), cabbage

**TABLE 1** Comparison between pulmonary arterial hypertension (PAH) and control subjects for smoking history, broccoli, cabbage, cauliflower, brussel sprouts consumption.

| Variables | Total ($N = 30$) | Control ($N = 10$) | PAH ($N = 20$) | $p$ value |
|---|---|---|---|---|
| Sex | | | | 0.384† |
| F | 22 (73.3) | 6 (60.0) | 16 (80.0) | |
| M | 8 (26.7) | 4 (40.0) | 4 (20.0) | |
| Smoking history | | | | 0.017† |
| Never | 20 (66.7) | 10 (100.0) | 10 (50.0) | |
| Ever | 9 (30.0) | 0 (0.0) | 9 (45.0) | |
| Current | 1 (3.3) | 0 (0.0) | 1 (5.0) | |
| Broccoli sprouts | | | | 0.020† |
| <2 times/mo | 16 (53.3) | 2 (20.0) | 14 (70.0) | |
| 2–4 time/mo | 10 (33.3) | 6 (60.0) | 4 (20.0) | |
| 5–8 times/mo | 1 (3.3) | 1 (10.0) | 0 (0.0) | |
| >8 times/mo | 2 (6.7) | 1 (10.0) | 1 (5.0) | |
| Missing | 1 | 0 | 1 | |
| Cabbage | | | | <0.001† |
| <2 times/mo | 19 (63.3) | 2 (20.0) | 17 (85.0) | |
| 2–4 time/mo | 5 (16.7) | 4 (40.0) | 1 (5.0) | |
| 5–8 times/mo | 4 (13.3) | 3 (30.0) | 1 (5.0) | |
| >8 times/mo | 1 (3.3) | 1 (10.0) | 0 (0.0) | |
| Missing | 1 | 0 | 1 | |
| Cauliflower | | | | 0.551† |
| <2 times/mo | 23 (76.7) | 7 (70.0) | 16 (80.0) | |
| 2–4 times/mo | 5 (16.7) | 3 (30.0) | 2 (10.0) | |
| >8 times/mo | 1 (3.3) | 0 (0.0) | 1 (5.0) | |
| Missing | 1 | 0 | 1 | |
| Brussel sprouts | | | | 0.002† |
| <2 times/mo | 24 (80.0) | 5 (50.0) | 19 (95.0) | |
| 2–4 times/mo | 4 (13.3) | 4 (40.0) | 0 (0.0) | |
| >8 times/mo | 1 (3.3) | 1 (10.0) | 0 (0.0) | |
| Age | | | | <0.001 |
| Frequency | 30 | 10 | 20 | |
| Mean (95% CI) | 50.7 (45.3–56.2) | 37.1 (29.9–44.3) | 57.6 (52.4–62.7) | |
| Median (min–max) | 54.5 (23–79) | 34 (23–56) | 59 (34–79) | |

*Note*: The † in the "$p$ value" column means the $p$ value calculated by the Fisher's exact test.
Abbreviation: CI, confidence interval.

($p = <0.001$), and brussel sprouts ($p = 0.002$) consumptions (Table 1). Smoking was associated with higher risk of PAH risk ratio [RR] = 2.0, CI = (1.3, 3.1)] and vegetables consumptions (broccoli [RR = 0.4, CI = (0.2, 0.9)], cabbage [RR = 0.2, CI = (0.1, 0.8)], and brussel sprouts [RR = 0.2, CI = (0.1, 0.5)]) were associated with lower risk of PAH.

## Metal levels and PAH

In the whole blood samples, copper levels ($p = 0.007$) were significantly higher, and thorium ($p < 0.001$) levels were significantly lower in PAH patients when compared to controls (Table 2). In the plasma samples, sodium ($p = 0.007$), potassium ($p = 0.002$), copper ($p = 0.002$),

**TABLE 2** Comparison of metal levels after logarithmic transformation in the whole blood, plasma, and urine samples between pulmonary arterial hypertension (PAH) and control subjects.

| | Total ($N = 30$) | Control ($N = 10$) | PAH ($N = 20$) | $p$ value |
|---|---|---|---|---|
| Whole blood metals (log ng/mL) | | | | |
| Copper | | | | 0.007 |
| Mean (95% CI) | 6.8 (6.7–6.9) | 6.7 (6.6–6.8) | 6.9 (6.8–7.0) | |
| Median (min–max) | 6.8 (6.4–7.3) | 6.7 (6.4–7.1) | 6.9 (6.6–7.3) | |
| Thorium | | | | <0.001 |
| Mean (95% CI) | −5.8 (−6.6 to −4.9) | −3.4 (−5.3 to −1.5) | −6.9 (−6.9 to −6.9) | |
| Median (min–max) | −6.9 (−6.9 to 0.5) | −2.0 (−6.9 to 0.5) | −6.9 (−6.9 to −6.9) | |
| Plasma metals (log ng/mL) | | | | |
| Sodium | | | | 0.007 |
| Mean (95% CI) | 15.0 (15.0–15.0) | 15.0 (14.9–15.0) | 15.0 (15.0–15.0) | |
| Median (min–max) | 15.0 (14.9–15.0) | 15.0 (14.9–15.0) | 15.0 (14.9–15.0) | |
| Potassium | | | | 0.002 |
| Mean (95% CI) | 12.3 (12.2–12.4) | 12.1 (12.0–12.1) | 12.4 (12.3–12.6) | |
| Median (min–max) | 12.2 (11.9–13.0) | 12.0 (11.9–12.2) | 12.4 (11.9–13.0) | |
| Copper | | | | 0.002 |
| Mean (95% CI) | 7.1 (7.0–7.2) | 6.9 (6.7–7.0) | 7.2 (7.1–7.3) | |
| Median (min–max) | 7.1 (6.4–7.5) | 6.8 (6.4–7.5) | 7.2 (6.8–7.5) | |
| Silver | | | | <0.001 |
| Mean (95% CI) | −3.1 (−4.1 to −2.1) | −5.7 (−7.3 to −4.0) | −1.9 (−2.7 to −1.0) | |
| Median (min–max) | −2.0 (−6.9 to 0.8) | −6.9 (−6.9 to 0.5) | −1.7 (−6.9 to 0.8) | |
| Urine metals (log ng/mL) | | | | |
| Thallium | | | | 0.035 |
| Mean (95% CI) | −1.5 (−1.9 to −1.0) | −0.7 (−1.2 to −0.2) | −1.8 (−2.4 to −1.2) | |
| Median (min–max) | −1.4 (−6.9 to 0.4) | −0.7 (−2.0 to 0.4) | −1.7 (−6.9 to −0.9) | |

*Note*: Only metals which showed significant differences are shown here.

Abbreviation: CI, confidence interval.

and silver ($p < 0.001$) levels were significantly higher in PAH patients (Table 2). In the urine samples, thallium levels ($p = 0.035$) were significantly lower in PAH patients.

## Idiopathic versus nonidiopathic PAH metals comparison

Of the 20 PAH patients, there were 6 idiopathic PAH and 14 nonidiopathic PAH patients. In the whole blood, iron levels were significantly higher ($p = 0.006$) and copper levels were significantly lower ($p = 0.01$) in idiopathic PAH when compared to nonidiopathic PAH (Table 3). In the plasma, potassium ($p = 0.002$), silver ($p < 0.001$), and lead ($p = 0.038$) were significantly higher; but copper levels ($p = 0.008$) were significantly lower in idiopathic PAH (Table 3).

## Differences between the lowest and highest metal levels in PAH and control

Range of the majority of the whole blood, plasma and urine metal levels were greater in PAH patients than those in control subjects (Table 4).

## Metal levels and PAH severity

There was significant positive correlation between mRAP and whole blood zinc ($r = 0.48771$, $p = 0.029$),

**TABLE 3** Comparison of metal levels after logarithmic transformation in the whole blood, plasma, and urine samples between idiopathic and nonidiopathic pulmonary arterial hypertension (PAH).

| | Total ($N = 30$) | Control ($N = 10$) | Idiopathic ($N = 6$) | Nonidiopathic ($N = 14$) | $p$ value |
|---|---|---|---|---|---|
| Whole blood metals (log ng/mL) | | | | | |
| Iron | | | | | 0.006 |
| Mean (95% CI) | 12.9 (12.9–13.0) | 13.0 (13.0–13.1) | 13.1 (13.0–13.2) | 12.8 (12.7–12.9) | |
| Median (min–max) | 13.0 (12.4–13.2) | 13.0 (12.9–13.2) | 13.1 (12.9–13.2) | 12.9 (12.4–13.1) | |
| Copper | | | | | 0.010 |
| Mean (95% CI) | 6.8 (6.7–6.9) | 6.7 (6.6–6.8) | 6.8 (6.7–6.9) | 6.9 (6.8–7.0) | |
| Median (min–max) | 6.8 (6.4–7.3) | 6.7 (6.4–7.1) | 6.8 (6.6–7.0) | 6.9 (6.6–7.3) | |
| Plasma metals (log ng/mL) | | | | | |
| Potassium | | | | | 0.002 |
| Mean (95% CI) | 12.3 (12.2–12.4) | 12.1 (12.0–12.1) | 12.6 (12.4–12.8) | 12.4 (12.2–12.6) | |
| Median (min–max) | 12.2 (11.9–13.0) | 12.0 (11.9–12.2) | 12.7 (12.3–12.9) | 12.3 (11.9–13.0) | |
| Copper | | | | | 0.008 |
| Mean (95% CI) | 7.1 (7.0–7.2) | 6.9 (6.7–7.0) | 7.1 (7.0–7.3) | 7.2 (7.1–7.3) | |
| Median (min–max) | 7.1 (6.4–7.5) | 6.8 (6.4–7.5) | 7.2 (6.8–7.3) | 7.2 (6.8–7.5) | |
| Silver | | | | | <0.001 |
| Mean (95% CI) | −3.1 (−4.1 to −2.1) | −5.7 (−7.3 to −4.0) | −2.1 (−4.1 to −0.2) | −1.7 (−2.7 to −0.8) | |
| Median (min–max) | −2.0 (−6.9 to 0.8) | −6.9 (−6.9 to 0.5) | −1.2 (−6.9 to −0.7) | −2.0 (−6.9 to 0.8) | |
| Lead | | | | | 0.038 |
| Mean (95% CI) | −1.5 (−2.3 to −0.8) | −2.0 (−3.1 to −0.9) | −2.9 (−5.5 to −0.3) | −0.6 (−1.1 to −0.2) | |
| Median (min–max) | −1.0 (−6.9 to 1.2) | −1.4 (−6.9 to −0.7) | −1.7 (−6.9 to 1.0) | −0.7 (−2.0 to 1.2) | |

*Note*: Only metals which showed significant differences are shown here.

Abbreviation: CI, confidence interval.

plasma levels of sodium ($r = 0.449$, $p = 0.047$) and calcium ($r = 0.543$, $p = 0.013$) (Table 5). There was significant positive correlation between PAWP and plasma levels of calcium ($r = 0.519$, $p = 0.018$) and selenium ($r = 0.444$, $p = 0.049$); and urine arsenic ($r = 0.46$, $p = 0.041$) (Table 5). There was significant positive correlation between CO and whole blood level of sodium ($r = 0.485$, $p = 0.03$), and plasma levels of silver ($r = 0.665$, $p = 0.001$); and negative association with whole blood potassium ($r = -0.448$, $p = 0.047$). There was significant positive correlation between CI and whole blood levels of sodium ($r = 0.555$, $p = 0.011$), calcium ($r = 0.487$, $p = 0.029$) and plasma levels of silver ($r = 0.678$, $p = 0.001$) with negative correlation with whole blood potassium ($r = -0.539$, $p = 0.014$), and iron ($r = -0.478$, $p = 0.032$). There was significant a negative correlation between PVR and plasma silver ($r = -0.467$, $p = 0.037$). There was significant negative correlation between SvO2 and whole blood levels of magnesium ($r = -0.618$, $p = 0.004$), plasma levels of magnesium ($r = -0.616$, $p = 0.004$) and chromium ($r = -0.52$, $p = 0.022$), and urine levels of chromium ($r = -0.476$, $p = 0.039$), manganese ($r = -0.661$, $p = 0.002$), barium ($r = -0.464$, $p = 0.045$) and uranium ($r = -0.725$, $p = 0.001$). There was a positive association between SvO2 and plasma silver levels ($r = 0.667$, $p = 0.001$).

There was significant negative correlation between REVEAL Lite 2 scores and urine levels of beryllium ($r = -0.462$, $p = 0.046$) and sodium ($r = -0.597$, $p = 0.006$). There was significant negative correlation between last 6MWD and plasma levels of chromium ($r = -0.55$, $p = 0.014$) and urine levels of uranium ($r = -0.55$, $p = 0.014$). There was significant positive association between last 6MWD and whole blood barium ($r = 0.555$, $p = 0.013$) and plasma silver ($r = 0.5815$, $p = 0.009$). There was significant positive correlation between last BNP and plasma levels of chromium ($r = 0.463$, $p = 0.039$) and urine level of uranium ($r = 0.514$, $p = 0.02$). There was significant negative association between last BNP and urine sodium ($r = -0.457$, $p = 0.042$) and calcium ($r = -0.444$, $p = 0.049$) levels. There was a negative association between Compera risk scores and plasma silver ($r = -0.555$, $p = 0.011$); a

**TABLE 4** Differences between the lowest and highest of metal levels after logarithmic transformation in the whole blood, plasma, and urine samples between pulmonary arterial hypertension (PAH) and control subjects.

| | Blood | | Plasma | | Urine | |
|---|---|---|---|---|---|---|
| Metal (log ng/mL) | PAH | Control | PAH | Control | PAH | Control |
| Beryllium | 7.94 | 6.80 | 6.80 | 6.80 | 7.57 | 7.57 |
| Sodium | 0.38 | 0.20 | 0.12 | 0.08 | 2.67 | 2.94 |
| Magnesium | 1.15 | 0.23 | 1.97 | 0.31 | 3.35 | 2.80 |
| Aluminum | 1.50 | 1.47 | 11.88 | 0.75 | 3.61 | 5.09 |
| Potassium | 0.49 | 0.23 | 1.11 | 0.31 | 2.71 | 1.99 |
| Calcium | 0.42 | 0.21 | 0.41 | 0.16 | 4.99 | 2.96 |
| Vanadium | 7.80 | 7.42 | 7.85 | 7.94 | 7.95 | 6.25 |
| Chromium | 7.99 | 0.00 | 7.05 | 0.00 | 8.94 | 6.25 |
| Manganese | 2.95 | 1.32 | 0.00 | 7.05 | 12.26 | 0.00 |
| Iron | 0.79 | 0.31 | 1.71 | 0.97 | 14.17 | 3.00 |
| Cobalt | 6.93 | 5.95 | 2.30 | 6.24 | 3.32 | 3.43 |
| Nickel | 9.23 | 8.11 | 8.44 | 7.49 | 2.77 | 1.88 |
| Copper | 0.74 | 0.66 | 0.76 | 1.06 | 3.53 | 2.90 |
| Zinc | 0.64 | 0.35 | 0.99 | 0.58 | 2.97 | 3.81 |
| Arsenic | 8.18 | 7.85 | 6.93 | 6.93 | 12.37 | 4.04 |
| Selenium | 1.14 | 0.74 | 1.47 | 0.65 | 12.06 | 12.60 |
| Molybdenum | 3.21 | 1.18 | 2.89 | 2.19 | 5.04 | 4.34 |
| Silver | 2.48 | 5.55 | 7.74 | 7.42 | 5.56 | 4.87 |
| Cadmium | 7.05 | 6.65 | 4.86 | 4.86 | 8.55 | 8.71 |
| Antimony | 2.99 | 2.63 | 2.28 | 1.43 | 7.75 | 0.00 |
| Barium | 10.35 | 9.36 | 8.66 | 2.56 | 4.72 | 2.74 |
| Thallium | 4.86 | 0.00 | 0.00 | 0.00 | 5.96 | 2.39 |
| Lead | 2.70 | 1.55 | 8.11 | 6.24 | 2.62 | 1.61 |
| Thorium | 0.00 | 7.42 | 4.86 | 4.86 | 4.87 | 4.87 |
| Uranium | 0.00 | – | – | – | 4.87 | – |

*Note*: "–" means missing value.

positive association with urine manganese ($r = 0.487$, $p = 0.029$) and uranium ($r = 0.489$, $p = 0.028$) levels.

## DISCUSSIONS

We performed a pilot study to evaluate the possible correlations between whole blood, plasma and urine metal levels and PAH etiology, classification, and clinical severity. The major findings include antioxidant rich vegetable consumptions are less in PAH patients; copper, thorium, sodium, potassium, silver, and thallium are different in PAH patients; idiopathic PAH patients have different iron, copper, potassium, silver and lead levels; heterogenicity of metal levels in PAH are much higher than controls; multiple metals show significant correlations with hemodynamic and clinical severity parameters in PAH patients.

The importance of oxidative stress and inflammation was recently demonstrated in a PAH murine model since sulforaphane, a Nrf2 activator, attenuated oxidative stress, prevented the pathological changes of the RV in PAH mice by increasing cardiac Nrf2 expression and its downstream antioxidants.[19] In addition, sulforaphane did not protect RV systolic and diastolic functions in Nrf2 knockout PAH mice verifying the essential role of Nrf2 in SFN-mediated prevention of RV dysfunction and PAH, and increasing Nrf2 activity in patients with PAH may

**TABLE 5** Evaluating the correlation (correlation coefficients, $p$ value) between pulmonary arterial hypertension (PAH) and metal levels after logarithmic transformation in the whole blood, plasma and urine samples ($n = 20$).

| | mRAP | PAWP | CO | CI | PVR | SvO2 | REVEAL Lite 2 | Last 6MWD | Last BNP | Compera risk score |
|---|---|---|---|---|---|---|---|---|---|---|
| **Whole blood** | | | | | | | | | | |
| Sodium | x | x | 0.485 (0.054, 0.763); 0.030 | 0.555 (0.150, 0.801); 0.011 | x | x | x | x | x | x |
| Magnesium | x | x | x | x | x | −0.618 (−0.837, −0.228); 0.004 | x | x | x | x |
| Potassium | x | | −0.448 (−0.743, −0.008); 0.047 | −0.539 (−0.792, −0.127); 0.014 | x | x | x | x | x | x |
| Calcium | x | x | x | 0.487 (0.057, 0.765); 0.029 | x | x | x | x | x | x |
| Iron | x | x | x | −0.478 (−0.760, −0.045); 0.032 | x | x | x | x | x | x |
| Zinc | 0.48771 (−0.277, 0.581); 0.029 | x | x | x | x | x | x | x | x | x |
| Barium | x | x | x | x | x | x | x | 0.555 (0.135, 0.806); 0.013 | x | x |
| **Plasma** | | | | | | | | | | |
| Sodium | 0.449 (0.008, 0.743); 0.047 | x | x | x | x | x | x | x | x | x |

**TABLE 5** (Continued)

| | mRAP | PAWP | CO | CI | PVR | SvO2 | REVEAL Lite 2 | Last 6MWD | Last BNP | Compera risk score |
|---|---|---|---|---|---|---|---|---|---|---|
| Magnesium | x | x | x | x | x | −0.616 (−0.836, −0.225); 0.004 | x | x | x | x |
| Calcium | 0.543 (0.133, 0.795); 0.013 | 0.519 (0.100, 0.782); 0.018 | x | x | x | x | x | x | x | x |
| Chromium | x | x | x | x | | −0.520 (−0.788, −0.087); 0.022 | x | −0.550 (−0.803, −0.128); 0.014 | 0.463 (0.026, 0.752); 0.039 | x |
| Selenium | | 0.444 (0.001, 0.740); 0.049 | | | | x | x | x | x | x |
| Silver | x | x | 0.665 (0.316, 0.856); 0.001 | 0.678 (0.337, 0.862); 0.001 | −0.467 (−0.754 −0.031); 0.037 | 0.667 (0.306, 0.860); 0.001 | x | 0.5815 (0.173, 0.819); 0.009 | | −0.555 (−0.800 −0.149); 0.011 |
| **Urine** | | | | | | | | | | |
| Beryllium | x | x | x | x | x | x | −0.462 (−0.757, −0.010); 0.046 | x | x | x |
| Sodium | x | x | x | x | x | x | −0.597 (−0.827 −0.196); 0.006 | x | −0.457 (−0.748, −0.018); 0.042 | x |
| Calcium | x | x | x | x | x | x | x | x | −0.444 (−0.741, −0.002); 0.049 | x |

(Continues)

**TABLE 5** (Continued)

| | mRAP | PAWP | CO | CI | PVR | SvO2 | REVEAL Lite 2 | Last 6MWD | Last BNP | Compera risk score |
|---|---|---|---|---|---|---|---|---|---|---|
| Chromium | x | x | x | x | x | −0.476 (−0.765, −0.028); 0.039 | x | x | x | x |
| Manganese | x | x | x | x | x | −0.661 (−0.857, −0.296); 0.002 | x | x | x | 0.487 (0.056, 0.764); 0.029 |
| Arsenic | | 0.460 (0.022, 0.750); 0.041 | x | x | x | x | x | x | x | x |
| Barium | x | x | x | x | x | −0.464 (−0.758, −0.013); 0.045 | x | x | x | x |
| Uranium | x | x | x | x | x | −0.725 (−0.887, −0.404); 0.001 | x | −0.550 (−0.803, −0.128); 0.014 | 0.514 (0.093, 0.779); 0.020 | 0.489 (0.060, 0.766); 0.028 |

*Note*: "x" means nonsignificance. Only metals which showed significant differences are shown here. Data are presented as correlation coefficient (95% confidence interval); *p* value.

Abbreviations: 6MWD, 6-min walk distance; BNP, brain natriuretic peptide; CI, cardiac index; CO, cardiac output; mRAP, mean right atrial pressure; PAWP, pulmonary arterial wedge pressure; PVR, pulmonary vascular resistance; SvO2, mixed venous oxygen saturation.

have therapeutic potential.[20] Clinical trials evaluating effects of Nrf2 activators in PAH are currently underway,[21] pointing to the potentials of Nrf2 activators in the management of PAH. In our study, consumption of sulforaphane rich vegetables such as broccoli, cabbage and brussel sprouts are significantly lower in PAH patients when compared to controls. However, whether and how much sulforaphane rich vegetables contribute to PAH pathogenesis and progression is unknown due the potential confounding factors including consumption amounts, cooking methods and other dietary intakes.

Copper level was significantly higher in the whole blood and plasma of PAH patients when compared to controls in this study. Cellular copper plays an important role in angiogenesis and extracellular matrix remodeling and increased copper in vascular smooth muscle cells has been demonstrated to be associated with atherosclerosis and hypertension in animal experiments. In a study of seven pulmonary hypertension patients, mean serum copper level was significantly higher in patients with pulmonary hypertension, indicating that increased serum copper may be a cause or a marker of PAH.[22] In addition, serum levels of copper were found to be higher in chronic obstructive lung disease patients with pulmonary hypertension compared to patients without pulmonary hypertension.[23] In animal studies, elevated serum copper concentration was found in monocrotaline induced pulmonary hypertension rats[24] and the severity of cardiopulmonary damage was accompanied by a dose-dependent elevation in serum copper concentration.[25] Furthermore, a copper-depleted diet prevented, and copper chelation with tetrathiomolybdate reversed, the development of severe SU5416 + hypoxia PAH rats[26] and increased copper transportation in pulmonary arteries and smooth muscle cells contributes to the development of hypoxia-induced pulmonary hypertension.[27] These findings suggest copper restricted diet or chelation therapy might be potential therapeutic targets for PAH.

The differences and correlations in essential metals such as sodium, potassium, calcium, and magnesium in PAH are more complex as many PAH patients are on diuretics, which can significantly affect these metal concentrations. Whether dysregulation of these metals is merely a marker of the PAH disease or is actually a causal or worsening element for PAH is unknown and deserves preclinical and clinical studies.

Our results on heterogenicity of metals in PAH and control patients showed a larger range of metal concentrations in PAH except a few metals. As PAH itself is a heterogenous disease and exact etiologies are unknown, direct metal toxicities or other factors affecting metal concentrations could be related to PAH and explain its progression. We also showed different metal levels between idiopathic and non-idiopathic PAH patients and metal dysregulation might be the causal agent in some idiopathic PAH cases.

Another metal that deserves attention in PAH is silver. In this study, silver level was found to be positively correlated with CO, CI, SvO2, and 6MWD; negatively correlated with PVR and Compera risk scores in PAH patients. Several previous studies have demonstrated effects of silver on hemodynamics of the left heart. Arozal et al.[28] found that silver has a cardioprotective effect against myocardial infarction through the amelioration of nuclear factor kappa B expression level induced by oxidative stress overproduction. Ramirez-Lee et al.[29] demonstrated sustained vasoconstriction and increased cardiac contractility in response to silver in diabetic rats. They also found that low concentrations of silver increased nitric oxide production without modifying cardiac parameters while high concentrations of silver promoted a sustained vasoconstriction and increased cardiac contractility related to oxidative stress in rat heart.[30] In addition, silver has been correlated with the initiation of atherosclerosis by evoking oxidative stress, damaging to cell membrane, apoptosis, and inhibiting cell proliferation. Furthermore, silver appear to suppress the expression of nitric oxide synthase, which in turn leads to a decrease in the production of nitrogen oxide, a potent vasodilator.[31] By suppressing levels of nitric oxide and creating an oxidative milieu, it becomes evident how high concentrations of silver might further exacerbate PAH hemodynamics.

Higher chromium levels showed correlations with worse mixed venous oxygenation, worse 6 min walk, and higher heart failure markers. A recent meta-analysis of 11 eligible randomized controlled trials with 637 participants indicated that supplementation with chromium significantly decrease blood pressures.[32] In another human study of 62 patients, chromium levels were found significantly higher in patients with systolic ischemic heart disease.[33] The exact mechanisms of how chromium contribute to PAH disease progression is unknown. Possible explanations could be worse RV function from chromium exposure or hypotension effects from chromium exacerbates RV ischemia and causes further deterioration.

There are several limitations of this study. The sample size is small with only 20 PAH patients from a single center. To better evaluate the association between metal exposure and PAH, and the correlations between clinical indicators and metal concentrations, power analysis needs to be performed in the future. Calculations of power and sample size are based on ANOVA and linear regression. We expect that more significant or correlated risk factors can be detected as the sample size

increases and a spurious association or a false correlation that was caused by cofounders can be removed. In addition, multiple factors could have contributed to metal levels in patients including diet, supplements, medications, or other disease processes. Furthermore, only correlations could be inferred from this study, not causal relationships. Preclinical and randomized control studies to evaluation mechanisms of metal and PAH interactions and chelation therapy in PAH patients with abnormal metal levels are needed.

In conclusion, there are significant differences between PAH and control groups in terms of vegetable consumptions and metal concentrations. Silver and chromium levels are correlated with clinical indicators of PAH severities.

## AUTHOR CONTRIBUTIONS

**Karim El-Kersh, C. Danielle Hopkins, Lu Cai, Jiapeng Huang**: study design, data collection, manuscript preparation. **Xiaoyong Wu, Shesh N Rai**: study design, statistical analysis, manuscript preparation. **Matthew C. Cave, M. Ryan Smith, Young-Mi Go, Dean P Jones**: study design, data analysis, manuscript preparation.

## ACKNOWLEDGMENTS

The authors were supported in part by the University of Louisville Executive Vice President for Research and Innovation Internal Grant (JH, LC); University of Louisville School of Medicine Basic Grant (JH, LC); National Institute of Environmental Health Sciences (P30ES030283 to JH, LC, MCC); Gilead Sciences COMMIT COVID-19 RFP Program grant (Gilead IN-US-983-6063 to JH); National Center for Advancing Translational Sciences grant (1U18TR003787-01 to JH); National Institute Heart and Lung (R01HL125877, R01HL160927 to LC). National Heart, Lung and Blood Institute (1R01HL15877-01 to JH). YMG is supported by National Institute of Health R01 ES031980 and R21 ES031814. RS is supported in part by the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1TR002378. National Institute of Environmental Health Sciences (R35ES028373, R01ES032189, P42ES023716, and R21ES031510 to MCC). National Institute of General Medical Sciences (P20GM113226 to MCC). National Institute of Health (NIDDK RC2 DK118619 and NIEHS P30 ES019776 to DPJ).

## CONFLICT OF INTEREST STATEMENT

EKK provided consultative services to Acceleron, Merck, United Therapeutics served on advisory boards for J&J, Actelion, and United Therapeutics, received institutional research funding from J&J Actelion and United Therapeutics, and participated in a United Therapeutics speaker's bureau (2018–2021).

## ETHICS STATEMENT

IRB approval (University of Louisville IRB# 20.0947) was obtained, and all the included patients signed an informed consent before recruitment.

## ORCID

*Karim El-Kersh* http://orcid.org/0000-0003-1285-3628
*Jiapeng Huang* http://orcid.org/0000-0002-4794-8400

## REFERENCES

1. Hassoun PM. Pulmonary arterial hypertension. N Engl J Med. 2021;385(25):2361–76.
2. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, Yaici A, Weitzenblum E, Cordier JF, Chabot F, Dromer C, Pison C, Reynaud-Gaubert M, Haloun A, Laurent M, Hachulla E, Simonneau G. Pulmonary arterial hypertension in France: results from a national registry. Am J Respir Crit Care Med. 2006;173(9):1023–30.
3. Colon Hidalgo D, Elajaili H, Suliman H, George MP, Delaney C, Nozik E. Metabolism, mitochondrial dysfunction, and redox homeostasis in pulmonary hypertension. Antioxidants. 2022;11(2):428.
4. Lin Q, Fan C, Gomez-Arroyo J, Van Raemdonck K, Meuchel LW, Skinner JT, Everett AD, Fang X, Macdonald AA, Yamaji-Kegan K, Johns RA. HIMF (hypoxia-induced mitogenic factor) signaling mediates the HMGB1 (high mobility group box 1)-Dependent endothelial and smooth muscle cell crosstalk in pulmonary hypertension. Arterioscler Thromb Vasc Biol. 2019;39(12):2505–19.
5. Bhatnagar A. Environmental cardiology: studying mechanistic links between pollution and heart disease. Circ Res. 2006;99(7):692–705.
6. Navas-Acien A, Selvin E, Sharrett AR, Calderon-Aranda E, Silbergeld E, Guallar E. Lead, cadmium, smoking, and increased risk of peripheral arterial disease. Circulation. 2004;109(25):3196–201.
7. Domingo-Relloso A, Grau-Perez M, Briongos-Figuero L, Gomez-Ariza JL, Garcia-Barrera T, Dueñas-Laita A, Bobb JF, Chaves FJ, Kioumourtzoglou MA, Navas-Acien A, Redon-Mas J, Martin-Escudero JC, Tellez-Plaza M. The association of urine metals and metal mixtures with cardiovascular incidence in an adult population from Spain: the Hortega follow-up study. Int J Epidemiol. 2019;48(6):1839–49.
8. Chowdhury R, Ramond A, O'Keeffe LM, Shahzad S, Kunutsor SK, Muka T, Gregson J, Willeit P, Warnakula S, Khan H, Chowdhury S, Gobin R, Franco OH, Di Angelantonio E. Environmental toxic metal contaminants and risk of cardiovascular disease: systematic review and meta-analysis. BMJ. 2018;362:k3310.
9. Nigra AE, Ruiz-Hernandez A, Redon J, Navas-Acien A, Tellez-Plaza M. Environmental metals and cardiovascular disease in adults: a systematic review beyond lead and cadmium. Curr Environ Health Rep. 2016;3(4):416–33.
10. Yang AM, Lo K, Zheng TZ, Yang JL, Bai YN, Feng YQ, Cheng N, Liu SM. Environmental heavy metals and

cardiovascular diseases: status and future direction. Chronic Dis Transl Med. 2020;6(4):251–9.
11. Lagat DK, DeLong AK, Wellenius GA, Carter EJ, Bloomfield GS, Velazquez EJ, Hogan J, Kimaiyo S, Sherman CB. Factors associated with isolated right heart failure in women: a pilot study from western Kenya. Global Heart. 2014;9(2): 249–54.
12. Emiroglu Y, Kargin R, Kargin F, Akcakoyun M, Pala S, Mutlu H, Akcay M, Aung SM, Baran R, Özdemir N. BNP levels in patients with long-term exposure to biomass fuel and its relation to right ventricular function. Pulm Pharmacol Ther. 2010;23(5):420–4.
13. Rich DQ, Freudenberger RS, Ohman-Strickland P, Cho Y, Kipen HM. Right heart pressure increases after acute increases in ambient particulate concentration. Environ Health Perspect. 2008;116(9):1167–71.
14. Paithankar JG, Saini S, Dwivedi S, Sharma A, Chowdhuri DK. Heavy metal associated health hazards: an interplay of oxidative stress and signal transduction. Chemosphere. 2021; 262:128350.
15. Sonnweber T, Pizzini A, Tancevski I, Löffler-Ragg J, Weiss G. Anaemia, iron homeostasis and pulmonary hypertension: a review. Intern Emerg Med. 2020;15(4):573–85.
16. Lakhal-Littleton S, Crosby A, Frise MC, Mohammad G, Carr CA, Loick PAM, Robbins PA. Intracellular iron deficiency in pulmonary arterial smooth muscle cells induces pulmonary arterial hypertension in mice. Proc Natl Acad Sci USA. 2019;116(26):13122–30.
17. El-Kersh K, Danielle Hopkins C, Wu X, Rai SN, Cai L, Huang J. Plasma level of antimony correlates with pulmonary arterial hypertension severity. Curr Res Toxicol. 2022;3:100080.
18. Karpievitch YV, Dabney AR, Smith RD. Normalization and missing value imputation for label-free LC-MS analysis. BMC Bioinformatics. 2012;13(Suppl 16):S5.
19. Kang Y, Zhang G, Huang EC, Huang J, Cai J, Cai L, Wang S, Keller BB. Sulforaphane prevents right ventricular injury and reduces pulmonary vascular remodeling in pulmonary arterial hypertension. Am J Physiol Heart Circ Physiol. 2020;318(4): H853–66.
20. Zhang G, Kang Y, Cathey D, LeBlanc AJ, Cai J, Cai L, Wang S, Huang J, Keller BB. Sulforaphane does not protect right ventricular systolic and diastolic functions in Nrf2 knockout pulmonary artery hypertension mice. Cardiovasc Drugs Ther. 2022;36(3):425–36.
21. Muralidharan P, Hayes D, Jr., Black SM, Mansour HM. Microparticulate/nanoparticulate powders of a novel Nrf2 activator and an aerosol performance enhancer for pulmonary delivery targeting the lung Nrf2/Keap-1 pathway. Mol Syst Des Eng. 2016;1(1):48–65.
22. Ahmed T, Sackner MA. Increased serum copper in primary pulmonary hypertension: a possible pathogenic link? Respiration. 1985;47(4):243–6.
23. Asker S, Asker M, Yeltekin AC, Aslan M, Ozbay B, Demir H, Turan H. Serum levels of trace minerals and heavy metals in severe COPD patients with and without pulmonary hypertension. Int J Chronic Obstruct Pulm Dis. 2018;13:1803–8.
24. Ganey PE, Roth RA. Elevated serum copper concentration in monocrotaline pyrrole treated rats with pulmonary hypertension. Biochem Pharmacol. 1987;36(20):3535–7.
25. Molteni A, Ward WF, Ts'ao CH, Fitzsimons EJ. Serum copper concentration as an index of cardiopulmonary injury in monocrotaline-treated rats. Ann Clin Lab Sci. 1988;18(6): 476–83.
26. Bogaard HJ, Mizuno S, Guignabert C, Al Hussaini AA, Farkas D, Ruiter G, Kraskauskas D, Fadel E, Allegood JC, Humbert M, Noordegraaf AV, Spiegel S, Farkas L, Voelkel NF. Copper dependence of angioproliferation in pulmonary arterial hypertension in rats and humans. Am J Respir Cell Mol Biol. 2012;46(5):582–91.
27. Zimnicka AM, Tang H, Guo Q, Kuhr FK, Oh MJ, Wan J, Chen J, Smith KA, Fraidenburg DR, Choudhury MSR, Levitan I, Machado RF, Kaplan JH, Yuan JXJ. Upregulated copper transporters in hypoxia-induced pulmonary hypertension. PLoS One. 2014;9(3):e90544.
28. Arozal W, Monayo ER, Barinda AJ, Perkasa DP, Soetikno V, Nafrialdi N, Louisa M. Protective effects of silver nanoparticles in isoproterenol-induced myocardial infarction in rats. Front Med. 2022;9:867497.
29. Ramirez-Lee MA, Espinosa-Tanguma R, Mejía-Elizondo R, Medina-Hernández A, Martinez-Castañon GA, Gonzalez C. Effect of silver nanoparticles upon the myocardial and coronary vascular function in isolated and perfused diabetic rat hearts. Nanomedicine: NBM. 2017;13(8):2587–96.
30. Ramirez-Lee Manuel A, Martinez-Cuevas PP, Rosas-Hernandez H, Oros-Ovalle C, Bravo-Sanchez M, Martinez-Castañon GA, Gonzalez C. Evaluation of vascular tone and cardiac contractility in response to silver nanoparticles, using Langendorff rat heart preparation. Nanomedicine: NBM. 2017;13(4):1507–18.
31. Younis NK, Ghoubaira JA, Bassil EP, Tantawi HN, Eid AH. Metal-based nanoparticles: promising tools for the management of cardiovascular diseases. Nanomedicine: NBM. 2021; 36:102433.
32. Lari A, Fatahi S, Sohouli MH, Shidfar F. The impact of chromium supplementation on blood pressure: a systematic review and dose-response meta-analysis of randomized-controlled trials. High Blood Press Cardiovasc Prev. 2021; 28(4):333–42.
33. Khan N, Hashmi S, Siddiqui AJ, Farooq S, Sami SA, Basir N, Bokhari SS, Sharif H, Junejo S, El-Seedi HR, Musharraf SG. Understanding of metals dysregulation in patients with systolic and diastolic dysfunction in ischemic heart disease. Sci Rep. 2020;10(1):13948.

**How to cite this article:** El-Kersh K, Hopkins CD, Wu X, Rai SN, Cave MC, Smith MR, Go Y-M, Jones DP, Cai L, Huang J. Metallomics in pulmonary arterial hypertension patients. Pulm Circ. 2023;13:e12202. https://doi.org/10.1002/pul2.12202