Writing Assignment: Literature Review

The Role of Mechanical Load in Cardiovascular Development, Health, and Disease: Current Knowledge and Future Perspectives

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Abstract

Mechanical load is a critical regulator of cardiac structure and function under both physiological and pathological conditions. Mechanotransduction pathways play a critical role to mediate mechanical load-induced myocardial remodeling, whereby the morphology and function of cardiomyocytes and the extracellular matrix are altered, resulting in changes in cardiac function. Mechanical loading not only governs the function of the adult heart but is also responsible for normal morphogenesis of the cardiovascular system during prenatal development. In this review, the role of mechanical load in development, health, and disease are highlighted. First, how mechanical load governs the development of the heart from a primitive heart tube to a functional organ, its role in the maturation of the vasculature, as well as the mechanisms which control load-induced cardiac alterations in the adult heart during exercise and pregnancy are discussed. Subsequently, the mechanisms that contribute to mechanical load-induced pathologic cardiac remodeling are underlined. Mechanical overloading of the heart due to pressure or volume cause different alterations in cardiac structure and function which lead to heart failure. These differences translate to differences in disease phenotype that are reflected on pressure-volume loops. Next, the therapeutic interventions for mechanical load-induced heart failure are explored with a focus on mechanical unloading by left ventricular assist devices. Insights into the various types and function of these devices, as well as the mechanical unloading-induced mechanisms that contribute to reverse remodeling are provided. Finally, the current *in vivo* and *in vitro* models of mechanical loading and unloading are presented. These models have allowed for various discoveries in the field of cardiac mechanobiology. However, although significant advances have been made in our knowledge of the function of cardiac mechanical load, several mechanisms underlying cardiac mechanical properties, load-induced pathologic remodeling, and unloading-induced reverse remodeling remain unexplored. Thus, future advancements of *in vivo* and *in vitro* models are necessary to fill the gaps in our current understanding of myocardial mechanical properties, mechanotransduction, and reverse remodeling.

Layman's Summary

The heart is governed by various mechanical forces that can alter the structure and function of the heart. Changes in mechanical load can activate several genes through signaling pathways to alter the shape and size of cardiomyocytes and influence the activity of fibroblasts. These changes lead to physiological or pathological adaptation of the heart which can have beneficial or deleterious effects on the body. In addition, to governing the function of adult heart, mechanical load contributes to normal heart formation during prenatal development. This review aims to highlight the role of mechanical load in cardiac development, health, and disease, as well as summarize the different experimental models that are currently utilized to mimic mechanical load conditions observed in humans. Mechanical loading is responsible for the activation of several genes that control the development of the heart from a primitive tube to a functional organ. Furthermore, it plays a role in the development of the vascular system. During adulthood, changes in mechanical load due to exercise or pregnancy also activate certain pathways which lead to changes in structure of the heart that enhance its function. However, mechanical load can lead to pathological alterations of cardiac structure and function as well. Similarly, the persistent increased mechanical load due to pressure or volume activates pathways which contribute to maladaptive changes of the heart and eventually lead to heart failure. Several therapies are present to treat heart failure through mechanical unloading. Left ventricle assist devices are currently used to unload the heart. These devices induce reverse remodeling by reducing the activation of pathways involved in pathologic remodeling and activating other pathways that counteract the maladaptive processes. The knowledge of the role of mechanical load in health and disease would not have been possible without the current experimental models of mechanical load. However, several shortcomings in our understanding of mechanical load persist. Therefore, further developments in experimental models are required to increase our understanding of the mechanisms that underly mechanical load-induced remodeling, unloading-induced reverse remodeling, and mechanical properties of the heart.

Introduction to Mechanical Loading of the Heart

Cardiac mechanical load is an underlying cause of several cardiovascular complications and is responsible for cardiac morphogenesis during development. Changes in mechanical load, such as in hypertension, can lead to valvular heart disease, arrhythmias, atrial fibrillation, and cardiac hypertrophy (Kjeldsen, 2018). In addition, mechanical load can induce mechanosensitive pathway activation during cardiac morphogenesis to allow normal formation of the cardiovascular system during prenatal development (Boselli, Freund, & Vermot, 2015).

The heart is the first functional organ to form during mammalian embryogenesis, circulating nutrients to the surrounding tissue to promote growth (Lindsey, Butcher, & Yalcin, 2014). The embryo grows rapidly, increasing its metabolic demands, as well as producing an increased amount of waste. Therefore, a functional circulatory system is important to secrete the waste and distribute the oxygen and nutrients from the placenta throughout the growing embryo (Lindsey, Butcher, & Yalcin, 2014). As the embryo grows into a fetus the metabolic demand further increases and thus the heart grows from a primary heart tube to a four-chambered heart (Andrés-Delgado & Mercader, 2016). Early embryonic cardiac contractions cause blood pressure and wall shear stress to build up, and these hemodynamic loads govern normal cardiac morphogenesis by activating gene networks through mechanotransduction feedback loops (Midgett & Rugonyi, 2014; Boselli, Freund, & Vermot, 2015). The fetal heart is both structurally and functionally different from the neonatal and adult hearts, as the fetus relies on the placenta for gas exchange. Therefore, blood is shunted away from the lungs to prevent them from developing prematurely (Tan & Lewandowski, 2020).

However, after birth, a complex series of biochemical and structural modifications occur to functionally modify or eliminate the cardiovascular fetal shunts and transition to a circulation that utilizes the lungs as the site for gas exchange (Tan & Lewandowski, 2020). The structural changes of the fetal shunts are driven by changes in hemodynamic loads upon birth. Cutting the umbilical cord increases systemic vascular resistance (afterload) as the low-resistance placental flow becomes eliminated and leads to the closure of fetal shunts (Tan & Lewandowski, 2020; Cavaliere, 2016). As the lungs become incorporated in circulation, both ventricles carry out their specialized functions, the right ventricle pumps deoxygenated blood to the lungs to become oxygenated, and the left ventricle pumps the oxygenated blood to the body (Hayashi, et al., 1996). The cardiac cycle can be divided into two phases: diastole, a phase of relaxation and filling, and systole, a phase of contraction (Voorhees & Han, 2015). During diastole when the ventricles fill with blood the cardiac myocytes become stretched. The amount the cells stretch at the end of diastole is referred to as preload. During systole, however, cardiomyocytes work against a certain amount of load to eject blood into the body. This load is called afterload (Schotola, et al., 2017). Preload is determined by the volume of blood that enters the ventricle while afterload is determined by the amount of pressure the ventricle must overcome to pump blood (Voorhees & Han, 2015). Both preload and afterload are affected by hemodynamic changes, such that an increase in vascular resistance increases the pressure the ventricle has to overcome and thus increases the afterload of the heart (Schotola, et al., 2017). The ventricles interact with the peripheral circulation and adapt to

the hemodynamic changes to ensure blood flow compatible with metabolic requirements for survival. These hemodynamic changes can arise due to normal physiological processes such as development, exercise, and pregnancy or pathological processes such as obstruction of blood flow, valvular insufficiency, and myocardial infarction (MI) (Hayashi, et al., 1996).

Changes in preload, afterload, as well as contractility of cardiomyocytes affect the stroke volume (SV), which is a major determinant of cardiac output (CO) (Voorhees & Han, 2015). CO is defined as the volume of blood the heart pumps per minute and is represented by the multiplication of SV with heart rate (HR) (Voorhees & Han, 2015). In normal physiological conditions when the body's metabolic demand increases, such as during exercise, the heart increases the heart rate and the stroke volume, through increased preload, to compensate for this demand. This adaptation does not influence the structure of the heart. However, chronic changes in preload or afterload can lead to either physiological or pathological remodeling, such that physiologic remodeling is adaptive and leads to improved cardiac function, whereas pathologic remodeling is maladaptive causing cardiomyocyte death and cardiac dysfunction (Pitoulis & Terracciano, 2020). A chronic increase in afterload, caused by aortic stenosis or hypertension, places the heart in a diseased state, wherein the heart becomes overloaded with pressure. This chronic pressure overload (PO) leads to pathologic remodeling of the heart, specifically it results in concentric hypertrophy, which is described by an increase in ventricular wall thickness with little or no chamber dilation (Hartmann, et al., 2022). However, in a chronic state of increased preload, the heart becomes overloaded with volume, and this volume overload (VO) leads to a form of remodeling different from that of PO. VO-driven hypertrophy has an eccentric pattern, such that a slight increase in wall thickness is observed. However, the chamber becomes disproportionally dilated (Hartmann, et al., 2022). In addition to chronic changes in mechanical load, cardiac remodeling can be observed when acute damage occurs to the myocardium, such as during myocardial infarction (MI). During a MI the myocardium becomes permanently damaged, as the heart has a limited ability to regenerate, often not enough to compensate for the acquired damage. Therefore, instead, the heart can alter its phenotype and adapt to changes in its environment. This feature of cardiac plasticity involves mechanical, structural, electrical, and metabolic modifications which help compensate for the damaged myocardium (Pitoulis & Terracciano, 2020). Following MI, a greater strain is placed on the undamaged myocardium which leads to an increase in metabolic demand of the surviving cardiomyocytes. Due to the irreversibility of MI-induced myocardial damage, this increase in metabolic demand becomes chronic, and thus the heart alters its function and structure to compensate for this demand (Pitoulis & Terracciano, 2020).

The process of cardiac remodeling is complex and dependent on multiple factors. The main drivers of remodeling are mechanical load (Pitoulis, et al., 2021), neurohormonal changes (Hartupee & Mann, 2016), inflammation (Anzai, 2018), and several autocrine and paracrine factors (Hodgkinson, Bareja, Gomez, & Dzau, 2016; Segers & Keulenaer, 2021). Although all these aspects stimulate cardiac remodeling, the most important initial stimulus is a change in mechanical load (Zou, et al., 2002). These factors communicate and directly or indirectly influence each other (Pitoulis & Terracciano, 2020). An increase in mechanical load can lead to increased local secretion of neurohormones from cardiomyocytes, fibroblasts, endothelial

cells, and smooth muscle cells of the heart (Sadoshima & Izumo, 1997). One example of such a neurohormone is angiotensin II, which is found to be partly stored in secretory granule-like structures in ventricular cardiomyocytes (Sadoshima & Izumo, 1997). Upon increase in mechanical load, the increased stretching of cardiomyocytes causes autocrine secretion of angiotensin II and activates intracellular signaling pathways which upregulate the expression of proteins involved in the cardiac renin-angiotensin system (Malhotra, Sadoshima, BrosiusIII, & Izumo, 1999). Multiple protein kinases are then activated by angiotensin II which leads to downstream activation of genes, increase in protein synthesis, and induction of cardiac remodeling (Zou, et al., 2002). Other neurohormones influenced by the mechanical load which further promote remodeling include norepinephrine and endothelin-1 (ET-1) (Briest, et al., 2001; Drawnel, Archer, & Roderick, 2013). The mechanical load can also activate protein kinases in a neurohormone-independent manner (Zou, et al., 2002). Thus, highlighting the importance of mechanical load as a potent driver of cardiac remodeling.

Cardiac remodeling is a phenomenon that is observed in multiple cardiovascular complications in addition to MI, such as dilated cardiomyopathy and heart failure. However, several studies investigating these diseases do not utilize protocols to simulate physiological or pathological mechanical load in their models; whereas the studies which do consider mechanical load-driven cardiac remodeling have several limitations in their models (Pitoulis & Terracciano, 2020). These shortcomings include the use of oversimplified *in vitro* assays and models with low experimental throughput and unverified relevance to the adult myocardium. Moreover, the current protocols which simulate mechanical load fail to reflect the complexity of *in vivo* mechanics of the heart (Pitoulis & Terracciano, 2020).

The aim of this review is to increase our understanding of mechanical load in physiological and pathological states, underline potentially novel solutions to pathologic cardiac remodeling through mechanical unloading, and highlight the advantages and disadvantages of the current mechanical loading models to uncover what is unkown in the field of cardiac mechanical load and extrapolate the findings from the literature to the situation of patients and allow the provision of personalized treatment. Thus, the role of mechanical load in development, health, and disease, mechanical unloading of the heart, the mechanotransduction mechanisms of pathologic remodeling and reverse remodeling, the different *in vivo* and *in vitro* models of mechanical load, as well as the gaps in our understanding of the function of mechanical load are discussed.

Mechanical Loading in Development and Health

Mechanical load is an essential driver of cardiovascular system development during embryogenesis. Several observations studying chick embryos show that cardiac contractions and blood flow begin before the circulation of oxygen and nutrients are required to meet the metabolic needs of embryonic tissue (Granados-Riveron & Brook, 2012). This suggests that the mechanical forces generated by blood flow are necessary to drive the normal development of the heart from an early stage. The heart undergoes morphological alterations as the embryo grows. This can be partly attributed to changes in the mechanical load of the primitive heart which arise due to an increase in metabolic demand of the rest of the embryo (Lindsey, Butcher, & Yalcin, 2014). The major morphological changes are separated into four

events: heart tube formation, looping, trabeculation, and valve formation/septation (Lindsey, Butcher, & Yalcin, 2014). During the initial stages of heart formation the primary heart fields of the mesoderm fuse together to form the tubular heart which is made up of an inner layer of endocardial cells and an outer layer of contractile myocytes, separated by an elastic acellular layer known as cardiac jelly (Lindsey, Butcher, & Yalcin, 2014; Boselli, Freund, & Vermot, 2015). Myocardial contractions can already be observed at this stage. However, they are uncoordinated and irregular (Andrés-Delgado & Mercader, 2016). Following tube formation, looping occurs, such that the primitive heart starts to take shape. Looping is initiated by expansion and elongation of the heart tube. Cardiac progenitor cells from the mesodermal second heart field move to both ends of the tube and proliferate, contributing to the development of the outflow tract, right ventricle, and interventricular septum at the arterial pole, as well as atria and atrial septum at the venous pole (Lindsey, Butcher, & Yalcin, 2014). Looping can be divided into three stages: c-looping, primitive s-loop formation, and mature s-loop formation. During c-looping, the heart tube bends outward from the body and twists to the right forming the primitive atrium, ventricle and outflow tract, while during s-looping the walls thicken and protrude inside the primitive atrioventricular canal (AVC) and outflow tract, forming endocardial cushions which act as valves by shutting off the lumen with every contraction, and allowing for unidirectional rhythmic blood flow (Lindsey, Butcher, & Yalcin, 2014; Taber, 2001). Trabeculation occurs after looping. During this stage, endocardial extensions grow to form a network of projections called trabeculae, which consist of myocardial cells enclosed by an endocardial layer (Tan & Lewandowski, 2020). This greatly increases myocardial mass, surface area, and wall stiffness, which in turn increases CO, contractility, and conductivity (Tan & Lewandowski, 2020). Finally, the four-chambered heart forms when the trabeculae undergo compaction, which, along with the fusion of endocardial cushions, leads to septation and valve formation (Lindsey, Butcher, & Yalcin, 2014).

All these developmental stages are influenced by the mechanical forces generated by blood flow (Andrés-Delgado & Mercader, 2016). The flow of blood through the heart chambers exerts a force parallel to the walls called shear stress. Moreover, the change in blood flow during a cardiac cycle alters the stress the cardiac cells are being subjected to. This strain is called mechanical loading and it is affected by the viscosity of blood (Andrés-Delgado & Mercader, 2016). These hemodynamic forces activate genes through mechanotransduction feedback loops and govern normal cardiac morphogenesis (Boselli, Freund, & Vermot, 2015). In zebra fish, as the beating heart tube loops to form the primitive atrium, ventricle, and AVC, the contraction timing and wave pattern change as the contraction significantly slows along the AVC and ventricle. This change, as well as the change in geometry of the tube, leads to an oscillatory flow of blood within the heart, such that blood is pushed back into the atrium by the contracting ventricle. This reversal in flow generates distinct hemodynamic stresses on the cells of the endocardial layer, which are essential for valve development. Transcription factor KLF2a is activated by flow reversal increasing shear stress. Studies have shown that the mechanotransduction pathway for this activation involves a series of phosphorylation steps ending with phosphorylation of class II histone deacetylase HDAC5 by protein kinase D2. Upon phosphorylation of HDAC5, its gene repression activity on KLF2a is inactivated and thus KLF2a is expressed (Lee, et al., 2006). This flow-dependent transcription factor was also found to be

essential in mice, as a knockdown of KLF2 in mice leads to valve deformation (Lee, et al., 2006; Chiplunkar, et al., 2013). A study by Chiplunkar *et al*. (2013) showed that KLF2 in mice plays a significant role in AVC endocardial cushion endothelial-to-mesenchymal transformation, synthesis of cardiac jelly, and atrial septation through the modulation of several cardiac genes, such as SOX9, TBX5, and GATA4. Indicating that KLF2 is not only specific for normal valve development, but has diverse roles in the progression of cardiac development. microRNAs (miRs) have also been observed to play a role in normal cardiac development. In zebrafish, hemodynamic forces were shown to govern miR-21 expression which was localized in areas of increased shear stress, such as at constrictions of AVC and outflow tract (Banjo, et al., 2013). The findings identified miR-21 as a crucial member of a flow-dependent pathway that regulates endocardial cell proliferation and gene expression required for valve development (Banjo, et al., 2013). Similarly, miR-143 was discovered to play a role in normal cardiogenesis (Miyasaka, et al., 2011). Flow-dependent expression of miR-143 was shown to downregulate retinoic acid activity in the endocardium of embryonic ventricles, and indirectly regulate the formation of ventricles (Miyasaka, et al., 2011). These findings demonstrate that in addition to transcription factor activation, miRs are pivotal for normal cardiac morphogenesis.

As the embryo grows mechanical loading is not only essential for the development of the heart itself but also the vasculature. As the developing heart pumps blood the vessels are constantly subjected to mechanical load which causes endothelial shear stress and circumferential wall stress (Lu & Kassab, 2011). These mechanical forces trigger biological and biochemical events which drive embryonic vasculature development through vascular remodeling, angiogenesis, and maintenance of vessel identity (Lu & Kassab, 2011; Roman & Pekkan, 2012). Initially, endothelial cells (ECs) from the mesoderm aggregate and fuse together to form cord-like structures. This process of vasculogenesis is not dependent on blood flow and is governed by neural guidance genes (le Noble, Klein, Tintu, Pries, & Buschmann, 2008; Roman & Pekkan, 2012). These cords connect to form a primitive network of vessels, which are not yet hollow (Roman & Pekkan, 2012). Endothelial tip cells at the ends of these primitive vessels are lined with receptors vascular endothelial growth factor receptor 2 (VEGF-R2) and UNC5B. These receptors sense guidance cues present in the surrounding and mediate the process of angiogenesis, guiding angiogenic sprouts through the ECM and establishing their final positions (le Noble, Klein, Tintu, Pries, & Buschmann, 2008). At later stages of development blood flow also plays a role in this angiogenic process, mostly by dictating the location of sprout initiation (Campinho, Vilfan, & Vermot, 2020). Specifically, an *in vitro* study revealed that in areas with high laminar shear stress sprouting is inhibited, whereas transvascular and intralaminar flow promotes sprouting (Akbari, Spychalski, Rangharajan, Prakash, & Song, 2019). Once the primitive vessels have formed they must differentiate into arteries or veins to facilitate proper circulation. Both genetic and epigenetic factors play a role in forming the vessel identity. Blood vessels that are predestined to form arteries or veins contain ECs which are genetically labeled with specific markers. For example, zebrafish, mouse and chicken arterial ECs express ephrin-B2, neuropilin-1 (NRP-1), and notch pathway members notch3, gridlock, and DLL4; whereas venous ECs express EphB4, NRP-2, and Coup-TFII. However, it was observed that these genetic markers alone can not lead to

vessel differentiation but hemodynamic forces are essential in facilitating this differentiation, though it is unclear as to which forces are responsible for this (le Noble, Klein, Tintu, Pries, & Buschmann, 2008). After the differentiation of vessels, blood flow controls the patterning and remodeling of the vasculature (le Noble, Klein, Tintu, Pries, & Buschmann, 2008; Campinho, Vilfan, & Vermot, 2020). The plastic nature of vascular ECs aids in this process. Hemodynamic forces can bring about vascular remodeling by influencing the diameter of the primitive blood vessels. A study investigating the fluid dynamics of pharyngeal arch artery development in chick embryos suggested that shear stress is the main driver of arterial growth, such that an increase in shear stress leads to an increase in vessel caliber (Lindsey, Butcher, & Vignon-Clementel, 2018; Hoefer, Adel, & Daemen, 2013). Similarly, shear stress was implicated to be the main force driving vascular remodeling in the mouse yolk sac, as a reduction in blood viscosity, a major determinant of shear stress, leads to impaired yolk sac vasculature remodeling (Lucitti, et al., 2007). Thus, two distinct methods were observed which explain how blood flow and shear stress lead to vessel caliber increase in the mouse yolk sac. In areas of high flow, neighboring vessels fuse to form larger arteries. Subsequently, ECs migrate towards the newly formed larger arteries and proliferate to support vessel growth (Udan, Vadakkan, & Dickinson, 2013). However, the mechanistic details of EC migration are not understood. Baeyens *et al*. (2015) propose that VEGFR3 regulates the response of these ECs to shear stress, such that above a certain threshold ECs promote vessel enlargement. This was supported by the observation that a decrease in the expression of VEGFR3 in zebrafish embryos leads to a decrease in vessel diameter. However, it is very likely that other flow-dependent genes can influence vessel diameter as well, though they are not identified yet (Baeyens, et al., 2015).

In addition to development, mechanical loading is important in regulating CO during increased metabolic demand in normal physiological conditions. Consistent exercise and pregnancy can alter the mechanical load of the heart and induce cardiac remodeling. In fact, they can induce physiologic cardiac hypertrophy. However, different exercise regiments lead to a different form of overload which in turn lead to different forms of hypertrophy. Specifically, aerobic exercises, such as running, increase the end-diastolic volume, causing VO and eccentric hypertrophy, whereas strength training causes PO and thus concentric hypertrophy (Fernandes, Soci, & Oliveira, 2011). Several different mechanisms have been observed to mediate the exercise induced cardioprotective remodeling of the heart. Signaling pathways involving phosphatidylinositol 3 phosphate kinase (PI3K), protein kinase B (AKT), mammalian target of rapamycin (mTOR), nitric oxide (NO) signaling, as well as microRNA (miR) mediated gene expression regulation have been observed to promote cardioprotective remodeling due to exercise (Schüttler, Clauss, Weckbach, & Brunner, 2019). Physical activity increases insulin-like growth factor 1 (IGF-1), locally. This activates PI3K which leads to the phosphorylation of AKT and subsequent suppression of transcription factor CCAAT/enhancer binding protein β (C/EBPβ), which is a regulator of several genes with roles in cardiac hypertrophy, such as GATA4, TBX5, and NKX2-5 (Lerchenmüller & Rosenzweig, 2014). Another activator of this pathway is neuregulin-1 (NRG-1) which is upregulated during exercise. NRG-1 binding to its receptor v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 4 (ErbB4) causes PI3K phosphorylation and its downstream activities (Lerchenmüller &

Rosenzweig, 2014; Schüttler, Clauss, Weckbach, & Brunner, 2019). The activation of IGF-1/PI3K/AKT has been observed in both murine models (Lerchenmüller & Rosenzweig, 2014; Schüttler, Clauss, Weckbach, & Brunner, 2019) and zebrafish models (Chen, et al., 2021). Certain miRs have also been observed to be upregulated during physical exercise. For example, miR-222 was upregulated during aerobic exercise and was found to be essential in inducing cardiac growth, as well as mediating cardioprotection in mice (Liu, et al., 2015). It is unclear whether the initial stimulus which activates these pathways is a change in mechanical load. However, IGF-1 levels were found to increase in response to pressure and volume overload (Castellano, Affuso, Di Conza, & Fazio, 2009). Moreover, ErbB4 expression was found to be upregulated during PO-induced compensatory cardiac hypertrophy (Galindo, Ryzhov, & Sawyer, 2014). This indicates that changes in mechanical loading of the heart during exercise play an important role in the activation of these pathways, which bring about physiologic cardiac hypertrophy.

Pregnancy is another factor that can lead to physiologic hypertrophy. During pregnancy blood volume and CO are increased. This leads to mild eccentric hypertrophy of the heart, which is mechanistically similar to exercise-induced hypertrophy (Chung & Leinwand, 2014). Although both forms share certain pathways, pregnancy-induced hypertrophy activates certain genes distinct from exercise-induced hypertrophy. A possible reason for this could be the duration of overload the heart is exposed to. During exercise, VO is intermittent, whereas during pregnancy this overload is continuous. Moreover, hormonal changes, specifically sex hormone changes are observed during pregnancy, which play a role in hypertrophy (Chung & Leinwand, 2014). In fact, an increase in estrogen was found to downregulate the cardiac Kv4.3 channel and increase c-Src activity which in turn prolonged QT interval and promoted hypertrophy (Eghbali, et al., 2005). Both pregnancy and exercise activate the PI3K/AKT pathway through mechanical loading. However, in mid-pregnancy ERK1/2 phosphorylation is significantly increased, which mainly occurs due to an increase in progesterone levels (Chung, Yeung, & Leinwand, 2012). Furthermore, calcineurin-1 activity has been observed to increase in early pregnancy, again partly due to increased progesterone, and is necessary to induce hypertrophy during pregnancy (Chung, Yeung, & Leinwald, 2013). Thus, an interplay between mechanical load and hormonal changes is responsible for the induction of physiologic hypertrophy during pregnancy. After birth, hormonal fluctuations and changes in mechanical load can lead to post partum cardiomyopathy, a fatal form of heart failure. However, the mechanism of post partum cardiomyopathy and the role of mechanical load in disease etiology is unclear, though vascular dysfunction seems to be a contributing factor (Hoes, et al., 2022).

Mechanical Loading in Heart Failure: Pressure and Volume Overload

Cardiac remodeling is dependent on the stimulus which initiates the adaptive response of the myocardium. On a cellular level, both the physiologic and pathologic adaptations show an increase in cardiomyocyte size, increased protein synthesis, and changes in sarcomeric structure. However, they are distinguished by the stimuli that induce the hypertrophy and how they influence cardiac function; such that left ventricular (LV) remodeling induced by the physiological stimulus is reversible and leads to enhanced LV function, increased

angiogenesis, lack of fibrosis, decreased mitochondrial dysfunction, and enhanced cardiomyocyte survival; whereas pathological LV remodeling is irreversible, decreases CO, and promotes fibrosis and apoptosis (Fernandes, Barauna, Negrao, Phillips, & Oliveira, 2015). Pathologic stimuli, such as hypertension, aortic stenosis, and valvular insufficiency cause pathologic LV remodeling and eventually lead to heart failure. Both pressure and volume overload can lead to heart failure as both overloads induce cardiac hypertrophy and changes in myocardial function. However, in response to these loads, distinct hypertrophic changes occur. It is well established that VO leads to eccentric hypertrophy, whereas PO promotes concentric hypertrophy (Nauta, et al., 2019). VO induces an increase in cardiomyocyte length as newly synthesized sarcomeres are added in series, whereas in concentric hypertrophy sarcomeres are added parallel to each other and thus, an increase in cardiomyocyte thickness is observed (Gjesdal, Bluemke, & Lima, 2011). In addition to VO, eccentric hypertrophy is observed during exercise-induced hypertrophy and is proposed to physiologically resemble normal cardiac growth, indicating a mechanistic overlap between the two processes. In fact, the AKT-mTOR pathway, an essential regulator of cell growth, has been observed to play a pivotal role in the regulation of VO-induced eccentric hypertrophy (Ikeda, et al., 2015). Ikeda *et al*. (2015) demonstrated that mTOR is directly regulated by left ventricular end-diastolic pressure (LVEDP) which is increased during VO. Upon increase in LVEDP (i.e. diastolic wall stress) AKT is phosphorylated and activates mTOR. Subsequently, mTOR leads to the hyperactivation of phosphorylated ribosomal protein S6, an essential mediator of protein synthesis and cell growth (Li, et al., 2020). The activation of mTOR determines the rate of eccentric hypertrophy progression. Thus, for the first time, this study revealed the quantitative relationship between LVEDP, mTOR activity, and eccentric hypertrophy (Ikeda, et al., 2015). To further confirm the role of mTOR in eccentric hypertrophy, mTOR inhibition during VO prevented cardiomyocyte elongation, preserved LV systolic function, and suppressed eccentric hypertrophy (Ikeda, et al., 2015; Li, et al., 2020).

mTOR activation and subsequent induction of cell growth alone do not explain why cardiomyocytes are elongated during VO. Kehat *et al*. (2010) suggest a model whereby ERK1/2 signaling activated by MEK-1 prevents eccentric hypertrophy and promotes concentric hypertrophy. This model is consistent with a study that used transgenic mice overexpressing Dusp6. These mice showed a complete loss of ERK1/2 activity, and when subjected to pressure overload, cardiac eccentric hypertrophy was observed, instead of concentric hypertrophy (Kehat, et al., 2010). This suggests that eccentric hypertrophy is the primary effect and that ERK1/2 signaling is necessary for facilitating concentric hypertrophy, while simultaneously inhibiting eccentric growth (Kehat, et al., 2010). Several studies have shown that ERK1/2 signaling is associated with pressure overload (Sciarretta & Sadoshima, 2010; Kehat, et al., 2010; Mutlak, et al., 2018). Thus, PO-induced activation of ERK1/2 drives the progression towards concentric hypertrophy. However, the downstream targets of ERK1/2 which drive parallel sarcomeric addition are not identified. In another study, Nicol *et al.* (2001) showed that the activation of ERK5 by hypertrophic stimuli results in the activation of the MEK5 pathway and subsequent serial assembly of sarcomeres in cardiomyocytes *in vitro;* induced by leukemia inhibitory factor (LIF) and cardiotrophin-1 (CT-1) (Nicol, et al., 2001). However, this study did not provide a mechanistic link between VO and ERK5 activation.

Conversely, a study investigating the mechanism of VO-induced hypertrophy in mice with aortic regurgitation did not observe ERK5 activation, suggesting that VO may not influence the ERK5-MEK5 pathway (You, et al., 2018).

Distinguishable adaptive changes in the extracellular matrix (ECM) of the myocardium can also be observed between PO and VO. VO promotes wall thinning and chamber dilation, while PO promotes wall thickening (Hutchinson, Stewart, & Lucchesi, 2010). These changes in wall thickness are partly governed by alteration in ECM, such that in VO, collagen is degraded. This degradation occurs due to an imbalance between matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs). MMP activity is increased and thus leads to progressive LV dilation and contractile dysfunction, in addition to ECM degradation (Hutchinson, Stewart, & Lucchesi, 2010). The mechanism behind VO-induced increase in MMP is not fully unraveled. However, in vitro cyclical stretch of neonatal rat cardiomyocytes (which models VO) resulted in the activation of JAK-STAT signaling pathways and subsequent increase in MMP2 and MMP14 expression (Wang, Yang, Chang, & Hung, 2004). Furthermore, Saygili *et al*. (2009) demonstrated that the calcineurin-NFAT pathway can mediate the stretch-induced activation of MMP2 and MMP9 (Saygili, et al., 2009). VO-induced LV remodeling also includes cardiomyocyte and endothelial cell apoptosis (Hutchinson, Stewart, & Lucchesi, 2010). This could be due to VO-induced increase in reactive oxygen species (ROS) which subsequently increases the phosphorylation of JNK and promotes apoptosis through the mitochondrial pathway (Fiorillo, et al., 2005).

Heart failure can be divided into two types depending on the changes in ejection fraction: Heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF) (Xie, Burchfield, & Hill, 2013). Acute pathological events, such as myocardial infarction promote the development of HFrEF, whereas prolonged PO leads to HFpEF, as observed in a porcine model of progressive LV pressure overload (LVPO) (Yarbrough, et al., 2012; Torres, et al., 2020). In HFrEF, an initial insult to the myocardium results in reduced cardiac output. Neurohormonal activation triggered by reduced cardiac output initially acts as a compensatory mechanism. However, the chronic activation of these processes eventually leads to pathologic hypertrophy and biochemical dysregulation, such as altered calcium homeostasis, downregulated β1-adrenergic receptor, and fetal gene expression (Rajapreyar, Lenneman, & Prabhu, 2020). This translates to an abnormal heart with dilated chambers, eccentric hypertrophy, and systolic as well as diastolic dysfunction (Rajapreyar, Lenneman, & Prabhu, 2020). These characteristics are reminiscent of cardiac remodeling induced by VO. In fact, fluid retention, a compensatory mechanism of reduced cardiac output, can lead to VO and advance the progression of pathologic cardiac remodeling and heart failure (Miller, 2016). Mechanistically, it was hypothesized that PO leads to an increase in myocardial stiffness and impaired filling, i.e. diastolic dysfunction due to enhanced expression of collagen in the ECM of the myocardium (Yarbrough, et al., 2012). Yarbrough *et al*. (2012) revealed that PO does induce myocardial stiffness due to the accumulation of collagen. However, this accumulation was not due to increased expression of collagen at the transcriptional level but to increased collagen stability and change in structure. PO was found to influence the post-translational modification of collagen by increasing the cross-linking of collagen fibers and upregulating the expression of TIMPs, specifically TIMP-1 and TIMP-4 (Yarbrough, et al., 2012). TIMP-1 and

TIMP-4 are regulators of fibroblast growth, proliferation, and activity. Thus, increased expression of TIMPs caused by LVPO alters the regulation of collagen through the reduction of MMP activity, induction of profibrotic pathways, and promotion of fibroblast function (Yarbrough, et al., 2012).

Several findings have illustrated the influence of mechanical load alterations in the pathophysiology of cardiac remodeling. However, it is also imperative to assess the mechanical load experienced by the heart after the development of hypertrophy and at later stages of heart failure. The loading conditions can be illustrated by pressure-volume (PV) loops which are graphed based on LV pressure on the y-axis and volume on the x-axis. A PV loop is rectangular in shape and represents the phases of a cardiac cycle: Isovolumetric contraction, blood ejection, isovolumetric relaxation, and blood filling (Bastos, et al., 2020). Changes in preload and afterload are reflected in these loops, which are depicted as alterations in end-diastolic and end-systolic PV relationships, EDPVR and ESPVR respectively. These relationships also help characterize LV chamber properties, such that EDPVR represents LV compliance/stiffness and ESPVR reflects LV contractility (Bastos, et al., 2020). During end-stage heart failure, significant differences in PV loops are observed. For example, in HFrEF systolic dysfunction occurs due to the reduction of myocardial contractility. The reduced contractility prevents the complete ejection of blood from the LV and leads to an increase in end-systolic volume (ESV). Subsequently, an increase in end-diastolic volume (EDV) occurs as the remaining blood in the LV after contraction is added to the venous return that enters the LV, signifying an increase in preload (Gimelli, et al., 2014; Miranda-Silva, Sequeira, Lourenco, & Falcao-Pires, 2022). This increase in EDV is not as large as the increase in ESV, thus the SV, represented by the area of the loop, is reduced. In the PV graph, these changes are depicted as a rightward shift of the PV loop with a decrease in the slope of ESPVR (Miranda-Silva, Sequeira, Lourenco, & Falcao-Pires, 2022). Furthermore, in end-stage HFrEF LV dilation is characteristic. LV dilation increases the ventricular radius, such that the myocardium must develop a greater inward force to generate the same systolic pressure as a non-dilated ventricle. This implies that during end-stage HFrEF, in addition to an increase in preload, elevated afterload is observed (Reddi, Shanmugam, & Fletcher, 2017; Vest, 2019). On the other hand, increased LV stiffness in HFpEF leads to diastolic dysfunction and reduced LV filling, resulting in a decreased EDV and a reduction in preload. On the PV graph, this is depicted as a leftward shift of the PV loop and an increase in the slope of EDPVR (Hajouli & Ludhwani, 2022; Miranda-Silva, Sequeira, Lourenco, & Falcao-Pires, 2022; Reddi, Shanmugam, & Fletcher, 2017). An increase in afterload is also observed in end-stage HFpEF, as compensatory mechanisms in the form of vasoconstriction attempt to elevate LV filling pressure and normalize CO (Altay & Pehlivanoglu, 2017; Reddi, Shanmugam, & Fletcher, 2017). The changes of PV loops associated with HFrEF and HFpEF are shown in Figure 1 obtained from the study by Miranda-Silva *et al.* (2022).

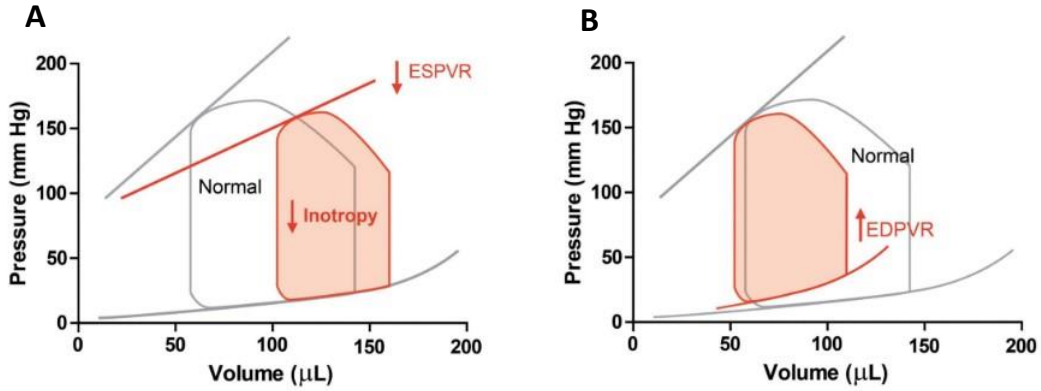

Figure 1: PV loop changes associated with HFrEF and HFpEF. The PV loops are plotted on pressure (y-axis) versus volume (x-axis) graphs. The PV loops of HFrEF (**A**) and HFpEF (**B**) patients are illustrated. In HFrEF, the loop is shifted to the right with a reduced slope of ESPVR, whereas in HFpEF the loop is shifted to the left with an increased slope of EDPVR. This figure is obtained from Miranda-Silva *et al.* (2022).

LVAD Supported Heart: Mechanical Unloading

Once heart failure develops there is no effective therapy that can lead to a cure (Lai & Chen, 2021). Current therapies for heart failure function to alleviate symptoms, reduce hospitalization, and prevent early death. To achieve this, angiotensin-converting enzyme (ACE) inhibitors, aldosterone antagonists, and beta-blockers are most commonly prescribed. Essentially, these drugs reduce blood pressure by dilating blood vessels and have been shown to induce a certain level of reverse remodeling (Tham, Bernardo, Ooi, Weeks, & McMullen, 2015). For example, Colucci *et al.* (2007) revealed that metoprolol, a beta blocker, leads to a reduction in LV ESV and improvement in LV ejection fraction in patients with LV systolic dysfunction. This implies that metoprolol can induce cardiac mechanical unloading and reverse remodeling (Colucci, et al., 2007). However, morbidity and mortality remain high as these therapeutic methods cannot lead to sufficient reverse remodeling. Several findings showed that left ventricular assist device (LVAD) implantation had the potential to dramatically reverse the process of remodeling by alleviating the power expenditure of the LV, through minimization of myocardial oxygen consumption and reduction of mechanical loading of the heart (Uriel, Sayer, Annamalai, Kapur, & Burkhoff, 2018; Marinescu, Uriel, Mann, & Burkhoff, 2016; Birks, 2013; Burkhoff, Topkara, Sayer, & Uriel, 2021). In fact, this reverse remodeling has been observed at the molecular, cellular, extracellular, and organ level in most patients with LVAD implantation. Moreover, time-dependent improvements in myocardial contractility, ventricular structure, hypertrophy, calcium transfer, beta-adrenergic signaling, cardiomyocyte survival, endothelial function, and microvasculature structure have all been documented (Marinescu, Uriel, Mann, & Burkhoff, 2016). These findings show that LVAD implantation is a promising therapeutic intervention to mechanically unload the heart and progress towards the reversal of heart failure. However, although LVAD can induce a significant level of reverse remodeling, its explantation often leads to relapse of the initial heart failure phenotype (Marinescu, Uriel, Mann, & Burkhoff, 2016).

LVADs were initially used during end-stage heart failure as a bridge to transplant. However, as LVAD technology advanced, its utility shifted towards a permanent alternative to transplantation, known as destination therapy, and occasionally a bridge to recovery.

Essentially, an LVAD is a pump attached to the LV which provides mechanical circulatory support. Functionally, LVADs pump blood from the left ventricle to the aortic root, reducing cardiac workload, preload, and neurohormonal activation while increasing systemic circulation and tissue perfusion (Burkhoff, Topkara, Sayer, & Uriel, 2021). LVADs can be separated into two types depending on their flow rhythm: Pulsatile or continuous flow. Moreover, continuous flow can be separated into two categories: Axial flow and centrifugal flow, which refer to how the blades in the pump rotate (Eisen, 2019). Both pulsatile and continuous flow LVADs have been associated with volume unloading and EDV reduction, with pulsatile flow LVADs showing a greater degree of unloading. However, continuous flow LVADs have been shown to exhibit left atrial volume unloading and improved function, in addition to LV volume unloading (Drakos, et al., 2011). Historically, the pulsatile LVAD was developed first to function as a bridge to transplant. A pulsatile LVAD called the HeartMate I was first investigated as destination therapy in the Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure (REMATCH) trial (Rose, et al., 2001). In this trial patients who received a LVAD had an improved quality of life compared to patients who received medical therapy. However, the survival rate one-year post-LVAD implantation was only 52% which was lower than the survival rate of cardiac transplantation. LVAD patients had severe adverse effects such as bleeding, infection, and device malfunction. Thus, the HeartMate I was not deemed an effective device for destination therapy (Rose, et al., 2001).

Further advancements in ventricular assist device design lead to the development of the HeartMate II, an axial continuous flow LVAD that contained a rotor that constantly propelled blood into the systemic circulation (Eisen, 2019). Due to their increased durability and smaller pump size, these LVADs were more effective than the HeartMate I. This was reflected in the survival rates of patients with the HeartMate II; which were 75% and 68%, six months and one-year post-implantation, respectively (Miller, et al., 2007). Moreover, another trial further illustrated the improved durability of HeartMate II as patients who received this device had a survival rate of 58% two years after implantation compared to the 24% survival rate of the HeartMate I. Furthermore, the HeartMate II recipients had a reduced risk of stroke and less likelihood to require device replacement (Slaughter, et al., 2009).

The next generation of LVAD, called the HeartWare ventricular assist device (HVAD), was designed to be even more durable than its preceding generation of LVADs. Similar to the HeartMate II, this device was a continuous flow LVAD. However, it functioned with a centrifugal flow rather than an axial flow, such that the flow in the device was perpendicular to the flow from the left ventricle. Furthermore, the device was smaller in size and its impeller was suspended by magnets to reduce the strain in the device (Eisen, 2019). The HeartWare Ventricular Assist System as Destination Therapy of Advanced Heart Failure (ENDURANCE) trial investigated the effectiveness of the HVAD compared to the HeartMate II (Pagani, et al., 2015). The trial illustrated that 54% of patients who received the HVAD survived two years without having a disabling stroke or removing the device due to failure or malfunction; whereas this was observed in almost 60% of the HeartMate II recipients (Pagani, et al., 2015). The HVAD recipients had much fewer instances of device malfunction or failure compared to the HeartMate II recipients (8.8% vs. 16.2%). However, the HVAD group had a higher incidence of stroke (29.7% vs. 12.1%) (Pagani, et al., 2015). Thus, although more durable, the

HVAD showed a higher risk of stroke. Another third-generation LVAD, the HeartMate III was not only found to be more durable than its predecessors, but also had a lower rate of stroke incidence (Mehra, et al., 2018). Moreover, patients implanted with the HeartMate III had a lower risk of arrhythmias, major infections and pump thrombosis compared to HVAD implanted patients at 2 years (Coyle, et al., 2020).

The observations of decreased LV size and reversal of morphology after LVAD implantation led to investigations that aimed to determine whether these changes were simply due to unloading of the heart and subsequent cessation or reversal of load-induced pathologic mechanisms, or rather due to activation of novel pathways which actively induced LV structural changes (Burkhoff, Topkara, Sayer, & Uriel, 2021). LVAD support can lead to improvements in neurohormonal activation, secondary to cardiac output recovery, and subsequent reduction in neurohormone-induced pathologic pathway activation (Kim, Uriel, & Berkhoff, 2017). Thus, implicating reverse remodeling as a passive process. However, aberrant gene expression associated with pathologic remodeling was found to persist during reverse remodeling, with only 5% of those genes normalizing after mechanical unloading, while novel gene expression changes were observed following reverse remodeling (Boulet, Mandeep, & Mehra, 2021). Therefore, the process of reverse remodeling can be considered as a combination of both passive, ie. cessation of pathological pathways, and active processes, ie. induction of cardioprotective pathways.

Following mechanical unloading through LVAD, an increase in expression of apoptosis-inhibiting proteins FasEx06del and B-cell lymphoma extra large (Bcl-XL), as well as reduced DNA fragmentation have been documented (Birks, 2013). Furthermore, a study by Chen *et al*. (2003) reported a substantial amount of transcription factors involved in stress control and cell growth were upregulated following LVAD support. Among these transcription factors forkhead box 3A (FOXO3a), hypoxia-inducible factor 1 (HIF-1), and cardiac-specific homeobox were of note (Chen, et al., 2003). Studies have shown that FOXO3a modulates the expression of different genes that regulate stress response at the G2-M checkpoint of the cell cycle (Tran, et al., 2002). Moreover, FOXO3a was found to act as a transcriptional activator of antioxidants catalase and superoxide dismutase (Chen, et al., 2003). Thus, contributing to an anti-apoptotic effect. With LVAD implantation, a reduction in expression of fetal genes, such as atrial natriuretic peptide (NPPA), brain natriuretic peptide (NPPB), β-myosin heavy chain (MYH7), and alpha-skeletal actin (SKA) was observed. Moreover, the expression of GATA4, a stress-induced regulator of cardiac hypertrophy, and the activation of ERK1/2, a major driver of cardiac hypertrophy, were also found to be reduced, indicating that mechanical unloading leads to a change in gene expression profile and mechanistic switch from pathological to a healthy state (Burkhoff, Topkara, Sayer, & Uriel, 2021). Calcium cycling was also observed to be improved in LVAD-supported hearts, leading to improved cardiomyocyte contractility associated with a significant increase in sarcoplasmic reticulum calcium content and increased entry of calcium through sarcolemma during the action potential (Burkhoff, Topkara, Sayer, & Uriel, 2021). Normalization of calcium handling genes Na/Ca exchanger (NCX), ryanodine receptor 2 (RyR2), and Sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) upon mechanical unloading were also reported to contribute to the improved cardiac contractility (Heerdt, et al., 2000). LVAD support can lead to the upregulation of several cytoskeletal genes

as well, including spectrin, β-actin, lamin A/C, α-tropomyosin, and α-filamin (Birks, et al., 2005). However, the dysfunctional arrangement of cytoskeletal protein seems to persist after mechanical unloading, as only minor improvements in the structural organization of tropomyosin, actin, and titin proteins were observed following LVAD support (de Jonge, et al., 2002).

Mechanical unloading not only provides beneficial effects for patients with end-stage heart failure but can also be used as a means to reduce infarct size upon reperfusion therapy following MI. In a study using the swine model of acute MI, Kapur *et al*. (2015) reported that initial unloading of the heart followed by delayed reperfusion reduced the infarct size. This study revealed that LV mechanical unloading led to the activation of the reperfusion injury salvage kinase (RISK) pathway and increased the stromal derived factor 1 alpha (SDF-1α) as well as its downstream effector glycogen synthase kinase 3 beta (GSK3β) (Kapur, et al., 2015). These pathways are involved in the ischemic conditioning of the myocardium, such that intermittent periods of ischemia in the nonischemic myocardium can lead to the activation of the RISK pathway which includes PI3K, AKT, and ERK. The RISK pathway prevents ROS-induced formation of mitochondrial permeability transition pores (mPTP), which induce cardiomyocyte death, and leads to reduced infarct size. SDF-1α is also involved in infarct size reduction. Upon increase of SDF-1α, AKT mediated phosphorylation and inactivation of GSK3β are inhibited leading to a reduced mPTP formation and infarct size (Uriel, Sayer, Annamalai, Kapur, & Burkhoff, 2018). These findings show that mechanical unloading does not only reverse the pathological changes present in adverse remodeling during heart failure but can also activate cardioprotective mechanisms to reduce the initial injury that eventually leads to heart failure development.

Mechanistic Insights of Pathologic Remodeling

In the previous chapter, the pathways involved in mechanical load-induced pathologic remodeling have been mentioned. However, they do not provide an in-depth view of the several intertwined pathways involved in this process. This chapter aims to summarize the various mechanotransduction pathways and mechanistic cross-talk involved in mechanical load-induced cardiac remodeling (Figure 2). Currently, several mechanosensors have been identified such as integrins, stretch-activated ion channels (SACs), and Guanine nucleotide-binding (G-) protein coupled receptors which are responsible for initiating mechanotransduction signaling pathways (Lammerding, Kamm, & Lee, 2009). Mechanical loading can activate several pathways downstream which eventually lead to pathological remodeling by either direct activation of these receptors, or by the release of paracrine and autocrine growth factors (Mann, 2004). The downstream effects occur through three main pathways: MAPK pathway, JAK/STAT pathway, and calcineurin-NFAT pathway (Ruwhof & Laarse, 2000).

The MAPK pathway involves three forms of kinases MAP kinases (MAPKs), MAPK/ERK kinases (MEKs), and MEK kinases (MEKKs) which are phosphorylated in the following order: MEKK→MEK→MAPK. In addition, the MAPKs are categorized into three subfamilies: Extracellular-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38 MAPKs, all of which contribute to mechanical load-induced cardiac hypertrophy (Ruwhof & Laarse, 2000).

The ERK pathway is stimulated by calcium influx, receptor tyrosine kinases (RTKs) and G-protein coupled receptors and leads to the activation of several transcription factors such as c-jun, 90kDa-s6 kinase, and activating transcription factor 3 (ATF3) (Wu, et al., 2017; Ruwhof & Laarse, 2000). The JNK pathway is activated by stress. However, the precise activators of JNK are unknown. This pathway leads to mechanical load induced hypertrophy by activating transcription factors c-Jun and ATF2 (Ruwhof & Laarse, 2000). Finally, the p38 MAPKs are also involved in load induced hypertrophy by activating ATF2 (Ruwhof & Laarse, 2000). Furthermore, p38 MAPK signaling is involved in cardiomyocyte apoptosis. Mechanical stress can induce pro-inflammatory cytokines which can bind to receptors on cardiomyocytes and activate apoptosis signaling kinase 1 (ASK1), this subsequently leads to activation of the p38 MAPK pathway and apoptosis (Wu, et al., 2017). Interestingly, p38 can also be activated by paracrine or autocrine factors binding to G-protein coupled receptors. Additionally, the phosphorylation of p38 by different kinases can lead to different transcriptional effects such that the activation of p38 MAPKα leads to apoptosis, whereas p38 MAPKβ activation leads to hypertrophy (Lammerding, Kamm, & Lee, 2009).

In rats, pressure overload was found to activate the JAK/STAT pathway through glycoprotein gp130 (Pan, et al., 1998). Upon increase in mechanical load, gp130 is phosphorylated which in turn phosphorylates JAKs. These tyrosine kinases phosphorylate STATs, which are latent transcription factors. Once activated, STATS dimerize and enter the nucleus to stimulate the transcription of genes associated with cardiac hypertrophy (Lammerding, Kamm, & Lee, 2009). Interestingly, in addition to JAK/STAT activation, gp130 has been observed to activate the ERK pathway, implicating that gp130 may play a significant role in mechanotransduction (Kunisada, et al., 1996).

Another pathway involved in mechanical load-induced cardiac hypertrophy is the calcineurin-NFAT pathway. Calcineurin is a Ca^{2+}/ calmodulin-dependent phosphatase that is activated upon calcium influx. Activation of calcineurin leads to the dephosphorylation of cytoplasmic transcription factor NFAT3, which subsequently enters the nucleus and activates GATA4. Activation of GATA4 eventually leads to the upregulation of hypertrophic genes, such as fetal genes (Molkentin, et al., 1998).

All of these pathways can be activated by the various mechanosensors mentioned earlier. Integrins are a class of cell-surface receptors that connect the cellular cytoskeleton to the ECM at regions called focal adhesion sites (Schwartz, Schaller, & Ginsberg, 1995). The cytoplasmic domain of integrins interacts with intracellular signaling molecules, such as focal adhesion kinases (FAKs) (Schwartz, Schaller, & Ginsberg, 1995). Studies have shown that stretch can activate the ERK and JNK pathways through integrins (MacKenna, Dolfi, Vuori, & Ruoslahti, 1998). Further studies demonstrated that integrins cluster at focal adhesion sites, recruiting non-receptor kinases FAK and Src, signal transducing molecules, and cytoskeletal proteins to form focal adhesion complexes (FACs) (MacKenna, Dolfi, Vuori, & Ruoslahti, 1998). In these FACs, integrins act as true receptors of mechanical load and induce the activation of FAK mediated by Src (Parsons & Parsons, 1997). The activation of FAK subsequently activates the ERK pathway either through Grb2-Sos-Ras or through the activation of PLC (Shyy & Chien, 1997; Zhang, et al., 1999). Integrins can also interact with growth factor receptors and

mediate the activation of ERK or JNK pathways upon ligand binding (Plopper, McNamee, Dike, Bojanowski, & Ingber, 1995). These findings illustrate the diverse ways integrins can lead to signal transduction upon changes in mechanical load. Moreover, integrins are observed to not only play a role in mechanotransduction in cardiomyocytes, but also in fibroblasts, leading to hypertrophic changes in cardiomyocytes and upregulation of profibrotic genes in fibroblasts (Ross, et al., 1998; MacKenna, Dolfi, Vuori, & Ruoslahti, 1998)

SACs are a group of mechanosensitive ion channels that can be activated upon changes in mechanical load and allow the passage of Ca^{2+}, Na^{+}, and K^{+} (Ruknudin, Sachs, & Bustamante, 1993). The increased influx of Ca^{2+}mediated by stretch-induced activation of SACs can lead to the activation of calcineurin and the subsequent cascade of the calcineurin-NFAT pathway. The contribution of SACs in mechanical load-induced pathologic remodeling remains controversial as several studies have observed that blocking of SACs did not inhibit the expression of fetal genes and subsequent hypertrophic responses in cardiomyocytes (Mann, 2004). However, a recent study has identified a novel ion channel resembling SACs called Piezo1 which can induce Ca^{2+} influx upon sensing of mechanical load. This Ca^{2+} influx activated the calcineurin-NFAT pathway and led to cardiomyocyte hypertrophy (Zhang, et al., 2021).

G-protein coupled receptors have also been implicated in mechanotransduction. The G-proteins combine cell surface receptors with their downstream effectors. Two forms of G-proteins are identified: small G-proteins and heterotrimeric G-proteins, both of which exist in an inactive guanosine-diphosphate (GDP) state and are activated by phosphorylation to active guanosine triphosphate (GTP) state (Hall, 1990). The small G-proteins, such as the Rho and Ras families are single polypeptides that act as signal transducers involved in the activation of MAPKs. Members of the Rho family have been shown to activate JNK and p38 MAPK pathways, whereas members of the Ras family are well-known activators of the ERK pathway, though they can mediate JNK pathway activation as well (Minden, Lin, Claret, Abo, & Karin, 1995; Minden, et al., 1994). The heterotrimeric G proteins have been associated with receptors at focal adhesion sites. Thus, they can be activated by changes in the stretch. Indeed, Gudi *et al.* (1998) demonstrated that stretching of neonatal rat fibroblasts led to the activation of G proteins within 1 minute. Moreover, other studies observed that the stretch-induced activation of G proteins led to cardiomyocyte hypertrophy through the activation of phospholipase C (PLC) and subsequently protein kinase C (PKC) (D'Angelo, et al., 1997; Jalili, Takeishi, & Walsh, 1999). Thus, linking integrins, heterotrimeric G proteins, PLC, and PKC in mechanotransduction (Ruwhof & Laarse, 2000).

The PI3K/Akt pathway is also involved in load-induced pathologic remodeling. This pathway is stimulated upon IGF-1 binding to its receptor (IGF-1R). Following mechanical loading, IGF-1 levels are increased and the enhanced stimulation of this pathway leads to increased protein synthesis and cardiomyocyte hypertrophy (Aoyagi & Matsui, 2011). Once activated IGF-1R phosphorylates Akt (p-Akt) through PI3K. Subsequently, p-Akt activates mTOR and deactivates GSK3β and FoxO. The activation of mTOR leads to an increase in protein synthesis and cell mass due to increased protein translation through p70S6K kinase and 4E-BP1 (Avruch, et al., 2006). GSK3β is a negative regulator of hypertrophy which is inactivated upon Akt activation. This inactivation leads to the activation of downstream targets of GSK3β, such as

GATA4, c-Jun, and NFAT, promoting hypertrophy (Sugden, Fuller, Weiss, & Clerk, 2008). p-Akt is also translocates into the nucleus where it phosphorylates transcription factor FoxO, inducing its translocation out of the nucleus and inactivation. This inhibition of FoxO further promotes cardiac hypertrophic changes (Ronnebaum & Patterson, 2010).

Mechanical load not only leads to cardiomyocyte hypertrophy but also ECM pathologic remodeling. Changes in the myocardial ECM are partly governed by fibroblasts that are activated in response to mechanical load either through direct activation by mechanical stress, or stress-induced secretion of paracrine and autocrine factors. Mechanical stress has been observed to increase the expression of, and dephosphorylate syndecan-4, a transmembrane proteoglycan and co-receptor of integrins. These changes lead to syndecan-4-induced activation of the calcineurin/NFAT pathway and subsequent induction of hypertrophic and profibrotic alterations in cardiomyocytes and fibroblasts, respectively (Herum, et al., 2015; Finsen, et al., 2011). In fibroblasts, syndecan-4 induces the production of collagen and matricellular proteins, such as osteopontin, and cross-linking of collagen (Herum, et al., 2015). Factors such as angiotensin II (Ang II), ET-1, TGFβ, and IGF-1 induce fibrosis by activating fibroblasts and inducing the expression of various ECM proteins (Wu, et al., 2017). In addition, the activation of fibroblasts produces fibroblast growth factor FGF-2 which can induce cardiomyocyte hypertrophy by MAPK pathway activation (Santiago, et al., 2011). All of these factors directly or indirectly lead to the activation of one of the MAPK pathways to induce their hypertrophic or profibrotic effects. Additionally, cytokines such as CT-1 or LIF also achieve such effects through the activation of the JAK/STAT pathway (Lammerding, Kamm, & Lee, 2009). Thus, a complex interaction between cardiomyocytes and fibroblasts through these autocrine and paracrine factors initiates and maintains hypertrophic and profibrotic responses to mechanical load (Wu, et al., 2017).

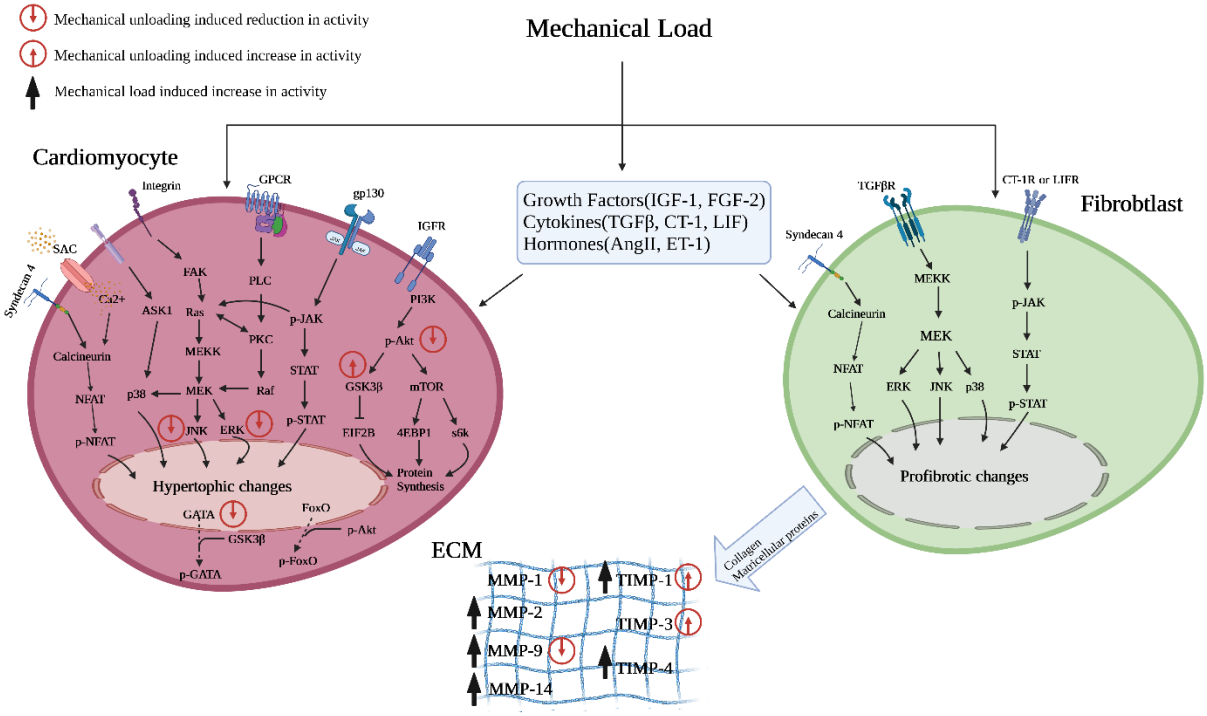

Figure 2: Mechanotransduction in cardiomyocytes and fibroblasts during pathologic and reverse remodeling. A summary of the mechanisms involved in mechanical load induced remodeling and mechanical unloading induced reverse remodeling.

The red arrows represent the alterations in the activity of signal transducers. Abbreviations: AngII, Angiotensin II; ASK1, apoptosis signal regulating kinase 1; Akt protein kinase; CT-1, Cardiotrophin-1; ET-1, endothlin 1; EIF2B, eukaryotic translation initiation factor 2B; FAK, Focal adhesion kinase; FGF-2, Fibroblast growth factor 2; FoxO, Forkhead box; GSK3β, Glycogen synthase kinase 3 beta; IGFR, Insulin-like growth factor receptor; JAK, Janus kinase; LIF, Leukemia inhibitory factor, MEKK, MAP kinase kinase kinase; MEK, MAPK/ERK kinase; mTOR, Mammalian target of rapamycin; MMP, Matrix metalloproteinase; NFAT, nuclear factor of activated T cells; PLC, Phospholipase C; PKC, Protein kinase C; PI3K, phosphatidylinositol-3-kinase; STAT, signal transduction and activator of transcription; S6K, ribosome protein subunit 6 kinase; TIMP, Tissue inhibitor of metalloproteinase; 4EBP1, eukaryotic translation initiation factor 4E-binding protein 1. Adapted from Wu *et al.* (2017).

Mechanistic Insights of Reverse Remodeling

Just as mechanical loading can induce myocardial pathologic remodeling, mechanical unloading can lead to reverse remodeling. Mechanical unloading-induced reverse remodeling can occur by either the blocking or reversal of pathways involved in pathologic remodeling or activation of novel cardioprotective pathways which lead to the reversal of cardiomyocyte hypertrophy and improvements in ECM structure as well as function. The activity of MEK/ERK pathway and Akt signal transduction are significantly decreased in cardiomyocytes upon mechanical unloading of failing hearts with LVAD support. This decrease in Akt activity leads to an increase in the activation of GSK3β. These alterations cause changes in transcription and cytoskeletal organization which promote reverse remodeling. The JNK and p38 MAPK pathways, however, were not affected by mechanical unloading in this study, thus LVAD induced mechanical unloading seems to specifically regulate certain kinases *in vivo* (Baba, et al., 2003). Similar findings were observed in a study that induced mechanical unloading by HHT in pressure-overloaded rats. In addition to changes in MEK/ERK and Akt/GSK3β, this study revealed that mechanical unloading also reduced NFκB activity, which is involved in cardiomyocyte hypertrophy and fibrosis (Xu, et al., 2010). In contrast, another study observed a decrease in JNK activity and an increase in p38 MAPK activity subsequent to mechanical unloading. JNK activity was inhibited due to a decrease in JNK protein levels. Furthermore, phosphorylated JNK was not detected during the study. Therefore, the inhibitory mechanism of JNK activity could be due to both effects and remains unclear. p38 MAPK phosphorylation was found to be increased following LVAD support, thus explaining the increased p38 activity (Flesch, et al., 2001). Figure 2 illustrates the effect of mechanical unloading on these pathways.

A consistent observation in several studies is the normalization of SERCA2a, which is known to be downregulated during mechanical load induced remodeling (Depre, Davies, Taegtmeyer, & Phil, 1999; Madigan, et al., 2001). The phosphorylation states of phospholamban, Ca^{2+}/calmodulin-dependent protein kinase II (CamKII), and calsequestrin influence the activity of SERCA2a (Rodrigues, Leite-Moreira, & Falcao-Pires, 2016). However, a direct link between mechanical loading/unloading and phosphorylation of these effectors currently remains unexplored.

A study investigating the effect of other mechanosensitive signal transducers which promote cardiac remodeling revealed that no alterations in the protein levels of G proteins and PLC isoforms were observed following LVAD support. Furthermore, translocation of different isoforms of PKC into the nucleus remained unchanged following mechanical unloading

(Takeishi, et al., 2000). Thus, indicating that mechanical load-induced reverse remodeling is independent of these pathways.

Mechanical unloading also promotes changes in myocardial ECM which counteract the pathologic alterations in heart failure. Collagen content was observed to decrease after LVAD support. However, this decrease only occurred after prolonged use of LVAD. Moreover, the collagen content was found to significantly decrease after combining LVAD support with ACE inhibitor therapy (Maybaum, et al., 2007; Bruggink, et al., 2006; Sakamuri, et al., 2016). The study by Sakamuri *et al.* (2016) revealed that the combination of LVAD support and ACE inhibitors improves the ratio of MMP/TIMP and leads to a reduction in ECM collagen content by reducing matricellular protein osteopontin. However, the study also illustrated that these therapies did not have an effect on another matricellular protein secreted protein, acidic, cysteine-rich (SPARC), and concluded that this persistent elevation of SPARC may contribute to the relapse of pathologic ECM remodeling following LVAD explantation (Sakamuri, et al., 2016). Several other investigations have illustrated that LVAD support leads to a reduction in various MMPs, such as MMP-1, MMP-9 and an increase in TIMPs, such as TIMP-1 and -3 (Li, et al., 2001; Klotz, et al., 2005). However, the mechanistic insights into the pathways of mechanical unloading induced ECM reverse remodeling remains unclear.

In Vivo Models of Mechanical Loading and Unloading

The findings pertaining to the various effects of mechanical loading on the heart and its contribution to the development of heart failure could not be discovered without the development of models mimicking this pathologic process both *in vivo* and *in vitro*. One of the most well-established and widely used models of mechanical loading is transaortic constriction (TAC). TAC is used to induce pressure overload in adult rodents by placing a constricting band around the aortic arch, between the left common carotid artery and brachiocephalic trunk (Schaefer, et al., 2016). TAC leads to stenosis of the aorta, pressure overload of the heart, and eventually, cardiac hypertrophy resembling that in HFrEF patients (Mohammadi, Abouissa, & Heineke, 2021). The degree of hypertrophy induced by TAC depends on the mouse strains used and the size of the needle that acts as a spacer around which the constricting band is tied. In most strains, pathologic responses are triggered around two weeks after surgery, resulting in a 20-50% increase in LV mass and reduction in cardiac function (Mohammadi, Abouissa, & Heineke, 2021). From 2 to 11 weeks post-TAC, a substantial level of LV concentric hypertrophy is observed with almost a doubling of LV mass. A progressive decline in LV function, increased LV chamber size, and fibrosis is also noted with most mice developing pulmonary congestion at the 11^{th} week (Lygate, 2006). An advantage of TAC is that it can allow for the measurement of pressure gradient across the stenosis, thus animals can be tested if they respond to the surgery. However, the reliability of this technique has been challenged due to the large variability in response to this standardized technique (Lygate, 2006). This variability of heart failure incidence can be due to the expertise of the operator. Therefore, advancements in TAC procedures have proposed the use of O-rings, instead of sutures, as a more optimized and simple method (Nakao, et al., 2022). Furthermore, Lygate *et al*. (2005) discovered that in their study, which used a 7–0 polypropylene suture as the constricting band, 25% of the mice internalized the band into the

aortic lumen. This allowed for an increase in the cross-sectional area of the stenosis and a reduction in pressure gradient (Lygate, et al., 2005). Thus, the material of the constricting band could be a variable in the effectiveness of TAC. However, it is unclear if the use of other types of constricting bands can also lead to band internalization. The use of both O-rings and sutures requires an open chest thoracotomy which involves the dissection of the ribs. Typically, the ribs are not reattached and thus can affect the breathing dynamics of the mouse (Eichhorn, et al., 2018). Eichhorn *et al*. (2018) established a minimally invasive closed chest approach to TAC, such that a lateral incision is performed through the second intercostal space without cutting through the ribs. Aortic banding is also commonly used in larger animal models, such as pigs. A study by Bikou *et al.* (2018) showed that the banding of the ascending aorta in pigs led to significant cardiac hypertrophy and myocardial fibrosis. Moreover, the pathologic LV remodeling in this model led to increased myocardial stiffness and subsequent diastolic dysfunction, however, systolic dysfunction was not observed, implying a resemblance to the HFpEF phenotype (Bikou, Miyashita, & Ishikawa, 2018). A less invasive model of HFpEF in pigs was developed by subcutaneously injecting deoxycorticosterone acetate (DOCA), an aldosterone agonist that promotes salt retention and induces hypertension, and feeding of a western type of diet. This model induced pressure overload, LV concentric hypertrophy, and diastolic dysfunction (Schwarzl, et al., 2015). It is worth mentioning that models of PO-induced HFpEF can eventually transition to HFrEF due to prolonged overload. Thus, this DOCA-WD pig model has a high translational potential to study HFpEF to HFrEF transition (Gyöngyösi, et al., 2017). Another model which aims to induce chronic hypertension and model HFrEF includes renal artery stenosis which can be performed in dogs, sheep, and pigs (Spannbauer, et al., 2019).

The aforementioned models cannot be utilized to study VO-induced LV remodeling, as they induce LV hypertrophy by PO. One model which can induce chronic VO is the aortocaval shunt model. An incision is performed through the abdominal midline to expose the inferior vena cava and abdominal aorta. A needle is then used to puncture the abdominal aorta and the adjacent vena cava to connect both. The needle is then removed, and the puncture is sealed (Garcia & Diebold, 1990; Scheuermann-Freestone, et al., 2001). This leads to the mixing of venous and arterial blood in the vena cava, increasing venous return and thus inducing VO (Scheuermann-Freestone, et al., 2001). Another similar model is the aortocaval fistula. In principle, it also involves the connecting of the abdominal aorta and inferior vena cava. However, instead of puncturing the walls of both blood vessels, they are connected with a longitudinal incision (Abassi, Goltsman, Karram, Winaver, & Hoffman, 2011). These models are commonly used in small animals. However, they are rarely used in large animal models (Spannbauer, et al., 2019). Moreover, although they lead to LV hypertrophy and heart failure (Garcia & Diebold, 1990; Scheuermann-Freestone, et al., 2001; Abassi, Goltsman, Karram, Winaver, & Hoffman, 2011), they do not physiologically represent VO-induced cardiac hypertrophy in humans. In humans, blood regurgitation can facilitate volume overload. Thus, animal models which mimic this process have been developed. One such model is aortic regurgitation (AR), which includes the puncture of aortic valves with a metal wire (You, et al., 2018). Similarly, a recently developed model, mitral regurgitation (MR), utilizes iridectomy scissors to damage the mitral valves and lead to volume overload through regurgitation (Li,

et al., 2020). Both models have been shown to induce LV hypertrophy, increased LV mass, and hypertrophic gene expression profile in both small and large animals (You, et al., 2018; Li, et al., 2020). However, The AR model lacks reproducibility and is difficult to perform in large animals. Therefore, models that utilize large animals more commonly perform MR to induce volume overload (Spannbauer, et al., 2019).

A model of mechanical unloading can be achieved following TAC. This model is called deTAC and is performed by simply removing the constricting band around the aorta. The TAC/deTAC model mimics LVAD implantation, such that at first mechanical overloading is present and then LV unloading is induced. Several studies have revealed that deTAC can induce regression of LV hypertrophy in mice (Hariharan, et al., 2013; Oyabu, et al., 2013; Goncalves-Rodrigues, Miranda-Silva, Leite Moreira, & Falcão-Pires, 2021). For example, Hariharan *et al*. (2013) demonstrated that 1 week of deTAC significantly reduced the increased LV mass and myocyte cross-sectional area induced by TAC. Moreover, the expression of fetal gene atrial natriuretic factor (ANF) was significantly downregulated with deTAC, indicating a reversal of hypertrophy. deTAC involving the removal of a suture-based constricting band has been met with complications as the inflammation around the aorta following TAC, hinders the easy removal of the band (Zhang, et al., 2013). To avoid these complications, Zhang *et al*. (2013) developed a highly reproducible and minimally invasive technique that involved the use of a titanium clip. The titanium clip was dimensionally stable and provided a stable constriction of the aorta, without loosening. Moreover, its removal simply required the squeezing of the clip perpendicular to the plane in which it was placed. Thus, the use of a titanium clip was not only advantageous for TAC, but also for deTAC. (Zhang, et al., 2013). Although the idea of deTAC is simple, it requires surgical access to the aorta for a second time. This exposes the animal to repeated trauma and increases the likelihood of performing an error during surgery. A model resembling deTAC has also been shown to induce cardiac reverse remodeling in large animals through regression of overload-induced gene expression (Walther, et al., 2002). In this model Walther *et al.* (2002) first induced pressure overload and LV hypertrophy by supracoronary banding of the ascending aorta in sheep. Eight months later the band was removed and a significant decrease in overload, as well as a reduction in expression of genes associated with the renin-angiotensin system (RAS), were observed implying a regression towards a healthy myocardial phenotype (Walther, et al., 2002).

Another model of mechanical unloading is heterotopic heart transplantation (HHT). HHT involves the transplantation of a donor's heart to alleviate the load on the recipient's heart. Two variations of HHT are present: Heterotopic abdominal heart transplantation (HAHT) and heterotopic abdominal heart-lung transplantation (HAHLT). In HAHT, the recipient's abdominal aorta and inferior vena cava (IVC) are anastomosed to the donor's ascending aorta and pulmonary artery, respectively. Subsequently, the donor's superior vena cava, IVC, and pulmonary veins are then ligated (Liu, et al., 2015). This provides complete unloading of the recipient's LV as the heart only receives venous return (Ibrahim, et al., 2013). However, in HAHLT only partial unloading occurs as the donor's ascending aorta is anastomosed to the recipient's abdominal aorta only and the pulmonary artery and veins are kept intact as the lungs are transplanted as well (Liu, et al., 2015). Thus, in this model the recipient's pulmonary blood flow is unchanged. HHT models LVAD-supported hearts and can be used to understand

the mechanisms underlying LVAD-induced reverse remodeling, as well as the optimal period of LVAD-mediated mechanical unloading. For example, a study by Oriyanhan *et al*. (2007) employed HAHT to investigate the optimal duration of mechanical unloading in rats with heart failure induced by coronary artery ligation. The results reported that HAHT-induced mechanical unloading normalized cardiac gene expression, cardiomyocyte hypertrophy, and cardiac function at 4 weeks, but negatively impacted these parameters with prolonged duration (Oriyanhan, et al., 2007). These findings were also observed in another study that utilized TAC to induce heart failure in rats (Schaefer, et al., 2019). This technique, however, is not as simple as the deTAC model, as it involves the transplantation of donor's hearts and the several complications which accompany this procedure, including infection, aortic regurgitation due to torsion of anastomosis, pulmonary embolism, intestinal obstruction, and donor bradycardia due to sinoatrial node damage during harvesting (Ibrahim, et al., 2013). HHT has been mostly used in small animals to study mechanical unloading and its effects on the pathologically remodeled heart. However, HHT in large animal models has not been studied extensively. In contrast, some preclinical studies have utilized LVADs in large animal models. For example, Nakamura *et al.* (1993) implanted LVADs into goats that had undergone LV infarction by coronary artery ligation. The findings revealed that out of the 8 goats only 3 recovered successfully with a significant reduction in overload (Nakamura, et al., 1993).

In Vitro Models of Mechanical Loading and Unloading

Early investigations examining mechanical load-induced cardiac remodeling and heart failure extensively utilized *in vivo* models. However, although more relevant for translation to clinical settings, these models have several limitations. First, differences in cardiovascular system biology exist between species. For example, small animals like mice or rats, which are extensively used in cardiovascular research, have smaller hearts, higher heart rates, and higher metabolism compared to humans (Jorba, et al., 2021). Moreover, certain ion channels differently contribute to repolarization currents between the two species. For example, unlike in humans, the current contributed by potassium ion channel protein Kv11.1 is negligible in rodents (Nerbonne, Nichols, Schwarz, & Escande, 2001). These differences can lead to discrepancies in the translation of electrophysiological findings, as repolarization times in rodent cardiomyocytes are shorter than in humans (Milani-Nejad & Janssen, 2013). Furthermore, cardiac structural dimensions are different between rodents and humans. For example, rodents' cardiac walls are thinner, thus mechanical strain patterns and ECM arrangement are dissimilar (Kusunose, et al., 2012). This can hamper the translation of mechanical load-induced pathologic ECM remodeling from rodents to humans (Jorba, et al., 2021). Moreover, the intraventricular pressure in the hearts of rodent models and humans is different (Kusunose, et al., 2012). Taken together with the observation that the heart rate in rodents is higher, the physiological loading conditions in rodents may not reflect that of humans. Finally, Larger animals have also been used more recently to study heart failure. However, certain limitations persist. Mainly, these models do not allow for the precise manipulation of mechanical and structural signals from the cardiac environment, thus hindering the precise examination of the role of mechanical load in cardiac pathophysiology (Jorba, et al., 2021).

With recent advancements in stem cell technology, cell culturing, and tissue engineering, *in vitro* models have become more commonly used to study cardiac mechanobiology. Initially, patient-derived primary cells and heart slices were used as the cell source of *in vitro* models (Guo & Huebsch, 2020; Jorba, et al., 2021). These cells are functionally mature and provide an accurate representation of the cardiac tissue they are derived from. However, upon isolation, these cells lose crucial functional and morphological characteristics, such that they begin to dedifferentiate, proliferate at a slower rate, and have a shorter life span (Banyasz, et al., 2008; Hoes, Bomer, & van der Meer, 2019). To circumvent these limitations biomimetic culture systems were developed to mimic the electromechanical stimulation and environment of the *in vivo* myocardial tissue (Watson, et al., 2019). A study by Watson *et al.* (2019) illustrated that the application of a physiologic degree of preload in the form of uniaxial static stretch to rat and rabbit myocardial slices maintains their functional, structural, and transcriptional properties at 24 hours and 5 days, respectively. Similar findings were observed in a biomimetic system with cardiac tissue slices from human failing hearts (Fischer, et al., 2019). Furthermore, Miller *et al.* (2022) developed a biomimetic cardiac tissue culture model (CTCM) from porcine heart slices experiencing cyclical stretch, that not only preserved the viability, structure, metabolic activity, and transcriptional profile of the tissue for 12 days, but also induced hypertrophic changes upon overstretching. However, these tissue slices are difficult to obtain and show differences between donors (Banyasz, et al., 2008; Hoes, Bomer, & van der Meer, 2019). Recently, human induced pluripotent stem cell (iPSC) derived cardiomyocytes have emerged as an alternative, as they show few differences between batches, allowing great reproducibility between studies, proliferate quickly, and have the potential for developing personalized medicine by using patient-specific iPSCs (Jorba, et al., 2021). However, a major drawback of iPSC-derived cardiomyocytes is that they lack the contractility and electrophysiologic phenotype of mature cardiomyocytes (Sheehy, et al., 2017).

Multiple *in vitro* models currently exist with different complexities. Ideally, these models are engineered to resemble the *in vivo* microenvironment of the heart. Two-dimensional models can be easily manipulated to create a synthetic environment with different ECM stiffness, structural organization, and external loads (Jorba, et al., 2021). The 2D models are advantageous as they allow for single-cell analyses and cell-cell interaction studies. Furthermore, they can be examined by simple techniques, such as microscopy and protein patterning techniques (Jorba, et al., 2021). To introduce mechanical loading in 2D models, cardiomyocytes can be cultured on stretchable elastomeric membranes which can be subjected to uniaxial or biaxial strain in a static or dynamic manner (Jorba, et al., 2021). Uniaxial strain can be achieved when the cells are stretched along a single axis, such as along the x-axis or y-axis only (Boulter, Tissot, Dilly, Pisano, & Feral, 2020), whereas biaxial stretching involves stretching along two axes (Tan, Scott, Belchenko, Qi, & Xiao, 2008). Moreover, static strain is applied by stretching the cells or tissue to a certain length and maintaining that position. This type of strain is simple to implement and can be performed for extended periods without tissue rupture (Zimmermann, 2013). On the other hand, dynamic strain in the form of cyclical stretch involves the repeated stretching of cells or tissue at different frequencies. This, however, can lead to tissue rupture and is more experimentally

demanding (Zimmermann, 2013). Applying stretch to the cultured cardiomyocytes mimics VO-induced strain exerted on *in vivo* cardiomyocytes, whereas altering the stiffness of the material on which the cardiomyocytes are cultured, can change the pressure the cells contract against, and thus help manipulate PO (Guo & Huebsch, 2020). Stretch can be applied in various ways to simulate different physiological responses to mechanical cues. For example, static stretch can be used to examine the effect of different degrees of preload, whereas dynamic stretch can be utilized to mimic changes in cyclical loading experienced by CMs *in vivo* (Jorba, et al., 2021). This stretch model, with its various stretch patterns, has been extensively used in the literature. In a study by McCain *et al*. (2013) rat cardiomyocytes were isolated from ventricles and cultured on polydimethylsiloxane (PDMS) coated elastic silicone membranes to form muscular thin films (MTFs). The MTFs were subjected to uniaxial and cyclical stretch at 10% strain and a frequency of 3Hz, using a custom-built multi-well system. The study revealed that uniaxial cyclic stretch altered cytoskeletal alignment, induced calcium cycling changes, and activated genes consistent with pathologic remodeling (McCain, Sheehy, Grosberg, Goss, & Parker, 2013). Similarly, biaxial cyclic stretching of neonatal rat ventricular cardiomyocytes induced hypertrophy and activated fetal genes associated with pathologic remodeling (Frank, et al., 2008). In addition to custom-built devices, cyclical stretch can be applied by commercial devices, such as the ARTEMIS ATMS Boxer (Wong, et al., 2018) or Flexcell FX-5000 Tension system (Banerjee, et al., 2015). Hypertrophic changes have also been observed in models of a static stretch (Yang, et al., 2016). Thus, irrespective of the stretching mode, cardiomyocytes tend to exhibit changes associated with pathologic remodeling upon experiencing various degrees of stretch-induced strain.

Stretch-induced adverse remodeling can also be observed in three dimensional models. 3D models are more complex as multiple components are involved to emulate in vivo cardiac tissue. Several materials have been explored to imitate the three-dimensional architecture, stiffness, and viscoelasticity of myocardial ECM (Jorba, et al., 2021). Polymeric hydrogels formed from networks of polyethylene glycol (PEG), gelatin methacryloyl, and alginate are commonly used as they allow for the control of their elasticity (Lee, et al., 2017). However, these hydrogels lack mechanosensitive ligands present in myocardial ECM, and the polymers can impair cell-mediated remodeling (Paik, Saito, Sugirtharaj, & Holmes, 2006). Therefore, to circumvent this drawback, hybrid biomaterials have been developed, such as polycaprolactone (PCL)/gelatin (Nguyen, et al., 2019). Nevertheless, only a few materials allow for the control of viscoelasticity, which is known to influence how cells recognize mechanical cues from their surrounding (Jorba, et al., 2021). The design of engineered heart tissue (EHT) aimed to integrate the mechanics of preload and afterload in 3D *in vitro* models (Eschenhagen, et al., 1997). Eschenhagen *et al*. (1997) developed this model which included a hydrogel scaffold mimicking myocardial ECM and containing cardiomyocytes, constructed between two stretching posts. The posts can be used to manipulate preload and afterload exerted on the EHT. Controlling the distance between the posts can alter the static stretch and influence the preload of the EHT, whereas altering the rigidity or stiffness of the posts can help manipulate afterload (Jorba, et al., 2021). For example, Hirt *et al*. (2012) reinforced silicone posts with metal braces to increase the afterload on the EHTs. This sustained increase in afterload was enough to induce adverse remodeling in the EHT, such that activation of fetal

genes, increased glucose consumption, and contractile dysfunction were reported (Hirt, et al., 2012). Moreover, cardiomyocyte hypertrophy was observed in EHT undergoing phasic unidirectional stretching (Fink, et al., 2000). In addition to controlling the posts, the mechanical properties of the EHT scaffold material can be manipulated. Thus, the EHT model allows for the independent manipulation of both external load and internal stiffness (Jorba, et al., 2021). However, the classic EHT model only allows for uniaxial stretch, which is not representative of the in vivo pathological strain pattern (Jorba, et al., 2021). This led to the development of in vitro EHT model with both a uniaxial and biaxial strain system, such that the EHT was attached to more than two stretching posts and stretched both horizontally and vertically (van Spreeuwel, et al., 2014).

The Gap in Our Understanding and Future Perspectives

Cardiovascular physiology and pathophysiology have been a subject of investigation for many years. Over this period, significant discoveries have been made and several effective therapies have been developed. However, the mortality rates of cardiovascular complications such as MI and heart failure remain high. Thus, our understanding of cardiovascular pathology remains incomplete, and further discoveries are necessary to develop novel therapies aimed at reducing mortality rates and improving prognoses. Cardiovascular mechanobiology has been frequently overlooked, with most studies focusing on underlying genetic and mechanistic causes, discovered through genome-wide association studies, proteomics, genomics, or multi-omics studies (Swiatlowska & Iskratsch, 2021). However, over the past decade, the persistent gap in our understanding led to a rise in studies examining the role of mechanical forces and mechanosensing mechanisms in cardiovascular development and disease (Swiatlowska & Iskratsch, 2021). These studies improved our knowledge of how mechanical load can induce embryonic and fetal cardiovascular development. Several mechanosensitive pathways were discovered involving transcription factor KLF2a and microRNAs such as miR-21 and miR-143, establishing a link between mechanical load and epigenetics for normal cardiac development (Banjo, et al., 2013; Chiplunkar, et al., 2013). Flow-induced activation of KLF2a was discovered to be mediated by protein kinase D2 (Lee, et al., 2006) and mechanosensing ion channels Trpp2 and Trpv4 (Heckel, et al., 2015) in zebrafish, and receptor P2X4 in humans (Sathanoori, et al., 2015). However, the mediators of mechanical load-induced miR activation remain unknown. Thus, further investigations are required to shed light on the upstream regulators of miRs. Furthermore, significant findings related to the mechanobiology of cardiac development were discovered in zebrafish. These findings, although beneficial, cannot be extrapolated to human cardiac development. Thus, future studies using patient-derived iPSC *in vitro* models may help determine the load-induced regulators of cardiac development in humans. Moreover, they could unravel the causality of abnormal epigenetics in cardiac malformations by studying epigenetic changes under specific loading conditions (Jarrell, Lennon, & Jacot, 2019). Lastly, although the effect of blood flow in cardiac development has been extensively studied, the role of cellular components of blood has not been often considered. For example, evidence shows that changes in flow shear stress, partly governed by blood viscosity, can trigger developmental changes. However, the direct role of red blood cells or other blood cells in

mechanotransduction during development has not been regularly examined (Boselli, Freund, & Vermot, 2015).

Unlike studies examining mechanical load during development, investigations of the role of mechanical load in exercise-induced cardiac hypertrophy have been performed more frequently in humans. However, such studies have failed to consider the sex differences in cardiomyocyte response to exercise, with most studies demonstrating that exercise induces myocardial hypertrophy in males (Scharhag, et al., 2002; Kokkinos, et al., 1995). Thus, further investigations should focus on identifying sex-specific mechanisms of hypertrophy which may also play a role in pathologic remodeling. However, these sex differences have been studied in animal models, although discrepancies have been observed between findings in animals and humans. For example, in humans, studies have illustrated that male cardiomyocytes have a higher hypertrophic potential than females (Bernardo, Weeks, Pretorius, & McMullen, 2010). Furthermore, male athletes were found to develop more pronounced LV hypertrophy when compared to females (Pelliccia, Maron, Culasso, Spataro, & Caselli, 1996). However, in rodent models of mice and rats, females were observed to display an increased hypertrophic response to exercise compared to males (Luczak & Leinwand, 2009; Schaible & Scheuer, 1979). These inconsistencies warrant the need for further investigations into the sex differences in exercise-induced cardiac remodeling in humans. Finally, it is important to note that several studies highlight the involvement of IGF-1/PI3K/AKT in exercise-induced hypertrophy, which is mainly observed in cardiomyocytes. However, the myocardium also comprises of non-cardiomyocyte cells such as endothelial cells and fibroblasts. Thus, further research is required to analyze the distinct functions of the various cell types and their crosstalk in response to exercise-induced mechanical load (Lerchenmüller & Rosenzweig, 2014).

Various explanations and potential mechanisms which regulate pregnancy-induced cardiac remodeling have been identified, with many of those mechanisms overlapping with exercise-induced hypertrophy, such as the PI3K/AKT pathway. However, loading patterns and durations, as well as hormonal alterations mechanistically distinguish pregnancy-, and exercise-induced cardiac remodeling. Recent evidence has shown that miRs are also involved in the structural and functional regulation of the pregnant heart (Szczerba, et al., 2020). However, mechanistic insights into the role of miRs in pregnancy-induced cardiac hypertrophy are lacking. Future studies with in vitro iPSC-derived cardiomyocytes can be utilized to induce continuous mechanical loading and hormonal stimulations to simulate pregnancy conditions and examine the expression and function of various miRs.

Mechanical load-induced pathologic remodeling has been extensively studied during the past decade. Several findings illustrated the mechanistic differences between pressure and volume overload and highlighted their phenotypic differences, such that VO was established to lead to eccentric hypertrophy, LV dilation, and systolic dysfunction, whereas PO was found to be responsible for driving concentric hypertrophy, LV wall thickening, and diastolic dysfunction (Hutchinson, Stewart, & Lucchesi, 2010; Nauta, et al., 2019). Ikeda *et al*. (2015) proposed a reason for these phenotypic differences to be ERK1/2 signaling that is observed in PO, but not in VO, suggesting that ERK1/2 was responsible for promoting concentric

hypertrophy while simultaneously suppressing eccentric hypertrophy. However, the downstream targets of ERK1/2 which drive these processes were not identified. Moreover, Zhang *et al*. (2010) demonstrated that VO could also induce the activation of ERK1/2, although much later than PO. Thus, further investigations are required to map the activation profile of ERK1/2 at different time points during eccentric and concentric hypertrophy to understand how temporal changes in ERK1/2 activation promote concentric and suppress eccentric remodeling (Ikeda, et al., 2015). Finally, although the activation of ERK1/2 is associated with PO, a direct causal link has not been identified. Thus, future studies must aim to establish a link between PO and ERK1/2 activation, and identify the mechanisms involved in ERK1/2-induced concentric remodeling.

In addition to cardiomyocyte hypertrophy, myocardial ECM alterations are observed upon mechanical overloading. PO and VO promote distinct changes in ECM, such that during VO MMP levels are increased which promotes collagen degradation, whereas in PO collagen stability and TIMP expression are increased (Hutchinson, Stewart, & Lucchesi, 2010; Yarbrough, et al., 2012). These studies investigated ECM changes at given time points during heart failure progression. Thus, these analyses represent both causative and compensatory changes in matrix regulatory proteins. Therefore, more in-depth temporal analyses are required to distinguish between causative and compensatory mechanisms. Moreover, examining the ratio between MMPs, TIMPs, and ECM protein content would provide clearer insights into the effect of mechanical loading, rather than studying each molecule alone (Hutchinson, Stewart, & Lucchesi, 2010).

As heart failure progresses, the mechanical forces and loading conditions experienced by the heart are altered. These changes can be depicted on PV graphs, such that during end-stage HFrEF the PV loops are shifted to the right with an increase in both preload and afterload, whereas in end-stage HFpEF they are shifted to the left with a decrease in preload and increase in afterload (Miranda-Silva, Sequeira, Lourenco, & Falcao-Pires, 2022; Reddi, Shanmugam, & Fletcher, 2017). The PV loops provide a substantial amount of information on cardiac function. However, they are not readily used in clinical trials or routine checks as they require invasive catheterization (Seemann, et al., 2019; Bastos, et al., 2020). Thus, future studies must aim to develop non-invasive PV analysis tools. Recently, Seemann *et al*. (2019) developed the first experimentally validated non-invasive technique to estimate LV PV loops based on data from ventricular volume curves and brachial pressure. This technique, although accurate, was not recommended for repeated use in humans and patients with atrial fibrillation due to its limitations (Seemann, et al., 2019). Thus, further advancements are required to develop non-invasive PV analytic tools that can be readily used in clinical settings. Several studies have identified mechanical load-induced mechanisms involved in the pathophysiology of HFrEF using animal models. However, a complete understanding of the effect of mechanical load in the pathology of HFpEF has not been drawn due to the limited availability of animal models that completely mimic human HFpEF (Conceição, Heinonen, Lourenco, Duncker, & Falcao-Pires, 2016). HFpEF is a multifactorial disease where several comorbidities contribute to its progression. However, the existing animal models do not recapitulate the multiple variables of human HFpEF. Therefore, future studies should employ a combination of existing HFpEF animal models or develop novel animal models with larger

animals that can incorporate the effects of aging, exercise, and associated comorbid conditions (Shah, et al., 2020; Withaar, Lam, Schiattarella, de Boer, & Meems, 2021). Moreover, recently, a novel type of heart failure has been categorized called heart failure with midrange ejection fraction (HFmrEF). The clinical characteristics of HFmrEF are similar to that of HFrEF. However, the prognosis of the disease resembles that of HFpEF. Although, the underlying pathophysiological mechanisms and the PV relationships associated with HFmrEF remain unknown (Li, et al., 2021). Thus, further investigations are required to understand the exact mechanisms in HFmrEF, how these mechanisms affect the prognosis of the disease, and if HFmrEF is an intermediate state between HFrEF and HFpEF (Li, et al., 2021).

Multiple pharmacological agents are employed to alleviate the symptoms of end-stage heart failure. However, therapeutic interventions that can effectively reverse pathological mechanisms and cure the disease remain to be developed. Advancements in LVAD therapy have provided promising results, with several studies reporting improvements in cardiac remodeling and function upon LVAD support. However, this therapy does not lead to a cure, as explantation of the LVAD causes relapse of the disease. Thus, implicating the need for adjuvant therapies in the form of pharmacological or cell therapy, to enhance the degree of reverse remodeling to continue post-LVAD explantation. Current pharmacological agents, such as ACE inhibitors, angiotensin receptor blockers (ARBs), and beta blockers induce a certain degree of reverse remodeling (Martens, Belien, Dupont, Vandervoort, & Mullens, 2018). However, the optimal pharmacological agent that can effectively enhance reverse remodeling to warrant LVAD explantation is currently unknown. Therefore, future investigations must test multiple combinations of pharmacological drugs with LVAD support to develop more effective therapies for heart failure. However, it is worth mentioning that LVAD support and the aforementioned pharmacological agents have been reported to be effective against HFrEF; and are considered to be ineffective against HFpEF (Miyagi, Miyamoto, Karimov, Starling, & Fukamachi, 2021). LVAD implantation in HFpEF patients is lacking due to the characteristic feature of small LV chamber size (Miyagi, Miyamoto, Karimov, Starling, & Fukamachi, 2021). However, a simulation study using a computer model of continuous flow LVAD and hemodynamics of HFpEF patients revealed that LVAD support can unload the LV, increase CO, provide hemodynamic benefits, and improve the quality of life of these patients (Moscato, et al., 2012). Therefore, advancements in LVAD technology must focus on circumventing the hurdles of implanting LVADs in small LV chambers.

Mechanistically, several mechanoreceptors and mechanosensory pathways have been identified. Integrins, SACs, pro-inflammatory cytokine receptors, and G-protein coupled receptors have been implicated to translate changes in mechanical load to signal transduction pathways. The PI3K/Akt, MAPK, calcineurin/NFAT, and JAK/STAT pathways have all been observed to influence load-induced pathological changes in the myocardium. However, most of these findings have been observed in cardiomyocytes, while the mechanosensitive pathways in fibroblasts and other non-cardiomyocyte cells, such as endothelial cells have remained unexplored.

LVAD-mediated unloading of the heart leads to a decrease in the activity of some mechanotransducer and partial reversal of pathways involved in cardiomyocyte hypertrophy

and fibrosis. The activity of the MEK/ERK pathways, as well Akt levels are reduced upon mechanical unloading counteracting their activation in load-induced remodeling. In addition, changes in the ratio of MMP/TIMP are observed favoring reverse remodeling. However, a better understanding of molecular mechanisms is necessary, which could shift LVAD function from bridge-to-transplant to bridge-to-remission.

A great proportion of studies have utilized in vivo animal models to discover the role of mechanical load in heart failure pathophysiology. Over decades of cardiovascular research, several techniques have been developed to model different cardiac loading conditions in both small and large animals. Aortic banding, specifically TAC, has been extensively performed to model PO while VO-induced heart failure has been induced by aortocaval shunting techniques or valvular regurgitation models. Both models of PO and VO were found to significantly induce LV hypertrophy, cardiac dysfunction, and heart failure in both large and small animals. However, it should be of note that the induction of PO and VO in these models does not represent the pattern or duration of mechanical overload experienced in humans, such that the induction of overload in the models is acute, whereas in humans the process is chronic. Indeed, Yarbrough *et al.* (2012) illustrated that the gradual and progressive constriction of the aorta provided a more accurate model of HFpEF than acute aortic constriction. Moreover, these models utilize relatively young animals, which does not reflect the age range at which humans begin to develop mechanical load-induced pathologic remodeling. Thus, future studies must consider using older animals, which would better represent the age of heart failure patients, and employing models of progressive mechanical load induction to more accurately reflect heart failure pathophysiology.

Advancements in biomaterials as well as cell isolation and culturing techniques have led to the development of several *in vitro* models of mechanical load. These models have allowed for the functional quantification of the impact of mechanobiological signals on cell and tissue behavior. Precise control of biomechanical properties such as ECM stiffness and mechanical strain in 2D models have provided insights into the mechanisms of load-induced cardiac remodeling. However, limitations in iPSC-derived cardiomyocyte maturity, cell diversity, and electrophysiological properties have challenged the findings from these models (Jorba, et al., 2021). Moreover, the development of 3D models has faced challenges and has been a subject of scrutiny as these models have been incapable of accurately recapitulating the complexity of cardiac tissue. Mimicking all the aspects of *in vivo* remodeled cardiac tissue is complex as several characteristics of the diseased tissue remain unknown. For example, the effect of inflammation on the ECM turnover and the mechanical properties of myocardium is not completely understood (Jorba, et al., 2021). Furthermore, there is a lack of experimental data on the nonlinear mechanics of the passive myocardium under different loading conditions (Sommer, et al., 2015). Therefore, further studies on the mechanical properties of passive myocardium can provide a blueprint for estimating material parameters, and allow for the development of biomaterials that can more accurately resemble the *in vivo* myocardium. Lastly, it should be noted that although advancements in *in vitro* models can increase their resemblance to the *in vivo* structure and function of the myocardium, these models are unable to reflect the cross-talk between organs and systems; and thus they lack the translational potential of *in vivo* models (Jorba, et al., 2021). Although significant advances in

2D and 3D *in vitro* models have been made, an ideal representation of myocardial tissue and the mechanical forces exerted by pressure and volume overload remains to be modeled (Jorba, et al., 2021).

Conclusion

In conclusion, the pathophysiology of heart failure has been a subject of interest for many years. Early research focused on genetic and proteomic investigations to unearth the underlying mechanisms. However, with novel insights into cardiac mechanobiology, the focus shifted towards understanding the underlying mechanotransduction and mechanosensing pathways of the disease. This review first discussed the importance of mechanical load throughout an individual's life by highlighting the role of mechanical load during development and health. Several studies revealed that mechanical load activates mechanotransduction pathways throughout prenatal development to activate genes and allow normal cardiac development. Under the influence of mechanical load KLF2, miR-21, miR-143, were found to be essential drivers of development of both the heart and the vasculature. Furthermore, the activation of the PI3K/Akt pathway was concluded to be central for exercise-induced hypertrophic effects in the heart. During pregnancy, however, the activation of PI3K/Akt and hormonal changes were responsible for cardiac physiologic hypertrophy. Subsequently, the role of mechanical (over)loading in pathologic myocardial remodeling and how LVAD support can reverse this remodeling were presented. PO and VO were revealed to induce different forms of hypertrophy due to their activation of different mechanotransduction pathways. VO was found to induce eccentric hypertrophy through the activation of the Akt/m-TOR pathway, whereas PO led to activation of the MAPK/ERK pathway and concentric hypertrophy. In addition, both PO and VO induced profibrotic changes in the myocardial ECM by influencing the ratio of MMPs to TIMPs. The pathological alterations in cardiomyocytes and ECM was found to develop two forms of heart failure: HFrEF and HFpEF which were differentiated by changes in the PV graphs, where HFrEF patients observed a shift in PV loop to the right, whereas HFpEF patients had a PV loop shifted to the left. The changes in PV loops, as well as mechanical load-induced pathologic remodeling were observed to be partially reversed upon LVAD implantation. LVAD support led to reversal of the activity of certain mechanotransducers, such as ERK and Akt, as well as the normalization of the MMP/TIMP ratio altering the cardiac phenotype from pathological to healthy. However, these devices did not provide a permanent solution, thus further investigations are required to enhance reverse remodeling enough to warrant LVAD explantation. Finally, the current *in vivo* and *in vitro* models of mechanical load which led to these findings were highlighted. The *in vivo* models, such as TAC/deTAC, DOCA-WD, aortocaval fistula, valvular regurgitation, and HHT all effectively led to load-induced remodeling and unloading-induced reverse remodeling. Similarly, the *in vitro* models, such as CTCM and EHT with their different complexities and modes of stretching also induced these load-induced changes. Our current knowledge of cardiac mechanobiology and its role in the physiology and pathophysiology of the heart would not have been possible without the developments in these models of mechanical load. The findings from these models have significantly impacted our view of cardiac mechanobiology. However, several limitations exist that challenge these findings, thus warranting the need for the development of novel, more accurate models. To summarize, investigations of cardiac

mechanobiology have improved our knowledge of cardiac mechanical load and how it induces functional and dysfunctional adaptations which promote health and disease, respectively. However, advancements of *in vivo* and *in vitro* models are still required to fill the gaps in our current understanding of myocardial mechanical properties, mechanotransduction, and reverse remodeling.

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