Experimental Physiology

Symposium Report

Pulmonary vascular mechanics: important contributors to the increased right ventricular afterload of pulmonary hypertension

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New Findings

- **What is the topic of this review?**
  This article reviews pulmonary vascular and right ventricular (RV) changes due to hypoxic pulmonary hypertension (HPH), which is a type of pulmonary hypertension (PH) found clinically and has been widely used to induce PH in animal models. As research into clinical PH progression broadens to include RV as well as pulmonary vascular remodelling, an improved understanding of the effects of HPH on the RV is required.
- **What advances does it highlight?**
  This article highlights the moderate, adaptive and reversible nature of RV and pulmonary vascular remodelling in HPH. Moreover, we show that increased haematocrit in HPH contributes significantly to RV overload, which warrants additional attention.

Chronic hypoxia causes pulmonary vasoconstriction and vascular remodelling, which lead to hypoxic pulmonary hypertension (HPH). Hypoxic pulmonary hypertension is associated with living at high altitudes and is a complication of many lung diseases, including chronic obstructive pulmonary disease, cystic fibrosis and obstructive sleep apnoea. Pulmonary vascular changes that occur with HPH include stiffening and narrowing of the pulmonary arteries that appear to involve all vascular cell types and sublayers of the arterial wall. Right ventricular (RV) changes that occur with HPH include RV hypertrophy and RV fibrosis, often with preserved systolic and diastolic function and ventricular–vascular coupling efficiency. Both vascular stiffening and vascular narrowing are important contributors to RV afterload via increases in oscillatory and steady ventricular work, respectively. The increased blood viscosity that occurs in HPH can be dramatic and is another important contributor to RV afterload. However, the viscosity, vascular mechanics and ventricular changes that occur with HPH are all reversible. Furthermore, even with continued hypoxia the vascular remodelling does not progress to the obliterative, plexiform lesions that are seen clinically in severe pulmonary hypertension. In animal models, the RV changes appear adaptive, not maladaptive. In summary, HPH-induced vascular mechanical changes affect ventricular function, but both are adaptive and reversible, which differentiates HPH from severe pulmonary hypertension. The mechanisms of adaptation

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and reversibility may provide useful insight into therapeutic targets for the clinical disease state.

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Introduction

Pulmonary hypertension (PH) is manifested as the elevation of pulmonary arterial pressure (P_{pa}) and is often haemodynamically defined as a mean resting P_{pa} of >25 mmHg. Pulmonary hypertension is a complex pulmonary disorder associated with a variety of causes; hypoxia-induced PH (HPH) is categorized in the third group of PH according to revised World Health Organization classifications (McLaughlin *et al.* 2009). Clinically, HPH can be caused by living at high altitudes and is a complication of many lung diseases, including chronic obstructive pulmonary disease, cystic fibrosis and obstructive sleep apnoea. The preclinical animal model of HPH was introduced in the 1970s (Zaiman *et al.* 2005) and is now widely used in rodents and calves to study the biological and functional changes in the pulmonary vasculature during the progression of PH.

While the pulmonary vascular changes in the most severe form of clinical PH, pulmonary arterial hypertension (PAH), can be dramatic, the cause of death is typically right ventricular (RV) failure. Therefore, biological and functional changes in the RV with PH progression, as well as the interactions between pulmonary vascular and RV dysfunction, have gained more attention recently. In this context, it is necessary to revisit the current understanding of HPH as well as its use as a model to study vascular and ventricular changes in the progression of PH.

Biological changes in pulmonary arteries during HPH

Hypoxia, the pathological condition in which one is deprived of adequate oxygen supply, can be achieved by exposing subjects to high altitude (which causes hypobaric hypoxia) or to a low-oxygen environment at normal barometric pressure (which causes normobaric hypoxia). Acutely, hypoxia leads to pulmonary vasoconstriction, mostly in the precapillary small pulmonary arteries (PAs), as evidenced by recent synchrotron radiation experiments (Schwenke *et al.* 2007). The cellular and molecular mechanisms of acute hypoxic pulmonary vasoconstriction have been extensively reviewed recently (Sylvester *et al.* 2012). Chronically, both continuous and intermittent hypoxia causes remodelling in large, proximal arteries and small, distal arterioles, as well as RV hypertrophy (Stenmark *et al.* 2006). These arterial changes involve all vascular cell types (i.e. endothelial cells, smooth muscle cells and adventitial fibroblasts) and include altered cell proliferation and apoptosis, expression of growth factors, cytokines and receptors, as well as inflammatory responses (Humbert *et al.* 2004; Stenmark *et al.* 2006; Zhang *et al.* 2012).

While the HPH model continues to serve as a model of human PH, and is especially suitable for studying forms of PH associated with respiratory disorders, it is also well recognized that the HPH-induced remodelling in PAs lacks the marked distal luminal reduction by intimal growth and complex vascular lesions found in severe PAH (Zaiman *et al.* 2005; Nicolls *et al.* 2012). The absence of this biological signature of PAH in HPH may explain the mild-to-moderate and reversible functional changes in PAs and RV that we will discuss in the section "HPH: a moderate and reversible type of PH".

Another major characteristic of PA remodelling during HPH is the accumulation of extracellular matrix (ECM), including elastin and collagen, especially in the proximal PAs (Poiani *et al.* 1990; Tozzi *et al.* 1994; Kobs *et al.* 2005; Drexler *et al.* 2008; Lammers *et al.* 2008; Estrada & Chesler, 2009; Ooi *et al.* 2010; Wang & Chesler, 2011*b*; Wang *et al.* 2013*b*). A recent study suggests that elastin remodelling contributes to proximal PA stiffening in response to HPH in neonatal calves (Lammers *et al.* 2008), but discrepant observations are also reported in other species in adults. For example, there is no change in elastin content in rodent large PAs after chronic hypoxia (Merklinger *et al.* 2005; Drexler *et al.* 2008; Ooi *et al.* 2010). Our group has found that in mouse HPH, the ECM changes are dominated by collagen and, in particular, the type I isoform is elevated significantly (Ooi *et al.* 2010; Wang & Chesler, 2011*b*; Wang *et al.* 2013*b*). Changes in collagen cross-linking also occur with progression of HPH and may affect blood flow dynamics (Wang & Chesler, 2011*b*; Wang *et al.* 2013*b*). Moreover, limiting collagen synthesis has been shown to limit the severity of HPH and RV dysfunction, although the mechanism is unclear (Kerr *et al.* 1984, 1987; Schreier *et al.* 2013). These studies suggest an important role of collagen in determining the severity and progression of HPH. The ECM remodelling that occurs with HPH has been postulated to be preceded by endothelial dysfunction, which in turn increases smooth muscle cell-mediated proteolysis (Budhiraja *et al.* 2004; Rabinovitch, 2012). The proteolytic enzymes include matrix metalloproteinases and their counteracting inhibitors (TIMPs), which are elevated in experimental HPH as well as in clinical PAH (Hassoun, 2005). Therefore, the exact regulatory mechanisms of ECM remodelling in PAs may be essential keys to potential therapeutic targets in PH.

Mechanical changes in pulmonary arteries during HPH

Functionally, acute and chronic HPH cause distal pulmonary arteriolar narrowing that increases pulmonary vascular resistance (PVR), which is defined as $(\mathrm{m}P_{\mathrm{pa}} - P_{\mathrm{la}})/\dot{Q}$, where $\mathrm{m}P_{\mathrm{pa}}$ is the mean P_{pa}, P_{la} is the left atrial pressure, and $\dot{Q}$ is the mean pulmonary flow (or cardiac output). Pulmonary vascular resistance is thus a useful parameter describing the degree of narrowing in the distal, small PAs and is markedly increased in HPH. From the mean pressure–flow relationship, one can also derive distal PA stiffness, assuming a fully recruited and dilated pulmonary vasculature (Linehan *et al.* 1992); in particular, the distal PA distensibility, which is the inverse of the distal PA stiffness, is given by the following equation:

$$\mathrm{m}P_{\mathrm{pa}} = \frac{[(1+\alpha P_{\mathrm{v}})^5 + 5\alpha R_0(\mathrm{Hct})(\mathrm{CO})^{1/5}] - 1}{\alpha}$$

where P_{v} is pulmonary venous pressure, CO is cardiac output, R_0 is the vascular resistance of the unstressed lung (when vascular pressure approaches zero) and is a function of the haematocrit, Hct, and α is the distal PA distensibility (expressed per millimetre of mercury), which is assumed to be constant throughout the pulmonary vascular bed. As the above equation cannot be solved explicitly for α, typically the distensibility (α) is obtained by curve-fitting the experimental data to this equation. *In vivo* (Reeves *et al.* 2005; Blyth *et al.* 2007) and *ex vivo* studies (Chesler *et al.* 2009) have demonstrated that chronic PH decreases α. However, no change in α has been observed in acute HPH (Reeves *et al.* 2005).

In proximal arteries, there is no evidence that acute HPH has an effect, but chronic HPH leads to remodelling and stiffening via increased ECM production and wall thickening. The stiffness of proximal, extralobar PAs is often measured by *ex vivo* or *in vivo* pressure–diameter relationships (Wang & Chesler, 2011*a*). In clinical settings, parameters that can be obtained non-invasively have been introduced, such as relative area change (RAC). The RAC is not a stiffness but an area strain and is calculated as the relative cross-sectional area change ($\Delta A/A$) of the proximal PA from systole to diastole; it is reduced significantly in PH patients (Gan *et al.* 2007). Another parameter frequently used in clinics to assess PA stiffening is the compliance (C), which is calculated as the ratio of stroke volume (SV) to pulse pressure (PP). In the systemic circulation, this metric reflects aortic stiffness because of the substantial length of the aorta before it branches into smaller arteries, whereas in the pulmonary circulation it may depend on intermediate and distal PA stiffness as well as proximal PA stiffness (Saouti *et al.* 2010). Our recent data show that in mouse models of HPH, C does not always correlate with large, extralobar PA stiffness measured *ex vivo* (Fig. 1; Wang *et al.* 2013*a*).

Haemodynamic consequences of pulmonary arterial mechanical changes during HPH

The haemodynamic consequences of changes in both distal and proximal PAs, as well as their interactions (e.g. pulse wave reflection), can be measured via the pulmonary vascular impedance (PVZ), which is derived from pulsatile pressure–flow relationships. Two approaches are commonly used to obtain PVZ, namely a frequency domain method and a time domain method (Wang & Chesler, 2011*a*). Both methods yield two important impedance metrics, the input impedance (Z_0), which is calculated either as the impedance magnitude at 0 Hz (in the frequency domain) or as total PVR (in the time domain), and the characteristic impedance (Z_{c}), which is calculated either as the impedance magnitude at high frequencies (in the frequency domain) or as the slope of the pressure–flow relationship in early systole (in the time domain).

The input impedance is essentially a measure of distal pulmonary constriction and increases with HPH progression; it is the PVZ in the absence of flow oscillations. The characteristic impedance depends principally on the ratio of stiffness of the proximal arteries to fluid inertia in the proximal arteries; it is PVZ in the absence of wave reflections. In animal studies, Z_{c} increases with PH in some species (Maggiorini *et al.* 1998; Wauthy

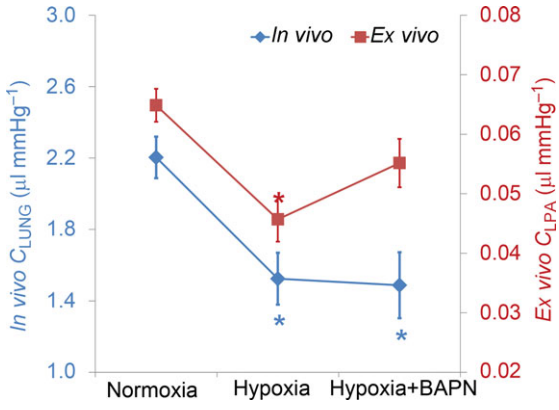

Figure 1. Compliance measured *in vivo* for whole lung (C_{LUNG}, by stroke volume divided by pulse pressure) and *ex vivo* for extralobar left pulmonary arteries (C_{LPA}, by change in volume divided by change in pressure) in mice with a collagen mutation exposed to normoxia, 10 days of hypoxia or 10 days of hypoxia with β-aminopropionitrile (BAPN)
See Wang *et al.* (2013*b*) for a description of this mouse strain and the experimental protocol for C_{LPA} measurements. The hypoxia + BAPN group showed persistently low *in vivo* compliance compared with the hypoxia group, suggesting similar levels of overall pulmonary vascular stiffening. However, extralobar left PA compliance tended to increase in the hypoxia + BAPN group compared with the hypoxia group, suggesting more compliant proximal large PAs with BAPN treatment. Results are shown as means ± SEM. $^*P < 0.05$ *versus* normoxia.

et al. 2004) but not in others (Ewalenko *et al.* 1997; Pagnamenta *et al.* 2003; Wauthy *et al.* 2004; Vanderpool *et al.* 2010*b*; Tabima *et al.* 2012), which probably reflects species-dependent differences in proximal PA stiffening *versus* dilatation in response to PH.

It is important to note also that PA stiffness itself is pressure dependent. That is, as pressure increases, the PAs distend and more collagen fibres engage such that stiffness increases. Thus, it can be difficult to separate the effects of remodelling-induced stiffening from strain- or dilatation-induced stiffening. Ideally, stiffness measurements should be made at multiple strain levels such that these two mechanisms of stiffening can be differentiated (Vanderpool *et al.* 2010*a*).

Changes in the right ventricle during HPH

Proximal and distal arterial stiffening and narrowing are important contributors to RV afterload via increases in steady and oscillatory ventricular work. The steady work of the RV is typically calculated as the product of mP_{pa} and stroke volume. Thus, it is the work required to overcome total PVR and to produce forward blood flow into pulmonary circulation; it depends greatly on distal PA narrowing. The oscillatory work of the RV is calculated as the difference between the total work (stroke work) and the steady work. The oscillatory work is the work required to produce zero-mean oscillations in blood flow; it largely depends on pulmonary arterial compliance. Typically, oscillatory work is considered 'wasted', so an increase in the ratio of oscillatory to total work is considered a sign of decreased RV efficiency. See Bellofiore & Chesler (2013) for a recent review of this topic. It is not currently known whether the RV remodels in a different manner in response to increased steady and oscillatory work demands.

Known consequences of increased RV afterload include RV hypertrophy and fibrosis. Right ventricular hypertrophy is typically quantified by the Fulton index, which is the weight ratio of the right ventricle to the sum of the left ventricle and septum [RV/(LV + S)]. An increased Fulton index is universally observed in HPH. Right ventricular fibrosis, which is a hallmark of a dysfunctional or failing RV, is seldom reported (or examined) in HPH. Our group has recently found a significant increase in collagen content in mouse RVs after 10 days of hypoxia (Schreier *et al.* 2013). However, the structural and functional changes in the RV with chronic HPH have been less well studied than those in the pulmonary circulation until recently (Tabima *et al.* 2010; Walker *et al.* 2011; Schreier *et al.* 2013).

Our group has successfully established methods to measure RV function in mice *in vivo* (Tabima *et al.* 2010). With HPH progression, we have consistently observed increased right ventricular systolic pressure, significant RV hypertrophy, and increased effective arterial elastance (E_a), which is an index of RV afterload (Tabima *et al.* 2010; Schreier *et al.* 2013). As a result of the pressure overload, RV contractility increases, as measured by preload recruitable stroke work and ventricular end-systolic elastance (E_{es}). The ratio of E_{es} to E_a, which is an index of ventricular–vascular coupling efficiency, is typically maintained (Tabima *et al.* 2010; Schreier *et al.* 2013). To date, we have seen no significant changes in cardiac output or ejection fraction in mice with chronic HPH. In large animals exposed to acute hypoxia, both E_{es} and E_a increase but E_{es}/E_a remains at control levels, which indicates preserved ventricular–vascular coupling (Wauthy *et al.* 2004). Therefore, RV functional changes during HPH seem to be adaptive and moderate, with preserved systolic and diastolic function.

Increases in haematocrit and blood viscosity

A unique change associated with HPH but not with other types of PH is the increased expression of erythropoietin, resulting in increased red blood cells, haematocrit (Hct) and haemoglobin levels. Haematocrit can increase from

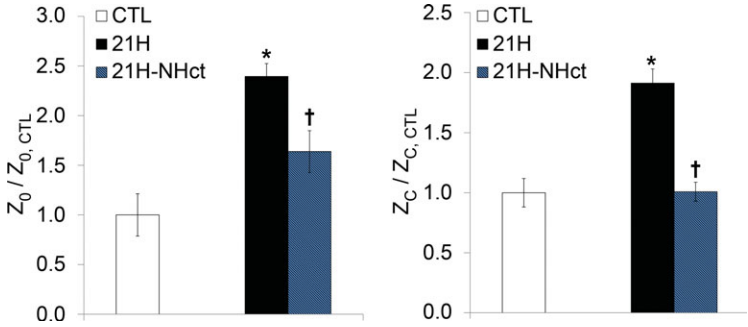

Figure 2. Pulmonary vascular input impedance (Z_0) and characteristic impedance (Z_c) in wild-type mice exposed to 21 days of hypoxia normalized to those measured in normoxic mice
Measurements were obtained in chronically hypoxic mice with elevated Hct (21H) as well as normal levels of Hct (21H-NHct) by blood dilution to show that decreased haematocrit led to significant reductions in Z_0 and Z_c. Results are shown as means ± SEM. *$P < 0.05$ *versus* normoxia; †$P < 0.05$ *versus* 21H.

~45% at normal levels to up to 80% after chronic exposure to hypoxia. This increases blood viscosity, which consequently increases pulmonary resistance. Whittaker and Winston found the following power-law relationship between PVR and haematocrit (Whittaker & Winton, 1933):

$$R_0(45\%) = R_0(\mathrm{Hct})\frac{1-\varphi^{1/3}}{0.234}$$

where R_0 is PVR at a Hct of 45% (normal) and φ is the measured Hct.

We recently measured PVZ in control mice and those exposed to 21 days of hypoxia at different haematocrits by partly replacing high-viscosity blood with hydroxyethylstarch (Schreier DS, Hacker TA, Song G and Chesler NC; unpublished data). By reducing the haemoglobin to normal levels (i.e. Hct ~42%), Z_0 (or PVR) decreased by about 31% and Z_c decreased by about 47% (Fig. 2). These data suggest that the increase in haematocrit is a significant contributor to the increased RV afterload in chronic HPH.

Hypoxic pulmonary hypertension: a moderate and reversible type of PH

It is well known that even with prolonged exposure to hypoxia, distal vascular remodelling does not progress to the obliterative, plexiform lesions that are seen clinically in severe PH (Gomez-Arroyo *et al.* 2012; Nicolls *et al.* 2012), suggesting that the vascular changes are only moderate in HPH. Another important characteristic of HPH that is different from severe PH is its reversibility. It has been shown in many studies that if allowed to recover in normoxic conditions, subjects will undergo a reverse remodelling in PAs and a decrease of P_{pa}, with a reduction in Hct as well (Rabinovitch *et al.* 1981; Liu, 1997; Tozzi *et al.* 1998; Riley *et al.* 2000; Li *et al.* 2004; Ooi *et al.* 2010; Tabima *et al.* 2012). In terms of vascular mechanics, the recovery process is accompanied by reduced proximal PA stiffening and reduced distal PA narrowing (Ooi *et al.* 2010; Tabima *et al.* 2012), which then significantly reduces the RV afterload. As a consequence, a regression in RV hypertrophy is often observed.

Given that HPH is a moderate and reversible type of PH, it does not capture the key features of severe, clinical PH. Recently, the appropriate usage of this model has been reconsidered (Gomez-Arroyo *et al.* 2012; Nicolls *et al.* 2012). Instead of a limitation, however, the reversibility of HPH may in fact be an advantage; that is, the contrasts between HPH and severe PH may shed light on key factors that determine the reversibility of RV and PA remodelling and the critically important transition from RV adaptation to RV failure.

Summary and conclusions

In summary, HPH-induced vascular mechanical changes affect ventricular function but both are adaptive and reversible, which differentiates HPH from severe pulmonary hypertension. The mechanisms of adaptation and reversibility may provide useful insight into therapeutic targets for the clinical disease state.

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Additional information

Competing interests

None declared.

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