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Metformin Therapy for Pulmonary Hypertension Associated with Heart Failure with Preserved Ejection Fraction versus Pulmonary Arterial Hypertension

To the Editor:

Pulmonary arterial hypertension (PAH; World Health Organization group 1) is a disease of the small pulmonary arteries, characterized by vasoconstriction, vascular proliferation, and remodeling. Although at present there are 14 drugs approved by the U.S. Food and Drug Administration for the treatment of PAH available on the U.S. market, the morbidity and mortality of PAH remain high. Pulmonary hypertension associated with heart failure with preserved ejection fraction (PH-HFpEF) (also referred to as combined pre- and postcapillary PH or CpcPH; World Health Organization group 2) is known to occur secondary to the left ventricular diastolic dysfunction and is recognized as a clinical complication of the metabolic syndrome (1). At present, there are no approved therapies for PH-HFpEF. We have recently reported that metformin, the first-line antidiabetic drug and the canonical AMP-activated protein kinase (AMPK) activator, exhibits therapeutic efficacy in the early treatment of PH-HFpEF in preclinical rat models (2). Because PH-HFpEF shares many pathophysiological characteristics with PAH, and many patients with PAH exhibit signs of insulin resistance and glucose intolerance in the absence of obesity and diabetes (3, 4), we evaluated metformin in the treatment of group 1 PH.

Although metformin has been found to prevent the development of PAH in hypoxia and monocrotaline rat models (5) and reverse PAH in SU5416/hypoxia (SuHx) rats (6), and is currently in a phase 2 clinical trial for the treatment of PAH (NCT01352026), our data showed that metformin treatment failed to reverse pulmonary pressures and vascular remodeling in mice with SuHx-induced PAH (Figure 1). In this experimental model, 6-week-old male C57BL/6J mice were injected subcutaneously with SU5416 (20 mg/kg) or vehicle buffer once per week during the first 3-week exposure to hypoxia (10% oxygen). Metformin (100 mg/kg) was given in drinking water and continued for 2-week exposure to hypoxia (Figure 1A). We observed no changes in right ventricular systolic pressure (RVSP) in metformin-

treated SuHx mice compared with untreated animals (Figure 1A), nor did we detect changes in right ventricular hypertrophy (Figure 1B) or pulmonary vascular remodeling in metformin-treated SuHx mice (Figure 1C).

To further validate these unexpected findings, we evaluated the preventative effect of metformin in the conventional SuHx rat model of PAH with a higher dose of metformin (300 mg/kg, the effective dose used in the rat model of PH-HFpEF, drinking water, starting at Day 1). In this experimental model, 8-week-old male Sprague Dawley rats were single-injected with SU5416 (20 mg/kg), followed by 3 weeks of exposure to hypoxia (10% oxygen) and an additional 3 weeks of exposure to normoxia. We further compared the effect of metformin with the effect of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR, an AMPK agonist) and nitrite (a drug that can be reduced to nitric oxide under hypoxia and that has been shown to improve hypoxia- and monocrotaline-induced PAH) (7) in the SuHx rat model of PAH. In line with our observations in mice with SuHx-induced PAH, no significant difference in RVSP (Figure 2A) was observed in metformin-treated SuHx rats compared with untreated animals. Right ventricular hypertrophy, fastest rate of pressure change in the right ventricle (RV dp/dt max), and pulmonary vascular remodeling were not affected in metformin-treated SuHx rats compared with untreated animals (Figures 2B–2D). In addition, the AMPK agonist AICAR (500 mg/kg, intraperitoneally, daily, starting at Day 1) did not reduce RVSP in SuHx rats, whereas nitrite (100 mg/L, drinking water, starting at Day 1) treatment significantly lowered RVSP and pulmonary vascular remodeling in rats with SuHx-induced PAH (Figures 2A and 2D). Collectively, these data demonstrate a lack of efficacy of metformin in prevention or reversal of PAH induced by SuHx, in contrast to clear beneficial effects in PH-HFpEF rat models with metabolic syndrome. These data suggest that metformin therapy for PH may be limited to PH associated with metabolic syndrome.

Recently, we have also reported that metformin limits PH-HFpEF by a mechanism involving, at least in part, activation of SIRT3 in skeletal muscle (2). SIRT3 is a member of the sirtuin family of protein deacetylases that is preferentially localized in mitochondria and known to regulate reactive oxygen species levels and global respiration. Dysregulation of SIRT3 has been implicated in the development of insulin resistance and diabetes both in humans and rodents (8). In addition, SIRT3 deficiency in pulmonary artery smooth muscle cells has been shown to promote PAH (4). Decreased levels of SIRT3 in skeletal muscle have been associated with the development of insulin resistance and diabetes, whereas caloric restriction- and exercise-induced increase in skeletal muscle SIRT3 leads to multiple health benefits within the cardiovascular and the musculoskeletal system (9). Our previously published data demonstrated that in rats with PH-HFpEF, the levels of activated SIRT3 are decreased specifically in skeletal muscle (not in the pulmonary vasculature and the heart), concomitant with robust increase in pulmonary pressures and vascular remodeling (2). Restoration of SIRT3 in skeletal muscle with nitrite and metformin improved insulin sensitivity and reduced pulmonary pressures (2). Because insulin resistance and exercise intolerance are key features of PAH (3, 4, 10), we next assessed the activation levels of skeletal muscle SIRT3 in SuHx rats. Consistent with our previous findings, our new data show that skeletal muscle SIRT3 activation is reduced in

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Figure 1. (A) Right ventricular systolic pressure, (B) Fulton index [weight of right ventricle/(weight of left ventricle + septum)], and (C, right) medial index [(mean medial wall thickness/mean external diameter) $\times$ 100] were measured in mice with SuHx-induced pulmonary arterial hypertension. (C, left) Representative images of hematoxylin and eosin–stained pulmonary arteries. Data are mean $\pm$ SEM. Statistical significance was assessed by one-way ANOVA followed by a Tukey *post hoc* test. *** $P < 0.001$; **** $P < 0.0001$. CTL = control; Met = metformin; ns. = not significant; RVSP = right ventricular systolic pressure; SuHx = SU5416/hypoxia.

rats with SuHx-induced PAH (Figure 2E), accompanied by increased pulmonary pressures (Figure 2A). Chronic oral nitrite supplementation, in turn, trended toward increasing SIRT3 activation in skeletal muscle with reduced pulmonary pressures, whereas metformin treatment failed to induce SIRT3 activation in SuHx rats (Figure 2E).

Collectively, our data suggest that reduction of skeletal muscle SIRT3 levels may be, at least in part, a unifying mechanism in the regulation of both PAH and PH-HFpEF. Notably, a recent report by Dean and colleagues showed the beneficial effect of metformin (100 mg/kg, daily oral gavage) on reversing PAH induced by SuHx in Wistar Kyoto female rats (6), in contrast to our studies performed using male mice and rats. Sex differences in the response to metformin have been described previously with respect to aging, life span, spontaneous tumorigenesis, and obesity (11, 12). In conclusion, our data suggest that metformin treatment may be preferentially beneficial in the treatment of PH-HFpEF, with limited efficacy for PAH. ■

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Figure 2. (A) Right ventricular systolic pressure, (B) Fulton index, (C) RV dP/dt max, (D) representative images of hematoxylin and eosin-stained pulmonary arteries and medial index, and (E) Western blot for SIRT3 activation levels in skeletal muscle ($n = 6$) obtained from SuHx rats treated or untreated with metformin, nitrite, or AICAR. Data are mean ± SEM. Statistical significance was assessed by one-way ANOVA followed by a Tukey *post hoc* test. * $P < 0.05$; *** $P < 0.001$; **** $P < 0.0001$. AICAR = 5-aminoimidazole-4-carboxamide ribonucleotide; CTL = control; met = metformin; nit = nitrite; ns. = not significant; RV dP/dt = fastest rate of pressure change in the right ventricle; RVSP = right ventricular systolic pressure; SuHx = SU5416/hypoxia.

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An Alternative Approach for the Analysis of Time-to-Event and Survival Outcomes in Pulmonary Medicine

To the Editor:

For many pulmonary illnesses, the time to an event, such as death, is of primary interest. Visually, the Kaplan-Meier survival

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curve is an essential tool to assess time-to-event data (1). However, summarizing the difference of a time-to-event outcome between study groups can be challenging. For example, although the log-rank test compares survival curves, this test does not provide an estimate of the effect of an exposure (i.e., treatment effect estimate) (2). The Cox proportional hazards regression model does provide an effect estimate in terms of the hazard ratio (HR), defined as the ratio of two instantaneous rates of an event at any time during follow-up (3). However, without knowing the event rate in the reference group, the HR can be difficult to interpret and place into context. Further, a key assumption of the Cox proportional hazards model is that the HR is constant between study groups over time (i.e., the proportional hazards assumption). When this assumption is violated, a situation that is not uncommon, results can become distorted and misleading (4). Another common metric, the median survival time, requires sufficient follow-up for survival to be less than 50% to be estimated.

The restricted mean survival time (RMST) estimate is an alternative approach that has not been widely applied in the field of pulmonary medicine. Graphically, the RMST represents the area under the survival curve and is interpreted in simple terms: the average time until an event occurs during a defined period ranging from time 0 (i.e., the start of follow-up) to a specific follow-up time point (τ) (4–10). Thus, the RMST is the τ -specific life expectancy for a study group. For example, the gray area under the Kaplan-Meier curve in Figure 1A demonstrates the 15-year RMST in our first example.

A comparison of the RMST between two study groups provides an estimate of the duration of time gained or lost that is associated with an exposure, and can be expressed on the absolute difference scale (Equation 1) or relative ratio scale (Equation 2):

$$\text{restricted life expectancy difference} = \text{RMST group 2} - \text{RMST group 1} \quad (1)$$

$$\text{restricted life expectancy ratio} = \text{RMST group 2} / \text{RMST group 1} \quad (2)$$

Methods

To illustrate the RMST approach, we examined unadjusted differences in survival in two cohorts of patients with pulmonary illnesses. These analyses were for illustrative purposes only and do not represent formal assessments of clinical hypotheses. The first sample included individuals in the London chronic obstructive pulmonary disease (COPD) cohort with moderate to very severe COPD, and we compared survival among those who chronically produced sputum with that among those who did not. The second sample included individuals who received a single lung transplant in the United States between 2005 and 2016 (after implementation of the lung allocation score in May 2005), using the United Network for Organ Sharing registry, and we compared posttransplant survival based on age at transplantation.

Results

In the first example, we analyzed 373 deaths among 661 patients in the London COPD cohort during a follow-up period of 20.4 years (ending in March 2016; Figure 1). The Kaplan-Meier plot showed that the survival curves crossed at Year 14 of follow-up, and the proportional hazards assumption was not met (Grambsch-Therneau test, $P = 0.026$; Figure 1B). Thus, we were