International Journal of Computational Vision and Biomechanics
Vol.1 No. 1 (January-June, 2015)

MUK PUBLICATIONS
Open Access Publisher

The Myocardium as a Composite Material: An Overview on the Dynamic Modulation of its Passive Mechanical Properties

Adelino Leite-Moreira & João Ferreira-Martins

Department of Physiology, Faculty of Medicine, University of Porto, Porto, Portugal

Cardiac muscle tissue can be regarded as composite material, with both cellular and matrix structures contributing to its overall physical properties and, consequently, its mechanical function. Notwithstanding the major importance of the latter structures on active myocardial mechanics, both clinicians and physiologists became increasingly interested on the mechanisms through which they may change myocardial passive properties as well. In this setting, the consistent association between increased myocardial stiffness and diastolic dysfunction has led to intense research in order to unravel the pathophysiological mechanisms driving the development of abnormal myocardial material properties. The current and still growing knowledge about the determinants of myocardial passive properties will allow the clinicians to diagnose earlier and to choose the best therapeutic strategies in order to prevent or even reverse pathological cardiac remodeling. Plus, a better understanding of the basic principles governing myocardial tissue function is also setting up the scaffolds to promote the development of cardiac tissue engineering and regenerative medicine, therefore providing new tools to improve patient care.

Key words: *cardiomyocytes; diastolic function; extra-cellular matrix; heart failure. myocardial stiffness;*

1. INTRODUCTION

Cardiac muscle tissue is a composite material consisting of cardiomyocytes, fibroblasts, blood vessels and extra-cellular matrix (ECM). Therefore, it is reasonable to postulate that changes in any of the elements within the myocardium may affect its material properties (1) and consequently, its mechanical function. Both cellular and matrix structures contribute to myocardial physical properties, based on their intrinsic mechanical features, spatial and functional relations with the surrounding structures and tissue geometry, which finally ensues in the adult heart. Thus, when considering heart biomechanical properties, we have to conceptually integrate not only the single contribution of the various cardiac muscle tissue elements, but also the complex interactions among them, as well as, their ability to modify their intrinsic mechanics under different pathophysiological conditions. In this way, mechanotransduction–the biomechanical response of cells to mechanical stimulation (2)–is one important mechanism through which cardiac muscle tissue may be continually adapted to different loading conditions. (2,3,4) However, although this adaptive mechanism induces changes in myocardial properties in order to achieve a new mechanical and biological equilibrium, it goes very often out of balance (5), making the perfect phenotype-environment matching for greatest adaptive value an insuperable strategy. (4)

Therefore, in this review paper, we propose to address: (i) the mechanical properties of myocardial tissue and heart, (ii) its structural, physiological and pathological determinants (iii) and the benefits that the current and still growing knowledge of those properties might yield by helping to design new therapeutic strategies, therefore providing new tools to improve patient care.

2. MECHANICAL PROPERTIES OF THE MYOCARDIUM

Mechanics is the branch of physics concerned with the behaviour of physical bodies when subjected to forces or displacements, and the subsequent effect of the bodies on their environment. In this setting, the mechanical activity of the heart can be regarded as composed of two continuous and interdependent functions: *systolic function*, which is related with the ability of the ventricle to contract and eject; and *diastolic function*, which refers to its ability to relax and fill. (6) Both are accomplished by means of active processes of contraction and relaxation but also by the maintenance of proper cardiac muscle's material properties that allow the cardiomyocytes to contract and relax/distend to their maximal extent. Although active properties of the myocardium are responsible for its motion and blood flow, it is only through the perfect match of both active and passive properties that its overall (systolic and diastolic) function is preserved. Although for long, heart muscle contraction was considered the core of heart mechanical function and over which major concerns had been focused, there is now a significant and still growing body of evidence emphasizing diastolic dysfunction as the target to treat patients with the evenly frequent diastolic heart failure (DHF). As stated above, diastolic function can be generally regarded as the ability of the ventricle to relax and fill. Therefore, diastole can be considered to depend on *active* myocardial relaxation, which is the process whereby the myocardium returns to an

unstressed length and force and on *passive* properties, which mostly influence the extent of muscle re-length and end-diastolic pressure-volume relationship. Besides the unquestionable importance of both active contraction and relaxation for ventricular mechanics, physiologists and cardiologists became increasingly interested in the determinants of material properties of the myocardium as their changes have been consistently associated to various forms of heart disease. (7)

2.1 Passive Mechanical Properties of the Myocardium

For conceptual purposes, "passive" mechanical properties are generally considered as following "active" diastolic ones because they are generally assessed after complete muscle relaxation. However, two remarks should be done: *first*, although those properties are commonly named as "passive", they can also be actively modulated; (8,9) *second*, even if the term "diastolic" instead of "passive" properties is preferred (both because of their active modulation and their major influence in diastolic distensibility), we should keep in mind that myocardial material properties may be also regarded as *constitutive properties*, a concept that underscores their possible presence throughout the two phases of the cardiac cycle and hence modulating both. (10)

The determinants of "passive" properties of the ventricular wall are myocardial stiffness, wall thickness and chamber geometry.

2.1.1 Myocardial Stiffness

Although in solid mechanics E is commonly regarded as the elastic or Young's modulus, we will refer to it as *stiffness*, given both the purpose of the present paper and the biomedical language used herein. Myocardial stiffness (E) can be assessed by examination of myocardial stress-strain relationship during diastole (11, 12) as cardiac muscle generates tension to oppose the stretch imposed by the preload. (8,13) *Stress* (σ) is defined as the force per unit cross-sectional area of a material and is usually expressed as dynes or grams per square centimeter. *Strain* (ε) is defined as the deformation of a material that is produced by the application of a force (stress) and is commonly expressed as a percentage change from the unstressed dimension. Langragian strain (ε_L) is defined as $(l - l_0)/l_0$, where l_0 is the length corresponding to a state of zero stress and l is the instantaneous length. (10, 12, 14) Therefore, myocardial stiffness (E) is defined as the change in stress (σ) with respect to a change in strain (ε), as shown in the following equation:

$$(E) = d\sigma/d\varepsilon \qquad \text{(equation 1)}$$

As shown in figure 1, the stress-strain curve of biological tissue is curvilinear and the slope of any tangent to this line represents myocardial stiffness ($d\sigma/d\varepsilon$). (1, 11, 12, 15, 16).

The slope and position of the cardiomyocytes stress-strain relation are affected by three major determinants: (i) the *passive elastic spring*, which consists of all cellular elements that resist to stretch in a time-independent manner;

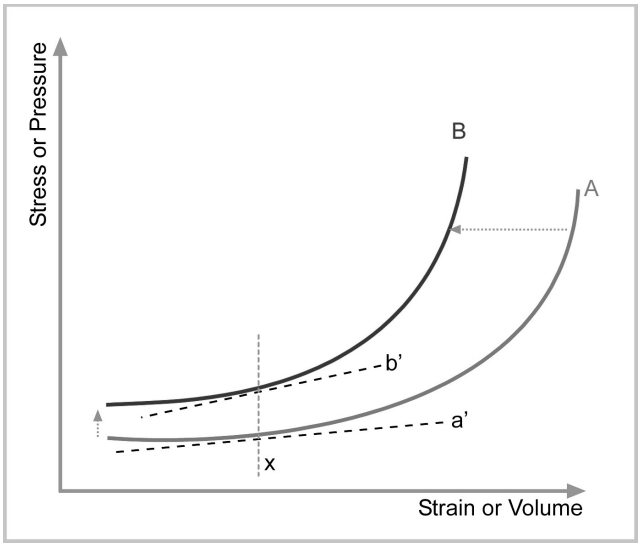

Figure 1: Stress-strain (or pressure-volume) relation. The transition from curve A to curve B indicates an increased cardiac muscle stiffness (higher stress at any given strain, x) or increased ventricle chamber stiffness (higher pressure at any given volume, x). The slope of the tangent lines (a' and b'), at any given strain or volume represents myocardial stiffness or ventricular chamber stiffness, respectively.

(ii) the *viscous damping*, which consists of all cellular structures that resist to stretch in a time-dependent manner, i.e., resist more when stretched faster; and (iii) *myofilament activation*, which represents the forces that result from cross-bridge cycling and actin-myosin interaction that act to resist stretch. (1) The latter underscore the concept that, like most biological materials, the myocardium is viscoelastic so that stresses in the heart wall depend on the rate at which it deforms (strain rate, ε) as well as on the magnitude of the deformation, (strain, ε). (1,18) Viscoelastic properties are manifested by specific features such as: *stress-relaxation*, which describes stress relief under constant strain; *creep* which is used to describe the tendency of a material to move or to deform permanently to relieve stresses; and *hysteresis*, in which for a given length variation, the force measured during stretch is greater than force measured during release. Because viscous forces increase with filling rate, these are greatest during the rapid filling phase in early diastole and atrial systole. Although there is no doubt that viscous forces contribute to cardiac muscle's resistance to change its length upon a force application and diastolic pressure, this contribution is probably small under physiological conditions. (18)

2.1.2 Wall thickness and Chamber Geometry

In the same way that an applied force (stress) can change muscle length (strain) depending on myocardial stiffness, a given rise in ventricular pressure can also increase chamber volume to an extent that will depend on overall chamber stiffness. Plotting the lower right corner of multiple pressure-volume (PV) loops obtained at various preloads, an

exponential curve is obtained and the slope of a tangent drawn to the curve at any point is the operating stiffness. (6) The end-diastolic pressure-volume relation changes with the heart's operating environment and depends on the balance between the pressure in the left ventricle and the stresses in the myocardium. These stresses, in turn, depend on the size and thickness of the ventricle and the stiffness of the myocardium. (6,11,18)

In this regard, Laplace law equation can be applied and relates wall stress (σ) to pressure (p) and geometry (radius, r and wall thickness, h):

$$\sigma = pr/2h \text{ (equation 2)}$$

According to the latter equation, ventricular wall stress increases in direct proportion to the internal pressure and radius and decreases in inverse proportion to the wall thickness. When overall chamber stiffness is increased, the EDPVR is shifted upwards and to the left. (figure 1, curve A to B).

In the setting of pathological situations where wall tension may be significantly increased (as in volume or pressure overloads), the adaptive response of the heart will be to change its geometry so that wall tension returns back to normal levels. The Laplace law provides the theoretical framework to understand the changes that take place in the aforementioned situations. When the ventricle is chronically subjected to volume overload, the resultant eccentric hypertrophy is characterized by an increase in end-diastolic volume, which results from elongation of cardiomyocytes. The latter makes the larger end-diastolic volume to correspond to a lower strain and, therefore to a lower wall stress and diastolic pressure. On the other hand, pressure overload results in concentric hypertrophy, in which there is an increase in wall thickness with a decrease or no change in chamber radius. (18) The latter effect is due to addition of new sarcomeres in parallel with the existing ones, lowering the average wall stress (i.e., force development in individual sarcomeres). However, in addition to these purely geometric effects, pressure-overload hypertrophy also results in changes in cardiomyocytes' cytoskeleton (10,14) and in a higher interstitial collagen deposition, thus increasing ventricular chamber stiffness and the end-diastolic pressure at any given volume. (15).

As myocardial tissue can be regarded as a composite material, it is reasonable to assume that changes in any of its elements might determine overall myocardial material properties and consequently, change the slope and position of the passive stress-strain and end-diastolic pressure-volume relations.

Besides the contribution of the intrinsic myocardial elements, it should be noted that extramyocardial factors such as hemodynamic load (20, 21) and the pericardium might be involved in changing these relations as well. However, they are beyond the scope of this work.

We will now discuss the current knowledge about the role played by the cardiomyocytes and the ECM in myocardial material properties, both in physiological and pathological settings.

3. CARDIOMYOCYTES AND MYOCARDIAL MATERIAL PROPERTIES

Several studies have shown that changes in myocardial material properties can be caused by mechanisms intrinsic

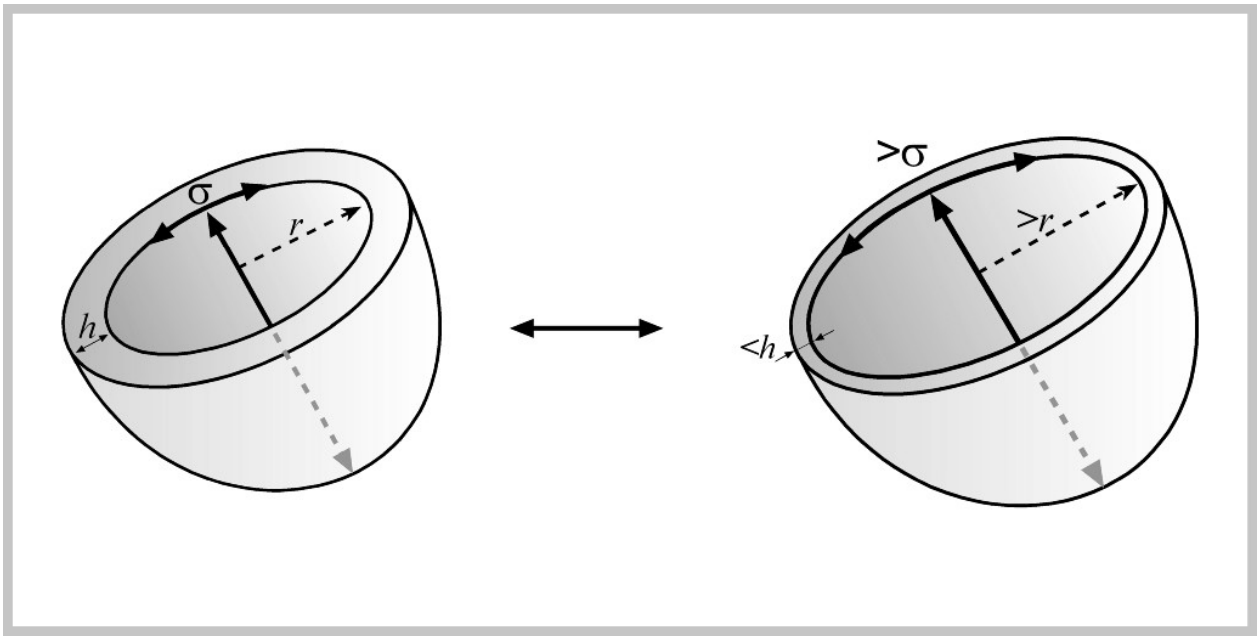

Figure 2: Schematic representation of the Laplace law. From left to right: according to the Laplace equation, an acute increase in ventricular end-diastolic pressure leads to an increased internal radius (>*r*), ventricular wall thinning (<*h*) and a higher ventricular wall stress (σ).

to the cardiomyocytes themselves. (1, 7, 15, 18, 20, 22, 24, 25, 26, 27). These include mechanisms that may alter (i) the relative content, (ii) the isoforms expression, (iii) post-translational modifications and/or (iv) active interactions among the cytoskeletal structures of cardiac cells, thus affecting the overall resistance to changes in shape.

The cardiomyocyte's cytoskeleton is composed of microtubules (tubulin), intermediate filaments (desmin), microfilaments (actin) and endosarcomeric proteins, of which titin has lately received particular attention.

3.1 Microtubules and Intermediate Filaments

At operating sarcomere lengths (1.9-2,2μm), microtubules and intermediate filaments were found to contribute less than 10% to passive tension. (27) However, in the setting of right-ventricular pressure overload hypertrophy (RVPOH) and at physiological rates of muscle length variation, there is a higher resistance to changes in cardiomyocytes shape, a result that is significantly attenuated when microtubules are chemically or physically depolymerized. Thus, although microtubules may not significantly contribute to myocardial stiffness both in healthy states and in slowly stretched muscles, their increased density may play a role under pressure-overloaded conditions and at physiological rates of contraction (15,16). In this way, increased microtubules density was found to alter the *viscous damping* of cardiomyocytes, i.e., an increase in resistance when rapidly stretched.

3.2 Myofilaments

Since the interaction between actin and myosin was shown to occur even at low diastolic calcium levels, it was hypothesized that the establishment of residual diastolic cross-bridges might as well mediate myocardial passive stiffness. (9) As described for microtubules, Zile and colleagues also showed that, in pressure-overloaded conditions, there is a correlation between increased myocardial stiffness, higher intracellular calcium concentration and increased number of cross-bridge interactions (1). Therefore, changes in calcium transients or myofilament's calcium-sensitivity may increase myocardial stiffness (9,28) even if these are most commonly regarded as determinants of myocardial relaxation rate. The latter findings mean that beyond the biophysical properties of structural cytoskeletal proteins, cardiomyocytes' stiffness may also be under active control, thus evoking the concept of active muscle tone. (9)

3.3 Titin

Over the last decade, the role of the endosarcomeric protein, titin, in the modulation of myocardial stiffness has been elucidated. Titin spans the half-sarcomere from the Z-disk to M-line and its ability to generate passive tension is associated with an extensible segment in the I-band of sarcomeres, comprised of serially linked but distinct domains: PEVK and Immunoglobulin (Ig)-like domains. (7, 9, 17, 22, 29). It is expressed as two different isoforms, the smaller and stiffer N2B and the larger and more compliant N2BA. While the former expresses only the cardiac specific N2B domain, the latter expresses both N2B and N2A, as well as additional PEVK and Ig domains. Relative expression of titin isoforms differs among species, with N2B predominating in rodents and greater amounts of N2BA being observed in larger mammals. (9) Accordingly, a correlation between titin isoforms expression ratio and muscle stiffness was shown to be consistent among species (9, 29), with those expressing higher proportions of N2B titin also presenting both increased myocardial as well as ventricular chamber stiffness. (7) Titin is the main determinant of cardiomyocyte passive tension over the physiologic sarcomere lengths and its role in diastolic tension is achieved not only through structural differences between its isoforms but also by post-translational modifications. The latter, either through phosphorylation or calcium binding effects, are important mechanisms for rapid adjustments of titin's mechanics. N2B titin's segment was shown to be the target for protein kinase A (PKA) phosphorylation (17, 30, 31, 32), which possibly destabilizes its structure and induces an increase in N2B segment length, thus reducing cardiomyocytes' passive tension. Because N2B element is included in all cardiac titin isoforms, its extension is predicted to reduce passive stiffness of both N2B and N2BA titins. However, the magnitude of the phosphorylation-induced decrease in passive stiffness is isoform dependent, because the shorter N2B titin makes its increase in length to have a greater impact on its fractional extention.

Besides titin extention, diastolic stiffness may be also produced by titin-calcium interactions in an isoform dependent manner. Calcium binding, directly increases the passive stiffness of N2BA expressing myocardium by changes in its structure, but has no effect on exclusively N2B expressing myocardium. In the latter case, calcium exerts an indirect effect by promoting titin-actin interaction, which may serve to retard thin filament sliding and contribute to myocyte passive stiffness. (7) Several reports have shown that in the setting of acquired heart diseases, there may be a switch in titin isoforms expression ratio. However, its direction is not consistent among studies and the mechanisms that may lead to one type of switch over another in heart failure remain unclear. (9, 7) Some argue that the expression ratio of titin isoforms is the mechanism whereby cardiomyocyte passive stiffness is altered both in human dilated cardiomyopathy and in hearts of spontaneously hypertensive rats (7, 17, 33, 35). However, two recent reports (26, 28) demonstrated that cardiomyocytes from patients with non-dilated diastolic heart failure had a higher expression of the stiff N2B titin isoform and a correspondent increase in passive stiffness, and that the latter could be normalized after treatment of cardiomyocytes with PKA. Therefore, these results suggest that different mechanisms, such as titin's isoforms expression ratio and its phosphorylation status may act in synergism and increase

myocardial passive stiffness, both in physiological and pathological settings.

4. EXTRA-CELLULAR MATRIX AND MYOCARDIAL MATERIAL PROPERTIES

Besides cardiomyocytes, the ECM is another major determinant of the myocardial material properties. (35) Among the proteins within the ECM, fibrillar proteins such as collagen and elastin, proteoglycans, and the basement membrane proteins each may play a role in determining the constitutive properties of the myocardium. (1) However, collagen molecules have been hypothesized to be the most important component within the ECM contributing to myocardial stiffness and diastolic heart failure (36) as it is a relatively stiff material with high tensile strength (35). However, its influence on the stress-strain relation of the myocardium depends on many factors including its concentration, fibril and fiber diameter, degree of crosslinking, spatial alignment and collagen types. In physiological conditions and at sarcomere lengths less than 2.2 μm, the combined passive fiber stiffness of the myocardium has been predominantly attributed to intracellular structures, notably titin. However, at longer sarcomere lengths, parallel collagen fibres bear an increasing fraction of the axial stress as collagen perimysial fibers untwist and straighten. (11) Interestingly enough, the increase in myocardial stiffness across different species and heart chambers is associated not only to a higher expression of the stiff N2B titin isoform but also by a parallel increase in collagen content, which suggests coordination between collagen synthesis and titin isoform expression. This might preserve the relative stiffness contributions of titin (lower sarcomere lengths) and collagen (higher sarcomere lengths) among species. However, this relationship may not be preserved in pathological states (9) such as arterial hypertension, diabetes (35,36) or myocardial infarction, (39,40) because of their role in disrupting the balance between collagen biosynthesis, degradation and post-translational processing. (36) However, even if the latter pathological states may result in increased collagen-based myocardial stiffness, (36, 37, 38) it seems prudent to rule out a possible and coordinated expression of stiff titin isoforms with a simultaneous interstitial fibrosis. (9)

5. NEUROHUMORAL MODULATION OF MYOCARDIAL MECHANICAL PROPERTIES

Besides mechanical inputs, growing evidence points to neurohumoral agents such as nitric oxide (NO), angiotensin II, and endothelin-1 to acutely alter myocardial mechanical properties as well. In isolated cardiomyocytes, NO was shown to shift the stress-strain relation down and to the right. Accordingly, intracoronary infusion of the exogenous NO donor, sodium nitroprusside, resulted in a similar displacement of the end-diastolic pressure-volume relation, both in normal and in hypertrophied human hearts as a result PKG-mediated phosphorylation of myofilaments. (8) In further experiments, substance P was shown to promote the release of endogenous NO, decreasing ventricular stiffness in patients with dilated cardiomyopathy. (6,40) Although some previous reports (42) stated that higher coronary perfusion pressures might cause an upward displacement of EDPVR by increasing myocardial vascular engorgement, the latter results also suggest that a simultaneous increase in endothelial shear-stress may enhance the release of endogenous NO and attenuate the aforementioned effect.

Chronic activation of the renin-angiotensin system is also a well recognized mechanism that leads to increased myocardial stiffness by promoting structural remodeling in both cardiomyocytes and ECM. However, acute activation of this system was recently shown to decrease myocardial and ventricular chamber stiffness in a time frame that was too short to alter the ECM. (43) Therefore, its effects on myocardial tissue must be caused by direct action on the cardiomyocytes to alter one or more determinants of its mechanical properties. Interestingly, a similar finding was also ascribed to endothelin-1 in isolated papillary muscles. (44)

6. CONCLUSION

The maintenance of normal myocardial mechanical properties is only possible through the appropriate expression of cellular and matrix phenotypes, which in turn are dependent on the perfect match between the myocardial inputs and its biological responses. As described along this paper, myocardial tissue is regarded as composite material whose properties depend on each of its specific constituent elements and the dynamic interplay between their structure and function. In addition, the biomechanical sensing features of the myocardium allow it to alter its structural and functional phenotypes when subjected to physiological inputs, thus achieving a new biological equilibrium that preserves overall ventricular function. In pathological settings, however, such equilibrium can be easily disrupted, with both cardiomyocytes and ECM exhibiting changes that may increase overall myocardium and ventricular chamber stiffness. The growing knowledge about the mechanisms driving the development of abnormal mechanical properties will possibly allows us to diagnose earlier and choose the best therapeutic strategies, thus preventing or even reversing the pathological cardiac remodeling. Plus, a better understanding of the basic principles governing myocardial tissue function is also setting up the scaffolds to promote the development of cardiac tissue engineering and regenerative medicine, thus providing the rationale to assemble cells and biomaterials into multi-dimensional structures that mimic the architecture and function of native heart muscle. (45, 46, 47)

REFERENCES

[1] Harris, T. S., Baicu C.F., Conrad C.H., Koide M., Buckeley J.M., Barnes M., Cooper G IV; Zile M.R., Constitutive properties of hypertrophied myocardium:cellular contribution

to changes in myocardial stiffness; Am J. Physiol Heart Circ Physiol. 282: H2173–H2182, 2002.

[2] Lammerding J., Kamm R.D., Lee R.T., Mechanotransduction in cardiac myocytes; Ann. N.Y. Acad. Sci. 1015: 53-70, 2004.

[3] Orr A.W., Helmke B.P., Blackman B.R., Schwartz M.A., Mechanisms of Mechanotransduction; Dev Cell. 10, 11–20, January, 2006.

[4] Swynghedauw B., Phenotypic plasticity of adult myocardium: molecular mechanisms; J. Exp. Biol. 209, 2320-2327

[5] Sussman M.A., Andrew M., Borg T.K., Dance band on the Titanic: biomechanical signaling in cardiac hypertrophy; Circ. Res. 2002; 91; 888-898.

[6] Leite-Moreira A.F., Current perspectives in diastolic dysfunction and diastolic heart failure; Heart 2006; 92; 712-718.

[7] LeWinter M.M., Wu Y., Labeit S,, Granzier H., Cardiac titin: structure, functions and role in disease; *Clin Chim Acta*., 2007 Jan; 375(1-2): 1-9.

[8] Bronzwaer JGF; Paulus WJ; Matrix, Cytoskeleton, or myofilaments: which one to blame for diastolic left ventricular dysfunction?; Prog Cardiovasc Dis.; vol. 47, No. 4, 2005, 276-284.

[9] Kass, D.A., Bronzwaer JGF; Paulus W.J., What mechanisms underlie diastolic dysfunction in heart failure?; Circ. Res. 2004; 94; 1533-1542.

[10] Cooper G., Cardiocyte cytoskeleton in hypertrophied myocardium; Heart Fail Rev., 5, 187-201, 2000.

[11] Brutsaert D.L., Zile M.R., New concepts in diastolic dysfunction and diastolic heart failure: Part I, diagnosis prognosis, and measurements of diastolic function; Circulation 2002; 105: 1387-1393.

[12] Gaash W.H., Passive elastic properties of the left ventricle; In: Gaash W.H., LeWinter M.M., eds, Left Ventricular diastolic dysfunction and heart failure. Philadelphia, Pa: Lea & Febiger; 1994: 3-24.

[13] Fung, Y. C. Foundations of Solid Mechanics, Prentice Hall, New York, 1965.

[14] Zile M.R., Richardson K., Cowles M.K., Buckley, J.M., Koide M., Cowles; Gharpuray V., Cooper G. IV; Constitutive Properties of Adult Mammalian Cardiac Muscle Cells; Circulation, 1998; 98: 567-579.

[15] Shintani H., Glantz S.A., The left ventricular diastolic pressure-volume relation, relaxation and filling; In: Gaash W.H., LeWinter M.M., eds, Left Ventricular diastolic dysfunction and heart failure. Philadelphia, Pa: Lea & Febiger; 1994: 3-24.

[16] J. D. Humphrey, Cardiovascular Solid Mechanics, Springer-Verlag, N.Y. 2002.

[17] Granzier H.L., Labeit S., The giant protein titin: a major player in myocardial mechanics, signaling and disease; Circ. Res. 2004; 94; 284-295.

[18] Glantz S.A., Gilbert J.C., Determinants of left ventricular filling and of the diastolic pressure-volume relation; Circ Res. 1989 May; 64 (5): 827-52.

[19] Zile M.R., Katz A.M., New molecular mechanisms in diastolic heart failure; Circulation 2006; 113; 1922-1925.

[20] Leite-Moreira A.F., Correia-Pinto J., Load as na acute determinant of end-diastolic pressure-volume relation; Am J. Physiol Heart Circ Physiol, 280: H51-H59, 2001.

[21] Leite-Moreira A.F., Correia-Pinto J., Gillebert T.C., afterload induced changes in myocardial relaxation: a mechanism for diastolic dysfunction; Cardiovasc. Res., 43 (1999) 322-353.

[22] Brady A.J., Length dependence of passive stiffness in single cardiac myocytes; Am J. Physiol Heart Circ Physiol. 260: H1062-H1071, 1991.

[23] Helmes M., Lim C.C., Liao R., Cui L., Sawyer D.B., Titin determines the Frank-Starling relation in early diastole; J. Gen Physiol; Vol. 121 February 2003; 97-110.

[24] Trombitas K., Redkar A., Centner T., Wu Y., Labeit S., Granzier H., Extensibility of isoforms of cardiac titin: variation in contour length of molecular subsegments provides a basis for cellular passive stiffness diversity; Biophys J. vol. 79 December 2000; 3226-3234.

[25] Nishimura S., Yasuda S., Katoh M., Yamada K.P., Yamashita H., Saeki Y., Sunagawa K., Nagai R., Hisada T., Sugiura S., Single cell mechanics of rat cardiomyocytes under isometric, unloaded and physiologically loaded conditions; Am J. Physiol Heart Circ. Physiol. 287: H196-H202, 2004.

[26] Borbély A., van der Velden J., Papp Z., Bronzwaer JGF, Edes I., Stienen GJM; Paulus W.J., Cariomyocyte stiffness in diastolic Herat failure; Circulation 2005; 111; 774-781.

[27] Granzier H.L., Irving T.C., Passive Tension in Cardiac Muscle: Contribution of Collagen, Titin, Microtubules, and Intermediate Filaments; Biophys J. Volume 68 March 1995 1027-1044.

[28] van Heerebeek L., Borbély A., Niessen H.W.M., Bronzwaer J.G.F., van der Velden J., Stienen G.J.M., Linke W.A., Laarman G.J., Paulus W.J., Circulation 2006; 113; 1966-1973

[29] Cazorla O., Freiburg A., Helmes M., Centner T., McNabb M., Trombitas K., Labeit S., Granzier H., differential expression of cardiac titin isoform and modulation of cellular stiffness; Circ. Res. 2000; 86: 59-67.

[30] Fukuda N., Wu Y., Nair P., Granzier H., Phosphorylation of titin modulates passive stiffness of cardiac muscle in a titin isoform-dependent manner; J. Gen Physiol; vol 125 March 2005; 257-271.

[31] Yamasaki R., Wu Y., McNaabb M., Greaser M., Labeit S., Granzier H.L., Protein Kinase A Phosphorylates Titin's Cardiac-specific N2B domain and reduces passive tension in rat cardiac myocytes; Circ Res. 2002 Jun 14;90(11): 1181-8.

[32] Kruger M., Linke W.A., Protein Kinase A phosphorylates titin in human heart muscle and reduces myofibrillar passive tension; J. Muscle Res Cell Motil; 2006, 27; 435-444.

[33] Makarenko I., Opitz C.A., Leake M.C., Kulke M., Gwathmey J.K., del Monte F., Hajjar R.J., Linke W.A., Passive stiffness changes caused by upregulation of compliant isoforms in human dilated cardiomyopathy hearts; Circ. Res. 2004, 95: 708-716.

[34] Wu Y., Labeit S., leWinter M.M., Granzier H.L., Titin: an endosarcomeric protein that modulates myocardial stiffness in DCM; J Card Fail.; vol. 8 No. 6 Suppl. 2002.

[35] Janicki J.S., Matsubara B.B., Myocardial collagen and left ventricular diastolic dysfunction; In: Gaash W.H., LeWinter M.M., eds, Left Ventricular diastolic dysfunction and heart failure. Philadelphia, Pa: Lea & Febiger; 1994: 3-24.

[36] Zile M.R., Brutseart D.L., New concepts in diastolic dysfunction and diastolic heart failure: Part II, causal mechanisms and treatment; Circulation 2002; 105: 1503-1508.

[37] Little W.C., Zile M.R., Kitzman D.W., Hundley W.G., O'Brien T.X., Degroof R.C., The effect of alagebrium chloride (ALT-711), a novel glucose cross-link breaker, in the treatment of elderly patients with diastolic heart failure; J. Card Fail., vol. 11 No 3, 2005.

[38] Asbun J., Villarreal F.J., The Pathogenesis of Myocardial Fibrosisin the Setting of Diabetic Cardiomyopathy; J. Am Coll Cardiol. Vol. 47, No. 4, 2006.

[39] Sun Y., Weber K.T., Infarct scar: a dynamic tissue; Cardiovas. Res. 46 (2000) 250–256.

[40] Holmes J.W., Borg T.K., Covell J.W., Structure and mechanics of healing myocardial infarcts**,** Annu. Rev. Biomed. Eng. 2005. 7: 223–53.

[41] Paulus J.W., Beneficial effects of nitric oxide on cardiac diastolic function: 'the flip side of the coin'; Heart Fail. Rev. 5, 337-344, 2000.

[42] Apstein C.S., Influence of the coronary vasculature on left ventricular diastolic chamber stiffness: the erectile properties of the myocardium; In: Gaash W.H., LeWinter M.M., eds, Left Ventricular diastolic dysfunction and heart failure. Philadelphia, Pa: Lea & Febiger; 1994: 3-24.

[43] Leite-Moreira A.F., Castro-Chaves P., Pimentel-Nunes P, Lima-Carneiro A., Guerra M.S., Soares J.B., Ferreira-Martins J. Angiotensin II acutely decreases myocardial stiffness: a novel AT1, PKC and Na+/H+ exchanger-mediated effect. Br J Pharmacol. 2006 Mar; 147(6): 690-7.

[44] Leite-Moreira A.F., Bras-Silva C., Pedrosa C.A., Rocha-Sousa A.A. ET-1 increases distensibility of acutely loaded myocardium: a novel ETA and Na+/H+ exchanger-mediated effect. Am J Physiol Heart Circ Physiol. 2003 Apr; 284 (4): H1332-9.

[45] Leor J., Amsalem Y., Cohen S. Cells, scaffolds, and molecules for myocardial tissue engineering; Pharmacol Ther. 105 (2005) 151–163.

[46] Gaudette G.R., Cohen I.S., Cardiac regeneration, materials can improve the passive properties of myocardium, but cell therapy must do more; Circulation 2006; 114: 2575-2577.

[47] Wall S.T., Walker J.C., Healy K.E., Ratcliffe M.B., Guccione J.M., Theoretical impact of the injection of material into the myocardium: a finite element model simulation; Circulation 2006; 114: 2627-2635.