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Biomechanical and mechanobiological drivers of the transition from post-capillary pulmonary hypertension to combined pre-/post-capillary pulmonary hypertension

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Running Title: Biomechanics of the transition from Ipc-PH to Cpc-PH

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Abstract

Combined pre-/post-capillary pulmonary hypertension (Cpc-PH), a complication of left heart failure (LHF), is associated with higher mortality rates than isolated post-capillary PH (Ipc-PH) alone. Currently, knowledge gaps persist regarding the mechanisms responsible for the progression of Ipc-PH to Cpc-PH. Here, we review the biomechanical and mechanobiological impact of LHF on the pulmonary circulation, including mechanotransduction of these pathological forces, which lead to altered biological signaling and detrimental remodeling driving the progression to Cpc-PH. We focus on pathologically increased cyclic stretch and decreased wall shear stress; mechanotransduction by endothelial cells, smooth muscle cells, and pulmonary arterial fibroblasts; and signaling-stimulated remodeling of the pulmonary veins, capillaries, and arteries that propel the transition from Ipc-PH to Cpc-PH. Identifying biomechanical and mechanobiological mechanisms of Cpc-PH progression may highlight potential pharmacologic avenues to prevent right heart failure (RHF) and subsequent mortality.

Glossary

BAECs – bovine arterial endothelial cells
Cpc-PH – combined pre-/post-capillary pulmonary hypertension
ECM – extracellular matrix
ECs – endothelial cells
eNOS – endothelial nitric oxide synthase
ET-1 – endothelin-1
HAECs – human arterial endothelial cells
HFpEF – Heart failure with preserved ejection fraction
HUVECs – human umbilical vein endothelial cells
ICAM - intercellular adhesion molecule
Ipc-PH – isolated post-capillary pulmonary hypertension
L-NMMA – L-NG-monomethyl arginine acetate
LAP – left atrial pressure
LHF – left heart disease
LV – left ventricle
MAPK – mitogen-activated protein kinase
MLCK – myosin light chain kinase
MMP – matrix metalloproteinase
mPAP – mean pulmonary artery pressure
NO – nitric oxide
PA – pulmonary artery
PAC – pulmonary artery compliance
PAECs – pulmonary artery endothelial cells
PAAF – pulmonary artery adventitial fibroblasts
PAH – pulmonary arterial hypertension
PASMCs – pulmonary arterial smooth muscle cells
PAWP – pulmonary artery wedge pressure
PDGF – platelet derived growth factor
PH-LHF – pulmonary hypertension-left heart disease
PP- pulse pressure
PVR- pulmonary vascular resistance
ROS – reactive oxygen species
RV – right ventricle
TGF-β – transforming growth factor-β
TSP-1 – Thrombospondin 1
VCAM – vascular cell adhesion molecule
VEGF – vascular endothelial growth factor
VSMCs – vascular smooth muscle cells
WSS – wall shear stress
WU – woods unit

Introduction

Left heart failure (LHF) impacts nearly 5.9 million adults and contributes to 1 out of every 9 deaths in the U.S[1]. Pulmonary hypertension (PH) occurs in 36-83% of those with LHF (PH-LHF)[2] and dramatically increases morbidity and mortality[3,4]. PH-LHF begins as a passive process termed isolated post-capillary PH (Ipc-PH), diagnosed by elevated mean pulmonary artery pressure (mPAP) with normal pulmonary vascular resistance (PVR). Mortality significantly increases once Ipc-PH transitions to combined pre-/post-capillary PH (Cpc-PH), with increased PVR, which also typically mark a change from reversible to irreversible disease [5]. While genetic, environmental, and metabolic factors likely impact disease progression in individual patients, biomechanical forces and mechanobiological signaling may be common drivers of this key pathophysiological transition.

Vascular biomechanical forces such as cyclic stretch, which acts on all cells in the vascular wall, and shear stress, which acts on the cells that line the lumen of the vascular wall, are transduced into biological signals in a process termed mechanotransduction. Mechanotransduction pathways contribute to the pathophysiology of cardiovascular diseases including atherosclerosis, arteriovenous malformations, and diabetes mellitus type II among others [6-9]. Specifically, mechanotransduction induces biological signals that drive vascular remodeling including hypertrophy, hyperplasia, apoptosis, and extracellular matrix (ECM) synthesis and degradation [10]. This remodeling in turn alters the mechanical function of the vessels [11]. For example, increased collagen synthesis (and less degradation) in the vessel wall will decrease vessel compliance and pulse wave dampening [12]. Within a less compliant vessel, cells stretch less with each pressure pulse, which alters the biomechanical forces on those cells. While this self-perpetuating process

-- biomechanical forces transduced into biological signals that cause vascular remodeling, which in turn change biomechanical forces -- can be homeostatic and adaptive, it can also be maladaptive, especially for tissues upstream and downstream. In pulmonary hypertension due to LHF, the ultimate outcome is right heart failure (RHF) (Figure 1).

This review will examine the known and suspected roles of LHF-induced hemodynamic changes in altering two key vascular biomechanical forces: cyclic stretch and wall shear stress (WSS), which activate mechanotransduction pathways, drive pulmonary venous, capillary, and arterial remodeling, and characterize the transition from Ipc-PH to Cpc-PH and subsequent RHF.

Clinical Definitions of Ipc- and Cpc-PH

As left ventricular (LV) function declines, LV filling pressures rise, inducing a concomitant elevation in left atrial pressure (LAP). The passive transmission of elevated LAP into the pulmonary veins is characteristic of Ipc-PH. Increased pulmonary venous pressures are transmitted across the capillaries and arteries in one-to-one fashion such that the rise in mPAP is proportional to the rise in LAP. The 2022 ESC/ERS Guidelines for the Diagnosis and Treatment of Pulmonary Hypertension defined Ipc-PH as mPAP >20mmHg, pulmonary artery wedge pressure (PAWP) >15mmHg with PVR<2 WU[13]. As the disease progresses, pulmonary vasoconstriction contributes to a greater than one-to-one rise in mPAP [14]. In this reactive phase, the increase in mPAP can be reversed with adequate LV afterload reduction [15].

Twelve to 38% of patients with PH-LHF progress through this reversible reactive phase of Ipc-PH to irreversible Cpc-PH [15]. The clinical definition of Cpc-PH is mPAP>20mmHg, PAWP>15mmHg, and PVR>2 WU[13]. The elevation in PVR in Cpc-PH

is associated with a greater risk of RHF and mortality in comparison to patients with Ipc-PH alone [15]. Post-mortem studies have demonstrated that increases in PVR is associated with pulmonary vascular remodeling, including pulmonary venous and arterial medial hypertrophy, diffuse lung intimal fibrosis, and distal arterial luminal occlusion [16].

In addition to increasing PVR, Cpc-PH is associated with decreased pulmonary arterial compliance (PAC), which is calculated as the pulse pressure (sPAP-dPAP) divided by stroke volume (SV) and represents the ability of the pulmonary arterial compartment to absorb and dampen hemodynamic pulsatility [17]. Large clinical studies have found that PAC is more predictive of mortality than mPAP or PVR in PH-LHF [18-20]. Importantly, decreased PAC has consequences distinct from increased PVR on the mechanical forces imposed on the upstream right ventricle and downstream pulmonary capillaries via altered pulse wave reflection and transmission, respectively [17].

Biomechanics: The missing link

The initial insult to the pulmonary vasculature in Ipc-PH is increased pulmonary venous, capillary, and arterial pressure. The impact of increased pressure on the two key vascular biomechanical forces, cyclic stretch and wall shear stress, in the pulmonary vasculature depends, in part, on the mechanical properties of the pulmonary vasculature. Since each compartment in the pulmonary vasculature – arteries, capillaries, and veins – has different structure [21], the cells in each compartment will be exposed to different biomechanical stimuli.

Healthy pulmonary arteries are rich in elastin and have concentric layers of smooth muscle cells (SMC), yielding a highly compliant structure [22]. They are populated with

pulmonary arterial fibroblasts (PAF) in the adventitia, which are responsible for ECM remodeling. As distance from the main PA increases, the relative amount of ECM protein decreases and percentage of SMC increases, reaching a maximum at the arterioles [23]. The capillary system is a vast but fragile network consisting only of endothelial cells (ECs) and ECM. Although thin-walled and lacking in SMCs and elastic fibers, the capillaries derive tensile strength from type IV collagen [24]. The pulmonary venules consist of elastic fibers and connective tissue with minimal SMCs. As pulmonary venules become veins approaching the left atrium, the amount of SMCs and elastin increases [25]. Overall, pulmonary veins are less compliant than pulmonary arteries [26].

Cyclic stretch occurs in a compliant vessel in which pressure is pulsatile; it is defined as the change in diameter from systole to diastole divided by the diameter at diastole (Figure 2). Thus, increased pulse pressure (PP) (defined as systolic pressure minus diastolic pressure) can drive increased cyclic stretch. In the pulmonary vasculature, PP is highest in large proximal arteries and drops precipitously at the pulmonary arterioles [27]. While difficult to measure, PP (i.e. pulsatility) in the capillaries in a healthy state is thought to be minimal; pulsatility in the pulmonary veins is also low. Therefore, cyclic stretch in the capillaries and veins in the healthy state are likely negligible.

As noted above, with Ipc-PH, increased LAP is transmitted from the veins across the capillaries to arteries in one-to-one fashion such that the rise in mPAP is proportional to the rise in LAP. For no change in cardiac output or pulmonary vascular resistance, the increase in mPAP is equal to the increase in LAP. How this affects cyclic stretch in the three compartments is not precisely known. A common feature of all vessels is nonlinear compliance; arteries, capillaries, and veins are more compliant at low pressures than at

high pressures [28]. Uniquely, in the pulmonary circulation, there is a linear relationship between mPAP and PP such that as mPAP increases, PP increases proportionally [29]. Thus, increased mean pressure decreases compliance and increases PP, which increases cyclic stretch on vascular cells [30]. In 2000, West hypothesized that high mPAP injures pulmonary capillaries and leads to stress failure [31]; alternatively, excessive cyclic stretch could be the mechanism. Whether this putatively increased pulsatility is then transmitted downstream of capillaries to pulmonary veins is unknown. In sum, the cyclic stretch imposed on cells in the pulmonary vasculature depends on PP (which itself depends on mean pressure), and vessel wall structure, both of which depends on compartment (artery, capillary, vein) and distance from the heart.

Wall shear stress (WSS) in the pulmonary vasculature also depends on compartment and distance from the heart. On the basis of Poiseuille's Law, WSS is proportional to blood flow velocity and blood viscosity, and inversely proportional to the lumen radius [32] (Figure 2). The branching pattern of the pulmonary arteries and veins is thought to keep time-averaged WSS relatively constant with distance from the heart, because flow rate decreases in proportion to radius cubed [33]. The WSS in the capillaries are difficult to define and measure. With Ipc-PH, increased mean pressure increases diameter, which should decrease WSS in all compartments (again, dependent on vascular compliance). Moreover, when cardiac output decreases due to LHF, blood flow velocities, and thus WSS, will decrease in all compartments. Decreased pulmonary venous systolic velocity has been found in subjects with LHF [34], which supports decreased WSS in the pulmonary veins.

Cyclic stretch, which acts on directly ECs, SMCs, and fibroblasts in the vessel wall, and WSS, which acts directly on ECs and can have consequences for SMCs and fibroblasts, are potent mechanical stimuli for vascular remodeling. Below, we review the pathways through which these biomechanical stimuli act on each cell type and provide evidence that biomechanics and mechanobiology incite key pulmonary vascular events in the transition from Ipc-PH to Cpc-PH.

Mechanotransduction of cyclic stretch and wall shear stress

Increased cyclic stretch

Increases in pulmonary pressure due to LHF and Ipc-PH increase can increase cyclic stretch, which stimulates EC signaling pathways that cause vasodilation, inflammation, and pathologic vessel remodeling through ECM turnover and SMC proliferation (Figure 3). Since both pressures and wall mechanical properties vary throughout the pulmonary vasculature – with pressures decreasing from the arteries to the capillaries and veins and mechanical properties reflecting their differing functions – the impact of Ipc-PH on cyclic stretch is distinct in each compartment and not entirely known. In the systemic circulation, a low magnitude cyclic stretch in the range of 5-10% is considered to be physiological and a high magnitude stretch greater than 20% is considered pathological [8]. The physiological and pathological stretch levels in the pulmonary vasculature are not well defined. In particular, the mechanical forces in PH-LHF have not been characterized and further investigations are needed to fully understand the abnormal biomechanical forces generated by this disease. Recent computational modeling simulations from Bartolo et al. suggest that physiological cyclic

stretch is 15-20% in the pulmonary arteries and under 5% in the capillaries and veins; in the setting of Ipc-PH (an increase in LAP from 2 mmHg to 20 mmHg), cyclic stretch is predicted to increase to up to 60% in the arteries, 10% in the capillaries, and 40% in the veins [35]. To understand the impact of these mechanical stimuli on biological changes in each cell type in the pulmonary vasculature, below we review key findings from *in vitro* studies examining the impact of cyclic stretch on ECs, SMCs, and fibroblasts, with the caveat that most of these studies use non-pulmonary cell sources. Table 1 provides the subset of these study results conducted using pulmonary vascular ECs, SMCs, and fibroblasts.

Impact of cyclic stretch on ECs in veins, capillaries, and arteries

High cyclic stretch on HUVECs *in vitro* increases eNOS phosphorylation through the PKA and P13K/Akt pathways [36,37]. Consequent vasodilation may contribute to decreased pulmonary blood flow velocities found in LHF [34]. HUVECs subjected to pathological cyclic stretch also increase release of interleukin-6 (IL-6) via nuclear factor-κB (NF-κB) dependent pathway [38]. Increased activation of the NF-κB pathway triggered by EC cyclic stretch leads to reactive oxygen species (ROS) stress and cytokine release resulting in inflammation [39,40]. Cyclic stretch triggered ROS production has been further shown to lead to ET-1 production, a potent vasoconstrictor, in both HUVEC and BAEC [41]. Cyclic stretch also triggers production of inflammatory cytokines including myocyte chemoattractant protein-1 (MCP-1) and IL-8 [42,43]. In addition to vasodilation and inflammation, increased cyclic stretch stimulates ECM remodeling through MMP production and activation[44-46]. Stretch leads to EC stiffening via cytoskeleton remodeling

characterized by increased actin fiber bundle thickness and fiber reorientation [47,48]. This remodeling could contribute to increased intimal thickness, which is a key histologic feature of PH-LHF remodeling and has been demonstrated throughout the pulmonary vascular bed in human patients[16,49,50]. These findings highlight the potentially important role of EC-stimulated remodeling in Cpc-PH progression.

In the capillary bed, increased EC cyclic stretch has been hypothesized to cause alveolar-capillary stress failure [24]. As first proposed by West, alveolar-capillary stress failure is the physical disruption of the alveolar-capillary membrane in response to elevated capillary pressure and volume [3]. Mechanical breakdown of the capillary membrane is theorized to increase permeability, stimulate remodeling, and release factors that further alter the function of the membrane[51]. Indeed, microvascular remodeling and dysfunction could be a critical link in the transition of Ipc-PH to Cpc-PH. Consistent with this hypothesis, pulmonary microvascular remodeling, including thickened alveolar septa and collapsed airspaces, were demonstrated in a mouse model of LHF [52]. Human lung microvascular ECs subjected to cyclic stretch demonstrated an increase in MMP-2 leading to degradation of the basement membrane, which can result in leakage of intraluminal fluid [53]. This mechanism is consistent with increased systemic levels of MMP-2 and MMP-9 in subjects with HFpEF [54]. Additionally, components of the EC cytoskeleton undergo rearrangement in response to pathologic stretch, which weakens junctional protein complexes with neighboring cells and reduces the integrity of the endothelium [55]. A rat model of LHF provided further support for the alveolar-capillary stress failure theory as altered capillary EC membrane permeability and cytoskeletal rearrangement were revealed as additional signs of capillary EC dysfunction [56]. These

studies demonstrate that cyclic stretch stimulates ECM turnover, cytoskeleton rearrangement, and endothelial dysfunction that is similar to the irreversible pathological remodeling demonstrated in animal models of LHF and subsequent Cpc-PH [52,57,58]. Thus, mechanotransduction of increased cyclic stretch occurring in the capillaries due to Ipc-PH likely drives pathologic remodeling that characterizes the transition to Cpc-PH. The overall effects of increased cyclic stretch leading to altered capillary EC function, ECM remodeling, and inflammation, which contribute to further pulmonary vascular remodeling, may be key to inciting the development of Cpc-PH from Ipc-PH [59].

In the arterial compartment, increased cyclic stretch similarly triggers vasodilation, inflammation, and ECM remodeling, though through some different pathways than in the venous and capillary compartments. Unlike in HUVECs, cyclic stretch applied to PAECs increased proliferation and eNOS phosphorylation while reducing NO via the P13K pathway [37,60]. In arterial ECs cyclic stretch activates VEGFR2, which leads to Src-dependent VE-cadherin tyrosine phosphorylation resulting in proliferation and migration [61,62]. Dynamically stretching human PAECs (20% vs 5%) for 24 hours led to time-dependent increases in IL-8 production [63]. Cyclically stretching PAECs also activates the IL-6 release pathways observed in the venous cells in addition to JNK, Erk, and P38 pathways unique to the arterial cells [64]. Cyclically stretching PAEC further induces the upregulation of TSP-1, which inhibits NO-stimulated PASMC growth and proliferation [65]. Similar to in venous and capillary ECs, in BAECs, 10% cyclic stretch induced a nine-fold increase in MMP-2 compared to static culture [46]. This study identified the stimulation of p38- and ERK-dependent pathways as the mechanisms responsible for the MMP increase [46]. Additional studies have demonstrated that when vascular ECs experience

pathological cyclic stretch, the cytoskeleton transmits the force to the subcellular mitochondria. Dynamically stretched PAECs increase mitochondrial release of ROS and activation of protein kinase C and focal adhesion kinase (FAK), a key regulator of angiogenesis [66,67]. Given these findings, it is likely that the increased cyclic stretch associated with LHF contributes to intracellular mitochondrial driven metabolic dysfunction.

Previous reviews compiled systemic and umbilical endothelial cell response to cyclic stretch [8,68], but EC characteristics depend on location [69]. Non-pulmonary cell studies present targetable pathways, which should be investigated in pulmonary EC specific experiments. The differing stretch-induced vasodilation, inflammation, and remodeling pathways observed in venous, capillary, and arterial EC further motivates studies to understand the biomechanical complexity of Ipc-PH progression to Cpc-PH. Key knowledge gaps include mechanotransduction pathways in pulmonary specific cell lines as well as the mechanical forces imposed on these cell types in both the physiological and pathological conditions in each compartment of the pulmonary circulation.

Impact of cyclic stretch on SMCs in veins and arteries

Cyclic stretch is a key regulator of SMC function impacting gene expression and cell signaling pathways to regulate proliferation, apoptosis, and remodeling [70]. Like ECs, pulmonary SMCs respond to pathologic cyclic stretch by increasing proliferation, ECM remodeling, and inflammation (Figure 4). In the systemic venous compartment, cyclic stretch activates the insulin-like growth factor 1 (IGF1) pathway to induce SMC

proliferation [71]. In the pulmonary arterial compartment, 20% biaxial stretch induces a RhoA-dependent increase in SMC proliferation [72]. Additionally, pulmonary arterial SMCs demonstrate stretch-induced dysfunction through increased VEGF expression via ROS-dependent TGF-β1 signaling promoting angiogenesis and inflammation [73]. Beyond VEGF, cyclic stretch stimulates overexpression of other growth factors and their receptors in PASMC including PDGF and PDGF-R via phosphorylation of focal adhesion kinase (FAK). Overexpression of PDGF-R is also present in rat models of pulmonary artery hypertension (PAH) indicating its likely role in pathological pulmonary vascular remodeling [74,75]. Dynamically stretched rat aortic VSMCs show significant time-dependent increases in L-arginine activity and transport velocity as well as L-arginine-dependent products such as L-proline, putrescine, and L-ornithine [76]. Increased levels of L-proline products result in decreased NO and increased collagen deposition by SMC [76], a combination of effects that could drive remodeling associated with the transition from Ipc-PH to Cpc-PH. Similar to ECs, when PASMCs experience pathological stretch, activity of mitochondrial complex III increases, leading to elevated cytosolic ROS and NADPH oxidase activity (NOX4), both of which contribute to vascular remodeling [77]. Pathological remodeling of PASMC due to increased cyclic stretch is characterized by proliferation, collagen deposition and inflammation, which likely contribute to the decreased pulmonary arterial compliance associated with the progression to Cpc-PH. Thus, arterial stiffening due to cyclic stretch-stimulated pathological remodeling by SMC and EC may compound the dysfunction and remodeling in the pulmonary capillaries and veins, driving the progression from Ipc-PH to Cpc-PH.

Impact of cyclic stretch on fibroblasts in arteries

The activation of fibroblasts and their differentiation into pro-inflammatory myofibroblasts is a major contributor to the arterial stiffening, alveolar membrane thickening, and interstitial fibrosis, which characterize the irreversible remodeling seen in Cpc-PH [51]. Several studies have shown pulmonary artery adventitial fibroblasts (PAAF) act as direct biomechanical transducers in response to stretch and injury. Increased cyclic stretch stimulates PAAF differentiation into myofibroblasts and increases expression of collagen and elastin messenger RNAs *in vitro* [78]. Cyclic stretch directly activates latent TGF-β1, which sustains the myofibroblast phenotype [79] via activation of Smad proteins [80] and the MAPK/ERK signaling pathway [81]. Indirectly, fibroblasts can act as mediators of pathological stress from SMCs and ECs induced by altered biomechanics. For example, cyclic stretch increases the expression of fibroblast growth factor (FGF-2) [75] and NOX-4 [77] in pulmonary vascular SMCs. Notably, NOX-4 has been shown to regulate TGF- β1 [82], which may act as a feedback mechanism for myofibroblast differentiation. Additionally, uniaxial cyclic stretch has been shown upregulate COX-2 in fibroblasts via an increase in intracellular Ca^{2+} [83], introducing yet another mechanism of indirect biomechanical transduction via NF-κB activation. Thus, the differentiation of fibroblasts into myofibroblasts and their proliferation stimulated by cyclic stretch that lead to upregulation of ROS and other pro-inflammatory proteins and cytokines are important drivers of pathological fibrosis and remodeling observed in Cpc-PH.

Decreased wall shear stress

WSS is the drag force (per unit area) exerted by blood on ECs throughout the vasculature [60]. Endothelial shear stress is a key regulator of vascular tone, structure, gene

expression, and remodeling [84-86]. Regional variations in shear stress are associated with systemic vascular pathologies such as atherosclerosis and aneurysm development [85-87]. Physiological WSS in the pulmonary arteries of healthy adults has been estimated to range from 15-20 dyn/cm^2 whereas patients with severe pulmonary hypertension have lower WSS in the range of 5-8 dyn/cm^2 [88]. Altered WSS has been shown to occur in a rat model of LHF; echocardiography showed increased PA luminal size and blood flow analysis found reduced WSS [89]. Bartolo et al predicted a 50% or more decrease in WSS in PH-LHF from 10-25 dyn/cm^2 (in the healthy state) to 2-5 dyn/cm^2 (in PH-LHF) using a computational fluid dynamics model [35]. Moreover, in humans with PH, computational fluid dynamics and phase-contrast cardiac MRI have demonstrated lower WSS in the proximal arteries than controls [90]. Pathological alterations in WSS activate EC mechanotransduction pathways leading to a chronic pro-inflammatory state characterized by disorganized alignment, vasoconstriction, increased vascular permeability, and maladaptive ECM remodeling [85,87].

In vitro studies have investigated the mechanisms by which altered shear stress triggers EC-driven remodeling. The molecular pathways involved in the resulting pathological inflammation, vasoconstriction, and vessel remodeling are illustrated in Figure 4. As with cyclic stretch, the majority of studies evaluating the impact of WSS have used HUVECs or arterial ECs as the prototype EC; however, some studies have specifically evaluated the impact of this mechanical stimulus in PAECs and these are detailed in Table 2. Findings so far highlight WSS as a potent mechanical stimulus that is transduced into a wide array of biological signals influencing intracellular energetics, cytoskeletal structure, and vascular tone[91-93]. Future work specifically evaluating these

mechanotransduction pathways in EC from throughout the pulmonary vascular bed is essential to understanding disease progression and identifying therapeutic targets.

Impact of WSS on ECs in veins and arteries

Vascular ECs sense WSS and transduce it into biochemical signals resulting in synthesis and release of the potent vasodilator nitric oxide [94,95]. Under physiological WSS, the production of NO is regulated through both calcium-independent eNOS phosphorylation and calcium-dependent pathways in ECs [91,96-98]. However, this process is dysregulated under pathologically low WSS (Figure 4). Compared to physiological WSS, pathologically low WSS reduces the release of vasodilators such as NO and prostaglandin F1-α (PGF1α), while increasing the release of vasoconstrictors such as endothelin-1 (ET-1) by up to 40% in cultured PAECs [88] and HUVECs [99]. Moreover, total eNOS expression under pathologically low WSS is reduced by 65% compared to physiological WSS and downstream Akt phosphorylation is reduced by 81% [88]. Consistent with this mechanism, patients with WHO Group 2 PH, including PH-LHF, have reduced PA eNOS expression, which correlates with the degree of vascular remodeling. [100]. Infusion of NOS inhibitors such as L-NMMA into the pulmonary arteries of subjects with LHF caused a dose-dependent reduction in pulmonary blood flow velocity with no change in PA pressure [101], demonstrating that NO-dependent pulmonary vasoconstriction was a key contributor to increased PVR in this cohort. These studies suggest that low WSS drives increased ET-1 and reduced NO, both of which have been demonstrated in subjects with PH-LHF [102-104].

Beyond vasoconstriction, chronically low WSS can drive pathological remodeling via altered EC, and subsequently altered SMC, structure and function. Under physiological WSS conditions, EC alignment is parallel to flow. However, low WSS is associated with disorganized EC cytoskeletal alignment in bovine PAECs [88]. Low WSS, as modeled by *in vivo* and *in vitro* cessation of flow in mouse pulmonary microvasculature, triggers ROS production via PECAM-1 and NADPH oxidase, leading to inflammation and angiogenesis [105,106]. In co-culture of rat aortic EC and SMC, low WSS upregulates platelet derived growth factor (PDGF) release in ECs, which increases SMC proliferation and migration [107], two hallmarks of pathological pulmonary arteriole remodeling [108]. Additionally, ECs exposed to low WSS stimulate SMC migration via MMP-2 activation and increased PDGF [109,110]. Since MMP-2 activation degrades the extracellular matrix through type IV collagen proteolysis, increased expression promotes integrin detachment and SMC migration [111]. ECM remodeling driven by MMP activation is a key feature of pathologic changes in PH [108,112]. Both low WSS and elevated MMPs have been found in PH-LHF [54,89]. The *in vitro* studies cited above provide a potential mechanism that links the observed pathological mechanical stimulus of low WSS resulting from LHF to the observed molecular changes that result in the pathological vascular remodeling observed in the transition from Ipc-PH to Cpc-PH.

Both chronically impaired NO production and ECM remodeling driven by chronically low WSS in PH-LHF result in increased arterial stiffness. Arterial stiffening ultimately causes key hemodynamic changes that are the hallmark of Cpc-PH, such as decreased PAC [17]. Reduced compliance in the arterial compartment results in highly pulsatile flow downstream in pulmonary arterioles, which further stimulates EC

dysregulation and SMC hypertrophy, ultimately driving the cycle of disease progression (Figure 1). In a vascular mimetic co-culture model with PAECs and SMCs, high pulsatility flow increased SMC size and elevated expression of contractile proteins, such as smooth muscle actin (SMA) and SM-MHC [113]. Moreover, high pulsatility flow reduced eNOS expression and increased ET-1, angiotensin converting enzyme (ACE), and transforming growth factor (TGF-β1), all of which have been associated with SMC hypertrophy and vasoconstriction [113] that drive increased PVR, the pathological development marking the transition from Ipc-PH to Cpc-PH.

Role of Biomechanics in Transition to Cpc-PH and RVF

The presence of pulmonary arterial remodeling is the defining characteristic of Cpc-PH. Irreversible pulmonary arterial muscularization, diffuse vascular fibrosis, and distal arterial luminal narrowing characterize Cpc-PH [16]. Multiple animal studies have demonstrated that these structural features are associated with the functional change -- elevation in PVR -- in Cpc-PH [114,115]. As chronic, elevated pulmonary pressure and decreased flow change capillary and venous mechanical properties downstream, these same altered biomechanical factors can induce further pulmonary arterial remodeling. Because the structural and functional changes that occur in the pulmonary vasculature with Cpc-PH are similar to those that occur with PAH, computational simulations of pulmonary vascular blood flow dynamics in PAH subjects have been used to shed light on the impact of altered biomechanics in Cpc-PH subjects. Using a combined MRI-computational fluid dynamics approach, Tang and colleagues demonstrated a profound reduction in WSS by a factor of six in the proximal and distal pulmonary arteries of

subjects with PAH compared to control subjects [116]. Similarly, a significant inverse relationship was found among WSS, mPAP, and PVR, and a significant positive relationship was found between WSS and capacitance in the main PA in subjects with PH [90]. Extensive muscularization of proximal and distal PAs associated with increased PVR and TPG has been confirmed in post-mortem human studies [114]. Thus, once Ipc-PH occurs, pressures increase in the pulmonary arteries, leading to dilation and decreased shear stress. Subsequent remodeling increases PVR and lowers compliance, which in turn alters hemodynamics downstream and promotes a vicious cycle of disease progression (Figure 1).

The irreversible pulmonary arterial remodeling that marks the transition to Cpc-PH is associated with and defined by PVR. In the setting of LHF, increased PVR dramatically increases risk of RV failure and mortality [117]. In a clinical study measuring RV function and mortality in Ipc-PH and Cpc-PH subjects, the prevalence of RV enlargement, RV dysfunction, and all cause-mortality increased with higher PVR [118]. Moreover, reduced PAC contributes to RV decline [17,119]. As the clinical outcomes of subjects with Cpc-PH depend on RV function, understanding the mechanisms by which mechanotransduction drives the transition from Ipc-PH to Cpc-PH is critical in determining therapeutic targets.

Therapeutic Implications

Currently, there are no FDA approved pharmacotherapies in the clinician's armamentarium for Cpc-PH. Therapeutic strategies beyond treatment of LHF are limited and largely have consisted of trials of pharmacologics developed for PAH, the vast majority of which target the altered biomechanics of PH. Current clinical management of

combined pre-/post-capillary pulmonary hypertension involves the use of vasodilators, diuretics, ACEI, ARBs, and PDE5 inhibitors. PAH drugs, including endothelin-1 antagonists, have shown variable effectiveness due to their potent systemic effects, which are generally not tolerated in the setting of LHF [120,121]. The purpose of current treatments are to relieve dyspnea, improve exercise capacity, and define eligibility for heart transplantation [122]. Targeting the mechanotransduction pathways in PH-LHF is a novel and potentially powerful therapeutic strategy that could disrupt a critical link in the transition from Ipc-PH to Cpc-PH. Thus, biomechanical and mechanobiological mechanisms of disease progression and their transduction pathways should be future targets for Cpc-PH therapies.

Bridging the Knowledge Gaps: Directions for future work

Here we have reviewed the known and hypothesized altered biomechanical forces in pulmonary veins, capillaries and arteries that occur due to Ipc-PH and the mechanobiological mechanisms that may drive transition to Cpc-PH and subsequent RHF. There is an urgent clinical need for improved understanding of this disease pathophysiology and progression as well as for novel therapeutic interventions to improve patient outcomes. As highlighted in this review, critical knowledge gaps remain both in our understanding of pulmonary biomechanics in PH-LHF as well as in mechanotransduction of these signals in the context of the three pulmonary vascular compartments.

Robust clinical and animal studies that quantify the mechanical forces acting on ECs and SMCs in both Ipc-PH and Cpc-PH are integral to understanding the WSS and

cyclic stretch distribution in Ipc-PH and transition to Cpc-PH. Invasive pressure measurements coupled with non-invasive flow and anatomic imaging with sufficient resolution will enable computation or estimation of these mechanical stimuli. In turn, knowledge of these mechanical stimuli will facilitate high quality, impactful *in vitro* mechanistic studies that clarify the mechanotransduction pathways in this disease process.

Critically, these mechanistic studies should be performed in pulmonary cell types. Research to date has been concentrated in systemic vascular-derived cell lines with mechanical stimuli that model disturbed and oscillatory flow conditions that drive atherosclerosis but are not relevant to either Ipc-PH or Cpc-PH. Beyond *in vitro* studies modeling physiological and pathological WSS or cyclic stretch in pulmonary vascular cells, both stresses need to be applied simultaneously. A limited number of studies have evaluated the impact of combined WSS and cyclic stretch. One such study, performed with systemic artery-derived cells, demonstrated potentiation of some mechanotransduction responses and inhibition of others, highlighting the need to study both mechanical forces together [123]. Importantly, future studies should also consider the effects of the biological environment, including sex, sex hormone status, age, and systemic diseases such as metabolic syndrome and diabetes on mechanotransduction.

Indeed, *in vitro* studies that interrogate the intersections between known risk factors and pulmonary vascular cell mechanotransduction will accelerate research breakthroughs. We posit that patient-specific factors such as sex, genetics, and comorbidities impact cellular mechanotransduction and may be key to outcomes and responses to treatment. Women are known to be at higher risk of developing PAH than

men [124] although in other contexts estrogen is considered vasculo-protective [125,126]. While sex differences in mechanotransduction have not been identified in vascular and EC and SMC, estrogen has been shown to affect mechanotransduction in bone cell networks [127]. In terms of genetics, CAV1, a gene responsible for encoding caveolin-1 protein, regulates mechanotransduction of vascular shear stress [128]. in pulmonary vascular ECs and has been shown to be dysfunctional in PH [129]. CAV1 mutations are also associated with lipid disorders such as type 2 diabetes [130], which itself has been shown to be an independent risk factor for PH [131]. Previous studies have shown that diabetes may cause defects in the mechanotransduction of arterial SMCs via alterations in ECM composition which lead to increased stiffness and decreased arteriolar compliance [132]. Other comorbidities of Cpc-PH such as obesity and age have also been shown to alter mechanotransduction in EC and SMC [133,134]. Thus, sex and its consequences for sex steroid hormones, certain genetic mutations, obesity, and age may modulate mechanotransduction and thereby drive the transition from Ipc-PH to Cpc-PH. These relationships warrant further investigation as both mechanisms of disease progression and potential therapeutic targets.

Conclusion

PH-LHF alters pulmonary vascular biomechanical forces resulting in increased cyclic stretch and decreased WSS, which may drive transition from Ipc-PH to Cpc-PH. These mechanical stimuli and their biological consequences need further investigation to identify targetable mechanisms to prevent progression of this disease. Understanding the

mechanisms of mechanotransduction in the pulmonary circulation will deepen our understanding of Ipc-PH and Cpc-PH and could open doors to new pharmacologic therapies.

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Tables

Table 1: Cyclic Stretch in Pulmonary Vascular EC, SMC, and Fibroblasts

Cell Type	Stimulus vs. Static	Mechanotransduction	Biological Response	Reference
Bovine PAEC and PASMC	20% cyclic stretch at 1 Hz for 0-24h	VE-cadherin and Rac1 dependent EC proliferation. RhoA kinase dependent SMC proliferation	Vessel Remodeling	Liu, 2007 [72]
Bovine PAEC	25% cyclic stretch at 0.25Hz for 24h	Mitochondrial complex III stimulated increase in ROS leading to increased FAK activation	Angiogenesis	Ali, 2006 [66]
Human PMVEC	20% cyclic stretch at 0.83 Hz for 24h	Increase of IL-8 synthesis and release via p38 activation	Inflammation	Iwaki, 2009 [63]
Human PMVEC	18% cyclic stretch at 0.5Hz for 4 days	Increased MMP 2, 14, increased activity of TIMP2	ECM Remodeling	Haseneen, 2003 [53]
Rabbit PASMC	15% cyclic stretch at 1Hz for 24h	Increased tyrosine kinase phosphorylation of FAK leading to increased PDGF and PDGF-R expression	SMC Proliferation	Tanabe, 2000 [74]
Ovine PASMC	5-25% cyclic stretch at 1Hz 48h	Increased VEGF and Fibroblast Growth Factor-2	SMC Proliferation/Angiogenesis	Quinn, 2002 [75]
Ovine PASMC	20% cyclic stretch at 1 Hz for 8h	Increased TGFβ lead to NADPH oxidase and ROS dependent increase in VEGF	Angiogenesis	Mata-Greenwood, 2005 [73]

Ovine PASMC	15% cyclic stretch at 1Hz for 24h	Increased ROS via NOX4	SMC Proliferation and Migration	Wedgwood, 2015 [77]
Rat PAAF	10% equibiaxial static stretch for 24h	Increased myofibroblast differentiation and increased *Col1a1, Col3a1, Eln*	Vessel Remodeling	Wang 2021[85]
Human Lung Fibroblasts	20% cyclic stretch at 1Hz for 30 min	Increased intracellular Ca^{2+}, increased production of ROS leading to NF-κB activation and increased COX-2	Inflammation	Amma 2005[92]

Table 2: Shear Stress in Pulmonary Vascular EC

Cell Type	**Stimulus vs Control**	**Mechanotransduction**	**Biological Response**	**Reference**
Physiological Shear Stress				
Human PAEC	3-8 dyn/cm^2 vs static for 10min	Caveolin mediated mitochondrial ATP generation	EC Homeostasis	Yamamoto, 2018 [92]
Bovine and Human PAEC	10 dyn/cm^2 vs static for 24h	Rac/PAK dependent myosin light chain phosphorylation and actin polymerization	EC Cytoskeleton Rearrangement	Birukov, 2002 [93]
Ovine PAED	20 dyn/cm^2 vs static for 8h	Akt dependent eNOS phosphorylation and NO production	Vasodilation	Wedgwood, 2003 [91]
Low Shear Stress				
Bovine PAECs	5 vs 20-60 dyn/cm^2 for 20h	Reduced eNOS phosphorylation NO, PGF1α, and VEGF, increased ET-1, F-actin and VE-cadherin rearrangement	Vasoconstriction and cytoskeleton rearrangement	Li, 2009 [88]

Mouse PMVEC	Loss of flow vs 5 dyn/cm^2 for 1h	NADPH oxidase dependent ROS production	Inflammation and Angiogenesis	Milovanova, 2006 [106]
Mouse PMVEC	Loss of flow vs 5 dyn/cm^2 for 1h	PECAM-1 dependent ROS production and proliferation	Inflammation and Angiogenesis	Noel, 2013 [105]
High Shear Stress				
Ovine PAEC	30-100 dyn/cm^2 vs 5-20 dyn/cm^2 for 4h	Catalase inhibition increasing ROS, akt mediated eNOS phosphorylation increasing NO production	Inflammation and vasodilation	Kumar, 2010 [98]

Figures

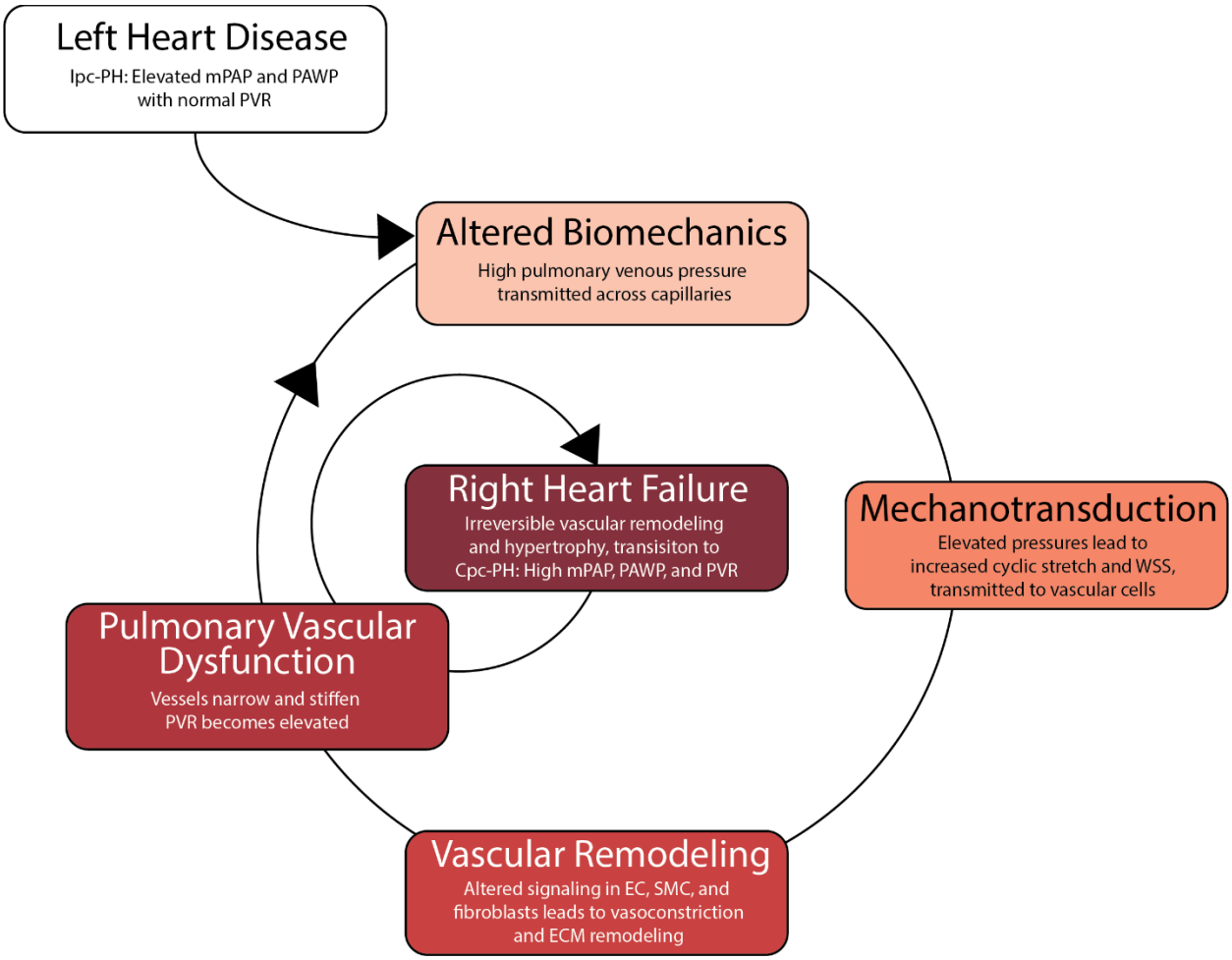

Figure 1: Mechanotransduction is the key step in the pathophysiologic progression of pulmonary vascular disease in the setting of left heart failure. The transition from isolated post-capillary pulmonary hypertension to combined pre-/post-capillary pulmonary hypertension is characterized by pulmonary vascular remodeling and results in right heart failure.

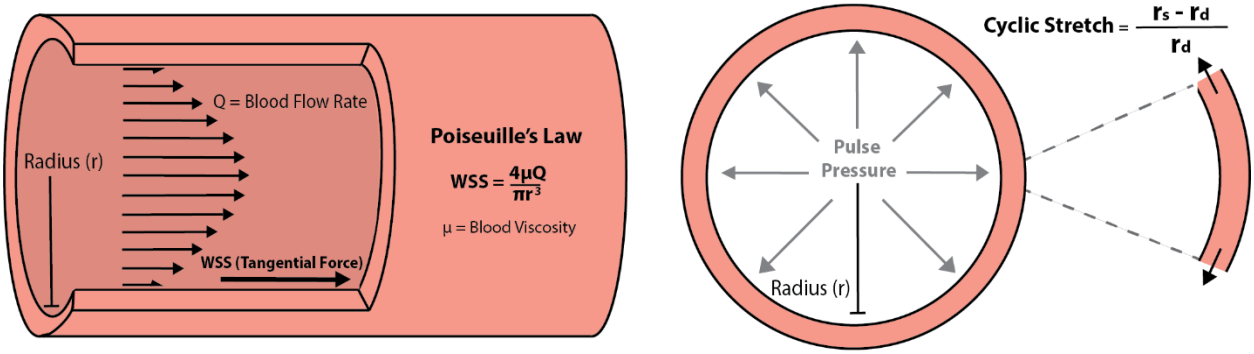

Figure 2: Schematic representation of WSS and cyclic stretch in a vessel. WSS is directly proportional to blood flow rate (Q) and blood viscosity (μ) and is inversely proportional to the radius of the vessel lumen (r). Cyclic stretch is the difference between lumen radius at systole (r_s) and the lumen radius at diastole (r_d) normalized by the radius at diastole.

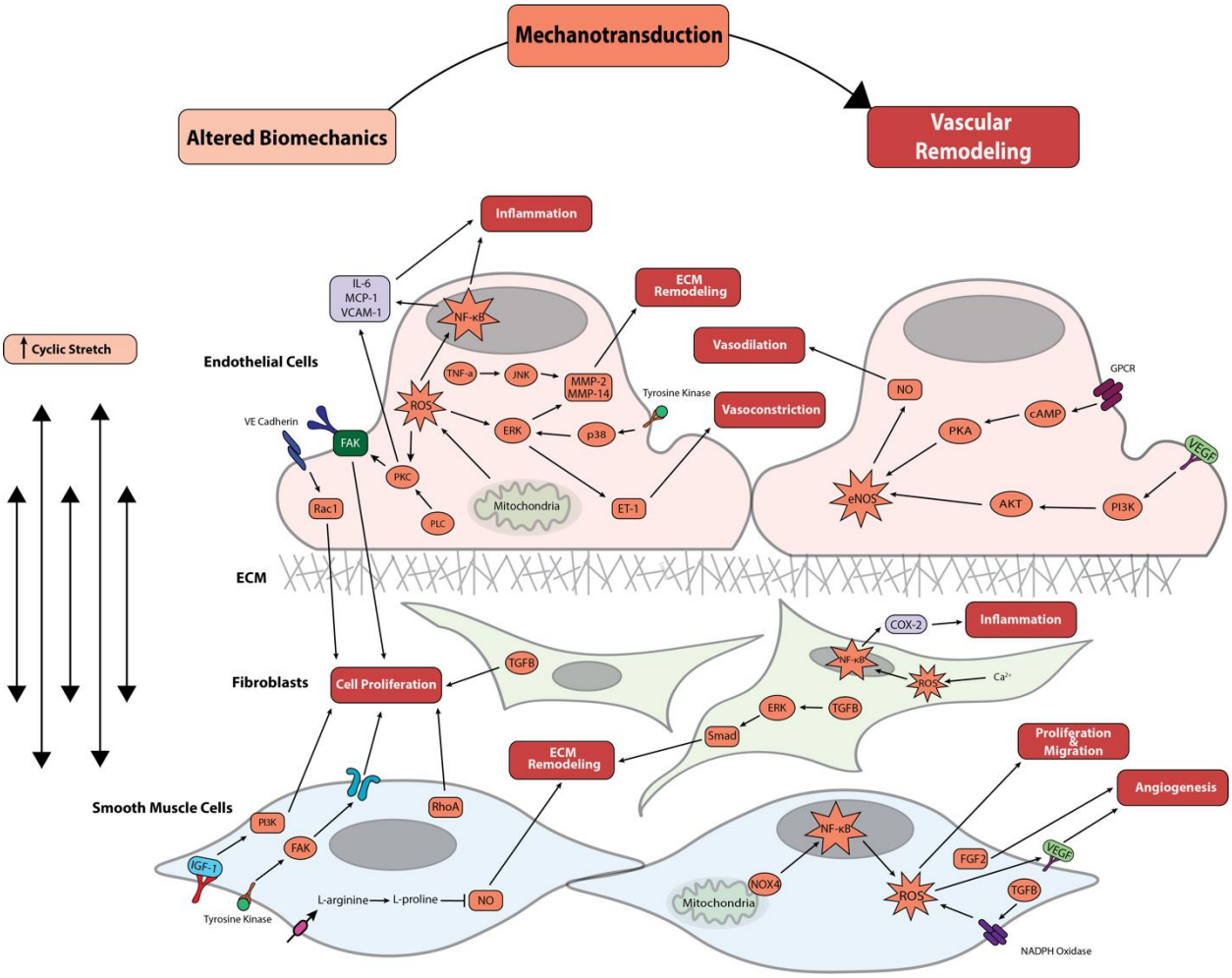

Figure 3: Mechanotransduction of increased cyclic stretch due to left heart failure in endothelial cells, fibroblasts, and smooth muscle cells triggers a cascade of pathologic vascular remodeling. Boxes within the cell signaling cascade are colored to match their corresponding box at the top of the figure (Altered Biomechanics, Mechanotransduction, or Vascular Remodeling).

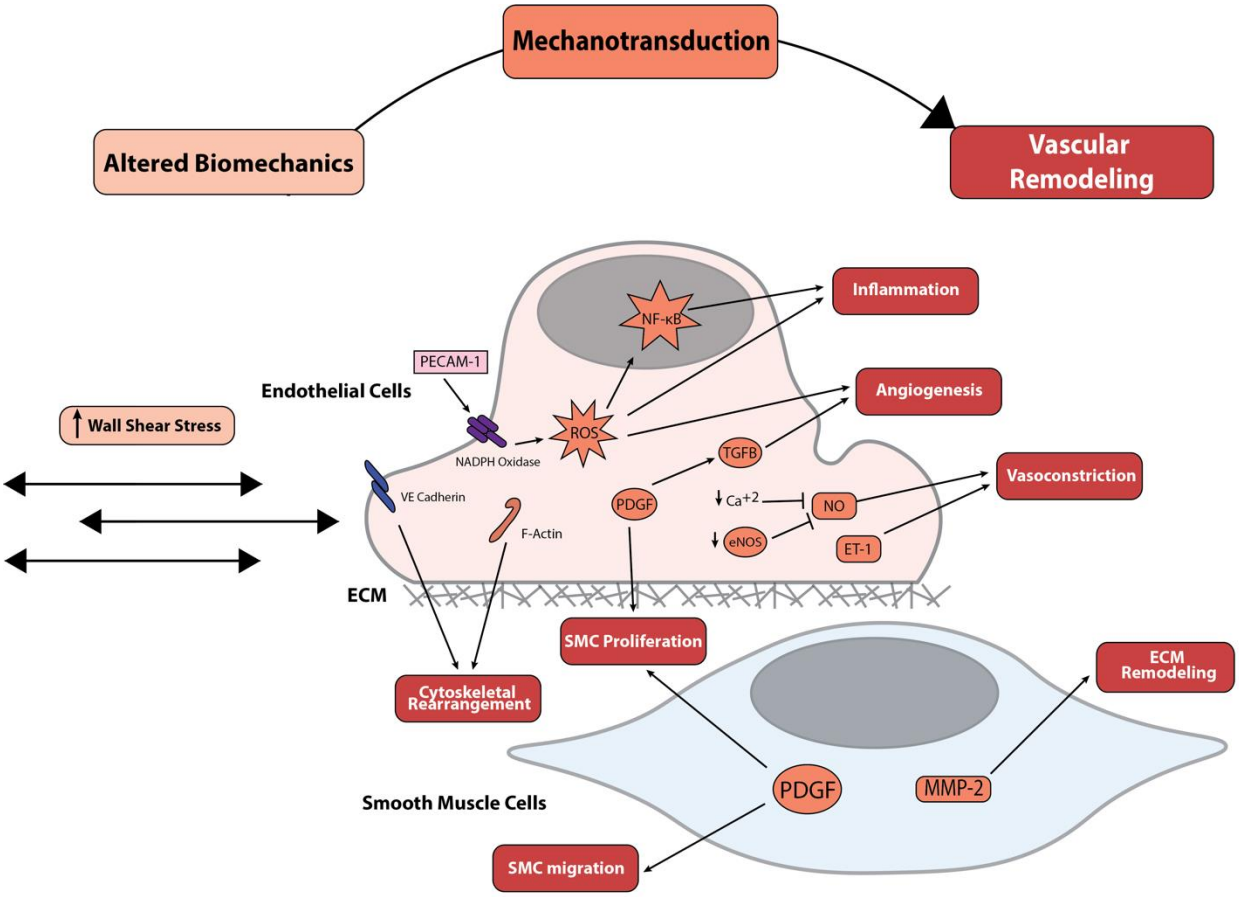

Figure 4: Mechanotransduction of low wall shear stress due to left heart failure in endothelial cells and smooth muscle cells results in pathologic vascular remodeling throughout the vessel wall. Boxes within the cell signaling cascade are colored to match their corresponding box at the top of the figure (Altered Biomechanics, Mechanotransduction, or Vascular Remodeling).