Mechanical stress and hypertrophy

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Abstract

There is accumulating evidence that mechanical stress on the left ventricle is a significant trigger for left ventricular hypertrophy. It has been known for sometime that mechanical stress activates a number of protein kinases and reprogramming of gene expression in the myocardium. A recent study has shown that there is a link between mechanical stress and the activation of angiotensin II (Ang II) receptors. Ang II has been known for a long time to initiate hypertrophy. Providing clinicians with stress maps of a patient's heart would be useful aid in diagnosis. Based on a patient's echo cardiogram the left ventricular (LV) characteristics of chamber wall velocity and position as a function of time and myocardium thickness are obtained. In addition Doppler measurements of velocity through the mitral valve are also extracted from the echo cardiogram. A simulation program is used to solve the equations of fluid flow in the LV during both diastole and systole. The velocity through the mitral valve is compared with the Doppler measurements as a check on the accuracy of the calculations. The flow velocities are used to calculate the shear stress on the myocardium as a function of time. It was found that the maximum stress occurs during the opening of the mitral valve near the valve. The regions of peak stress occur at different locations of the myocardium and septal wall over a heart cycle. It is not known if the average stress over a heart beat or the maximum shear stress that triggers the gene changes. There are large peaks in the stress and extended periods of low level stress which could play a significant role in the development of hypertrophy. Some initial tests on rabbits have shown that there is a variation in the development of certain biomarkers as a function of location.
Keywords: hypertrophy, stress, biomarkers, Angiotensin II.

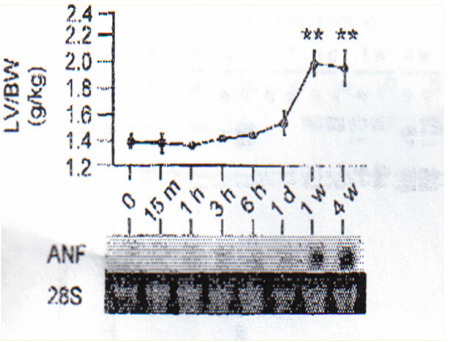
1 Introduction

Cardiac hypertrophy is a major cause for heart failure. There are many aspects of the causes known although there is not a clear mechanism established. The role of Angiotensin II has been known for sometime although there are a number of mechanisms for its generation established, one of which is stress. The molecular markers for the development of the disease have also been examined in a variety of animal models. The mechanisms required for the development of these markers are not clear. It could be that some markers are related to Angiotensin II while others could be due to stress. The aim of this study was to calculate the stress and stress related molecular changes in the signal transduction pathways and protein expression that lead to hypertrophy. The hypothesis is that stress results in molecular changes leading to hypertrophy and cardiac failure. If the development of hypertrophy has a number of paths then only some of markers may be found in locations of maximum stress.

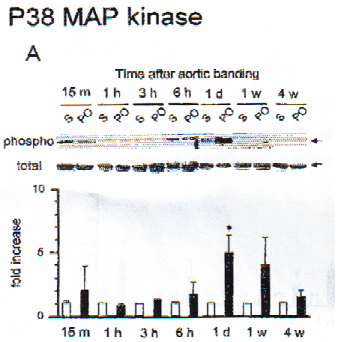
There exists considerable evidence that cardiac hypertrophy can be triggered by mechanical stress on the myocardium. In vivo the mechanical stress is generally a result of pressure or volume overload. It is found in both human and animal models that the resultant gene modifications usually lead to hypertrophy in the left ventricular myocardium. Two recent surveys reached the conclusions that [1] "Cardiac myocytes have the ability to sense stretch and convert it into intracellular growth signals, which lead to hypertrophy" [1] and " Mechanical stress is considered to be the trigger inducing a growth response in the overloaded myocardium" [2]. It is thought that mechanical stress is first perceived by integrins [3]. The integrins transmit signals by organizing the actin filaments through intermediary molecules such as α-actinin, talin, vinculin, paxilin and tensin. Further it has been shown [4] that intermediate filaments transmit mechanical stress to the chromatin and it is hypothesized that the modified chromatin induces modulation of gene expression. Another possible mechanism involves guanine nucleotide-binding proteins that join cell surface receptors to the appropriate effectors [5].

Rabbits are a suitable model for studying hypertrophy. To study human hypertrophic cardiomyopathy it has been possible to develop a transgenic rabbit as a suitable model [6]. These rabbits responded to treatment in a manner similar to that of humans. A study involving rabbits as a model for humans [7] for hypertrophic cardiomyopathy found that myofribllar ATPase activity was reduced very early. It was also found that stress related signaling kinases were activated. A significant paper for the present work [8] studied the development of hypertrophy in rabbits for both pressure and volume overload. The pressure overload was developed by using aortic banding. The rabbits were sacrificed at 15 min, 1 hr, 3 hr, 6hr, 1 day, 1 week and 4 weeks. The results showed signal transduction proteins were expressed after 1hr and that hypertrophy was significant after 1 week. Western blot analysis was undertaken for MAP kinase, Akt, GSK-3β, p70sk6 and JAK/Stat3 and that these signaling pathways showed significant activation at different times

The hypertrophy can be seen in both the increase of the left ventricular mass, figure 1a and the expression of P38 MAP kinase even after one day, figure 1b. The thoracic aortic banding was performed on the rabbits by tying a surgical silk ligature tightly around a stainless tube with an external diameter of 3mm. The tubing was then withdrawn. Sham operated rabbits were used as controls at each time point



(a)



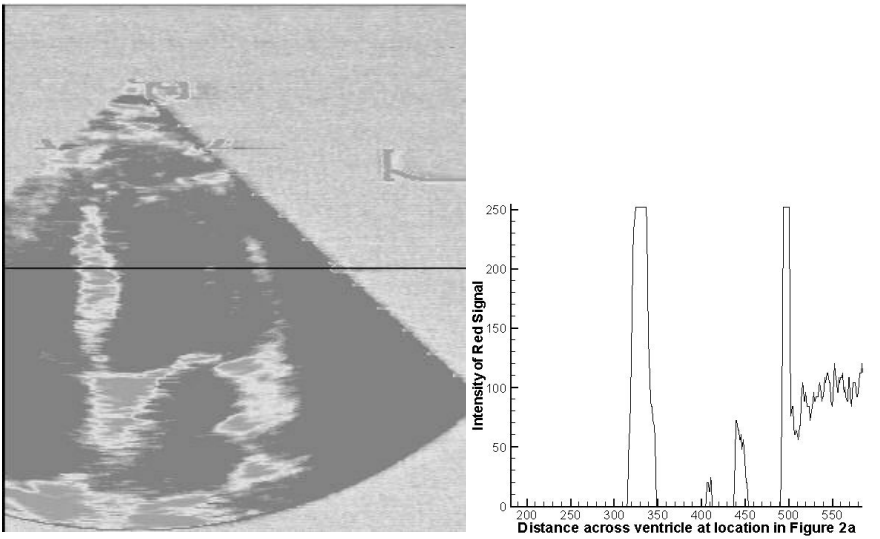
(b)

Figure 1: (a) Left Ventricle Weight. (b) Generation of P38 [8] change of rabbits.

2 Predictive methodology

Patient echocardiograms are analyzed to yield myocardial stress distributions. It is first necessary to discern the inside of the ventricle shape at various times during a heart beat. This readily obtained by examining the pixel colours and a typical echocardiogram and pixel plot are shown in figure 2. The right hand edge of the large peak is the septal wall and the next peak is the inside of the myocardium.

In the solution the bloodflow into the left atrium is simulated by a source distributed throughout the atrium. In order to conserve mass sinks are distribute around the periphery of the integration domain. The velocity through the mitral valve, obtained from the Doppler measurements is used to set the source strength such that the simulated ventricular volume changes match the experimental. The simulation is undertaken on both a Cartesian and Lagrangian grids simultaneously [9]. The valves have to be modelled as thicker than in reality as Lagranian integration must go around both sides of the valve. The Navier-Stokes equations are then solved with a predictor corrector scheme [9]. The simulation code calculates the fluid velocity using an equation for fluid flow known as the Navier Stokes equation which is based on Newton's law of motion.



(a) (b)

Figure 2: Echocardiogram and corresponding pixel count.

$$\rho\left(\frac{\partial \hat{u}}{\partial t} + \hat{u} \bullet \nabla \hat{u}\right) + \nabla p = \mu \nabla^2 \hat{u} + \hat{F} \tag{1}$$

$$\nabla \hat{u} = 0 \tag{2}$$

where $\hat{u}$ is the velocity vector, ρ is the density, t is the time , p is the pressure and the viscosity is μ.

The boundary force $\hat{F}$ arising from the heart muscles is

$$\hat{F}(\hat{x},t) = \int_0^L \hat{f}(s,t)\delta\left(\hat{x} - \hat{X}(s,t)\right)ds \tag{3}$$

Here $\hat{f}$ is the force on the boundary element at the point s defined on a Lagrangian system where $\hat{x}$ is defined on the Cartesian system and $\hat{X}^n$ is the *nth* point on the Lagrangian system. In the present work the boundary force is replaced in the finite difference analysis by the movement of the boundary.

The shear stress is calculated from the change of velocity near the wall. The first step in the solution involves obtaining the shape of the ventricle at various times as described above. Following a method often used by echocardiographers only six images in a cardiac cycle will be selected. The initial image when the mitral valve is closed, a second image when the mitral valve is fully open, a third just before the atrium starts to contract, one at the end of the ventricle filling (diastole) stage, a fourth when the mitral valve is closed and the aortic valve opens and a final image at the end of systole corresponding to the initial image. A linear variation was assumed between each image, time frame. It was assumed

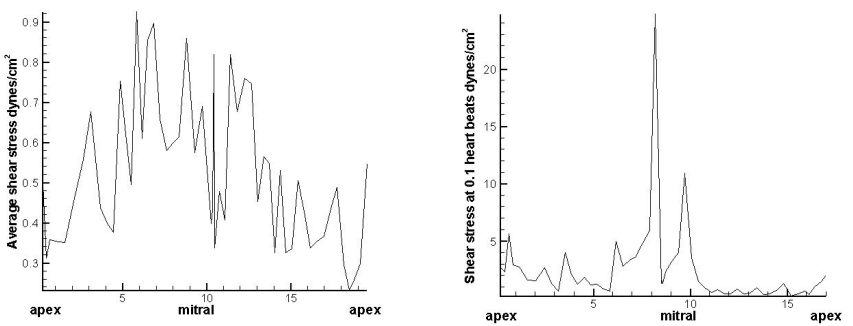
that the motion of the wall would be normal to the surface. The echocardiogram tracing is obtained as a digital image. A least squares curve fit was used to quantify the ventricular shapes at various times using quadratic forms where the constants are polynomials fitted at each digital point. If the source was allowed to start while the mitral valve was closed then the program would fail due to excessive pressure. Similarly the wall cannot be allowed to start moving until the source starts. Thus an initial short period is required without source or wall motion to allow the mitral valve to start opening (these events are independent of fluid motion and dependent on cardiac electrical signals).

The second step requires the simulation of the atrium. The atrium changes shape during the diastole stage and thus changes the pressure. However the use of a source in place of the correct inflow pattern to the atrium is an artifice which makes the actual atrium shape unimportant. Thus the calculations of stresses in the atrium are not meaningful and are not shown in the figures below. The atrium shape was fixed at close to a hemispherical shape with the mitral valve in the closed and early open positions. After some time the atrium contracts for a period before the mitral valve closes.. The shape was expanded and contracted as required for the different sized mitral valves. The source strength was increased slowly as the mitral valve opens in accordance with the increase in volume of the ventricle.

Once the calculation of the flow velocities and pressures are completed the stresses at the walls were calculated.. Two points are chosen as close to the wall as possible along a line normal to the surface. A finite difference method was used to obtain the derivative of the velocity along this line. Similarly the velocity normal to the wall was calculated along the same line.

3 Results

Echocardiographs for six patients were studied. One consideration is whether the average stress over a heart beat or the maximum shear stress triggers the gene changes. An example of each for a healthy human is shown in figure 3.

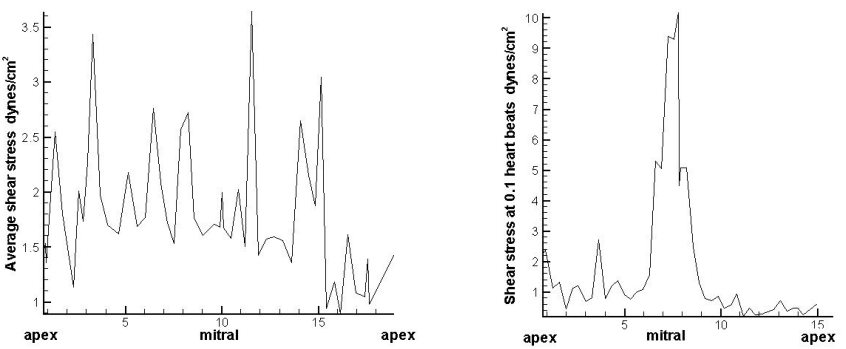


(a) (b)

Figure 3: (a) Shear stress aver. over part heart beat (b) Shear stress at 0.1 heart beat of patient M53 (healthy).

The left side of the figure depicts the myocardium and the right side depicts the septal wall. In figure 3(a) the shear stress has been averaged over the time that the mitral valves are not moving and in 3(b) the instantaneous shear stress at a time when the mitral valve is opening. In the latter case the stresses are much higher due to the motion of the mitral valve. As the valves are modeled as rigid leaves rather than flexible leaves it is possible that the stress is not as high as indicated. Thus considering the myocardium the shear stress has peaks of high stress. The values of the peaks in (a) are much lower than the average stress values which also peaks in the mid myocardium. One interpretation would be that the large instantaneous stress values during the opening initiates hypertrophy and that average stress is important during the development of the condition. As can be seen in the above case the maximum stress, excluding the mitral valve region, occurs at approximately the same location.

A similar stress calculation for a 70 year old male who did not necessarily have developed hypertrophy but had required bypass surgery the following day are shown in figure 4.



(a) (b)

Figure 4: (a) Shear stress average over part of heart beat. (b) Shear stress at 0.1 heart beat of patient M70.

It can be seen that the average shear stress over the septum is much higher than in the case of the healthy patient and the values are approximately four times larger. However the peak instantaneous stress at the mitral valve is much lower. This is due to the lower velocity during the E wave.

Some preliminary tests were undertaken on four rabbits which had hypertrophy induced by pacing. It is known [10] that pacing will produce stress on the left ventricle. The results are only for the long time after the induction of hypertrophy. Two rabbits were paced and another two were used as controls. The average ratio of LV weight to body mass was 2.5% when the animals were euthanized. The first paced rabbit (ID 55824) was euthanized at 8.5 weeks and the second rabbit (ID 55821) was allowed to proceed to heart failure at 9.5 weeks. Bio markers for #55824 were sort for STAT#, phospho, phosphor-Akt,

tubulin and α-Actin. Additional biopsies were obtained at the apex of the myocardium (LVA) and the midpoint of the septum (SW2). The blots were analysed using the Scion Corporation software and the results are shown in Table 1. The α-Actin signal was uniformly strong at each location. The largest increase was found in tubulin at the middle of the septal wall as shown in figures 5–8 and Table 1.

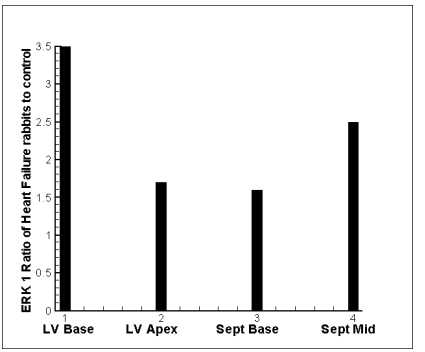


Figure 5: Ratio of ERK1 in heart failure rabbits to that in control rabbits.

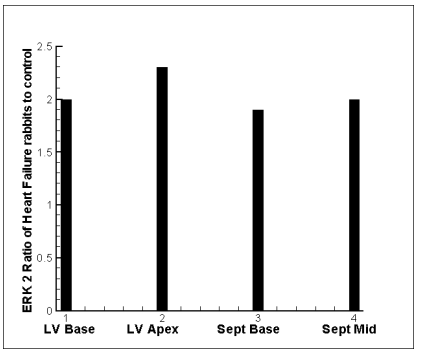


Figure 6: Ratio of ERK2 in heart failure rabbits to that in control rabbits.

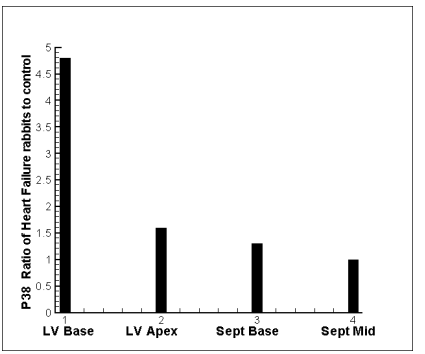


Figure 7: Ratio of P38 in heart failure rabbits to that in control rabbits.

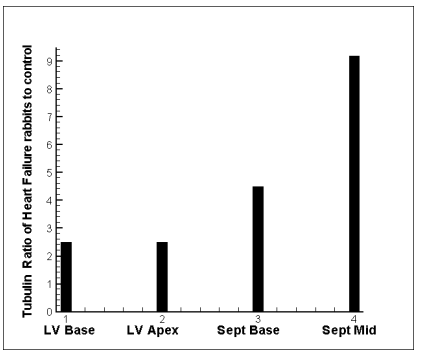


Figure 8: Ratio of Tubulin in heart failure rabbits to that in control rabbits.

Table 1: Ratio of protein expression in heart failure rabbits to control 8 weeks after surgery.

Base Rabbit ID	LV Apex	Sept Base	Sept Mid	Biomarker
3.5 ID 55821	1.7	1.6	2.5	ERK1
2.0 ID 55821	2.3	1.9	2.0	ERK2
4.8 ID 55821	1.6	1.3	1.0	P38 Kinase
2.5 ID 55824	2.5	4.5	9.2	Tubulin
1.0 ID 55824	1.0	1.0	1.0	Actin

4 Conclusions and future work

It can clearly be seen that even at the long times there is a variation in stress induced biomarkers indicative of hypertrophy around the ventricle. It is proposed to investigate if hypertrophy is initiated at certain locations by large stresses induced by pressure overload. The development with time of hypertrophy around the ventricle will be examined by taking biopsies and testing the protein expressions. As early times will also be involved the biomarkers will have to include proteins which were not included in the preliminary study. In addition biopsies taken from the inner part of the myocardium will be compared with those from the outer part of the myocardium to investigate if there is a transmural change in biomarkers which would indicate if the hypertrophy was initiated from the inside or outer region of the myocardium.

References

[1] Sadoshima J. and Izumo S. “The cellular and molecular response of cardiac myocytes to mechanical stress” Ann. Revs of Physiology, 59, 551-571, 1997.

[2] Ruwhof C,van der Laarse A. “Mechanical stress-induced cardiac hypertrophy:mechanisms and signal transduction pathways “Cardiovascular Research”, 47, 23-37, 2000.

[3] Wang,N., Butler JP,Ingher,DE “Mechanotransduction across the cell surface and through the cytoskeleton”,Science,260,1124-1127,1993.

[4] Bloom S, Lockard VG, Bloom M "Intermediate filament—mediated stretch-induced changes in chromatin: a hypothesis for growth initiation in cardiac myocytes", J. Mol. Cell Cardiol., 28, 2123-2127, 1996.
[5] Hall A, "The cellular functions of small GTP-binding proteins," Science 249, 635-640, 1990.
[6] Marian AJ, et al "A transgenic rabbit model for human hypertrophic cardiomyopathy", J Clin Invest., 1999, 104, 12, 1683-1692.
[7] Nagueh SF et al "Evolution of expression of cardiac phenotypes over a four year period in the β-myosin heavy chain –Q403 transgenic rabbit model of human hypertrophic cardiomyopathy" J Mol and Cell. Cardio., 36, 663-673, 2004
[8] Miyamoto,T, et al "Activation of distinct signal transduction pathways in hypertrophic hearts by pressure and volume overload", Basic Res Cardiol 2004, 99:328-337.
[9] Peskin CS and Printz BF, Improved Volume Conservation in the Computation of Flows with Immersed Elastic Boundaries *J.Comp. Phys,* 105, pp.33-46, 1993.
[10] van Wamel et al. "Stretch –induced paracine hypertrophic stimuli increase TGF-beta expression in cardiomyocytes", Mol cell biochem, 236 (1-2), 147-53, 2002