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The Role of Lrp5/6 in Cardiac Valve Disease: LDL-Density-Pressure Theory

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ABSTRACT

Atherosclerosis and osteoporosis are the leading causes of mortality and morbidity in the World. Recent epidemiologic studies have demonstrated that these disease processes develop in parallel. Evidence indicates that hyperlipidemia plays a paradoxical role in both disease processes. However, the mechanism is not understood. This prospectus hypothesizes the role of lipids activate atherosclerosis within the bone and the heart to initiate the development of diseases in both of these tissues. The Prospectus on the Lrp 5/6 receptors provides a foundation for the mechanisms involved in the Lrp5/6 mediated disease biology. The LDL-Density-Pressure theory: the Role of Lrp5/6 provides a biological and a hemodynamic approach towards understanding the development of valvular heart disease and the implications in the field of bone molecular biology. This prospectus will review the current literature, provide a basis for the development of valve disease and indicate future therapeutic pathways for this disease process in the future. J. Cell. Biochem. 112: 2222–2229, 2011.

KEY WORDS: LIPIDS; Lrp5; Wnt; OSTEOBLASTOGENESIS

The low-density lipoprotein (LDL)-related receptor 5 and 6 (Lrp5 and Lrp6) genes were cloned in 1998 based on their homology with the LDL receptor (LDLR) [Brown et al., 1998; Dong et al., 1998; Hey et al., 1998; Kim et al., 1998]. Mutations in either LRP5 or LRP6 proteins have caused a number of disease processed in the field of bone [Gong et al., 2001; Little et al., 2002], and have been associated with cardiovascular disease [Kim et al., 1998; Fujino et al., 2003; Rajamannan et al., 2005a; Caira et al., 2006]. The most recent perspectives in the field of Lrp5/6 [Williams and Insogna, 2009] in signaling in the bone and Lrp5 in the regulation of bone mass [Johnson and Summerfield, 2005] demonstrate the most comprehensive reviews in this field and provide the background for the structure and function of these co-receptors. The LDL-density-pressure theory combines the structure, function analysis of these co-receptors with the results from the genetic studies to provide a unique hypothesis for the role of these receptors in the heart. The focus of this prospectus is to further combine the structure, function, developmental biology with the genetics studies of the Lrp5/6 co-receptors to further define their role in the development of cardiovascular calcification as related to the bone formation within the heart.

Lrp5/6 AND CANONICAL Wnt SIGNALING IN OSTEOBLASTOGENESIS

The LDL co-receptor Lrp5/6 is a member of the family of structurally closely related cell surface LDLRs that have diverse biological functions in different organs, tissues, and cell types which are important in development and disease mechanisms. The most prominent role in this evolutionary ancient family is cholesterol homeostasis. In humans, cholesterol in the blood is captured by LDL and metabolized by the liver via endocytosis of the LDLR. There is recent evidence that members of the LDLR gene family are active in the cell signaling pathways between specialized cells in many multicellular organisms.

The LRP5 pathway regulates bone formation in different diseases of bone [Gong et al., 2001; Boyden et al., 2002]. The discovery of the LRP5 receptor in the gain of function [Boyden et al., 2002] and loss of function [Gong et al., 2001] mutations in the development of bone diseases, resulted in a number of studies which have shown that activation of the canonical Wnt pathway is important in osteoblastogenesis [Babij et al., 2003; Fujino et al., 2003; Holmen et al., 2004; Westendorf et al., 2004]. Three studies to date have confirmed the regulation of the LRP5/Wnt pathway for

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cardiovascular calcification in vivo and ex vivo [Shao et al., 2005; Rajamannan et al., 2005a; Caira et al., 2006]. The LRP5 receptor signaling in bone is mediated via the canonical Wnt pathway as shown in Figure 1. In this pathway, Wnt proteins bind to receptors composed of a frizzled protein and either of the LDLR-related proteins LRP5 or LRP6. Signaling via Disheveled and/or Axin then results in inactivation of a multiprotein complex including Axin, adenomatous polyposis coli (APC), and glycogen synthase kinase-3β that normally renders β-catenin unstable. By inhibiting this complex, Wnt signals lead to accumulation of β-catenin in the cytosol and its entry into the nucleus. Once in the nucleus, β-catenin binds to proteins of the T-cell factor/lymphoid enhancer factor-1 family and modulates the expression of several target genes which include Cyclin D, Msx2, Cbfa1, and Sox9. Bone and cartilage are major tissues in the vertebrate skeletal system, which is primarily composed of three cell types: osteoblasts, chrondrocytes, and osteoclasts. In the developing embryo, osteoblast and chrondrocytes both differentiate from common mesenchymal progenitors in situ, whereas osteoclasts are of hematopoietic origin and brought in later by invading blood vessels. Osteoblast differentiation and maturation lead to bone formation controlled by two distinct mechanisms: intramembranous and endochondral ossification, both starting from mesenchymal condensations.

Lrp5 has been shown to have an effect on bone mass via the mechanostat effect on regulating bone formation. The findings in the human of the high bone mass gain of function mutation [Little et al., 2002] led to a series of discoveries that Lrp5 regulates bone mass via the mechanical force effect on the receptor [Akhter et al., 2004; Johnson et al., 2004; Johnson and Summerfield, 2005]. Lrp6 also regulates bone but has been found to have a low bone mass effect in patients in which a putative partial loss-of-function mutation in LRP6 was associated with early cardiovascular-related death associated with increased plasma LDL, triglycerides, hypertension, diabetes, and osteoporosis [Mani et al., 2007]. The first paper for the proof of concept demonstrated that Lrp5 and Lrp6 both play an important role in bone formation in the Lrp5 null mice and the Lrp6 heterozygote mice [Holmen et al., 2004]. This paper demonstrated that both receptors are necessary for bone mass and limb development. These data were further corroborated with studies presented at the Sun Valley workshop in 2008 [Zylstra et al., 2008], which indicated that the homozygous LRP6^{flox} mice with the osteocalcin (OC) promoter had significantly low bone mass demonstrating that Lrp6 was required for normal bone acquisition. When the mice were mated with the Lrp5 null mice, the offspring developed severe osteopenia with reduced bone formation and increased bone resorption suggesting that Lrp5 and Lrp6 are required to fully activate β-catenin in mature osteoblasts.

THE STRUCTURAL ROLE OF Lrp5 AND Lrp6 AS SUBCLASS MEMBERS OF LDLR IN LIPOPROTEIN METABOLISM

The LDLR gene family consists of cell surface proteins involved receptor-mediated endocytosis of specific ligands. This class of gene family has similar protein structures and plays a role in lipid

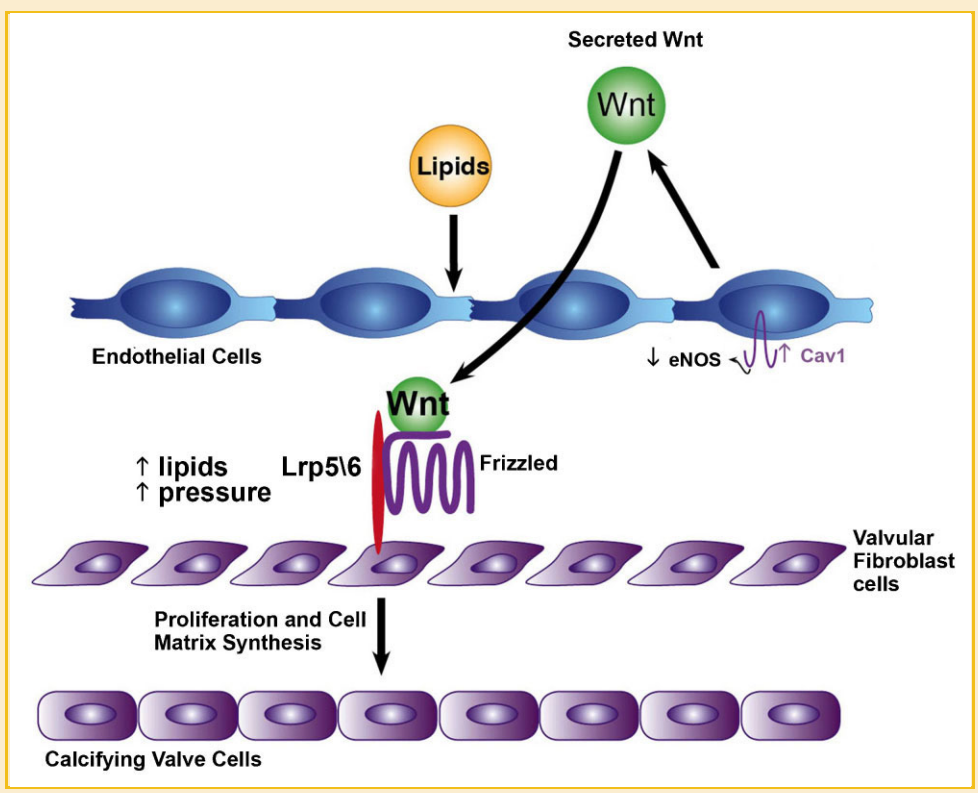

Fig. 1. Schematic for the mechanism of Lrp5/6 in Canonical Wnt Activation.

metabolism. However the genetic studies in the field of cardiovascular disease have revealed novel signaling and disease mechanisms for the various subclasses of this family of receptors. LDL is normally bound at the cell membrane and taken into the cell ending up in lysosomes where the protein is degraded and the cholesterol is made available for repression of microsomal enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the rate-limiting step in cholesterol synthesis. At the same time, a reciprocal stimulation of cholesterol ester synthesis takes place. Mutations in this gene cause the autosomal dominant disorder, familial hypercholesterolemia.

Lrp5 co-receptor in lipid metabolism encodes a transmembrane LDLR that binds and internalizes ligands in the process of receptor-mediated endocytosis. This protein also acts as a co-receptor with Frizzled protein family members for transducing signals by Wnt proteins and was originally cloned on the basis of its association with type 1 diabetes mellitus in humans. This protein plays a key role in skeletal homeostasis and many bone density related diseases are caused by mutations in this gene. Mutations in this gene also cause familial exudative vitreoretinopathy. Mutations results in increases in bone mass (gain of function) [Little et al., 2002] and losses in bone mass (loss of function) [Gong et al., 2001].

The role of lipid signaling of the LRP5 receptor has been defined in experimental in vitro and in vivo lipid models of vascular atherosclerosis. LRP5 binds apoE-containing lipoproteins in vitro and is widely expressed in many tissues including hepatocytes, adrenal gland, and pancreas [Kim et al., 1998]. The production of mice lacking LRP5 revealed that LRP5 deficiency led to increased plasma cholesterol levels in mice fed a high-fat diet, secondary to decreased hepatic clearance of chylomicron remants and also marked impaired glucose tolerance [Fujino et al., 2003]. In the LRP5 mice that were not fed the high cholesterol diet, the mice did not develop high cholesterol levels [Magoori et al., 2003]. The investigators went on to define the role of LRP5 in the lipoprotein metabolism by developing a double knockout mouse for ApoE:LRP5. They found that the double KO mouse had approximately 60% higher cholesterol levels compared with the age matched apoE knockout mice. High-performance liquid chromatography analysis of plasma lipoproteins revealed that their was no difference in the apoproteins but the cholesterol levels in the very low density and LDL fractions were markedly increased in the apoE:Lrp5 double KO mice. There was threefold increase in the atherosclerosis indicating that the Lrp5 mediates both apoE-dependent and apoE-independent catabolism of lipoproteins. In 1994, studies demonstrated that the plasma cholesterol levels in the double KO mice lacking both ApoE and LDLR were not significantly different from the levels in the ApoE knockout mice [Ishibashi et al., 1994]. The severe hypercholesterolemia developed in the double knockout mice lacking both apoE and Lrp5 suggests the presence of an alternative pathway for cholesterol catabolism mediated by Lrp5, which appears to be independent of the LDLR pathway. The LRP5 deficient islets also demonstrated a reduction of intracellular ATP and calcium in response to glucose, and thereby decreasing glucose induced insulin secretion [Fujino et al., 2003]. Furthermore, experimental hypercholesterolemia is associated with the increase in LRP5 receptor expression and activation of cell proliferation and extracellular matrix production critical in bone formation [Rajamannan et al., 2005a]. These studies provide evidence that lipoprotein metabolism is regulated by the fifth family member of the LDL co-receptor family LRP5 in these knockout mouse studies.

Lrp6 co-receptor also plays a role in bone formation and lipid metabolism. This gene encodes a member of the LDLR gene family. LDLRs are transmembrane cell surface proteins involved in receptor-mediated endocytosis of lipoprotein and protein ligands. The protein encoded by this gene functions as a receptor or, combines with Frizzled, as co-receptor for Wnt and thereby transmits the canonical Wnt/beta-catenin signaling cascade. Through its interaction with the Wnt/beta-catenin signaling cascade this gene plays a role in the regulation of cell differentiation, proliferation, and migration and the development of many cancer types. This protein undergoes gamma-secretase dependent RIP-(regulated intramembrane proteolysis) processing but the precise locations of the cleavage sites have not been determined. Mutations results in cardiovascular events related to increases in LDL cholesterol [Mani et al., 2007; Tomaszewski et al., 2009]. In 2008, studies demonstrate that mutations in the EGFP domain of the LDLR-related protein 6 impairs cellular LDL clearance. The LRP6 receptor has been extensively studied for it function in regulating embryogenesis and cell proliferation [Pinson et al., 2000; Tamai et al., 2004]. This protein has been localized to lipid rafts [Yamamoto et al., 2006; Komekado et al., 2007]. Furthermore, LRP6 is important in LDL clearance, as shown in in vitro and in vivo Lrp5/6$^{+/-}$ mice [Liu et al., 2008].

Lrp5/6 AND Wnt SIGNALING IN CARDIAC VALVE DEVELOPMENT

Wnt signaling and Lrp5/6 co-receptors are critical in the signaling process in cardiac development [Gessert and Kuhl, 2010]. A recent discovery in Lrp6 research is the role of kremen 2 (KRM2) for neural crest induction, and the in vivo [Hassler et al., 2007]. The study indicates that Kremen is required for neural crest induction in Xenopus and promotes LRP6-mediated Wnt Signaling. Wnt signaling has also been demonstrated in heart valve development, osteogenic gene induction at highly specific stages of valve development, remodeling of atrioventricular (AV) and semilunar valves [Alfieri et al., 2010]. All of the developmental data provide the importance of this evolutionary signaling pathway in heart valve and cardiac development. After birth, Lrp5/6 expression is low unless it the expression increases in disease processes.

Lrp5/6 SIGNALING IN OSTEOBLASTOGENESIS IN VALVULAR HEART DISEASE

Our group and others have demonstrated that osteoblastogenesis and chrondrogenesis is critical in the development of valvular heart disease. The presence of calcification in the aortic valve is responsible for valve stenosis. Severe aortic stenosis can result in symptomatic chest pain, as well as syncope and congestive heart failure in patients with severe aortic valve stenosis. For years, aortic valve stenosis was thought to be a degenerative process. However, the pathologic lesions of calcified aortic valves demonstrate indicate

the presence of complex calcification in these tissues. Furthermore, there are a growing number of descriptive studies delineating the presence of bone formation in the aortic valve [O'Brien et al., 1995a; Mohler et al., 1997, 2001].

Until recently the etiology of valvular heart disease has been thought to be a degenerative process related to the passive accumulation of calcium binding to the surface of the valve leaflet. Recent descriptive studies have demonstrated the critical features of aortic valve calcification, including osteoblast expression, cell proliferation, and atherosclerosis [O'Brien et al., 1995b; Mohler et al., 2001; Rajamannan et al., 2002, 2003b] and mitral valve degeneration, glycosaminoglycan accumulation, proteoglycan expression, and abnormal collagen expression [Whittaker et al., 1987; Wooley et al., 1991; Grande-Allen et al., 2004, 2005]. These studies define the biochemical and histological characterization of these valve lesions. Studies have also shown that specific bone cell phenotypes are present in calcifying valve specimens in human specimens [Jian et al., 2001; Caira et al., 2006]. These data provide the evidence that the aortic valve calcification follows the spectrum of bone formation in calcifying tissues. Genes which code for the bone extracellular matrix proteins in osteoblast cells include alkaline phosphatase (AP), osteopontin (OP), OC, and bone sialoprotein (BSP). These data support a potential regulatory mechanism that these matrix proteins play a role in the development of biomineralization. To date, many of these markers have been shown to be critical in the extracellular mineralization and bone formation that develops in normal osteoblast differentiation. Figure 1 demonstrates the dual role of Lrp5/6 in the activation of Wnt signaling: (1) lipid binding and (2) mechanostat effect in the activation of the Wnt signaling pathway.

CARDIOVASCULAR RISK FACTORS

SPECIFICALLY LIPID BIOCHEMISTRY IN VALVULAR HEART DISEASE

With the decline in the incidence of rheumatic carditis, calcific AS has become the most common indication for surgical valve replacement in the US. Numerous epidemiologic studies have identified risk factors for AS disease development, which are similar to those of vascular atherosclerosis, including smoking, male gender, body mass index, hypertension, elevated lipid and inflammatory markers, metabolic syndrome, and renal failure [Deutscher et al., 1984; Hoagland et al., 1985; Aronow et al., 1987; Mohler et al., 1991; Lindroos et al., 1994; Boon et al., 1997; Stewart et al., 1997; Wilmshurst et al., 1997; Otto et al., 1999; Palta et al., 2000; Chan et al., 2001; Chui et al., 2001; Pohle et al., 2001; Aronow et al., 2001b; Peltier et al., 2003; Briand et al., 2006; Faggiano et al., 2006]. For years this disease process was thought to be due to a degenerative phenomenon by which calcium attaches to the surface of the aortic valve leaflet. Understanding calcification, as the critical end-stage process which causes progression to severe stenosis and leads to poor outcomes [Rosenhek et al., 2000], is becoming important in the results of the randomized trials for treating aortic stenosis with medical therapy.

HEMODYNAMIC PHENOTYPE OF CARDIAC VALVE DISEASE

ROLE OF Lrp5/6 IN CARDIAC VALVE DISEASE

The development of heart valve disease occurs in the left side of the heart the aortic valve and the mitral valve, which manifests as calcific aortic valve disease and myxomatous mitral valve disease. The LDL-density-pressure theory provides a scientific explanation for the manifestation of this phenotype. In experimental hypercholesterolemia the left-sided heart valves aortic [Rajamannan et al., 2001, 2002, 2003a, 2005a,b] and the mitral valve [Makkena et al., 2005] develop the atherosclerotic lesion but not the right-sided heart valves: pulmonic and tricuspid valves. Figure 2 demonstrates the differences in the pressures in the heart and the expression of the phenotype of the heart valve. The lipids bind to the Lrp5/6 receptors to activate the Canonical Wnt pathway and the bone formation. Since the Lrp5 plays a role in the mechanostat theory [Johnson and Summerfield, 2005] this mechanism provides the foundation for the

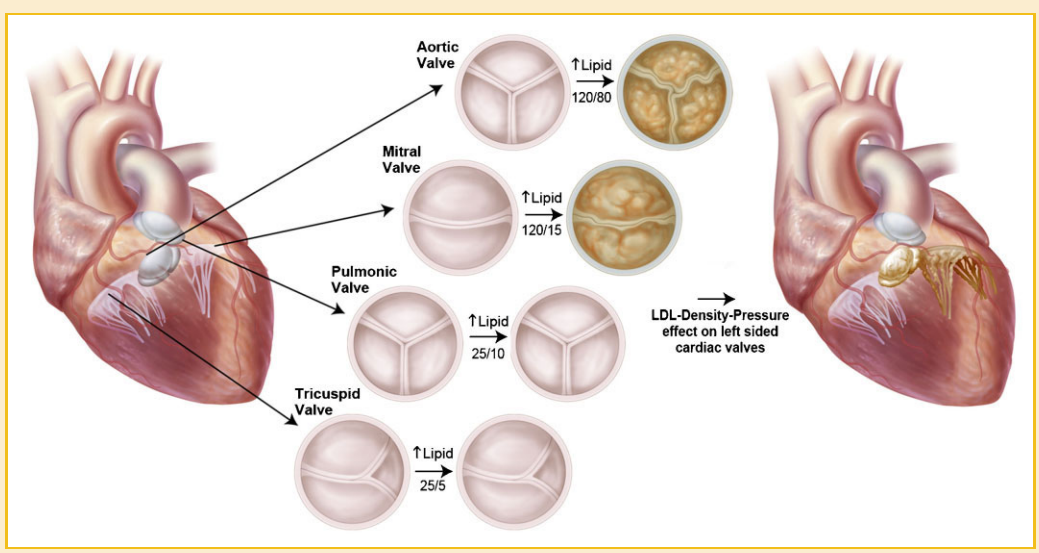

Fig. 2. Schematic for the role of hemodynamic pressures in the heart in the lipid–pressure activation of Lrp5/6 in the valves.

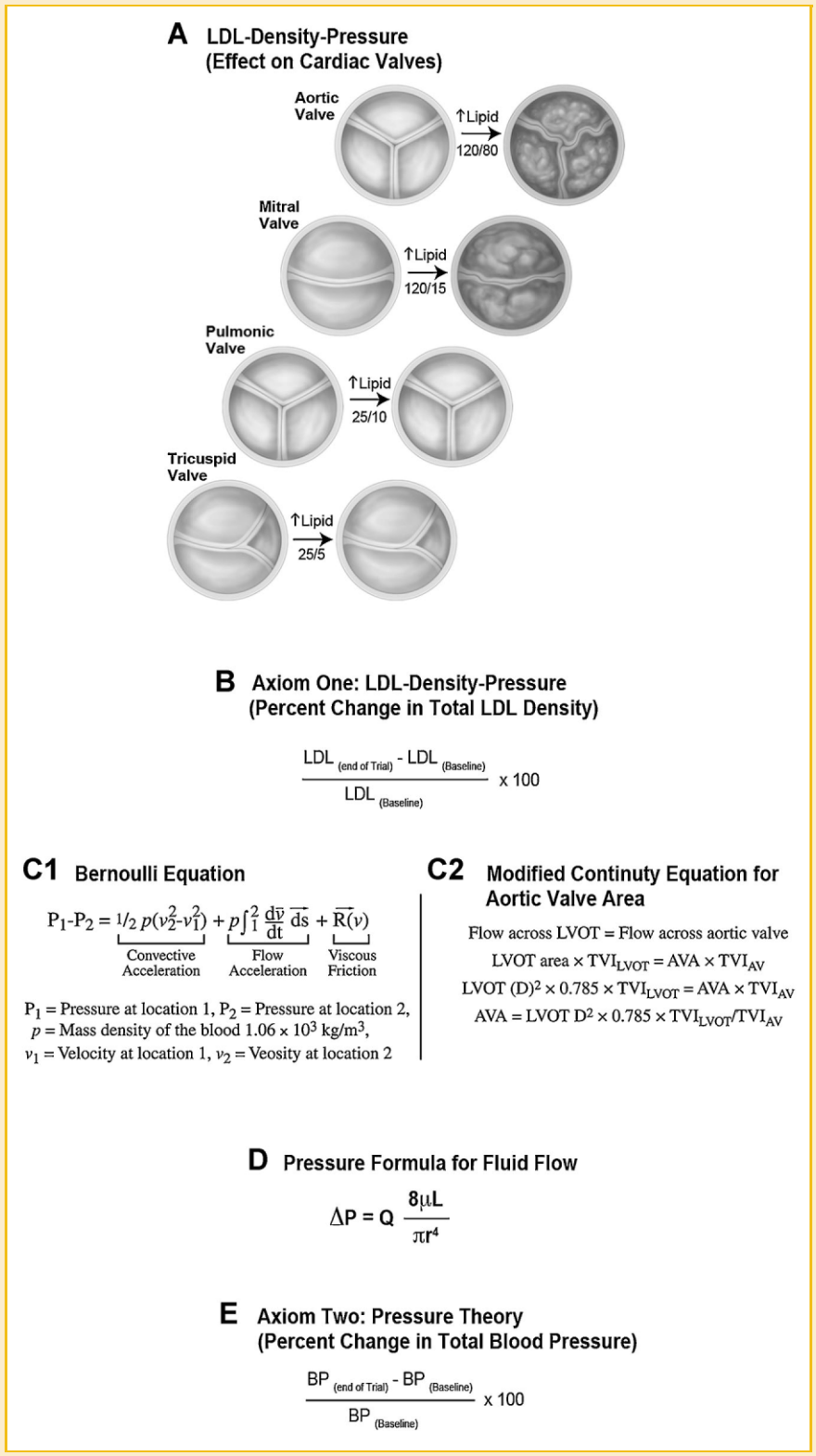

Fig. 3. The LDL-Density-Pressure Theory (Effect on Cardiac Valves). The serum lipid levels affect all four heart valves as the lipids circulate throughout the heart. However, the pressure is different depending on the chamber and location in the heart. Panel A: The effect of lipids and the different pressures in the heart. Panel B: Axiom One: LDL-Density-Pressure- percent change in the LDL density. Panel C1: Bernoulli Equation Panel C2: Modified Continuity Equation for Aortic Valve Area Panel D: Pressure Formula for Fluid Flow. Panel E: Axiom Two: Pressure Theory(Percent Change in Total Blood Pressure).

pressure theory on mechanical effects on Lrp5 in the heart. Normal pressures in the heart increase from the right atrium, to the right ventricle, to the left atrium, and finally to the left ventricle for normal cardiac physiology as shown in Figure 2. However, this theory hypothesizes that in the presence of hyperlipidemia the phenotypic expression of the valve changes in response to the different pressures in the heart. Human phenotypic studies of cardiac valve disease have demonstrated that the aortic valve expresses an osteoblast phenotype [Rajamannan et al., 2003c; Caira et al., 2006] and the mitral valve expresses a chondrogenic phenotype [Caira et al., 2006]. It is known that the mitral valve has a lower pressure present in the left atrium as compared to the aorta. Therefore the pressure on the mitral valve is enough to produce cartilage and the pressure on the aortic valve is higher to drive the Lrp5 mechanostat mechanism to form bone. The mitral annulus which has slightly higher pressures than the valve leaflet can form bone if the pressures are high enough causing mitral annular calcification [Boon et al., 1997; Aronow et al., 2001a]. The results of these studies further confirm the mechanostat theory for the role of Lrp5 in the presence of the different pressures in the heart. The highest pressure aortic valve differentiates to form bone and the mitral valve which has lower pressures only develops calcification at the mitral annulus and in the leaflets develops a cartilage phenotype. Even though the lipids are present throughout the systemic circulation, the right-sided valves with the lowest pressures in the hearts do not calcify or form cartilage as shown in Figure 2. The human study [Caira et al., 2006] further confirms the hemodynamic pressures correlating with the expression of Lrp5/6 in the human diseased valves by immunohistochemistry staining with the Lrp5/6 antibody.

LDL-DENSITY-PRESSURE THEORY

HYPOTHESIS FOR THE ROLE OF Lrp5/6 SIGNALING IN VALVULAR HEART DISEASE

This hypothesis evaluates the effect of pressure in the development of calcification is dependent on two axioms, biology and hemodynamics. The experimental data demonstrate that lipids activate the bone differentiation [Rajamannan et al., 2005a] in the valve. The first axiom is the effect of LDL in atherosclerotic biology. LDL affects the left-sided heart valves, as shown in Figure 3A. In the presence of experimental hypercholesterolemia the LDL only manifests disease in the left-sided valves where the pressure is higher in the heart is overtime, the leaflets fuse which to occlude the aortic valve Figure 3B. Calculation of the percent lowering of LDL density will target specifically the biologic effect of LDL on this disease. Figure 3C demonstrates a formula to calculate the percent reduction of the LDL density before and after therapy.

The second axiom is the effect of the radius on fluid flow. The Bernoulli equation [Bernoulli, 1738] Figure 3D1, the formula for flow through a pipe, was modified [Hatle et al., 1980] Figure 3D2, to calculate aortic valve areas by echocardiography using the Doppler technique. The modification of this equation for aortic valve areas includes the deletion for the calculation for the flow acceleration and the viscous friction because the velocity profile in the center of the lumen is usually flat. Thus the viscous friction factor can be ignored in the clinical setting of aortic valve disease. However, the flow in the lumen of a vessel is not flat due to smaller radius, therefore, the viscous friction factor must be taken into account. The importance of the smaller radius is shown in Figure 3E, which is the calculation of resistance of fluid through a pipe. This size of the radius becomes important in the calculation of flow as the inverse r^4 dependence of the resistance to fluid flow will increase viscosity by a factor of 16 requiring the effect of viscous friction to become important with smaller radii. Reductions of the LDL density will therefore, have a quicker effect in the reduction of the vascular lesion as it affects the lumen circumference directly as shown in Figure 3A. To measure the treatment effect for blood pressure on aortic valve disease: Figure 3F, is the calculation for the percent improvement in the blood pressure as an effect on the progression of the valve disease. This hypothesis provides a mathematical foundation for treating this disease in the heart.

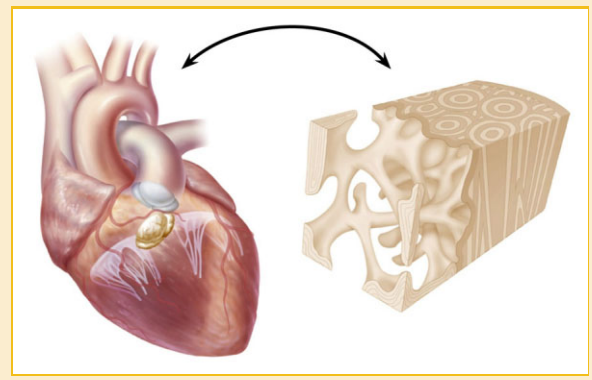

Fig. 4. The Cardiovascular-Bone Paradox: Atherosclerotic Calcification and Atherosclerotic Osteoporosis.

SUMMARY

Mathematically and biologically, medical therapy for aortic valve disease can consider the following two axioms for targeting the disease biology in terms of the magnitude of the LDL density to activate the atherosclerotic process and the change in blood pressure in aortic valve disease. This theory provides a biologic-hemodynamic foundation for the mechanism of Lrp5/6 activation in the heart. Figure 4 demonstrates the cardiovascular-bone paradox. This prospectus provides a novel foundation for the role of lipids and blood pressure in the development of bone formation in the heart.

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