Diastolic Left Ventricular Dysfunction

A Clinical Appraisal

Jean G.F. Bronzwaer

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Diastolic Left Ventricular Dysfunction

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door

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The mind is like a parachute,
it only functions when it's open.
(Anonymous)

Voor Sylvia, Willemijn, Ties en Kasper

Voor mijn ouders

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Diastolic Left Ventricular Dysfunction: The Epicenter of the Heart Failure Syndrome

A pathophysiological perspective

1.1 General Introduction

Heart failure may be caused by either diastolic or systolic dysfunction, known as diastolic heart failure or systolic heart failure(9). Diastolic heart failure is a clinical syndrome characterized by symptoms and signs of heart failure, a preserved left ventricular ejection fraction, and abnormal diastolic function(8,409,410). Systolic heart failure is a clinical syndrome characterized by symptoms and signs of heart failure, and a reduced left ventricular ejection fraction, as a manifestation of abnormal systolic function. Approximately one third of patients with congestive heart failure have predominantly diastolic heart failure. Another third have impairment of both systolic and diastolic function, and the remainder primarily have disordered systolic function(67).

Recently the existence of pure diastolic dysfunction as a cause of chronic heart failure is called in question, because of subtle derangements of systolic performance in the presence of a normal left ventricular ejection fraction(153,276,403-405). Thus, so called diastolic heart failure is possibly caused by a combination of both diastolic and systolic dysfunction. To discriminate diastolic from systolic heart failure, the use of *resting* left ventricular ejection fraction is however widely accepted. Because most heart failure patients are symptomatic on exertion, a correlation between resting left ventricular ejection fraction and symptoms is absent(282). The widespread use of left ventricular ejection fraction to describe left ventricular performance results more from its easy measurability than from conceptual soundness as it is a pre- and afterload, and contractility dependent parameter.

The purpose of this thesis is to provide a pathophysiological perspective, based on new clinical findings on the mechanisms by which left ventricular dysfunction may lead to heart failure.

1.2 Heart failure and left ventricular ejection fraction

Left ventricular contractility, pre- and afterload

In order to generate an adequate cardiac output at rest and during exercise, while operating at normal left ventricular filling pressures, the left ventricle must generate enough pressure to overcome the resistance offered by the systemic circulation. This vital pump function is best described by Ohm's law as it applies to the circulation: Blood Pressure (BP) = Cardiac Output (CO) × Total Peripheral Resistance (TPR). Cardiac output is maintained by the cyclic

contraction and relaxation of the left ventricle, which generates arterial pressure and propels blood forward depending on pre- and afterload and myocardial contractility(54). Contractility or inotropic state is the inherent capacity of the myocardium to contract independently of changes in pre- or afterload. At a molecular level, an increased inotropic state can be explained by enhanced interaction between calcium ions and the contractile proteins and an increase in the calcium transient. Factors that increase contractility include exercise and adrenergic stimulation. An increase in contractility may be associated with enhanced rates of relaxation, called a positive lusitropic effect. Preload is defined as the wall stress at the end of diastole which determines the maximal resting length of the sarcomere. Measurement of wall stress in vivo is difficult because the radius of the left ventricle neglects the confounding influence of the complex anatomy of the left ventricle. Surrogate measurements of the indices of preload include left ventricular end-diastolic pressure or dimensions(258). Left ventricular systolic load or afterload is a function of both internal (cardiac) properties and external (vascular) factors that impede the forward flow of blood(38). Wall stress develops when tension is applied to a cross-sectional area, and the units are force per unit area(258). According to LaPlace's law:

$$\text{LV Wall Stress} = \frac{\text{LV Pressure x LV Radius}}{\text{2 x LV Wall Thickness}}$$

The afterload during ejection varies with decreasing left ventricular volume according LaPlace's law, i.e., the left ventricular pressures generated during ejection interact with systolic left ventricular geometry and wall thickness to determine left ventricular afterload(38).

Aortic impedance (= arterial input impedance) gives another accurate measure of the afterload. Systemic vascular resistance is the most widely used vascular impedance parameter. However, systemic vascular resistance alone is an inadequate measurement of afterload. The vascular contribution to left ventricular afterload is the product of a complex interaction between a variety of vascular parameters, which, in addition to systemic vascular resistance, include arterial compliance, viscoelasticity, and blood inertia. These factors, acting directly and through reflected waves, produce the arterial pressure wave form. During left ventricular systole, when the aortic valve is open, the left ventricular cavity and the systemic arterial system are in continuity. Blood flow and blood pressure are the physiological mediators that connect the heart and the systemic circulation(38). End-systolic wall stress reflects the three major components of the afterload, namely, the peripheral resistance, the arterial compliance, and the peak intraventricular pressure(258).

Myocardial damage and its sequellae

An index event either may cause damage of the myocardium, with loss of functioning

cardiomyocytes, or failure of the cardiomyocytes to contract or relax properly. Irrespective the nature of the index event, left ventricular dysfunction ensues with a reduction in pumping capacity of the heart. Often a subclinical course follows with the patient remaining asymptomatic for even long periods of time(43,151,233). The adrenergic system, the renin angiotensin system(83,259) and the cytokine system(218,268) initially may reduce the sequellae of an impaired left ventricular pumping capacity following left ventricular dysfunction. Thus, in the absence of abnormal loading conditions (e.g. acute mitral- or aortic regurgitation), left ventricular dysfunction is a prerequisite for the development of the syndrome of heart failure, but is in itself not sufficient to render the patient symptomatic(217,291). The neurohormonal system, together with a process which is called 'ventricular remodeling', following an index event, are thought to be responsible for the development and progression of the syndrome of heart failure. The process of left ventricular remodeling affects both left ventricular size and geometry as well as the functioning of the individual cardiomyocytes.

Due to this remodeling process, functional reserve in heart failure is hampered, i.e. the force frequency response is blunted(243,244). The failing heart is therefore not able to sufficiently increase stroke volume by a decrease in end-systolic volume secondary to an exercise-induced increase in heart rate. Also in case of symptomatic non-dilated left ventricular hypertrophy with normal resting left ventricular ejection fraction and decreased left ventricular distensibility, a blunted force-frequency response may contribute to signs and symptoms of heart failure(142,209,343,354). Thus the dysfunctional left ventricle, irrespective of its dimensions or ejection fraction, shows a blunted force-frequency response and depends on its ability to utilize preload reserve to increase stroke volume at increased demand. The concomitant increase in filling pressure totally depends on left ventricular distensibility as will be discussed below.

In symptomatic dilated cardiomyopathy patients the same pathophysiological mechanisms ensue as a cause of increased filling pressures during exercise(7,280,337). A fall in left ventricular diastolic distensibility causes heart failure symptoms due to reduced preload reserve and a rise in left ventricular filling pressure(203). Factors that influence left ventricular diastolic distensibility can be categorized as extrinsic or intrinsic to the left ventricular chamber, the latter affecting the dynamic process of myocardial contraction and diastolic relaxation, and structural components of the left ventricular chamber wall.

Myocardial contraction and diastolic relaxation

Dynamic changes in left ventricular distensibility depend on the amount of persisting diastolic cross bridge interactions determining diastolic myocardial tone. As early as in 1927 Meek(232) defined tone as *"a sustained partial contraction, independent of the systolic contractions, by virtue of which the muscle fibers resist distension during diastole more than they would because*

of their mere physical properties". If the extent of myocardial relaxation decreases, a larger amount of diastolic cross bridges remain present with a concomitant decrease in left ventricular wall distensibility. Only recently clinical evidence emerged that nitric oxide (NO), derived from NO-donors or from coronary and endocardial endothelium and myocardium, following receptor-mediated stimulation, increases diastolic left ventricular distensibility by means of a relaxation hastening effect(267).

During the diastolic phase of the cardiac cycle a compliant myocardium enables the ventricles to collect blood at low filling pressures and determines the ability of the myocardium to stretch. Adequate myocardial relaxation is the result of a low cytosolic calcium concentration ($[Ca^{2+}]_i$), 10.000 fold lower than outside the cell, attained by the energy consuming process of cytosolic Ca^{2+} removal. Myocardial contraction follows electrically activated Ca^{2+} transients. Electrical depolarization of the sarcolemma enables Ca^{2+} entry from the extracellular space into the cardiomyocytes via L-type Ca^{2+} channels(152). A relatively small amount of extracellular calcium releases a larger amount of calcium from the sarcoplasmatic reticulum through the ryanodine receptor.The ryanodine receptor, a sarcoplasmatic reticulum Ca^{2+} release channel, is required for excitation-contraction coupling(75,123,220,223,402). Cytosolic calcium concentration rises and increases the binding of calcium to troponin C. Troponin is the key component of the calcium-dependent switch of the contractile apparatus in striated muscle like myocardium. There are three subunits of troponin: troponin C, a Ca^{2+}-binding calmodulin-like protein; troponin T, which attaches the complex to tropomyosin, anchoring it to the thin filament as well as having a regulatory role; and troponin I, named for its ability to inhibit actin-myosin interactions at diastolic levels of Ca^{2+}. As cytosolic Ca^{2+} increases in systole, it binds to a regulatory Ca^{2+}-binding site on troponin C, leading to increasing affinity of troponin C for troponin I and weakening the interactions of troponin I and actin. This permits movement of tropomyosin-troponin on the thin filament such that the inhibition of actin-myosin interaction is diminished, increasing the probability of crossbridge cycling and muscle shortening and tension development. The aortic valve opens and blood is ejected from the left ventricle(29,246).

The contraction cycle ends when calcium is actively removed from the cytosol against a steep concentration gradient back into the sarcoplasmatic reticulum and extracellular space. Calcium removal is followed by myocardial relaxation and ventricular diastolic filling. The transition from contraction to relaxation occurs simultaneously with the plateau phase of the cellular action potential and simultaneously with the descending limb of the cytoplasmic calcium transient. This transition from contraction to relaxation occurs during early ejection in healthy hearts, and even prior to aortic valve opening in diseased hearts. In normal hearts and with normal load, myocardial relaxation is completed at minimal left ventricular pressure during the rapid filling period(106). The further course of diastolic filling with the later part of rapid filling, with slow filling and with atrial contraction is not a passive phase of the cardiac cycle, but is a phase during which ventricular stiffness is further modulated by several factors, including ongoing myocardial relaxation. In addition to passive myocardial and ventricular properties, these ongoing active

processes determine diastolic left ventricular pressure and the development of pulmonary congestion at rest and during exercise(106).

Depolarization of the cell membrane and subsequent contraction of the myocardium may be seen as a gross distortion of a delicate equilibrium of relatively low cytosolic calcium content accomplished during diastole. A low diastolic cytosolic calcium concentration in combination with a sufficiently high ATP concentration is mandatory to detach enough systolic actin-myosin cross-bridges. Compared with contraction (a 'downhill' process) relaxation (an 'uphill' process) is more prone to disturbances in energy supply. It is therefore of no surprise that this process of diastolic relaxation is the first to be affected in various disease states(29).

Relaxation abnormalities decrease the ability of the myocardium to stretch. Diastolic relaxation is prolonged in heart failure, together with an increase in diastolic cytosolic Ca^{2+} content(30,80,122,321). The dynamic process of myocardial relaxation depends on how the cardiomyocyte handles Ca^{2+} fluxes. The cardiomyocyte functions optimally when a high peak of the Ca^{2+} transient is combined with a low diastolic cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$)(86). An increase in diastolic $[Ca^{2+}]_i$ results in an increase in residual cross-bridge interactions causing increased diastolic myocardial stiffness and decreased diastolic stretch. In case of an increased diastolic $[Ca^{2+}]_i$, compensatory mechanisms are not optimally available to the myocardium when the contraction load increases. In failing myocardium the Ca^{2+} transient is wider, its upstroke and decay shallower and diastolic $[Ca^{2+}]_i$ higher(138,158,222,278). Beta-adrenergic blockade, however may restore calcium dynamics and normalizes expression of calcium-handling proteins, eventually leading to improved hemodynamic function in mammalian cardiomyopathic hearts(42,285).

Some controversy exists about the role of this altered Ca^{2+} handling by failing cardiomyocytes(137,275,338). Is this altered Ca^{2+} handling causing impaired cardiomyocytal diastolic and systolic function? Or is the way the failing cardiomyocyte handles its Ca^{2+} an answer to the different phenotype of contractile protein composition(275)? Structural myocardial changes may also impair its ability to stretch, as will be discussed below.

Recently it was shown, that in the end-stage failing human heart, basal contractility is well preserved but "contractility reserve" (the ability to increase contractility with heart rate or sympathetic stimulation) is severely depressed. These fundamental changes in myocardial performance may play a role in the deterioration of pump function which causes reduced exercise capacity of the heart failure patient(see below)(138).

Also recently, the contractile response to the myosin light chain 2 dephosphorylation was found to be enhanced in failing hearts, despite the reduced level of basal myosin light chain 2 phosphorylation. The enhanced response to myosin light chain 2 dephosphorylation in failing cardiomyocytes might result from differences in basal phosphorylation of other thin and thick filament proteins between donor and failing hearts. Regulation of calcium sensitivity via myosin light chain 2 phosphorylation may be a potential compensatory mechanism to reverse the

detrimental effects of increased calcium sensitivity and impaired calcium handling on diastolic function in human heart failure(318,371,372).

Myocardial remodeling and hypertrophy

A fall in cardiac output after cardiac muscle damage from myocardial infarction (MI), viral infection, or pressure overload due to hypertension or valvular heart disease results in activation of signaling pathways including adrenergic, renin angiotensin (including angiotensin-(1-7))(87,88,210,359), and cytokine, designed as stress responses(28,35,72,78,218,221). The chronic activation of these pathways in heart failure results in defects in excitation-contraction coupling including: (A) Protein kinase-A hyperphosphorylation of the cardiac ryanodine receptor that results in depletion of FK506 (tacrolimus) binding protein (FKBP12.6) (which helps keep the channel closed in diastole) from the channel macromolecular complex, causing a diastolic Ca^{2+} leak that depletes sarcoplasmic reticulum (SR) Ca^{2+}(123,290,294,402); and (B) reduced SR Ca^{2+} reuptake via sarcoplasmic reticulum Ca^{2+}-ATPase(22,222,223,234,294). Other defects in the response in heart failure include alterations in the cytoskeleton(101,377,398) and the extracellular matrix (imbalance between matrix metalloproteinases and tissue inhibitors of metalloproteinase) (167,168,221,231,251,345,379).

A decrease in cardiac output decreases pulse pressure, which is sensed by high pressure baroreceptors as a decrease in the fullness of the arterial system. Arterial under filling sensed by arterial baroreceptors determines the “integrity” of the circulation (i.e. cardiac output and total peripheral resistance) and activates neurohormonal systems to maintain cardiac output(322). This neurohormonal activation, together with mechanical myocardial overload following myocardial damage, triggers myocardial molecular and cellular remodeling and hypertrophy.

The remodeling process follows cardiomyocytic contractile and relaxation abnormalities, hypertrophy, apoptosis(248,335) and necrosis, together with interstitial changes, including the extracellular matrix. Two recent experimental studies(387,401) demonstrate that cardiomyocyte apoptosis can be a major component of the failing heart. Although not analyzed directly, the phenotype and even the reduced survival of these transgenic mice can only be explained by a concomitant reduction of cardiomyocyte regeneration. Taken together with other available data, the results presented in these two papers make a strong case for the need to develop a new understanding of normal and pathological cardiac homeostasis in which both cardiomyocyte death and cardiomyocyte renewal are essential for the maintenance of cardiac function and its adaptation to different physiological and pathological demands (10,27,248,249,255,292).

The remodeling process may be reversible provided the cause of the remodeling has been either suppressed or attenuated (11,19,45,82,120,125,149,194,202,231,253,362,363,406). Elegant animal experiments showed reverse remodeling with reduced systolic wall stress and improved

adrenergic signaling by passive external support that does not generate diastolic constriction(309). Myocardial remodeling initially may restore cardiac output with a subsequent deactivation of the neurohormonal systems(233). The left ventricular remodeling process, however, is not a new steady state, but in itself progressive and leading to further deterioration of left ventricular pump function and progression of heart failure(66,136,217).

Ventricular dilatation and a decrease in ejection fraction in heart failure may not be the result of contractile failure per se, but primary the result of the structural cardiomyocytic and interstitial changes. In other words, to generate the same stroke volume, an increase in end diastolic left ventricular volume (not related to a preload-mediated increase in sarcomere length) mandates a decrease in left ventricular ejection fraction(5,6,66,138,370). Left ventricular stroke volume, despite a reduced ejection fraction, is indeed often normal(66,147).

In dilated cardiomyopathy the left ventricle dilates (with a secondary fall in left ventricular ejection fraction) due to impaired cardiomyocytic function and marked interstitial fibrosis. These myocardial changes influence overall pump performance of the heart(173,225). Recently it was shown in patients with dilated cardiomyopathy, that NO augments left ventricular stroke volume and left ventricular stroke work because of a NO-mediated rightward shift of the diastolic left ventricular pressure-volume relation and a concomitant increase in left ventricular preload reserve(267). These new findings indicate the potential existence of a diastolic stroke volume reserve, caused by an increase in myocardial distensibility which enables utilization of the Frank-Starling mechanism at relatively low filling pressures. In the failing heart, a high left ventricular wall stress in the presence of a high left ventricular diastolic volume, and, more importantly, a high left ventricular filling pressure, initiates signaling cascades leading to hypertrophy and remodeling(136). Left ventricular pressure changes do affect wall stress to a greater extent as compared to changes in left ventricular diastolic volume. In other words, according to LaPlace's law, doubling filling pressure doubles wall stress, whereas doubling end-diastolic volume, causes an increase in wall stress of only 1,3 times.

Myocardial hypertrophy has long been recognized as one of the heart's mechanisms for compensating a pressure or volume overload(94). This compensation has often been viewed as a feedback loop in which the concentric hypertrophy, which develops in pressure overload, normalizes wall stress while the eccentric hypertrophy, which develops in volume overload, allows for an increase in total stroke volume to compensate that which is lost from regurgitation(55). While the concentric hypertrophy of pressure overload often is compensatory, many examples are noted where either too little hypertrophy occurs to normalize stress or in other cases, in which hypertrophy exceeds the amount needed for normalization. These observations invoke nonmechanical mechanisms which modulate the degree to which the mechanical signal of pressure overload is translated into an increase in myocardial mass. Hypertrophy develops when the rate of myocardial protein synthesis exceeds that of protein degradation. It now appears

that in pressure overload this imbalance is created as synthesis rate increases while in volume overload hypertrophy appears to accrue because degradation rate decreases(55).

The fact that the failing heart, during exercise, depends on the Frank-Starling mechanism as its functional reserve mechanism per se, calls upon different potentially harmful signaling pathways for hypertrophy and myocardial remodeling if filling pressures and wall stress remain elevated(2,135,136,141,169,347). The prognosis of chronic cardiac hypertrophy patients can be affected significantly by modulation of the signaling mechanisms rather than reduction of cardiac hypertrophy itself. Therefore, what should be treated in patients with chronic cardiac hypertrophy may be the signaling mechanisms mediating cardiac hypertrophy, which have more pronounced effects upon cell survival and death of individual cardiomyocytes(135,240).

Myocardial hypertrophic changes, secondary to increased myocardial stretch, are able to compensate for decreased myocardial contractility or increased load of any cause or both. Hypertrophy may be able to increase contractile myocardial function initially, however hypertrophy by itself has eventually a plethora of effects on dynamic and structural properties of the cardiomyocyte(121,212,256). The process of myocardial hypertrophy may be physiologic as in athletes, or causes damage on the long run after an initial period of cardiac compensation. Intrinsic myocardial compensatory mechanisms, elicited by myocardial stretch, are influenced by autocrine, paracrine, and endocrine mediated effects of nitric oxide, angiotensin II, endothelin 1, insulin like growth factor, transforming growth factor-β, fibroblast growth factor, cardiotrophin-1, aldosterone, and B-type natriuretic peptide (46,308,311,323,373). Myocardial stretch induces, apart from the length-dependant activation, myocardial gene expression of different autocrine and paracrine working substances utilizing some sort of mechanotransduction of the cardiomyocytal sarcolemma influencing the cell nucleus(20,41,143,247,284). These substances have different effects on myocardial stiffness. Mechanotransduction of course, is compromised in case the extracellular compartment prevents the cardiomyocyte to stretch(15,320).

Myocardial stretch is, apart from systemic and exogenous reflex mechanisms, able to increase myocardial contractile force as will be discussed below. Important in this respect is that the amount of stretch, which depends on the myocardial stiffness, determines the height of the force increase. In other words, the easier myocardium stretches, the better it is able to cope with an increase in contraction load. Implicitly this statement tells us that systolic function importantly depends on diastolic myocardial stiffness. This holds true for intrinsic compensatory myocardial mechanism as the 'Frank-Starling mechanism' and 'Von Anrep effect' and not for systemic and other exogenous reflex mechanisms. In order to preserve pump function, it is therefore of utmost importance that the myocardium is protected against every cause of a decrease in myocardial stiffness. If an increased wall stress sustains, and is not lowered by the intrinsic myocardial compensatory mechanisms, a process of myocardial hypertrophy is initiated(212).

Heart failure symptoms and left ventricular ejection fraction

Despite intense investigation of thousands of heart failure patients in many clinical trials, we still do not understand the exact pathophysiological mechanisms of heart failure symptoms(16,84,92,131,206,208,260,261,288,344,349). Inclusion of heart failure patients in most clinical trials was based on the presence of a reduced ejection fraction. In the ACC/AHA guidelines on heart failure(140). the following statement has been made: *"The mechanisms responsible for exercise intolerance in patients with chronic heart failure have not been clearly defined. Patients with a very low ejection fraction may be asymptomatic, whereas patients with preserved left ventricular systolic function may have severe disability. The apparent discordance between the severity of systolic dysfunction and the degree of functional impairment is not well understood despite intense investigation"*.

Several factors may contribute to the above mentioned lack of understanding.

1. In case of a dilated left ventricle (high end-diastolic volume) with a normal stroke volume (i.e. low EF) during rest and exercise, the human body will not notice the presence of left ventricular dysfunction because of a near normal overall pumping capacity of the heart despite a reduced EF. The human body is not aware of a low EF but perceives low minute volume and high filling pressures(322). Unfortunately their is no relation between EF and these two parameters. A reduced minute volume activates systemic neuro humoral reflex mechanisms, an increase in left ventricular filling pressure reduces the compliance of the lungs causing dyspnea.
2. The use of left ventricular ejection fraction as the clinical standard to evaluate left ventricular systolic performance(56). Ejection fraction may be reduced exclusively by an increase in end-diastolic volume and not necessarily by a decrease in stroke volume. In heart failure a reduced left ventricular ejection fraction usually is the result of dilatation of a remodeled left ventricle and has a poor correlation with symptoms in the presence of a normal left ventricular stroke volume(66,178).
3. It is assumed that a normal resting left ventricular ejection fraction is synonymous with preserved left ventricular systolic function. This may not be the case, since in diastolic heart failure patients, despite their normal resting left ventricular ejection fraction, left ventricular systolic function is probably impaired as evident from other measures of left ventricular performance(153,276,403-405). One aspect of systolic left ventricular dysfunction is its incompetence to increase contractility with an increase in heart rate on exertion. This so called blunted or negative force-frequency response of failing myocardium causes a lack of exertional decrease in end-systolic left ventricular volume to increase left ventricular stroke volume(2). To increase left ventricular stroke volume, the failing myocardium therefore depends on its preload recruitable stroke work, i.e. the Frank-Starling mechanism. In case of diastolic dysfunction, and blunted left ventricular preload

reserve, subtle systolic dysfunction may therefore lead to heart failure irrespective of a normal or low ejection fraction, i.e. a non-dilated or dilated left ventricle respectively. The use of ejection fraction to distinguish systolic from diastolic heart failure thus may not be acceptable anymore because heart failure patients with either a normal or low ejection fraction probably have both diastolic and systolic dysfunction(170).

4. For practical purposes *resting* left ventricular ejection fraction is used in the large clinical trials as a function of left ventricular performance. Because most patients with heart failure only are symptomatic on exertion, *resting* left ventricular ejection fraction has a poor correlation with symptoms during exercise(282).
5. Systolic heart failure (low left ventricular ejection fraction) is discriminated from diastolic heart failure (normal left ventricular ejection fraction) because systolic heart failure is thought to have a worse prognosis than diastolic heart failure. Diastolic heart failure was associated with a lower annual mortality rate of approximately 8% as compared to annual mortality of 19% in heart failure with systolic dysfunction(109,216). More recently, however, careful examination of the available studies raises the possibility that the natural history of patients with diastolic heart failure may not be different from that observed in patients with congestive heart failure and reduced systolic function(18,150,151,326,339,375). So, as far as prognosis is concerned, systolic heart failure and diastolic heart failure with so called preserved systolic function, i.e. normal left ventricular ejection fraction, may be two of a kind(374).
6. Furthermore, the use of left ventricular ejection fraction does not give information on diastolic left ventricular function in heart failure. Therefore the presence of diastolic dysfunction can not be excluded on the basis of a reduced left ventricular ejection fraction per se.
7. Symptoms of heart failure like dyspnea on exertion may also occur in a variety of unrelated conditions, including obesity, lung disease, and myocardial ischemia. This could lead to a false diagnosis of heart failure with preserved left ventricular ejection fraction.
8. In the absence of significant mitral regurgitation, the increase in stroke volume during dynamic exercise is largely the result of a combined increase in preload and contractility. In the presence of mitral regurgitation, however, dynamic changes in the severity of regurgitation during exercise may compromise the normal increase in forward stroke volume and hence reduce maximal cardiac output and exercise capacity(187). In patients with heart failure due to left ventricular systolic dysfunction, exercise-induced changes in forward stroke volume during exercise are strongly influenced by exercise-induced changes in the severity of functional mitral regurgitation. Exercise-induced changes in the severity of functional mitral regurgitation may contribute to limit exercise capacity in heart failure patients(187).
9. EF is neither a measure of contractility nor a load-independent measurement of systolic function(47,410).

10. Increased aortic stiffness may limit exercise tolerance in patients with dilated cardiomyopathy. A stiffer aorta may interfere with both systolic and diastolic left ventricular function. The combination of a stiffer left ventricle with a stiffer central vasculature can further reduce exercise performance in patients with an already depressed left ventricular function. Changes in aortic stiffness may be a clinically important parameter in predicting heart failure symptomatology(36,159,312).

From 1 to 10 it becomes clear why some patients with reduced left ventricular ejection fraction do not have signs and symptoms of heart failure and why patients with normal left ventricular ejection fraction are symptomatic. A more detailed analysis of systolic and diastolic left ventricular function instead of a simple left ventricular ejection fraction measurement therefore is necessary.

Defining left ventricular function.

Cardiovascular physiologists have been researching ways to characterize the two fundamental properties of the left ventricle. First, its passive aspects, that is how easily the left ventricle can be filled, depending on left ventricular diastolic distensibility, and second, left ventricular contractility, or how strong it gets when activated. Left ventricular pressure-volume analysis has proven to be the best way to assess both aspects(154,211).The analysis is performed by simultaneously measuring the pressure and volume(13,346) of blood inside the left ventricle(356). These two signals are measured as curves in time, but then plotted with left ventricular volume on the x-axis and left ventricular pressure on the y-axis, so that each time the left ventricle contracts and relaxes, it creates a so called left ventricular pressure-volume loop. Points along the pressure-volume loop indicate the changes in left ventricular volume and pressure.

Pressure-volume relationships have provided extensive insight into left ventricular pumping characteristics(154,301). As advances in noninvasive methods continue to evolve, reliance on invasive methodologies to characterize diastolic left ventricular properties will continue to fade into the background. At present, however, simultaneously acquired left ventricular pressure and volume remains the gold standard for the two primary myocardial properties which determine stiffness (myocardial relaxation and myocardial material properties), and have clearly played a central role in the evolution of our understanding of cardiac diastolic disease and its treatment(154,317).

1.3 Left ventricular diastolic distensibility and heart failure

Structure of the left ventricular wall

The healthy left ventricle, with its remarkable mechanical efficiency, has a gothic architecture, which results from the disposition of the myocardial fibers supported and maintained by a normal collagen matrix scaffold. The myocardial fibers of the left ventricle have an oblique and spiral orientation of 60°(12). Despite the sarcomere shortening of only 15%, the global left ventricular ejection fraction is around 60% due to this myocardial fiber orientation. As evident from three-dimensional MRI images and from measurements of the curvature and thickness of the ventricular walls, the normal left ventricle was recently compared to a gothic building(65). In this model, the remarkable structural strength of the gothic left ventricle, that allows it to withstand the important stresses in its wall associated with the cardiac pumping function, is the result of its geometry, i.e. left ventricular volume, left ventricular shape and left ventricular wall thickness, and of the structure of the myocardium. A Romanesque transformation often characterizes the diseased left ventricle of heart failure patients.

The myocardium is composed of muscle fibers, a functional syncytium of cardiomyocytes, embedded in an extracellular matrix containing endothelial cells, fibrillar collagen, fibroblasts, and macrophages. The total amount of non-cardiomyocytes in the myocardium outnumbers the amount (not the volume) of cardiomyocytes.

Endothelial cells

Experimental work during the past 15 years has demonstrated that endothelial cells in the heart play an obligatory role in regulating and maintaining cardiac function, in particular, at the endocardium and in the myocardial capillaries where endothelial cells directly interact with adjacent cardiomyocytes(48,388). Removing the endocardial layer experimentally showed the same calcium desensitizing effect as muscle shortening does, but differs as compared to all other inotropic interventions(348,381). Endothelium- mediated auto-/paracrine signaling by nitric oxide interacts with other cardiopulmonary pathways, such as beta-adrenergic or cholinergic pathways in the heart, atrial and B-type natriuretic peptide activity(77,215,228), and circulating thyroid and aldosterone hormones(48,245). Nitric oxide is released from the coronary and endocardial endothelium possibly as a result of flow-induced shear stress and the cyclic mechanical deformation that occurs during the cardiac cycle(328). Both coronary flow and minute volume usually increase during exercise with a secondary increase in nitric oxide release.

Nitric oxide induces an early onset of ventricular relaxation, thereby enhancing ventricular

relaxation, early rapid filling, and diastolic left ventricular distensibility. In many cases, this effect may be accompanied by a slight decrease in peak systolic pressure despite the often unaltered rate of pressure development and unaltered ejection properties(71).

Such apparently negative inotropic effects may be regarded by many as potentially detrimental; rather, they should be considered as potentially beneficial to cardiac function (272), acting as a compensatory feedback, when the physiological effects on contraction duration of enhanced ventricular preload and/or afterload are superimposed on the pathological prolongation of contraction duration in ventricular hypertrophy, especially if tachycardia intrudes on diastolic filling time(48). Interestingly, Pinsky et al.(283) have demonstrated that there is a cyclical release of nitric oxide in the beating heart, most marked subendocardially, which peaks at the time of ventricular relaxation and early rapid filling. These appropriately timed, brief bursts of nitric oxide release would provide important beat-to-beat modulation of ventricular relaxation, early filling, and diastolic coronary perfusion. The subendocardial localization would suggest endocardial endothelial cells as its major source(48).

An increase in left ventricular diastolic distensibility, (i.e., low left ventricular end-diastolic pressures at high left ventricular end-diastolic volume), with its preload induced increase in stroke volume, constitutes one of the most fundamental modulators of cardiac systolic function (23,48,51,52,146,197,272,273,286,289,328).

In addition to endothelial nitric oxide production with its paracrine effect on the myocardium, nitric oxide is also produced within the cardiomyocyte in the presence of three isoforms of nitric oxide synthase(NOS): eNOS(NOS3), iNOS(NOS2), or nNOS(NOS1), exerting autocrine effects (26,163,325,396,415). Recently, basal release of nitric oxide is reported in the isolated heart, which significantly augments preload-induced increase of cardiac output, i.e. the Frank-Starling mechanism(289).

To quantify the relationship between applied force and nitric oxide synthesis, intermittent compressive or distending forces applied to ex vivo non beating hearts were shown to cause bursts of nitric oxide synthesis, with peak nitric oxide levels linearly related to ventricular transmural pressure(283). Experimentally removing coronary and endocardial endothelial cells abolishes the nitric oxide signal, which may indicate that these cells transduce mechanical stimulation into nitric oxide production in the heart(283). Taken together, these studies may help explain load-dependent relaxation, cardiac memory for mechanical events of preceding beats, diseases associated with myocardial distension, autoregulation of myocardial perfusion and protection from thrombosis in the turbulent flow environment within the beating heart(283).

These results, together with previous findings that nitric oxide modulates the force-frequency relation, beta-adrenergic inotropic response(23), myocardial relaxation(272,273), and possibly heart rate, indicate the potential importance of nitric oxide in the regulation of myocardial performance(289).

The effects of nitric oxide on myocardial function are postulated to be mediated by the intracellular messenger cGMP via a depression of myofilament sensitivity to intracellular Ca^{2+} (17,95,146,273,332,340,365). Recently data became available that effects of the nitric oxide donor sodium nitroprusside on cell contraction are mediated by changes in intracellular pH (pH_i)(146).

Bioassay studies showed the tonic release of a stable substance with potent myocardial activity: "myofilament desensitizing agent"(330). Like nitric oxide, this "myofilament desensitizing agent" did show a reduction in the amplitude of cardiomyocyte shortening, an earlier onset of cardiomyocyte relaxation and an increase in diastolic length. These effects are, like nitric oxide, not accompanied by changes in the cytosolic Ca^{2+} transient, indicating that they result from a reduced Ca^{2+} sensitivity of the contractile proteins. Thus both nitric oxide and this "myofilament desensitizing agent" enhance myocardial relaxation and increase active diastolic elasticity by reducing diastolic tone(328).

Endothelial modulation of myocardial function can now be seen as an important regulatory pathway influencing heterometric and homeometric autoregulatory mechanisms (see below). Eventually, modulation of the myofilamentary affinity for Ca^{2+} is the major component of the underlying basis of the Frank-Starling mechanism(182). Cardiac endothelial cells release different factors that exert important paracrine effects on myocardial systolic and diastolic function(49,50,130,329). It is estimated that no cardiomyocyte is more than 3 to 5μ from an endothelial cell(328,329). Results of experimental studies (91,105,201,235,327,331) are confirmed in human subjects(272,273). These studies have demonstrated that vascular as well as endocardial endothelial cells exert a paracrine influence on cardiomyocytal function. Cardioactive factors released by the cardiac endothelium include nitric oxide, endothelin, adenylpurines and other substances as yet chemically unidentified(328). The cardiac endothelial cells also possess enzymatic activities which contribute to changes in local levels of substances as angiotensin II and bradykinin(328). These endothelial paracrine factors influence myocardial systolic and diastolic function predominantly by modifying cardiac myofilament properties rather than altering cytosolic Ca^{2+} transients(307).

Fibrillar collagen

Another important constituent of the ‘non-cardiomyocytal compartment’ is fibrillar collagen. Fibrillar collagen (type I and type III, with a shift in collagen III to I in heart failure) is the main structural protein of the extracellular matrix occupying 4% of the interstitial space. Type III collagen is far less abundant and more distensible than type I collagen which has the tensile strength of steel. A collagen network (endo-, peri-, and epimysium) scaffolds the cardiomyocytes to maintain tissue architecture and cardiomyocyte alignment during the cardiac cycle. The

amount of collagen in the extracellular matrix(400), but to a greater extent, collagen cross-linking and the ratio of type III and type I collagen are important determinants of myocardial stiffness(15,179,392).

The cardiomyocyte: structural properties

The cytosol is the fluid region of the cell cytoplasm that lies outside of the cell organelles. Initially, the cytosol was thought to be a fairly homogeneous "soup" in which all of the organelles floated. It is now known that the cytosol of all cells contains a cytoskeleton. In general, the cytoskeleton helps to maintain cell shape and mobility and provides anchoring points for other cellular structures(3,128,129,200). The cardiomyocyte cytoskeleton is composed of myofibrillar and extramyofibrillar or cytoplasmic compartments. In contrast to our knowledge of the importance of alterations in proteins that actively participate in cardiomyocyte contraction, relaxation and growth, there is less information regarding the role of cytoarchitectural components of the cardiomyocyte in normal and pathological states.

A myofibril comprises a chain of sarcomeres. A sarcomere is the segment from one Z-disc to the next and consists of two halves of an I-band and one A-band. A sarcomere is both the structural and functional unit of cardiac muscle and contains two types of filaments: thick filaments, composed of myosin, and thin filaments, containing actin. Myosin is an unique enzyme because it can move along an actin myofilament by coupling the hydrolysis of ATP to conformational changes. Such an enzyme, which converts chemical energy into mechanical energy, is called a mechanochemical enzyme or motor protein. Myosin is the motor, actin filaments are the tracks along which myosin moves, and ATP is the fuel that powers the motility. Coordinated, synchronous contraction of the heart is facilitated by mechanical coupling between sarcomeres at Z-discs, adjacent cardiomyocytes at intercalated discs, and cardiomyocytes and the extracellular matrix at costameres and dystrophin-glycoprotein complexes(257).

The myofibrillar cytoskeleton includes *titin*(127,383,393,395) (the largest protein known and the third most abundant cardiac protein), *myosin binding protein C*(226,298,389,390) (a protein located within sarcomeric A-bands), and *desmin*(124,139,351,382) (a longitudinally oriented intermediate filament (IF) bridging between the Z-discs(37) in the same myofibril). Its alignment with the sarcomere is held in place by numerous intermediate filament-associated proteins (IFAP) including *skelemin* at the M line and *synemin* at the Z-disc, and *vinculin* (an adapter protein which attach actin filaments to integrins)(20,41,85,90,103,104,257,305). *Nebulette* is an actin binding Z-disc protein, extending approximately 25% of the thin filament length(239,254,315).

Transduction of mechanical stress into biochemical signals is largely mediated by a group of integral membrane proteins called *integrins*(306). They link the extracellular matrix to the

cellular cytoskeleton to provide physical integration between the outside and the inside of the cardiomyocyte. Extracellular ligands, like collagen, fibronectin or laminin, activate integrins which initiate signaling in multiple intracellular pathways through the integrin bound protein melusin. Melusin interacts with the cytosolic domain of integrin and acts as a biomechanical sensor which regulates gene expression, growth and survival of cardiomyocytes(20,41,60). It is suggested(20), that progression to cardiac failure does not depend on the phenotype of hypertrophy, but may be related to the balance between the activation of protective and deleterious pathways. In other words, as discussed above, it is not the hypertrophy per se that determines a detrimental outcome(172), but rather the different signaling pathways (integrin mediated versus activation of Gq-coupled receptors(299) or cytokine receptors) that are activated in response to the chronic overload state of the heart, with a potential protective role for the integrin mediated hypertrophy signaling pathway(20,302,333).

The extramyofibrillar cytoskeleton is composed of at least three classes of fibers: *tubulin-containing microtubules* (24 nm) (microtubules are polymers of α- and β-tubulin that can polymerize and depolymerize to participate in multiple cellular processes, including growth and proliferation)(413), *actin microfilaments* (7 nm) and *intermediate filaments* (10 nm).

The ability to modulate the amount and degree of polymerization of tubulin may have important therapeutic implications. On the levels of the sarcomere and the cardiomyocyte a persistent increase in microtubule density accounts to a remarkable degree for the contractile dysfunction seen in pressure-overload right ventricular hypertrophy(413). A persistent increase both in microtubules and in their biosynthetic precursors exists in pressure-hypertrophied myocardium. Increased microtubule density constitutes primarily a viscous load on the cardiomyocyte contractile apparatus in pressure-overload cardiac hypertrophy(412).

Biophysical interactions among sarcolemmal integrin receptors and the cytoplasmic (extramyofibrillar) and myofibrillar cytoskeleton are still largely unknown(350). The integrin-cytoskeleton complex may act as a mechanoreceptor translating signals from the extracellular matrix and inducing biochemical signals within the cell that regulate gene expression and cellular growth(20,41,90,305). The extracellular matrix together with cytoskeletal proteins are the major determinant for *passive* myocardial properties. An *active* component of myocardial stiffness depends on the intensity of diastolic cross-bridge interaction, which determines diastolic myocardial tone as was discussed above.

The *passive* myocardial stiffness was initially thought to originate from the extracellular collagen matrix of the endo-, peri-, and epimysium. Investigation on isolated cardiomyocytes made it however clear, that stiff structures are located within the cell as well. The stiff *extra*cellular or interstitial elements, which help prevent overstretch of muscle tissue, may be relevant only at more extreme, pathological, stresses and strains(394). and the *intra*cellular elements, produce myocardial stiffness in the more physiological range of diastole(112). These

elements, the cytoskeletal proteins, e.g., titin, store elastic energy during cardiomyocyte contraction below the equilibrium length (restoring force) and develop passive force when the cardiomyocyte is stretched(110-112,205).

The cardiomyocyte: functional properties

Despite early reports of dynamic changes in diastolic distensibility of the left ventricular chamber(232,316) it was not before the 1970's that clinicians became aware of this important myocardial property. Myocardial distensibility was initially thought to be dependent on myocardial material properties, left ventricular wall thickness and geometry. This implied that the relation between left ventricular diastolic pressure and volume could change only as a result of chronic myocardial changes (i.e., myocardial scarring or hypertrophy). In line with this assumption left ventricular pressure was used as a surrogate for left ventricular volume(336). However, clinical observations, that angina pectoris caused a change in wedge pressure in patients with indwelling, right sided catheters, changed this traditional view. Nowadays we understand clearly that intrinsic dynamic factors, like myocardial relaxation, coronary vascular perfusion, and residual diastolic left ventricular myocardial tone, can instantaneously influence diastolic left ventricular distensibility (262).

Animal experiments showed that the myocardium does not relax to a totally passive “flaccid” state, but instead rapidly relaxes to a lower level of residual cross bridge cycling associated with a basal level of energy use and heat production, as was discussed above (68,184,211,317,342,353,414). In both animal and human myocardium, diastolic volume at a given diastolic pressure, is partly dependent on weakly bound cross bridges as a function of the diastolic calcium concentration in the cardiomyocyte(160,317,342). This residual diastolic cross bridge cycling, therefore actually may modify the Frank-Starling mechanism, a modification which simply would not exist if the diastolic or resting mechanical myocardial properties were completely passive(160,342).

Influencing these *active* diastolic myocardial properties, in a way that left ventricular diastolic distensibility increases (i.e. a downward and rightward shift of the left ventricular diastolic pressure-volume curve) results in an increase in end-diastolic sarcomere length at a given end-diastolic pressure and improved left ventricular systolic performance by increasing preload reserve(272,273,289). The left ventricular wall stress (‘afterload’) in this case will only be minimally increased because of a larger radius in the setting of unchanged pressure prior to contraction.

Preload effects on rate of left ventricular pressure fall, however, is still subject to debate

(98,99,106,107,189,411). Afterload is an acute determinant of the end-diastolic pressure-volume relation, which, therefore, does not provide a load-independent assessment of diastolic function(189).

The active diastolic myocardial properties are determined by residual diastolic interaction of contractile proteins related to changes in rate and extent of myocardial relaxation, among other factors. Relaxation refers to the process by which the myocardium returns to its initial length and tension following a contraction. The onset of left ventricular filling occurs before the end of relaxation from the previous systole. The ventricular diastolic pressure-volume relation is influenced by the rate and extent of myocardial relaxation from the previous systole(106,189,191,265).

The rate of relaxation affects the atrioventricular pressure difference, which is the driving force behind early ventricular filling. The efficient and complete reuptake of activator calcium from the sarcomeres by the sarcolemma and the sarcoplasmatic reticulum produces a fast rate of relaxation to a low minimum ventricular pressure, which facilitates high left ventricular filling rates. The rate and extent of relaxation are influenced by changes in load and depend on the intracellular processes controlling cross-bridge inactivation, and any regional dyssynchrony of the contraction-relaxation sequence(69,211).

Other factors change the rate and extent of relaxation, or influence active diastolic tone: stretch sensitive calcium channels, diastolic sarcoplasmic calcium release, the influence of neuro hormones and endothelial modulation of active diastolic tone(108,221,287,391).

An increased number of force producing cross-bridges during diastole contributes to reduced distensibility of the cardiomyocyte compartment and reduced sarcomere length at a given diastolic pressure impairing left ventricular systolic(108,183,407) and diastolic(31,397) performance. This reduction in end-diastolic sarcomere length causes a decrease in left ventricular equilibrium volume with a strain independent diastolic dysfunction: i.e. an upward shift of the diastolic left ventricular pressure-volume relationship (Figure 4).

A complete evaluation of the left ventricular diastolic properties includes the determination of left ventricular myocardial and chamber stiffness constants, end-diastolic pressure, end-diastolic wall stress, end-diastolic stiffness, and the dynamic processes of rate and extent of left ventricular myocardial relaxation. The use of echocardiographic or radionuclide techniques to quantify the rate of blood flow or volume changes in the left ventricle during diastole generates parameters that are influenced by loading conditions in the heart and therefore cannot be viewed as specific indexes of diastolic distensibility(53). The only way to demonstrate left ventricular diastolic dysfunction or to calculate stiffness, is to show that the left ventricular end-diastolic pressure-volume relation is altered compared with normal (i.e., steeper than normal or displaced upward in the left ventricular pressure-volume plane). By characterizing the left ventricular end-diastolic pressure-volume relation, it is possible to show not only that left ventricular

diastolic pressure is elevated, but also that such a pressure increase is not the result of increased left ventricular diastolic volume as by increased preload but follows an upward shift of the left ventricular end-diastolic pressure-volume relation(53).

Diastolic stress-strain versus pressure-volume relationships

Both structural and dynamic myocardial factors ultimately determine left ventricular chamber- and myocardial stiffness. Chamber stiffness has to be distinguished from myocardial stiffness. Left ventricular chamber stiffness is derived from left ventricular pressure and volume data, whereas myocardial stiffness is derived from left ventricular wall stress and strain data. Left ventricular chamber stiffness increases either with each increment in left ventricular filling pressure: so called preload- or strain dependent increase in chamber stiffness, or through a leftward and upward shift of the entire pressure-volume relation, often referred to as a decrease in diastolic left ventricular distensibility (i.e. a strain independent increase in left ventricular chamber stiffness). The diastolic left ventricular pressure-volume relation can be expressed as an exponential function. A modulus of left ventricular chamber stiffness (K_C) is derived from the slope of the linear relation between left ventricular chamber stiffness (dP/dV) and diastolic left ventricular pressures(198).

A myocardial stiffness constant (K_M) is determined by the slope of the linear relation between left ventricular myocardial stiffness ($d\sigma/d\varepsilon$) and left ventricular myocardial wall stress for instance at the end of diastole(98,198). These definitions of left ventricular chamber- and myocardial stiffness ignore the existence of stress relaxation (a time dependent decline in stress when a material is held at a constant length after a rapid stretch), creep (a gradual increase in length when a material is held at constant stress after a rapid stretch), viscoelasticity (causing a higher level of stress when a material is stretched rapidly), and elastic recoil(98,198).

Elastic recoil causes diastolic suction which is created by the preceding systolic contraction. The more forceful systolic contraction and concomitant decrease in end-systolic volume, the greater elastic recoil and suction early in diastole(89). Diastolic suction may facilitate left ventricular filling during exercise in the presence of an intact force-frequency response with a decrease in the end-systolic volume below the left ventricular equilibrium volume(144,199). If these experimental findings play a significant role clinically, is doubtful (271). Both stress relaxation and creep probably have clinically little significance in the intact heart as well.

In summary, the diastolic left ventricular pressure-volume relationship is determined both by the material properties of the left ventricular wall, and by left ventricular geometry (i.e. left ventricular shape, left ventricular volume and left ventricular wall thickness). The material properties of the myocardium dictate the strain that follows a given stress, and determine position and shape of the myocardial stress-strain relationship. These material properties, together with

the left ventricular geometry also determine position and shape of the diastolic left ventricular pressure-volume relationship. The link between the one dimensional myocardial stress-strain relationship and the left ventricular pressure-volume relationship is LaPlace's Law, which takes into account left ventricular geometry. At low filling pressures the structural determinants of the diastolic left ventricular pressure-volume relationship are the cytoskeleton titin(115,162), together with the (residual) diastolic cross-bridges interactions of contractile elements after a previous contraction. At very high filling pressures the structural determinants of the diastolic left ventricular pressure-volume relationship are determined by collagen fibers of the extracellular matrix, superimposed on cytoskeletal properties and on the (residual) diastolic cross-bridges interactions of contractile elements after a previous contraction. The importance of diastolic left ventricular distensibility will become even more obvious when the sequellae of systolic dysfunction on heart failure symptoms will be discussed below.

1.4 Stroke volume reserve and heart failure symptoms

Frank, Starling, Bowditch, Von Anrep and Gregg

'Heterometric autoregulation'

Although the contractile performance of the heart is under continuous nervous, hormonal, and electrophysiological influence, the heart has intrinsic mechanisms by which it can adapt its output to changing systemic demands. An increase in left ventricular end-diastolic volume following an increase in venous return or an increase in aortic input impedance, instantly leads to a more powerful contraction. This is called the Frank-Starling mechanism which allows the heart to cope with an increase in venous return by an increased stroke volume or maintaining stroke volume despite an increase in aortic input impedance. This is labeled 'heterometric autoregulation' and corresponds to an increase in contractile performance following an increase in left ventricular end-diastolic volume.

Frank and **Starling** carried out fundamentally different experiments. Frank measured the isovolumic pressure developed by frog heart at different volumes(93). He therefore discovered the pressure-volume-volume relationship which depends directly on the force-length relationship of the sarcomeres. Starling studied cardiac shortening as manifest by cardiac output and its relationship to end-diastolic conditions as manifest by right atrial pressure(266).

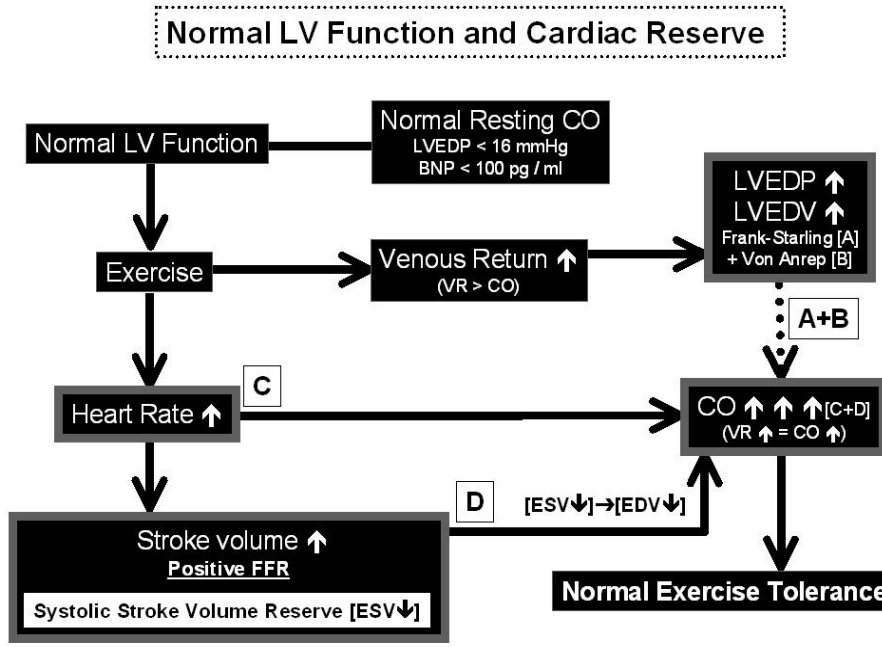

Figure 1. Diagram illustrating cardiac reserve mechanisms during exercise in the presence of intact cardiac function. Exercise initially increases venous return(VR) and left ventricular(LV) end-diastolic volume (EDV), causing an increase in LV stroke volume[A] ('Fast force response' or Frank-Starling mechanism).To date, no data are available on the role of the 'slow force response'(Von Anrep effect)[B] during exercise, in the presence of an intact force-frequency response (FFR). Subsequently a heart rate increase per se[C] increases cardiac output(CO). A high heart rate increases myocardial contractility (positive (FFR)[D], causing a reduction in end-systolic volume (ESV) and EDV. This increase in contractility compensates for a loss of the Frank-Starling mechanism. The increase in CO matches the increased VR, resulting in normal exercise tolerance. B-type natriuretic peptide(BNP) < 100 pg / ml is in the normal range, indicating normal LV filling pressures and LV wall strain(228). LVEDP: left ventricular end-diastolic pressure; LVEDV: left ventricular end-diastolic volume.

Thus he was studying the ability of cardiac muscle to shorten more at a given load from a greater initial length. Starling in the promulgations of his law implied a common mechanism for these two phenomena and spoke of the "energy liberated" being a function of initial muscle fiber length(157,357). The existence of the Frank-Starling mechanism, disputed by some(174,324), is reported to be operational in the dilated and failing left ventricle (117,133,134,155,156,334,360,366,367,369,385,399).

At low levels of exercise, when heart rate is only slightly increased, minute volume is mainly

increased by an increase in venous return which forces end-diastolic volume and stroke volume to increase (Frank-Starling mechanism)(Figure 1). Whereas, at higher levels of exercise different cardiac reserve mechanisms will be operable, as will be discussed below.

(Patho)Physiology of Exercise

The inability to perform exercise without discomfort may be one of the first symptoms experienced by patients with heart failure and is often the principal reason for seeking medical care. Therefore, exercise intolerance is inextricably linked to the diagnosis of heart failure(282). Increased venous return stretches the left ventricle with a concomitant length-dependent activation and increase in ejection pressure or stroke volume or both. There is abundant experimental evidence for a biphasic increase in contractile force production after myocardial stretch: an instantaneous increase in contractile force (without changing contractility(368)) due to an increased Ca^{2+} sensitivity of contractile elements (**Frank-Starling** mechanism) (96,145,355) and a slow increase in contractility mediated by a stretch induced increase in the intracellular Ca^{2+} transient (**Von Anrep** effect) (4,62-64,185,274,355,378). The peak of the Ca^{2+} transient after a length change does not change immediately(264,368). A rise in sarcomere length, causes an initial increase in contractile force, followed by a slow force increase(264,296). The slow force increase corresponds to a higher peak of the Ca^{2+} transient(73). This rise in the peak of the Ca^{2+} transient means an increase in contractility. Contractility or inotropic state is a more stable descriptor characterizing the cardiac muscle behavior as long as chemical environment and temperature do not change (138,278,368). The amplitude of the Ca^{2+} transient is determined by the rate at which the sarcoplasmic reticulum releases Ca^{2+} and the rate at which troponin C binds free Ca^{2+}. Interventions or states that increase the affinity of troponin C to Ca^{2+} decrease the amplitude of the intracellular Ca^{2+} signal(30). Furthermore, the Ca^{2+}, released from the sarcoplasmic reticulum, does not activate the contractile proteins maximally, allowing the myocardium to have a large contractile reserve(30). On the cellular level cardiac contractility depends mainly on the amount of activator Ca^{2+} that reaches the myofilaments, the amount of contractile protein, its Ca^{2+} affinity, adenosine triphosphatase (ATPase) activity, and the rate of actin-myosin cross-bridge cycling(30,102). Thus at a constant temperature and unchanged Ca^{2+} sensitivity, it is the peak of the Ca^{2+} transient that determines myocardial contractility. Since instantaneous sarcomere length changes do not influence the peak of the Ca^{2+} transient, contractility is thus separated from the consequences of an instantaneous change of sarcomere length, i.e. an increased Ca^{2+} affinity of troponin C(73,74). Thus the slow effect of a change in length on force development is concurrent with a change in the peak of the Ca^{2+} transient and may consequently be considered as an inotropic intervention(4,368). Notably, in case a change in cardiac muscle length is applied in order to measure contractile state, a change in contractile state is caused eventually by that change in muscle length itself, and measurements therefore should

be made promptly after the length change is applied(4,368).

To date, no data are available on the role of the 'slow force response'(Von Anrep effect) during exercise, in the presence of an intact force-frequency response(61) (see below). An exercise induced increase in heart rate, eliciting the force frequency response, possibly causes a decrease in end-diastolic volume before the slow force response gets operational, which takes a couple of minutes(63).

Exercise increases contractility further by a combination of β-adrenergic stimulation and a positive force-frequency relation or '**Bowditch** frequency treppe' (2,39,44,236,295,304,319). Increased stroke volume and heart rate on exercise enable the heart to increase the resting cardiac output three to four times(2). A positive force-frequency response may be seen as a systolic stroke volume reserve, responsible for an additional 30% increase in minute volume at high heart rates, as compared to heart rate increase alone(2). β -adrenergic stimulation of the myocardium increases the Ca^{2+} transient and speeds up relaxation. A positive force-frequency relation increases the sarcoplasmic reticulum (SR) Ca^{2+} content due to a reduced inhibition of SR Ca^{2+}-ATPase (SERCA2) by phospholamban(33,34,236,237). An increase in SR Ca^{2+} content causes a higher Ca^{2+} transient(44,295,304). The force-frequency effects are amplified by β-adrenergic stimulation(303,304). Experimentally heart rate was shown to modulate the slow force response(364).

A positive force-frequency relationship results in a decrease in end-systolic volume thereby decreasing end-diastolic volume which initially was increased due to an increase in venous return(204,408). During exercise, before heart rate increases, the Frank-Starling mechanism is the predominant compensatory mechanism to increase cardiac output(286). In case of a complete heart block(219) or fixed heart rate(252) and during autonomic blockade(161) the Frank-Starling mechanism and possibly the slow-force response (Von Anrep effect) are the principal compensatory mechanisms available.

However, when endurance athletes (148,195,196,361), elite bicyclists(81), or endurance trained older man(118) exercise, they all have signs of a lower diastolic chamber stiffness and a smaller decrease of end-diastolic volume when heart rate increases, as compared to sedentary men. This smaller decrease in end-diastolic volume during exercise in trained subjects may be explained by an increased utilization of the Frank-Starling mechanism(118) during high levels of exercise, notwithstanding β-adrenergic stimulation of the myocardium, the force-frequency relation, and β-adrenergic amplification of the force-frequency relation(304). In the failing heart however, an increase in heart rate during exercise results in a decrease in active tension development (negative force-frequency relationship)(2,188,242,280,337,343).

β-adrenergic stimulation of the myocardium and β-adrenergic amplification of the force-frequency relation in heart failure is not significant(32,59,304). The decrease in end-diastolic volume at increased heart rate, therefore will be smaller than normal(203,237,242), as is the case in athletes. However, heart failure patients, in contrast to athletes, depend chiefly

on the Frank-Starling mechanism to increase cardiac output during exercise because of a negative force-frequency relationship and blunted β-adrenergic effects on myocardial contractility (Figure 2)(169,242,304). In heart failure patients with left ventricular hypertrophy and normal EF, often referred to as diastolic heart failure, also show a blunted or negative force-frequency response, with sometimes even an increase in end-systolic volume, with increasing heart rate(21). Diastolic dysfunction present at rest did not worsen during increased heart rates(142,153,169,281,343).

Assuming the importance of the Frank-Starling mechanism as a compensatory mechanism in heart failure, irrespective of resting systolic function or EF, myocardial stiffness plays a momentous role. The amount of increase of the left ventricular stroke volume or stroke work is related to the amount of increase in the left ventricular end-diastolic volume. The amount a given end-diastolic pressure increases end-diastolic volume depends mainly on myocardial stiffness. A low stiffness results in a relatively higher increase in end-diastolic volume and thus in left ventricular stroke work. Hence a low myocardial stiffness is expected to result in lower left ventricular filling pressures and wall stresses when DCM patients exercise, beneficially affecting their exercise capacity (diastolic stroke volume reserve) (272)(Figure 2).

However, in the failing heart, a stiff ventricle is prone to continuous high myocardial wall stress when during exertion end-diastolic volume tends to increase as a result of a negative force-frequency relation of dilated cardiomyopathy. A high left ventricular stiffness promotes the induction of high left ventricular wall stress, who's sequellae play an important role in the clinical picture of human heart failure. First, high filling pressure causes signs and symptoms of dyspnea on exertion. Second, high diastolic wall stress exposes the myocardium to stimuli initiating myocardial hypertrophy and remodeling with its specific deleterious effects on myocardial structure and function(230,310,311,341). Third, high myocardial stiffness reduces the ability of the ventricle to utilize the predominant compensatory mechanism of a stretch induced increase in contraction force to regulate stroke work (Frank-Starling mechanism and Anrep effect) (Figure 3)(4).

Important in this respect is that myocardial stiffness determines the amount of stretch for a given amount of force. In other words, the easier myocardium stretches, the better it is able to cope with an increase in contraction load at a given end-diastolic pressure. Implicitly this statement tells us that systolic function importantly depends on diastolic myocardial stiffness. This holds true for intrinsic compensatory myocardial mechanism as the length dependent activation (Frank-Starling mechanism) and 'Von Anrep effect' and not for systemic and other exogenous reflex mechanisms. But why possesses myocardium a high diastolic stiffness? As was discussed above, diastolic stiffness depends, apart from changes in structural myocardial properties, on how the myocardium recovers from a systolic 'insult', which determines intensity of residual diastolic cross-bridge interaction, as was discussed above.

Von Anrep effect versus Gregg effect

Von Anrep showed in 1912 that a heart dilatation induced by clamping the heart outflow was followed by a decline in heart volume toward the initial volume(380). Rosenblueth in 1959 (300) reported that an increase in heart rate (Treppe effect or force frequency response(39)) and an increase in afterload both increase contractility of the isolated canine right ventricle. They used the expression 'the two-staircase phenomenon'. Sarnoff in 1960(314) used the term 'homeometric autoregulation' to define a decrease in left ventricular end-diastolic volume occurring after an initial increase in left ventricular end-diastolic volume secondary to an increase in afterload. Both experiments (300,314) were performed in an isolated heart preparation which rules out a possible influence of systemically released catecholamines. In 1973 Parmley(264) showed that if the length of an isolated strip of myocardial tissue was increased, there was a corresponding rapid (Frank-Starling mechanism) and a slow (Von Anrep effect) increase in developed force.

The mechanism leading to the slow force response to stretch (Von Anrep effect) is calcium entry through the reverse mode of sodium-calcium exchange causing a progressive increase in the calcium transient(14,63,64,164,274,279). Others found however, nitric oxide produced by activation of endothelial nitric oxide synthase (NOS) to act as a second messenger of stretch by enhancing ryanodine receptor Ca^{2+}-release channels activity, contributing to myocardial contractile activation of the slow force response(277). Thus, lengthening of the muscle, longitudinal stretch of the myocardium, causes an immediate increase in developed force (Frank-Starling mechanism) followed by a slow increase in developed force (Von Anrep effect).

An increase in coronary perfusion, however, causes transversal stretch of the myocardium, which increases developed force as well (**Gregg** effect(114,166)) through activation of stretch-activated ion channels(1,186). Stretch-activated ion channels blockade in isometrically contracting perfused rat papillary muscle completely blunted the increase of developed force and of peak intracellular calcium concentration induced by the Gregg effect; however, it did not affect the Von Anrep effect. The slow force response was also augmented by an increase in coronary perfusion. This suggests an increase of developed force via the Von Anrep effect on top of the Gregg effect. Therefore, increased coronary perfusion causing transversal stretch of the myocardium, and muscle lengthening causing longitudinal stretch of the myocardium, increase myocardial contraction through different stretch-triggered mechanisms(185).

Cardiomyocytes and the vasculature are aligned in parallel. Increasing coronary perfusion experimentally deforms the myocardium perpendicular to the alignment of the cardiomyocytes (transversal stretch) through an increase in blood vessel diameter and papillary muscle diameter(186). This activates stretch-activated ion channels with an initial, however transient, increase in calcium influx, subsequently followed by an increase in calcium sensitivity, both contributing to a more forceful cardiac contraction. Because the immediate stretch-activated ion

channel-sensitive influx of Ca^{2+} at the onset of the Gregg effect is transient, the intracellular [Ca^{2+}] elevation of the Von Anrep effect is similar at low and high perfusion. In the steady state, the Gregg effect results in increased sensitivity of the myofilaments for calcium. Together with the Von Anrep-induced slow intracellular [Ca^{2+}] elevation, this leads to an augmented Von Anrep effect at high perfusion(186).

Myocardial stretch by lengthening , however, results mainly in fiber lengthening and longitudinal stretch of cardiomyocytes with an instant increase in calcium sensitivity of contractile elements and a slow increase of intracellular calcium concentration, not via stretch-activated ion channels or L-type calcium channels, but likely via angiotensin II induced endothelin-1 release and activation of the sodium-hydrogen exchanger(274) and/or via endogenous nitric oxide mechanisms(277).

Frank-Starling relationship: Do we know its mechanism?

The increase in myofilament Ca^{2+} responsiveness on an increase in sarcomere length is, in part, the cellular basis for Frank-Starling's law of the heart(79). It has been suggested that a decrease in myofilament lattice spacing in response to an increase in sarcomere length underlies this phenomenon. This hypothesis is supported by previous studies in which reduced muscle width induced by osmotic compression was associated with an increase in Ca^{2+} sensitivity, mimicking those changes observed with an increase in sarcomere length. However alterations in myofilament lattice spacing may not be the mechanism that underlies the sarcomere length-induced alteration of calcium sensitivity in skinned myocardium(175-177,241).

Recent evidence suggests that titin may play a role in regulating active force. Titin-based passive force enhances length-dependent activation of cardiomyocytes(58). Degradation of titin was found to significantly increase lattice spacing. Interfilament lattice spacing is widely held as a determinant of the probability of actomyosin interaction at a given calcium concentration (96,229), and the effect of titin on lattice spacing may thus explain the effect of titin's passive tension on the length dependence of calcium sensitivity. However, considering the recent work that showed that lattice spacing and calcium sensitivity are not well correlated (176,177), it is also worth considering that titin influences active force via an earlier proposed mechanism (113) in which the likelihood of cross-bridge interaction is enhanced by passive force-induced thick filament strain. This mechanism also provides an explanation for the reported length-dependent effect of titin on maximal active tension in rat cardiac trabeculae(97). Thus the Frank-Starling mechanism of the heart may in part be due to an effect of titin-based force on the length dependence of maximal active tension and calcium sensitivity.

Left ventricular dysfunction and cardiac reserve

In the presence of left ventricular dysfunction and blunted force-frequency response, the systolic stroke volume reserve is not sufficient or even absent(2). Notwithstanding, many patients with left ventricular dysfunction are mildly symptomatic or even asymptomatic on exertion, suggesting an adequate rise in stroke volume. But how? Exercise increases venous return, which forces ventricular filling and diastolic volumes to increase. An increase in ventricular diastolic volume increases stroke volume (Frank-Starling mechanism and Von Anrep effect). If, in the presence of left ventricular dysfunction with blunted systolic stroke volume reserve, there is an adequate rise in minute volume at high levels of exercise, there must be an adequate increase in both heart rate and stroke volume(Figure 2). If patients with left ventricular dysfunction are able to recruit an adequate stroke work increase(169), they may exercise with reasonable few symptoms. In these patients, stroke volume increase during both low and high levels of exercise is the result of increased ventricular diastolic filling. A distensible left ventricular wall enables them to utilize both the Frank-Starling mechanism and Von Anrep effect (Figure 2). As discussed above, in the absence of left ventricular dysfunction, stroke volume will increase at low levels of exercise because of an increased ventricular diastolic filling (Frank Starling mechanism) and at high levels of exercise because of an increased ventricular emptying (positive force-frequency response)(132) (Figure 1).

The absence of a positive force-frequency response in left ventricular dysfunction is thus compensated for by a facilitation of ventricular filling to increase stroke volume at high levels of exercise.

This aspect of left ventricular dysfunction may be easily anticipated in patients encountering low left ventricular ejection fractions, with increased end-systolic and end-diastolic left ventricular volumes. These patients show a blunted or even negative force-frequency response and, in case they have an easily distensible left ventricular myocardium, they may be mildly symptomatic even at higher levels of exercise(270,289) (Figure 2). But when failing hearts are studied with a normal left ventricular systolic function or ejection fraction at rest, it may be of some surprise that these hearts also lack a positive force-frequency response at high levels of exercise(343,352). Thus failing hearts, with a so called normal systolic function at rest, prove to have an inadequate increase in systolic performance at high heart rates as well. So, what is called a normal systolic function at rest, does not preclude systolic dysfunction at high heart rates. These, mostly hypertrophic, hearts are not able to intensify their systolic and diastolic calcium fluxes and are in this respect as myopathic as dilated hearts with poor contractility.

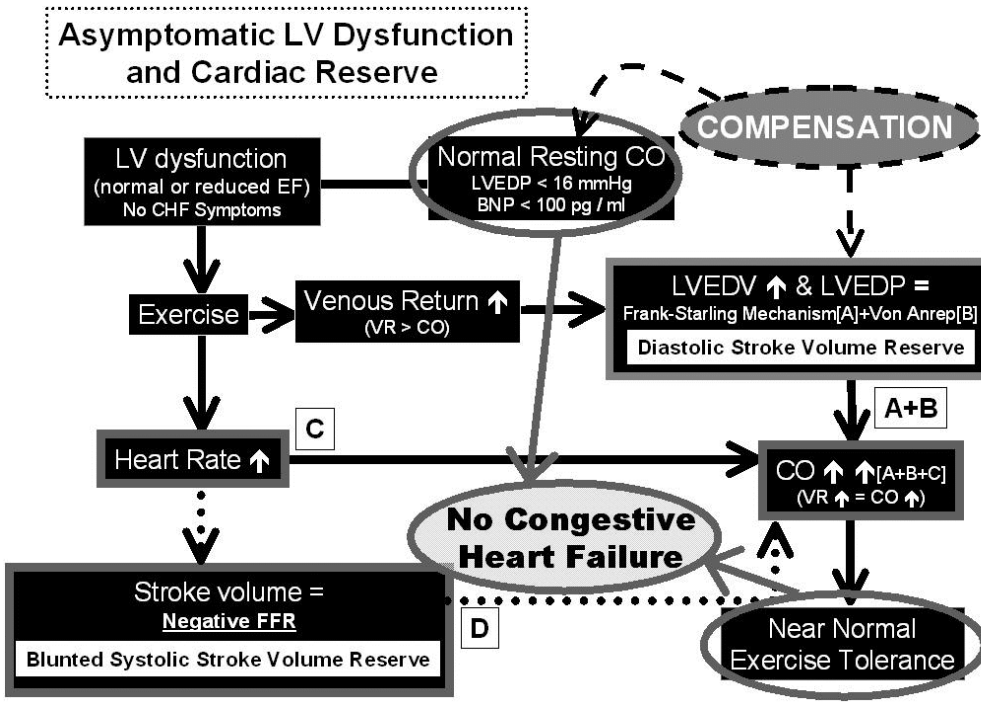

Figure 2. Diagram illustrating cardiac reserve mechanisms during exercise in the presence of left ventricular (LV) dysfunction, with either a normal or reduced LV ejection fraction (EF), without congestive heart failure (CHF) symptoms. Compensatory mechanisms (neurohumoral stimulation, myocardial remodeling) are able to maintain normal resting cardiac output at normal LV filling pressures (BNP< 100 pg / ml) and a normal exercise tolerance. Exercise initially causes an increases in venous return(VR) and in left ventricular (LV) end-diastolic volume (EDV), with an increase in LV stroke volume[A] ('Fast force response' or Frank-Starling mechanism).The Frank-Starling mechanism[A] as well as the 'slow force response' or Von Anrep effect[B] has been demonstrated to be existent in the failing and hypertrophic human myocardium(133,369). Dysfunctional myocardium, however, lacks a positive force-frequency response (FFR), but the heart rate increase per se[C] increases cardiac output(CO). To compensate for a blunted systolic stroke volume reserve[D], diastolic distensibility should be high enough to generate an adequate increase in stroke volume on exertion. High myocardial distensibility enables an adequate preload recruitable LV stroke work at normal LV filling pressures (B-type natriuretic peptide(BNP) < 100 pg / ml). The increase in CO[A+B+C] matches the increased VR, resulting in near normal exercise tolerance. LVEDP: left ventricular end-diastolic pressure; LVEDV: left ventricular end-diastolic volume.

Recently Yu et al reported systolic abnormalities to be evident in patients previously labeled as diastolic heart failure. Their findings underline the possible common coexistence of systolic and diastolic dysfunction in heart failure(276,403-405). In conclusion, the mechanism to increase

stroke volume at high heart rates both in hearts with poor or normal resting contractility and blunted force-frequency response (i.e. systolic stroke volume reserve) is the ability to utilize preload recruitable stroke work (Frank-Starling mechanism and Von Anrep effect, i.e. diastolic stroke volume reserve), in the presence of high diastolic myocardial distensibility.

Heart failure and filling pressure

Heart failure symptoms may be caused by pure diastolic dysfunction(100), but more often result from a combination of systolic and diastolic dysfunction. What all heart failure patients have in common, however, is the difficulty in left ventricular diastolic filling and increased left ventricular filling pressure because of diastolic dysfunction. Increased left ventricular filling pressures and wall stress increase wall strain which results in increased production of B-type natriuretic protein(213,313,358).

Circulating concentrations of adrenomedullin are elevated in cardiovascular disease in proportion to the severity of cardiac and hemodynamic impairment(293). Adrenomedullin, discovered nearly a decade ago, has undergone intensive investigation in the ensuing years, especially in regard to participation in the regulation of cardiovascular and pressure/volume homeostasis, and a potential role in the pathophysiology of heart disease. Raised plasma adrenomedullin levels following acute cardiac injury and in heart failure provide prognostic information on adverse outcomes.Administration of adrenomedullin in experimental and human heart failure induces reductions in arterial pressure and cardiac filling pressures, and improves cardiac output, in association with inhibition of plasma aldosterone (despite increased renin release) and sustained (or augmented) renal glomerular filtration and sodium excretion(293).

The biomechanical stimulation of a novel heart failure biomarker ST2, an interleukin-1 receptor family member, in vitro is similar to the mechanical induction of B-type natriuretic peptide, which is a useful diagnostic and prognostic marker in human heart failure. The change in serum levels of ST2 over time possiblyprovides prognostic information in patients with severe heartfailure independent of plasma B-type natriuretic protein(386).The plasma B-type natriuretic peptide is a useful alternative to left ventricular function parameters (e.g. left ventricular ejection fraction) to confirm the diagnosis of heart failure. B-type natriuretic protein was accurate in making the diagnosis of congestive heart failure, and levels correlated with severity of disease (24,25,171,181,193,213-215,227,250,263,297). Increased B-type natriuretic peptide levels were, understandably, unable to differentiate between systolic and diastolic dysfunction because B-type natriuretic protein production depends only on raised left ventricular filling pressure with a subsequent raise in left ventricular wall strain(40,180,213,224,238).This indicates the epicenter of the heart failure syndrome: a decrease in left ventricular diastolic distensibility raises left ventricular filling pressure irrespective of a normal or low left ventricular ejection fraction(170)

(Figure 3).

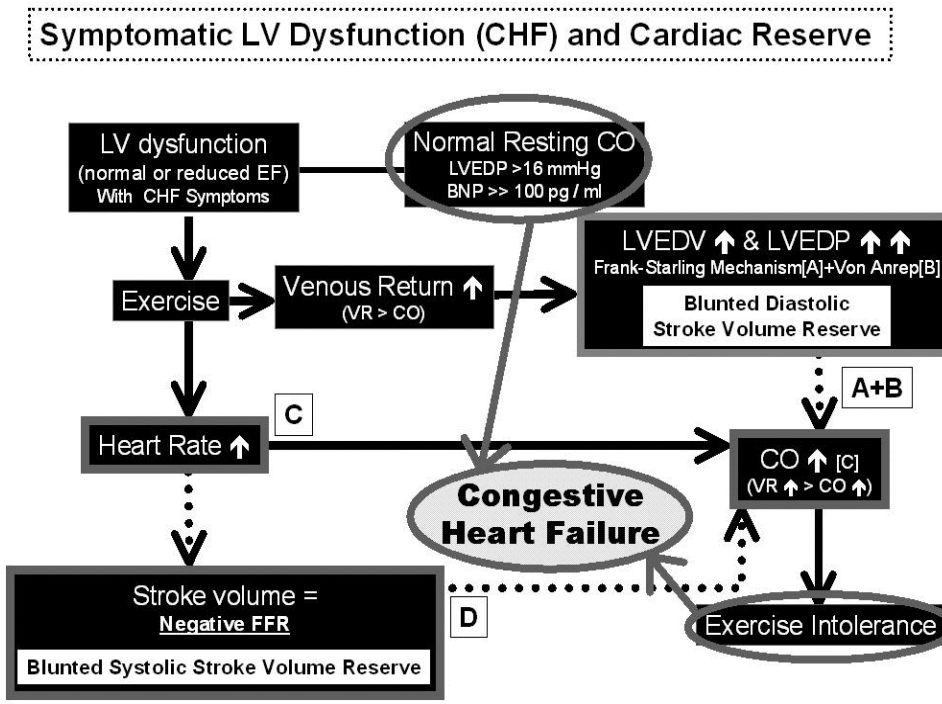

*Figure 3. Diagram illustrating cardiac reserve mechanisms during exercise in the presence of symptomatic left ventricular (LV) dysfunction (Congestive Heart Failure (CHF)), with either a normal or reduced LV ejection fraction (EF). Resting filling pressures often are elevated. (B-type natriuretic peptide (BNP) >> 100 pg / ml). High resting LVEDP may predict a low preload recruitable LV stroke work, i.e., an insufficient rise in LV stroke volume (blunted diastolic stroke volume reserve)[A+B]. Following an increase in venous return(VR), exercise raises LVEDP even further. A heart rate increase per se increases cardiac output(CO)[C]. But, together with an insufficient rise in LV stroke volume (blunted **systolic** (Negative force frequency response (FFR)) and blunted **diastolic** stroke volume reserve [A+B+D]), the increase in CO does not match the increased VR, resulting in reduced exercise tolerance. LVEDP: left ventricular end-diastolic pressure; LVEDV: left ventricular end-diastolic volume.*

Left ventricular diastolic distensibility determines left ventricular filling pressure when left ventricular end-diastolic volume rises on exertion, thereby determining the functional class of the heart failure patient. Despite the possibility of pure diastolic dysfunction as a cause of heart failure symptoms(100), discriminating between so called systolic and diastolic heart failure is misleading and distracts attention from the fact that heart failure symptoms follow a final common pathway, i.e. decreased diastolic distensibility, irrespective of resting left ventricular

ejection fraction. Changes in diastolic properties depend upon the underlying pathological mechanisms or disease states with their subsequent changes in shape and position of the diastolic pressure-volume relationships. The different types of left ventricular diastolic dysfunction and concomitant shift of the diastolic left ventricular pressure-volume relationships, will be discussed in chapter 1.5.

1.5 Diastolic pressure-volume relationship and left ventricular diastolic dysfunction

Left ventricular volume, shape and wall thickness, together with left ventricular myocardial material properties, dictate position and shape of the left ventricular end-diastolic pressure-volume relationship as a representation of left ventricular diastolic properties(116). The material properties of the left ventricular wall may change instantly or chronically. An instant increase in myocardial stiffness may be strain dependent (Figure 4: A➜B) or strain independent (Figure 4:A➜C). A chronic increase in myocardial stiffness causes an increase in diastolic filling pressure especially at a higher range of diastolic volumes (Figure 4: thick arrow and dotted line).

Myocardial ischemia instantaneously influences left ventricular diastolic distensibility with an upward and leftward, or rightward shift of the left ventricular diastolic pressure-volume relationship, depending on the type of ischemia(31,376,397) (Figure 5).

Recent findings in patients with hypertensive pulmonary edema, unchanged end-systolic and end-diastolic volumes before and after treatment of their hypertensive crises, suggest that this pulmonary edema was due to the exacerbation of diastolic dysfunction by hypertension and not the result of transient systolic dysfunction with a secondary increase in left ventricular preload (Figure 6)(100). Afterload elevations were recently reported to induce an upward shift of the diastolic pressure-volume relation(189,192). These findings indicate diastolic dysfunction in the presence of normal systolic function following positive inotropic stimulation by the sympathetic nervous system (Figure 6). An unchanged end-diastolic volume indicates a strain independent increase in myocardial stiffness by an upward shift of the left ventricular end-diastolic pressure-volume relationship.

An increase in myocardial contractility, and not a preload recruited stroke work increase, enables an unchanged left ventricular stroke volume, despite high afterload (Figure 6). In these patients, an increase in afterload obviously profoundly altered the process of myocardial relaxation, causing this upward shift of the end-diastolic pressure-volume relationship with high left ventricular end-diastolic pressure and pulmonary edema, notwithstanding preserved left ventricular stroke volume(189,190,207). These findings of intact systolic function in the phase

of pulmonary edema during a hypertensive crisis, do not rule out systolic dysfunction at increased hearts rates, because all patients described, did not show a significant change in heart rate before and after treatment of their hypertensive crisis(100).

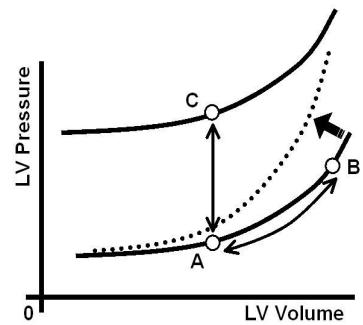

Figure 4. Three LV end-diastolic pressure-volume relationships. Increased venous return or increased afterload forces an increase in end-diastolic volume (A➔B) (Frank-Starling mechanism). An acute (A➔C➔A) and reversible increase in myocardial stiffness causes an increase in LV filling pressures at each diastolic volume (e.g. demand ischemia(31,397)). A chronic increase in myocardial stiffness causes an increase in diastolic filling pressure especially at a higher range of diastolic volumes (thick arrow and dotted line).

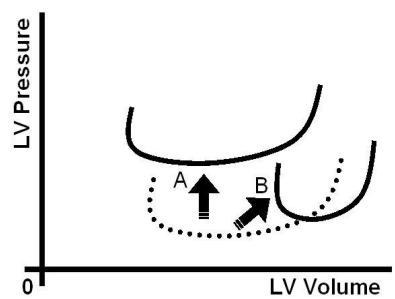

Figure 5. The influence of demand (A) and supply (B) ischemia on the shift of the diastolic pressure-volume relationship(269).

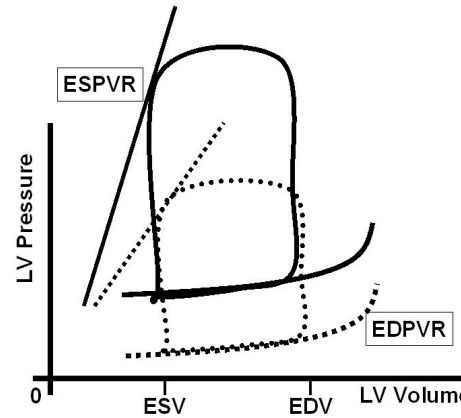

Figure 6. Proposed mechanism of hypertensive pulmonary edema in the elderly. In these patients two abnormalities may coexist: a steepening of the ESPVR, indicating increased contractility, and an upward shift of the EDPVR, indicating impaired rate and extent of myocardial relaxation(100). ESV: end-systolic volume. EDV: end-diastolic volume. ESPVR: end-systolic pressure-volume relationship. EDPVR: end-diastolic pressure volume relationship.

Otherwise, an increase in left ventricular afterload, may recruit preload reserve to sustain this increase in afterload while maintaining stroke volume. As opposed to patients with preserved stroke volume and unchanged left ventricular end-systolic volume and left ventricular end-diastolic volume(190), other patients show left ventricular dilatation, with both an increase in left ventricular end-systolic volume and left ventricular end-diastolic volume (Figure 7)(70,76). One can only speculate on why two different mechanisms, secondary to an increase in afterload, exist. Does older age with a concomitant stiffer myocardium play a role(100)? Or is myocardial subendocardial ischemia possibly responsible for an afterload mismatch and left ventricular dilatation(76)?

Chronic changes in myocardial stiffness follow changes in the myocardial extracellular matrix (Figure 4 thick arrow and dotted line). Hypertrophy often shows an increase in myocardial collagen content, a change in collagen cross linking and/or a change in the ratio between type I and type III collagen, which all influence myocardial stiffness(126,179,384).

In dilated cardiomyopathy the left ventricular dilates (with a secondary fall in ejection fraction (EF)) due to impaired cardiomyocytic function and marked changes in the extracellular matrix. These myocardial changes influence overall pump performance of the heart. Recently it was shown, that an increase in intracardiac production of nitric oxide augments left ventricular stroke

volume and left ventricular stroke work because of increased diastolic distensibility and a concomitant increase in left ventricular preload reserve(57,119,165,270,289) (Figure 8).

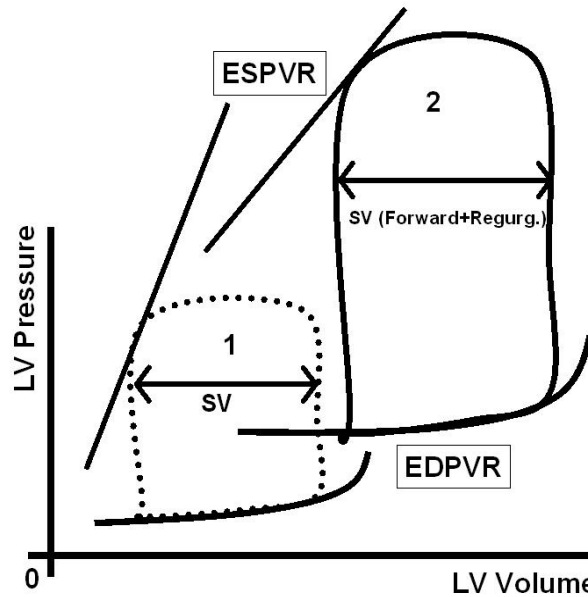

Figure 7. Proposed mechanism of hypertensive pulmonary edema in an occasional young patient. A shallowing of the end-systolic pressure-volume relationship (ESPVR) indicating decreased contractility and exhaustion of the rightward shift of the end-diastolic pressure-volume relationship (EDPVR), resulting in increased end-diastolic filling pressure and lung edema(76). SV: Stroke volume

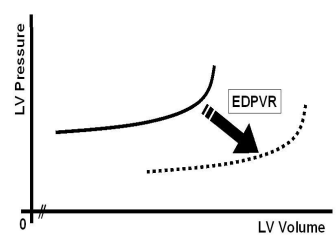

Figure 8. Two end-diastolic pressure-volume relations (EDPVR). A rightward shift of the EDPVR in patients with dilated cardiomyopathy is positively correlated with a higher nitric oxide (NO) synthase 2 (NOS2) gene expression. An increase in NOS2 and of NO may result in an increase in preload recruitable stroke work at normal filling pressures(270).

These findings indicate the potential existence of a ***diastolic*** stroke volume reserve, causing an increase in myocardial distensibility which enables utilization of left ventricular preload reserve (Frank-Starling mechanism, Von Anrep effect) at relatively low filling pressures. Low filling pressures in heart failure are important to reduce the initiation of signaling cascades leading to hypertrophy and remodeling. The fact that the failing heart, during exercise, depends on the ability of the left ventricle to recruit preload reserve as its functional reserve mechanism per se, induces the risk of triggering different potentially harmful signaling pathways for hypertrophy and myocardial remodeling if filling pressures and wall stress remain elevated(2,136).

1.6 Brief outline of this thesis

This thesis covers different clinical investigations on left ventricular dysfunction with special attention to three types of shift of the left ventricular diastolic pressure-volume relationship. Diastolic left ventricular dysfunction and an upward shift of the left ventricular diastolic pressure-volume relationship is covered in chapter 2, diastolic left ventricular dysfunction and a lack of rightward shift of the left ventricular diastolic pressure-volume relationship in chapter 3 and diastolic left ventricular dysfunction and a steeper slope of the left ventricular diastolic pressure-volume relationship is covered in chapter 4 of this thesis.

References

1. **Allaart CP, and Westerhof N**. Effect of length and contraction on coronary perfusion in isolated perfused papillary muscle of rat heart. *Am J Physiol* 271: H447-H454, 1996.
2. **Alpert NR, Leavitt BJ, Ittleman FP, Hasenfuss G, Pieske B, and Mulieri LA**. A mechanistic analysis of the force-frequency relation in non-failing and progressively failing human myocardium. *Basic Res Cardiol* 93 Suppl 1: 23-32, 1998.
3. **Alpert NR, Mulieri LA, and Warshaw D**. The failing human heart. *Cardiovasc Res* 54: 1-10, 2002.
4. **Alvarez BV, Perez NG, Ennis IL, de Hurtado MCC, and Cingolani HE**. Mechanisms underlying the increase in force and Ca2+ transient that follow stretch of cardiac muscle - A possible explanation of the Anrep effect. *Circ Res* 85: 716-722, 1999.
5. **Anand IS**. Ventricular remodeling without cellular contractile dysfunction. *J Card Fail* 8: S401-S408, 2002.
6. **Anand IS, Liu D, Chugh SS, Prahash AJ, Gupta S, John R, Popescu F, and Chandrashekhar Y**. Isolated myocyte contractile function is normal in postinfarct remodeled rat heart with systolic dysfunction. *Circulation* 96: 3974-3984, 1997.

7. **Andersson B, Stromblad SO, Lomsky M, and Waagstein F**. Heart rate dependency of cardiac performance in heart failure patients treated with metoprolol. *Eur Heart J* 20: 575-583, 1999.
8. **Angeja BG, and Grossman W**. Evaluation and management of diastolic heart failure. *Circulation* 107: 659-663, 2003.
9. **Ansari M, and Massie BM**. Heart failure: how big is the problem? Who are the patients? What does the future hold? *Am Heart J* 146: 1-4, 2003.
10. **Anversa P, and Nadal-Ginard B**. Myocyte renewal and ventricular remodelling. *Nature* 415: 240-243, 2002.
11. **Arnold RH, Kotlyar E, Hayward C, Keogh AM, and Macdonald PS**. Relation between heart rate, heart rhythm, and reverse left ventricular remodelling in response to carvedilol in patients with chronic heart failure: a single centre, observational study. *Heart* 89: 293-298, 2003.
12. **Arts T, Bovendeerd P, Delhaas T, and Prinzen F**. Modeling the relation between cardiac pump function and myofiber mechanics. *J Biomech* 36: 731-736, 2003.
13. **Baan J, van der Velde ET, de Bruin HG, Smeenk GJ, Koops J, van Dijk AD, Temmerman D, Senden J, and Buis B**. Continuous measurement of left ventricular volume in animals and humans by conductance catheter. *Circulation* 70: 812-823, 1984.
14. **Baartscheer A, Schumacher CA, Belterman CN, Coronel R, and Fiolet JW**. [Na+]i and the driving force of the Na+/Ca2+-exchanger in heart failure. *Cardiovasc Res* 57: 986-995, 2003.
15. **Badenhorst D, Maseko M, Tsotetsi OJ, Naidoo A, Brooksbank R, Norton GR, and Woodiwiss AJ**. Cross-linking influences the impact of quantitative changes in myocardial collagen on cardiac stiffness and remodelling in hypertension in rats. *Cardiovasc Res* 57: 632-641, 2003.
16. **Baker BJ, Wilen MM, Boyd CM, Dinh H, and Franciosa JA**. Relation of right ventricular ejection fraction to exercise capacity in chronic left ventricular failure. *Am J Cardiol* 54: 596-599, 1984.
17. **Balligand JL, Ungureanu D, Kelly RA, Kobzik L, Pimental D, Michel T, and Smith TW**. Abnormal contractile function due to induction of nitric oxide synthesis in rat cardiac myocytes follows exposure to activated macrophage-conditioned medium. *J Clin Invest* 91: 2314-2319, 1993.
18. **Banerjee P, Banerjee T, Khand A, Clark AL, and Cleland JG**. Diastolic heart failure: neglected or misdiagnosed? *J Am Coll Cardiol* 39: 138-141, 2002.
19. **Barbone A, Oz MC, Burkhoff D, and Holmes JW**. Normalized diastolic properties after left ventricular assist result from reverse remodeling of chamber geometry. *Circulation* 104: I229-I232, 2001.
20. **Barki-Harrington L, and Rockman HA**. Sensing heart stress. *Nat Med* 9: 19-20, 2003.
21. **Barry WH**. Na(+)-Ca(2+) exchange in failing myocardium: friend or foe? *Circ Res* 87: 529-531, 2000.
22. **Barry WH, and Gilbert EM**. How do beta-blockers improve ventricular function in patients with congestive heart failure? *Circulation* 107: 2395-2397, 2003.
23. **Bartunek J, Shah AM, Vanderheyden M, and Paulus WJ**. Dobutamine enhances cardiodepressant effects of receptor-mediated coronary endothelial stimulation. *Circulation* 95: 90-96, 1997.
24. **Baughman KL**. B-type natriuretic peptide -- a window to the heart. *N Engl J Med* 347: 158-159, 2002.
25. **Bay M, Kirk V, Parner J, Hassager C, Nielsen H, Krogsgaard K, Trawinski J, Boesgaard S, and Aldershvile J**. NT-proBNP: a new diagnostic screening tool to differentiate between patients with normal and reduced left ventricular systolic function. *Heart* 89: 150-154, 2003.
26. **Belhassen L, Kelly RA, Smith TW, and Balligand JL**. Nitric oxide synthase (NOS3) and contractile responsiveness to adrenergic and cholinergic agonists in the heart. Regulation of NOS3 transcription in vitro and in vivo by cyclic adenosine monophosphate in rat cardiac myocytes. *J Clin Invest* 97: 1908-1915, 1996.
27. **Beltrami AP, Urbanek K, Kajstura J, Yan SM, Finato N, Bussani R, Nadal-Ginard B, Silvestri**

F, Leri A, Beltrami CA, and Anversa P. Evidence that human cardiac myocytes divide after myocardial infarction. *N Engl J Med* 344: 1750-1757, 2001.

28. **Bernstein KE**. Two ACEs and a heart. *Nature* 417: 799-802, 2002.
29. **Bers DM**. Calcium fluxes involved in control of cardiac myocyte contraction. *Circ Res* 87: 275-281, 2000.
30. **Bers DM**. Cardiac excitation-contraction coupling. *Nature* 415: 198-205, 2002.
31. **Bertrand ME, Lablanche JM, Fourrier JL, Traisnel G, and Mirsky I**. Left ventricular systolic and diastolic function during acute coronary artery balloon occlusion in humans. *J Am Coll Cardiol* 12: 341-347, 1988.
32. **Bhargava V, Shabetai R, Mathiasen RA, Dalton N, Hunter JJ, and Ross J, Jr.** Loss of adrenergic control of the force-frequency relation in heart failure secondary to idiopathic or ischemic cardiomyopathy. *Am J Cardiol* 81: 1130-1137, 1998.
33. **Bluhm WF, Kranias EG, Dillmann WH, and Meyer M**. Phospholamban: a major determinant of the cardiac force-frequency relationship. *Am J Physiol Heart Circ Physiol* 278: H249-H255, 2000.
34. **Bluhm WF, Kranias EG, Dillmann WH, and Meyer M**. Phospholamban: a major determinant of the cardiac force-frequency relationship. *Am J Physiol Heart Circ Physiol* 278: H249-H255, 2000.
35. **Boehm M, and Nabel EG**. Angiotensin-converting enzyme 2--a new cardiac regulator. *N Engl J Med* 347: 1795-1797, 2002.
36. **Bonapace S, Rossi A, Cicoira M, Franceschini L, Golia G, Zanolla L, Marino P, and Zardini P**. Aortic distensibility independently affects exercise tolerance in patients with dilated cardiomyopathy. *Circulation* 107: 1603-1608, 2003.
37. **Borg TK, Goldsmith EC, Price R, Carver W, Terracio L, and Samarel AM**. Specialization at the Z line of cardiac myocytes. *Cardiovasc Res* 46: 277-285, 2000.
38. **Borow, K. M., R. H. Marcus, A. Neumann, and R. M. Lang.** Modern noninvasive techniques for the assessment of left ventricular systolic performance. In Braunwald, E., ed. Update to Heart disease. 1992, 389-398.
39. **Bowditch HP**. Über die eigenthümlichkeiten der reizbarkeit welche die muskelfasern des herzens zeigen. *Arb Physiol Aust* 1: 139-176, 1871.
40. **Bozkurt B, and Mann DL**. Use of biomarkers in the management of heart failure: are we there yet? *Circulation* 107: 1231-1233, 2003.
41. **Brancaccio M, Fratta L, Notte A, Hirsch E, Poulet R, Guazzone S, De Acetis M, Vecchione C, Marino G, Altruda F, Silengo L, Tarone G, and Lembo G**. Melusin, a muscle-specific integrin beta1-interacting protein, is required to prevent cardiac failure in response to chronic pressure overload. *Nat Med* 9: 68-75, 2003.
42. **Braunwald E, and Bristow MR**. Congestive heart failure: fifty years of progress. *Circulation* 102: IV14-IV23, 2000.
43. **Bristow MR**. Why does the myocardium fail? Insights from basic science. *Lancet* 352 Suppl 1: SI8-14, 1998.
44. **Brixius K, Pietsch M, and Schwinger RH**. The intracellular Ca(2+)-homeostasis influences the frequency-dependent force-generation in man. *Basic Res Cardiol* 94: 152-158, 1999.
45. **Bruckner BA, Stetson SJ, Perez-Verdia A, Youker KA, Radovancevic B, Connelly JH, Koerner MM, Entman ME, Frazier OH, Noon GP, and Torre-Amione G**. Regression of fibrosis and hypertrophy in failing myocardium following mechanical circulatory support. *J Heart Lung Transplant* 20: 457-464, 2001.
46. **Bruneau BG, Piazza LA, and de Bold AJ**. BNP gene expression is specifically modulated by stretch

and ET-1 in a new model of isolated rat atria. *Am J Physiol* 273: H2678-H2686, 1997.

47. **Brutsaert DL**. Diagnosing primary diastolic heart failure. *Eur Heart J* 21: 94-96, 2000.
48. **Brutsaert DL**. Cardiac endothelial-myocardial signaling: its role in cardiac growth, contractile performance, and rhythmicity. *Physiol Rev* 83: 59-115, 2003.
49. **Brutsaert DL, and Andries LJ**. The endocardial endothelium. *Am J Physiol* 263: H985-1002, 1992.
50. **Brutsaert DL, Meulemans AL, Sipido KR, and Sys SU**. Effects of damaging the endocardial surface on the mechanical performance of isolated cardiac muscle. *Circ Res* 62: 358-366, 1988.
51. **Brutsaert DL, and Sys SU**. Relaxation and diastole of the heart. *Physiol Rev* 69: 1228-1315, 1989.
52. **Brutsaert DL, and Sys SU**. Diastolic dysfunction in heart failure. *J Card Fail* 3: 225-242, 1997.
53. **Burkhoff D, Maurer MS, and Packer M**. Heart failure with a normal ejection fraction: is it really a disorder of diastolic function? *Circulation* 107: 656-658, 2003.
54. **Carabello, B. A.** Abnormalities in cardiac contraction: systolic dysfunction. In Hosenpud, J. D. and B. H. Greenberg, eds. Congestive Heart Failure - Pathophysiology, Diagnosis, and Comprehensive Approach to Management. Philadelphia, Lippincott Wiliams & Wilkins. 2000, 67-81.
55. **Carabello BA**. Concentric versus eccentric remodeling. *J Card Fail* 8: S258-S263, 2002.
56. **Carabello BA**. Evolution of the study of left ventricular function: everything old is new again. *Circulation* 105: 2701-2703, 2002.
57. **Casadei B, and Sears CE**. Nitric-oxide-mediated regulation of cardiac contractility and stretch responses. *Prog Biophys Mol Biol* 82: 67-80, 2003.
58. **Cazorla O, Wu Y, Irving TC, and Granzier H**. Titin-based modulation of calcium sensitivity of active tension in mouse skinned cardiac myocytes. *Circ Res* 88: 1028-1035, 2001.
59. **Chen X, Piacentino V, III, Furukawa S, Goldman B, Margulies KB, and Houser SR**. L-type Ca2+ channel density and regulation are altered in failing human ventricular myocytes and recover after support with mechanical assist devices. *Circ Res* 91: 517-524, 2002.
60. **Chien KR**. Stress pathways and heart failure. *Cell* 98: 555-558, 1999.
61. **Cingolani HE**. Slow force response and exercise. Personal Communication, 2003.
62. **Cingolani HE, Alvarez BV, Ennis IL, and Camilion dHM**. Stretch-induced alkalinization of feline papillary muscle: an autocrine-paracrine system. *Circ Res* 83: 775-780, 1998.
63. **Cingolani HE, Perez NG, and Camilion de Hurtado MC**. An autocrine/paracrine mechanism triggered by myocardial stretch induces changes in contractility. *News Physiol Sci* 16: 88-91, 2001.
64. **Cingolani HE, Perez NG, Pieske B, von Lewinski D, and Camilion de Hurtado MC**. Stretch-elicited Na(+)/H(+) exchanger activation: the autocrine/paracrine loop and its mechanical counterpart. *Cardiovasc Res* 57: 953-960, 2003.
65. **Coghlan HC, and Coghlan L**. Cardiac architecture: Gothic versus Romanesque. A cardiologist's view. *Semin Thorac Cardiovasc Surg* 13: 417-430, 2001.
66. **Cohn JN**. Structural basis for heart failure. Ventricular remodeling and its pharmacological inhibition. *Circulation* 91: 2504-2507, 1995.
67. **Colluci, W. S. and E. Braunwald.** Pathophysiology of heart failure. In Braunwald, E., ed. Heart Disease: A textbook of cardiovascular medicine. Philadelphia, W.B. Saunders Company. 2001, 503-528.
68. **Cooper G4**. Load and length regulation of cardiac energetics. *Annu Rev Physiol* 52: 505-522, 1990.
69. **Correia-Pinto J, Henriques-Coelho T, Oliveira SM, and Leite-Moreira AF**. Distinct load dependence of relaxation rate and diastolic function in Oryctolagus cuniculus and Ratus norvegicus. *J Comp Physiol [B]* 2003.
70. **Cotter G, Moshkovitz Y, Milovanov O, Salah A, Blatt A, Krakover R, Vered Z, and Kaluski E**. Acute heart failure: a novel approach to its pathogenesis and treatment. *Eur J Heart Fail* 4: 227-234,

2002.

71. **Cotton JM, Kearney MT, and Shah AM**. Nitric oxide and myocardial function in heart failure: friend or foe? *Heart* 88: 564-566, 2002.
72. **Crackower MA, Sarao R, Oudit GY, Yagil C, Kozieradzki I, Scanga SE, Oliveira-dos-Santos AJ, da Costa J, Zhang L, Pei Y, Scholey J, Ferrario CM, Manoukian AS, Chappell MC, Backx PH, Yagil Y, and Penninger JM**. Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature* 417: 822-828, 2002.
73. **Crozatier B**. Stretch-induced modifications of myocardial performance: from ventricular function to cellular and molecular mechanisms. *Cardiovasc Res* 32: 25-37, 1996.
74. **Crozatier B, and Delcayre C**. Cardiac myocyte stretch: an adaptative factor of myocardial function. *M S-Medecine Sciences* 15: 345-351, 1999.
75. **D'Agnolo A, Luciani GB, Mazzucco A, Gallucci V, and Salviati G**. Contractile properties and Ca2+ release activity of the sarcoplasmic reticulum in dilated cardiomyopathy. *Circulation* 85: 518-525, 1992.
76. **D'Aloia A, Fiorina C, Vizzardi E, Faggiano P, and Dei CL**. Hypertensive crisis and acute, reversible, left ventricular systolic dysfunction: a case report. *Eur J Heart Fail* 4: 655-660, 2002.
77. **D'Souza SP, and Baxter GF**. B Type natriuretic peptide: a good omen in myocardial ischaemia? *Heart* 89: 707-709, 2003.
78. **Danilczyk U, Eriksson U, Crackower MA, and Penninger JM**. A story of two ACEs. *J Mol Med* 81: 227-234, 2003.
79. **de Tombe PP**. Cardiac myofilaments: mechanics and regulation. *J Biomech* 36: 721-730, 2003.
80. **Del Monte F, Harding SE, Schmidt U, Matsui T, Kang ZB, Dec GW, Gwathmey JK, Rosenzweig A, and Hajjar RJ**. Restoration of contractile function in isolated cardiomyocytes from failing human hearts by gene transfer of SERCA2a. *Circulation* 100: 2308-2311, 1999.
81. **Di B, V, Talarico L, Di Muro C, Santoro G, Bertini A, Giorgi D, Caputo MT, Bianchi M, Cecchini L, and Giusti C** . Evaluation of maximal left ventricular performance in elite bicyclists. *Int J Sports Med* 16: 498-506, 1995.
82. **Dipla K, Mattiello JA, Jeevanandam V, Houser SR, and Margulies KB**. Myocyte recovery after mechanical circulatory support in humans with end-stage heart failure. *Circulation* 97: 2316-2322, 1998.
83. **Dostal DE, and Baker KM**. The cardiac renin-angiotensin system - Conceptual, or a regulator of cardiac function? [Review]. *Circ Res* 85: 643-650, 1999.
84. **Dougherty AH, Naccarelli GV, Gray EL, Hicks CH, and Goldstein RA**. Congestive heart failure with normal systolic function. *Am J Cardiol* 54: 778-782, 1984.
85. **Dudnakova TV, Lakomkin VL, Tsyplenkova VG, Shekhonin BV, Shirinsky VP, and Kapelko VI**. Alterations in myocardial cytoskeletal and regulatory protein expression following a single Doxorubicin injection. *J Cardiovasc Pharmacol* 41: 788-794, 2003.
86. **Eisner DA, Choi HS, Diaz ME, O'Neill SC, and Trafford AW**. Integrative analysis of calcium cycling in cardiac muscle. *Circ Res* 87: 1087-1094, 2000.
87. **Ferrario CM**. Does angiotensin-(1-7) contribute to cardiac adaptation and preservation of endothelial function in heart failure? *Circulation* 105: 1523-1525, 2002.
88. **Ferrario CM**. Contribution of angiotensin-(1-7) to cardiovascular physiology and pathology. *Curr Hypertens Rep* 5: 129-134, 2003.
89. **Firstenberg MS, Smedira NG, Greenberg NL, Prior DL, McCarthy PM, Garcia MJ, and Thomas JD**. Relationship between early diastolic intraventricular pressure gradients, an index of

elastic recoil, and improvements in systolic and diastolic function. *Circulation* 104: I330-I335, 2001.

90. **Force T, Michael A, Kilter H, and Haq S**. Stretch-activated pathways and left ventricular remodeling. *J Card Fail* 8: S351-S358, 2002.
91. **Fort S, Lewis MJ, and Shah AM**. The role of endocardial endothelium in the modulation of myocardial contraction in the isolated whole heart. *Cardioscience* 4: 217-223, 1993.
92. **Franciosa JA, Park M, and Levine TB**. Lack of correlation between exercise capacity and indexes of resting left ventricular performance in heart failure. *Am J Cardiol* 47: 33-39, 1981.
93. **Frank O**. Zur dynamik des Herzmuskels. *Zeitschrift fur Biologie* 32: 370-447, 1885.
94. **Frey N, and Olson EN**. Cardiac hypertrophy: the good, the bad, and the ugly. *Annu Rev Physiol* 65: 45-79, 2003.
95. **Friebe A, and Koesling D**. Regulation of nitric oxide-sensitive guanylyl cyclase. *Circ Res* 93: 96-105, 2003.
96. **Fuchs F, and Wang YP**. Sarcomere length versus interfilament spacing as determinants of cardiac myofilament Ca2+ sensitivity and Ca2+ binding. *J Mol Cell Cardiol* 28: 1375-1383, 1996.
97. **Fukuda N, Sasaki D, Ishiwata S, and Kurihara S**. Length dependence of tension generation in rat skinned cardiac muscle: role of titin in the Frank-Starling mechanism of the heart. *Circulation* 104: 1639-1645, 2001.
98. **Gaasch, W. H., C. A. Apstein, and H. J. Levine.** Diastolic properties of the left ventricle. In Levine, H. J. and W. H. Gaasch, eds. The Ventricle. Boston, Martinus Nijhoff Publishing. 1985, 143-170.
99. **Gaasch WH, Carroll JD, Blaustein AS, and Bing OH**. Myocardial relaxation: effects of preload on the time course of isovolumetric relaxation. *Circulation* 73: 1037-1041, 1986.
100. **Gandhi SK, Powers JC, Nomeir AM, Fowle K, Kitzman DW, Rankin KM, and Little WC**. The pathogenesis of acute pulmonary edema associated with hypertension. *N Engl J Med* 344: 17-22, 2001.
101. **Ganote CE, and Armstrong SC**. Dystrophin-associated protein complex and heart failure. *Lancet* 359: 905-906, 2002.
102. **Gao WD, Perez NG, and Marban E**. Calcium cycling and contractile activation in intact mouse cardiac muscle. *J Physiol* 507: 175-184, 1998.
103. **Giancotti FG**. A structural view of integrin activation and signaling. *Dev Cell* 4: 149-151, 2003.
104. **Giancotti FG, and Ruoslahti E** . Integrin signaling. *Science* 285: 1028-1032, 1999.
105. **Gillebert TC, De Hert SG, Andries LJ, Jageneau AH, and Brutsaert DL**. Intracavitary ultrasound impairs left ventricular performance: presumed role of endocardial endothelium. *Am J Physiol* 263: H857-H865, 1992.
106. **Gillebert TC, Leite-Moreira AF, and De Hert SG**. Load dependent diastolic dysfunction in heart failure. *Heart Fail Rev* 5: 345-355, 2000.
107. **Gillebert TC, and Raes DF**. Preload, length-tension relation, and isometric relaxation in cardiac muscle. *Am J Physiol* 267: H1872-9, 1994.
108. **Go LO, Moschella MC, Watras J, Handa KK, Fyfe BS, and Marks AR**. Differential regulation of two types of intracellular calcium release channels during end-stage heart failure. *J Clin Invest* 95: 888-894, 1995.
109. **Gottdiener JS, McClelland RL, Marshall R, Shemanski L, Furberg CD, Kitzman DW, Cushman M, Polak J, Gardin JM, Gersh BJ, Aurigemma GP, and Manolio TA**. Outcome of congestive heart failure in elderly persons: influence of left ventricular systolic function. The Cardiovascular Health Study. *Ann Intern Med* 137: 631-639, 2002.
110. **Gotthardt M, Hammer RE, Hubner N, Monti J, Witt CC, McNabb M, Richardson JA, Granzier H, Labeit S, and Herz J** . Conditional expression of mutant M-line titins results in cardiomyopathy with altered sarcomere structure. *J Biol Chem* 278: 6059-6065, 2003.

111. **Granzier H, Labeit D, Wu Y, and Labeit S**. Titin as a modular spring: emerging mechanisms for elasticity control by titin in cardiac physiology and pathophysiology. *J Muscle Res Cell Motil* 23: 457-471, 2002.
112. **Granzier H, and Labeit S**. Cardiac titin: an adjustable multi-functional spring. *J Physiol* 541: 335-342, 2002.
113. **Granzier HL, and Wang K**. Passive tension and stiffness of vertebrate skeletal and insect flight muscles: the contribution of weak cross-bridges and elastic filaments. *Biophys J* 65: 2141-2159, 1993.
114. **Gregg DE**. Effect of coronary perfusion pressure or coronary flow on oxygen usage of the myocardium. *Circ Res* 13: 497-500, 1963.
115. **Gregorio CC, Granzier H, Sorimachi H, and Labeit S**. Muscle assembly: a titanic achievement? *Curr Opin Cell Biol* 11: 18-25, 1999.
116. **Grossman W**. Diastolic dysfunction in congestive heart failure. *N Engl J Med* 325: 1557-1564, 1991.
117. **Guardigli G, Ansani L, Percoco GF, Toselli T, Spisani P, Braggion G, and Antonioli GE**. AV delay optimization and management of DDD paced patients with dilated cardiomyopathy. *Pacing Clin Electrophysiol* 17: 1984-1988, 1994.
118. **Hagberg JM, Goldberg AP, Lakatta L, O'Connor FC, Becker LC, Lakatta EG, and Fleg JL**. Expanded blood volumes contribute to the increased cardiovascular performance of endurance-trained older men. *J Appl Physiol* 85: 484-489, 1998.
119. **Hare JM**. Nitric oxide and excitation-contraction coupling. *J Mol Cell Cardiol* 35: 719-729, 2003.
120. **Hare JM, Shernan SK, Body SC, Graydon E, Colucci WS, and Couper GS**. Influence of inhaled nitric oxide on systemic flow and ventricular filling pressure in patients receiving mechanical circulatory assistance. *Circulation* 95: 2250-2253, 1997.
121. **Harris TS, Baicu CF, Conrad CH, Koide M, Buckley JM, Barnes M, Cooper G, and Zile MR**. Constitutive properties of hypertrophied myocardium: cellular contribution to changes in myocardial stiffness. *Am J Physiol Heart Circ Physiol* 282: H2173-H2182, 2002.
122. **Hasenfuss G, Schillinger W, Lehnart SE, Preuss M, Pieske B, Maier LS, Prestle J, Minami K, and Just H**. Relationship between Na+-Ca2+-exchanger protein levels and diastolic function of failing human myocardium. *Circulation* 99: 641-648, 1999.
123. **Hasenfuss G, and Seidler T**. Treatment of heart failure through stabilization of the cardiac ryanodine receptor. *Circulation* 107: 378-380, 2003.
124. **Haubold K, Herrmann H, Langer SJ, Evans RM, Leinwand LA, and Klymkowsky MW**. Acute effects of desmin mutations on cytoskeletal and cellular integrity in cardiac myocytes. *Cell Motil Cytoskeleton* 54: 105-121, 2003.
125. **Heerdt PM, Holmes JW, Cai B, Barbone A, Madigan JD, Reiken S, Lee DL, Oz MC, Marks AR, and Burkhoff D**. Chronic unloading by left ventricular assist device reverses contractile dysfunction and alters gene expression in end-stage heart failure. *Circulation* 102: 2713-2719, 2000.
126. **Hein S, Arnon E, Kostin S, Schonburg M, Elsasser A, Polyakova V, Bauer EP, Klovekorn WP, and Schaper J**. Progression from compensated hypertrophy to failure in the pressure-overloaded human heart: structural deterioration and compensatory mechanisms. *Circulation* 107: 984-991, 2003.
127. **Hein S, Gaasch WH, and Schaper J**. Giant molecule titin and myocardial stiffness. *Circulation* 106: 1302-1304, 2002.
128. **Hein S, Kostin S, Heling A, Maeno Y, and Schaper J**. The role of the cytoskeleton in heart failure. *Cardiovasc Res* 45: 273-278, 2000.
129. **Heling A, Zimmermann R, Kostin S, Maeno Y, Hein S, Devaux B, Bauer E, Klovekorn WP, Schlepper M, Schaper W, and Schaper J**. Increased expression of cytoskeletal, linkage, and

extracellular proteins in failing human myocardium. *Circ Res* 86: 846-853, 2000.

130. **Henderson AH, Lewis MJ, Shah AM, and Smith JA**. Endothelium, endocardium, and cardiac contraction. *Cardiovasc Res* 26: 305-308, 1992.
131. **Higginbotham MB, Morris KG, Conn EH, Coleman RE, and Cobb FR**. Determinants of variable exercise performance among patients with severe left ventricular dysfunction. *Am J Cardiol* 51: 52-60, 1983.
132. **Higginbotham MB, Morris KG, Williams RS, McHale PA, Coleman RE, and Cobb FR**. Regulation of stroke volume during submaximal and maximal upright exercise in normal man. *Circ Res* 58: 281-291, 1986.
133. **Holubarsch C, Ludemann J, Wiessner S, Ruf T, Schulte-Baukloh H, Schmidt-Schweda S, Pieske B, Posival H, and Just H**. Shortening versus isometric contractions in isolated human failing and non-failing left ventricular myocardium: dependency of external work and force on muscle length, heart rate and inotropic stimulation. *Cardiovasc Res* 37: 46-57, 1998.
134. **Holubarsch C, Ruf T, Goldstein DJ, Ashton RC, Nickl W, Pieske B, Pioch K, Ludemann J, Wiesner S, Hasenfuss G, Posival H, Just H, and Burkhoff D**. Existence of the Frank-Starling mechanism in the failing human heart. Investigations on the organ, tissue, and sarcomere levels. *Circulation* 94: 683-689, 1996.
135. **Homcy CJ**. Signaling hypertrophy: how many switches, how many wires. *Circulation* 97: 1890-1892, 1998.
136. **Hoshijima M, and Chien KR**. Mixed signals in heart failure: cancer rules. *J Clin Invest* 109: 849-855, 2002.
137. **Houser SR, and Lakatta EG**. Function of the cardiac myocyte in the conundrum of end-stage, dilated human heart failure. *Circulation* 99: 600-604, 1999.
138. **Houser SR, and Margulies KB**. Is depressed myocyte contractility centrally involved in heart failure? *Circ Res* 92: 350-358, 2003.
139. **Huang X, Li J, Foster D, Lemanski SL, Dube DK, Zhang C, and Lemanski LF**. Protein kinase C-mediated desmin phosphorylation is related to myofibril disarray in cardiomyopathic hamster heart. *Exp Biol Med (Maywood)* 227: 1039-1046, 2002.
140. **Hunt SA, Baker DW, Chin MH, Cinquegrani MP, Feldmanmd AM, Francis GS, Ganiats TG, Goldstein S, Gregoratos G, Jessup ML, Noble RJ, Packer M, Silver MA, Stevenson LW, Gibbons RJ, Antman EM, Alpert JS, Faxon DP, Fuster V, Gregoratos G, Jacobs AK, Hiratzka LF, Russell RO, and Smith SC, Jr.** ACC/AHA Guidelines for the Evaluation and Management of Chronic Heart Failure in the Adult: Executive Summary. *Circulation* 104: 2996-3007, 2001.
141. **Hunter JJ, and Chien KR**. Signaling pathways for cardiac hypertrophy and failure. *N Engl J Med* 341: 1276-1283, 1999.
142. **Inagaki M, Yokota M, Izawa H, Ishiki R, Nagata K, Iwase M, Yamada Y, Koide M, and Sobue T**. Impaired force-frequency relations in patients with hypertensive left ventricular hypertrophy. A possible physiological marker of the transition from physiological to pathological hypertrophy. *Circulation* 99: 1822-1830, 1999.
143. **Ingber DE**. Cellular basis of mechanotransduction. *Biol Bull* 194: 323-325, 1998.
144. **Ingels NB, Jr., Daughters GT, Nikolic SD, DeAnda A, Moon MR, Bolger AF, Komeda M, Derby GC, Yellin EL, and Miller DC**. Left ventricular diastolic suction with zero left atrial pressure in open-chest dogs. *Am J Physiol* 270: H1217-H1224, 1996.
145. **Irving TC, Konhilas J, Perry D, Fischetti R, and de Tombe PP**. Myofilament lattice spacing as a function of sarcomere length in isolated rat myocardium. *Am J Physiol Heart Circ Physiol* 279: H2568-H2573, 2000.

146. **Ito N, Bartunek J, Spitzer KW, and Lorell BH**. Effects of the nitric oxide donor sodium nitroprusside on intracellular pH and contraction in hypertrophied myocytes. *Circulation* 95: 2303-2311, 1997.
147. **Jacob R, and Gulch RW**. The functional significance of ventricular geometry for the transition from hypertrophy to cardiac failure. Does a critical degree of structural dilatation exist? *Basic Res Cardiol* 93: 423-429, 1998.
148. **Jensen-Urstad M, Bouvier F, Nejat M, Saltin B, and Brodin LA**. Left ventricular function in endurance runners during exercise. *Acta Physiol Scand* 164: 167-172, 1998.
149. **Jessup M**. Aldosterone blockade and heart failure. *N Engl J Med* 348: 1380-1382, 2003.
150. **Jessup M**. The less familiar face of heart failure. *J Am Coll Cardiol* 41: 224-226, 2003.
151. **Jessup M, and Brozena S**. Heart failure. *N Engl J Med* 348: 2007-2018, 2003.
152. **Kamp TJ, and He JQ**. L-type Ca2+ channels gaining respect in heart failure. *Circ Res* 91: 451-453, 2002.
153. **Kass DA**. Force-frequency relation in patients with left ventricular hypertrophy and failure. *Basic Res Cardiol* 93 Suppl 1: 108-116, 1998.
154. **Kass DA**. Assessment of diastolic dysfunction. Invasive modalities. *Cardiol Clin* 18: 571-586, 2000.
155. **Kataoka H**. Hemodynamic effect of physiological dual chamber pacing in a patient with end-stage dilated cardiomyopathy: a case report. *Pacing Clin Electrophysiol* 14: 1330-1335, 1991.
156. **Kataoka H**. Physiologic pacemaker therapy for drug-resistant heart failure in a patient with dilated cardiomyopathy and first-degree atrioventricular block: a case report. *J Cardiol* 23: 113-120, 1993.
157. **Katz AM**. Ernest Henry Starling, his predecessors, and the "Law of the Heart". *Circulation* 106: 2986-2992, 2002.
158. **Katz AM, and Lorell BH**. Regulation of cardiac contraction and relaxation. *Circulation* 102: IV69-IV74, 2000.
159. **Kawaguchi M, Hay I, Fetics B, and Kass DA**. Combined ventricular systolic and arterial stiffening in patients with heart failure and preserved ejection fraction: implications for systolic and diastolic reserve limitations. *Circulation* 107: 714-720, 2003.
160. **Kayhan N, Bodem J, Vahl C, and Hagl S**. The positive staircase (force-frequency relationship) and the Frank-Starling mechanism are altered in atrial myocardium of patients in end-stage heart failure transplanted for dilative cardiomyopathy. *Transplant Proc* 34: 2185, 2002.
161. **Kelbaek H, Hartling OJ, Gjorup T, Marving J, Christensen NJ, and Godtfredsen J**. Effects of autonomic blockade on cardiac function at rest and during upright exercise in humans. *J Appl Physiol* 63: 554-557, 1987.
162. **Kellermayer MS, and Grama L**. Stretching and visualizing titin molecules: combining structure, dynamics and mechanics. *J Muscle Res Cell Motil* 23: 499-511, 2002.
163. **Kelly RA, Balligand JL, and Smith TW**. Nitric oxide and cardiac function. *Circ Res* 79: 363-380, 1996.
164. **Kentish JC**. A role for the sarcolemmal Na+/H+ exchanger in the slow force response to myocardial stretch. *Circ Res* 85: 658-660, 1999.
165. **Khan SA, Skaf MW, Harrison RW, Lee K, Minhas KM, Kumar A, Fradley M, Shoukas AA, Berkowitz DE, and Hare JM**. Nitric oxide regulation of myocardial contractility and calcium cycling: independent impact of neuronal and endothelial nitric oxide synthases. *Circ Res* 92: 1322-1329, 2003.
166. **Khouri EM, Gregg DE, and Rayford CR**. Effect of exercise on cardiac output, left coronary flow and myocardial metabolism in the unanesthetized dog. *Circ Res* 17: 427-437, 1965.
167. **Kim HE, Dalal SS, Young E, Legato MJ, Weisfeldt ML, and D'Armiento J**. Disruption of the

myocardial extracellular matrix leads to cardiac dysfunction. *J Clin Invest* 106: 857-866, 2000.

168. **King MK, Coker ML, Goldberg A, McElmurray JH, III, Gunasinghe HR, Mukherjee R, Zile MR, O'Neill TP, and Spinale FG**. Selective matrix metalloproteinase inhibition with developing heart failure: effects on left ventricular function and structure. *Circ Res* 92: 177-185, 2003.
169. **Kitzman DW, Higginbotham MB, Cobb FR, Sheikh KH, and Sullivan MJ**. Exercise intolerance in patients with heart failure and preserved left ventricular systolic function: failure of the Frank-Starling mechanism . *J Am Coll Cardiol* 17: 1065-1072, 1991.
170. **Kitzman DW, Little WC, Brubaker PH, Anderson RT, Hundley WG, Marburger CT, Brosnihan B, Morgan TM, and Stewart KP**. Pathophysiological characterization of isolated diastolic heart failure in comparison to systolic heart failure. *JAMA* 288: 2144-2150, 2002.
171. **Koch A, and Singer H**. Normal values of B type natriuretic peptide in infants, children, and adolescents. *Heart* 89: 875-878, 2003.
172. **Koide M, Nagatsu M, Zile MR, Hamawaki M, Swindle MM, Keech G, DeFreyte G, Tagawa H, Cooper G, and Carabello BA**. Premorbid determinants of left ventricular dysfunction in a novel model of gradually induced pressure overload in the adult canine. *Circulation* 95: 1601-1610, 1997.
173. **Kokkonen JO, Lindstedt KA, and Kovanen PT**. Role for chymase in heart failure: angiotensin II-dependent or -independent mechanisms? *Circulation* 107: 2522-2524, 2003.
174. **Komamura K, Shannon RP, Ihara T, Shen YT, Mirsky I, Bishop SP, and Vatner SF**. Exhaustion of Frank-Starling mechanism in conscious dogs with heart failure. *Am J Physiol* 265: H1119-H1131, 1993.
175. **Konhilas JP, Irving TC, and de Tombe PP**. Length-dependent activation in three striated muscle types of the rat. *J Physiol* 544: 225-236, 2002.
176. **Konhilas JP, Irving TC, and de Tombe PP**. Myofilament calcium sensitivity in skinned rat cardiac trabeculae: role of interfilament spacing. *Circ Res* 90: 59-65, 2002.
177. **Konhilas JP, Irving TC, Wolska BM, Jweied EE, Martin AF, Solaro RJ, and de Tombe PP**. Troponin I in the murine myocardium: influence on length-dependent activation and interfilament spacing. *J Physiol* 547: 951-961, 2003.
178. **Konstam MA**. "Systolic and diastolic dysfunction" in heart failure? Time for a new paradigm. *J Card Fail* 9: 1-3, 2003.
179. **Koshy SK, Reddy HK, and Shukla HH**. Collagen cross-linking: new dimension to cardiac remodeling. *Cardiovasc Res* 57: 594-598, 2003.
180. **Krishnaswamy P, Lubien E, Clopton P, Koon J, Kazanegra R, Wanner E, Gardetto N, Garcia A, DeMaria A, and Maisel AS**. Utility of B-natriuretic peptide levels in identifying patients with left ventricular systolic or diastolic dysfunction. *Am J Med* 111: 274-279, 2001.
181. **Kruger S, Graf J, Kunz D, Stickel T, Hanrath P, and Janssens U**. brain natriuretic peptide levels predict functional capacity in patients with chronic heart failure. *J Am Coll Cardiol* 40: 718-722, 2002.
182. **Lakatta EG**. Starling's law of the heart is explained by an intimate interaction of muscle length and myofilament calcium activation. *J Am Coll Cardiol* 10: 1157-1164, 1987.
183. **Lakatta EG**. Functional implications of spontaneous sarcoplasmic reticulum Ca2+ release in the heart. *Cardiovasc Res* 26: 193-214, 1992.
184. **Lakatta EG, and Lappe DL**. Diastolic scattered light fluctuation, resting force and twitch force in mammalian cardiac muscle. *J Physiol* 315: 369-394, 1981.
185. **Lamberts RR, Van Rijen MH, Sipkema P, Fransen P, Sys SU, and Westerhof N**. Coronary perfusion and muscle lengthening increase cardiac contraction: different stretch-triggered mechanisms. *Am J Physiol Heart Circ Physiol* 283: H1515-H1522, 2002.
186. **Lamberts RR, Van Rijen MH, Sipkema P, Fransen P, Sys SU, and Westerhof N**. Increased

coronary perfusion augments cardiac contractility in the rat through stretch-activated ion channels. *Am J Physiol Heart Circ Physiol* 282: H1334-H1340, 2002.

187. **Lapu-Bula R, Robert A, Van Craeynest D, D'Hondt AM, Gerber BL, Pasquet A, Melin JA, De Kock M, and Vanoverschelde JL**. Contribution of exercise-induced mitral regurgitation to exercise stroke volume and exercise capacity in patients with left ventricular systolic dysfunction. *Circulation* 106: 1342-1348, 2002.
188. **Lehnart SE, Schillinger W, Pieske B, Prestle J, Just H, and Hasenfuss G**. Sarcoplasmic reticulum proteins in heart failure. *Ann N Y Acad Sci* 853: 220-230, 1998.
189. **Leite-Moreira AF, and Correia-Pinto J**. Load as an acute determinant of end-diastolic pressure-volume relation. *Am J Physiol Heart Circ Physiol* 280: H51-H59, 2001.
190. **Leite-Moreira AF, Correia-Pinto J, and Gillebert TC**. Afterload induced changes in myocardial relaxation: a mechanism for diastolic dysfunction. *Cardiovasc Res* 43: 344-353, 1999.
191. **Leite-Moreira AF, Correia-Pinto J, and Gillebert TC**. Load dependence of left ventricular contraction and relaxation. Effects of caffeine. *Basic Res Cardiol* 94: 284-293, 1999.
192. **Leite-Moreira AF, Correia-Pinto J, and Gillebert TC**. Diastolic dysfunction and hypertension. *N Engl J Med* 344: 1401, 2001.
193. **Levin ER, Gardner DG, and Samson WK**. Natriuretic peptides. *N Engl J Med* 339: 321-328, 1998.
194. **Levin HR, Oz MC, Chen JM, Packer M, Rose EA, and Burkhoff D**. Reversal of chronic ventricular dilation in patients with end-stage cardiomyopathy by prolonged mechanical unloading. *Circulation* 91: 2717-2720, 1995.
195. **Levine BD**. Regulation of central blood volume and cardiac filling in endurance athletes: the Frank-Starling mechanism as a determinant of orthostatic tolerance. *Med Sci Sports Exerc* 25: 727-732, 1993.
196. **Levine BD, Lane LD, Buckey JC, Friedman DB, and Blomqvist CG**. Left ventricular pressure-volume and Frank-Starling relations in endurance athletes. Implications for orthostatic tolerance and exercise performance. *Circulation* 84: 1016-1023, 1991.
197. **Levine BD, Zuckerman JH, and Pawelczyk JA**. Cardiac atrophy after bed-rest deconditioning: a nonneural mechanism for orthostatic intolerance. *Circulation* 96: 517-525, 1997.
198. **LeWinter, M. M., B. Decena, and M. D. Tischler.** Abnormalities of myocardial relaxation and filling: diastolic dysfunction. In Hosenpud, J. D. and B. H. Greenberg, eds. Congestive Heart Failure - Pathophysiology, Diagnosis, and Comprehensive Approach to Management. Philadelphia, Lippincott Wiliams & Wilkins. 2000, 67-81.
199. **LeWinter MM, Fabian J, and Bell SP**. Left ventricular restoring forces: modulation by heart rate and contractility. *Basic Res Cardiol* 93 Suppl 1: 143-147, 1998.
200. **LeWinter MM, and Vanburen P**. Myofilament remodeling during the progression of heart failure. *J Card Fail* 8: S271-S275, 2002.
201. **Li K, Stewart DJ, and Rouleau JL**. Myocardial contractile actions of endothelin-1 in rat and rabbit papillary muscles. Role of endocardial endothelium. *Circ Res* 69: 301-312, 1991.
202. **Li YY, Feng Y, McTiernan CF, Pei W, Moravec CS, Wang P, Rosenblum W, Kormos RL, and Feldman AM**. Downregulation of matrix metalloproteinases and reduction in collagen damage in the failing human heart after support with left ventricular assist devices. *Circulation* 104: 1147-1152, 2001.
203. **Linden RJ**. The size of the heart. *Cardioscience* 5: 225-233, 1994.
204. **Linden RJ**. Atrial receptors and heart volumes. *Adv Exp Med Biol* 381: 125-134, 1995.
205. **Linke WA, and Fernandez JM**. Cardiac titin: molecular basis of elasticity and cellular contribution to elastic and viscous stiffness components in myocardium. *J Muscle Res Cell Motil* 23: 483-497, 2002.

206. **Litchfield RL, Kerber RE, Benge JW, Mark AL, Sopko J, Bhatnagar RK, and Marcus ML**. Normal exercise capacity in patients with severe left ventricular dysfunction: compensatory mechanisms. *Circulation* 66: 129-134, 1982.
207. **Little WC**. Hypertensive pulmonary oedema is due to diastolic dysfunction. *Eur Heart J* 22: 1961-1964, 2001.
208. **Little WC, Kitzman DW, and Cheng CP**. Diastolic dysfunction as a cause of exercise intolerance. *Heart Fail Rev* 5: 301-306, 2000.
209. **Liu CP, Ting CT, Lawrence W, Maughan WL, Chang MS, and Kass DA**. Diminished contractile response to increased heart rate in intact human left ventricular hypertrophy. Systolic versus diastolic determinants. *Circulation* 88: 1893-1906, 1993.
210. **Loot AE, Roks AJ, Henning RH, Tio RA, Suurmeijer AJ, Boomsma F, and van Gilst WH**. Angiotensin-(1-7) attenuates the development of heart failure after myocardial infarction in rats. *Circulation* 105: 1548-1550, 2002.
211. **Lorell BH**. Significance of diastolic dysfunction of the heart. *Annu Rev Med* 42: 411-436, 1991.
212. **Lorell BH, and Carabello BA**. Left ventricular hypertrophy: pathogenesis, detection, and prognosis. *Circulation* 102: 470-479, 2000.
213. **Maisel A**. B-type natriuretic peptide levels: diagnostic and prognostic in congestive heart failure: what's next? *Circulation* 105: 2328-2331, 2002.
214. **Maisel AS, Krishnaswamy P, Nowak RM, McCord J, Hollander JE, Duc P, Omland T, Storrow AB, Abraham WT, Wu AH, Clopton P, Steg PG, Westheim A, Knudsen CW, Perez A, Kazanegra R, Herrmann HC, and McCullough PA**. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. *N Engl J Med* 347: 161-167, 2002.
215. **Maisel AS, McCord J, Nowak RM, Hollander JE, Wu AH, Duc P, Omland T, Storrow AB, Krishnaswamy P, Abraham WT, Clopton P, Steg G, Aumont MC, Westheim A, Knudsen CW, Perez A, Kamin R, Kazanegra R, Herrmann HC, and McCullough PA**. Bedside B-Type natriuretic peptide in the emergency diagnosis of heart failure with reduced or preserved ejection fraction. Results from the Breathing Not Properly Multinational Study. *J Am Coll Cardiol* 41: 2010-2017, 2003.
216. **Mandinov L, Eberli FR, Seiler C, and Hess OM**. Diastolic heart failure. *Cardiovasc Res* 45: 813-825, 2000.
217. **Mann DL**. Mechanisms and models in heart failure - A combinatorial approach. *Circulation* 100: 999-1008, 1999.
218. **Mann DL**. Stress-activated cytokines and the heart: From Adaptation to Maladaptation. *Annu Rev Physiol* 65: 81-101, 2003.
219. **Manno BV, Hakki AH, Eshaghpour E, and Iskandrian AS**. Left ventricular function at rest and during exercise in congenital complete heart block: a radionuclide angiographic evaluation. *Am J Cardiol* 52: 92-94, 1983.
220. **Markhasin VS, Solovyova O, Katsnelson LB, Protsenko Y, Kohl P, and Noble D**. Mechano-electric interactions in heterogeneous myocardium: development of fundamental experimental and theoretical models. *Prog Biophys Mol Biol* 82: 207-220, 2003.
221. **Marks AR**. A guide for the perplexed: towards an understanding of the molecular basis of heart failure. *Circulation* 107: 1456-1459, 2003.
222. **Marks AR**. Calcium and the heart: a question of life and death. *J Clin Invest* 111: 597-600, 2003.
223. **Marx SO, and Marks AR**. Regulation of the ryanodine receptor in heart failure. *Basic Res Cardiol* 97 Suppl 1: I49-I51, 2002.
224. **Massie BM**. Natriuretic peptide measurements for the diagnosis of "nonsystolic" heart failure: good news and bad. *J Am Coll Cardiol* 41: 2018-2021, 2003.

225. **Matsumoto T, Wada A, Tsutamoto T, Ohnishi M, Isono T, and Kinoshita M**. Chymase inhibition prevents cardiac fibrosis and improves diastolic dysfunction in the progression of heart failure. *Circulation* 107: 2555-2558, 2003.
226. **McClellan G, Kulikovskaya I, and Winegrad S**. Changes in cardiac contractility related to calcium-mediated changes in phosphorylation of myosin-binding protein C. *Biophys J* 81: 1083-1092, 2001.
227. **McCullough PA, Nowak RM, McCord J, Hollander JE, Herrmann HC, Steg PG, Duc P, Westheim A, Omland T, Knudsen CW, Storrow AB, Abraham WT, Lamba S, Wu AH, Perez A, Clopton P, Krishnaswamy P, Kazanegra R, and Maisel AS**. B-type natriuretic peptide and clinical judgment in emergency diagnosis of heart failure: analysis from Breathing Not Properly (BNP) Multinational Study. *Circulation* 106: 416-422, 2002.
228. **McCullough PA, Omland T, and Maisel AS**. B-type natriuretic peptides: a diagnostic breakthrough for clinicians. *Rev Cardiovasc Med* 4: 72-80, 2003.
229. **McDonald KS, and Moss RL**. Osmotic compression of single cardiac myocytes eliminates the reduction in Ca2+ sensitivity of tension at short sarcomere length. *Circ Res* 77: 199-205, 1995.
230. **McMurray J, and Pfeffer MA**. New therapeutic options in congestive heart failure: Part I. *Circulation* 105: 2099-2106, 2002.
231. **McMurray J, and Pfeffer MA**. New therapeutic options in congestive heart failure: Part II. *Circulation* 105: 2223-2228, 2002.
232. **Meek WJ**. Cardiac Tonus. *Physiol Rev* 7: 259-287, 1927.
233. **Mercadier, J. J.** Progression from cardiac hypertrophy to heart failure. In Hosenpud, J. D. and B. H. Greenberg, eds. Congestive Heart Failure - Pathophysiology, Diagnosis, and Comprehensive Approach to Management. Philadelphia, Lippincott Wiliams & Wilkins. 2000, 67-81.
234. **Mercadier JJ, Lompre AM, Duc P, Boheler KR, Fraysse JB, Wisnewsky C, Allen PD, Komajda M, and Schwartz K**. Altered sarcoplasmic reticulum Ca2(+)-ATPase gene expression in the human ventricle during end-stage heart failure. *J Clin Invest* 85: 305-309, 1990.
235. **Meulemans AL, Andries LJ, and Brutsaert DL**. Endocardial endothelium mediates positive inotropic response to alpha 1-adrenoceptor agonist in mammalian heart. *J Mol Cell Cardiol* 22: 667-685, 1990.
236. **Meyer M, Bluhm WF, He H, Post SR, Giordano FJ, Lew WY, and Dillmann WH**. Phospholamban-to-SERCA2 ratio controls the force-frequency relationship. *Am J Physiol* 276: H779-H785, 1999.
237. **Minamisawa S, Hoshijima M, Chu G, Ward CA, Frank K, Gu Y, Martone ME, Wang Y, Ross J, Jr., Kranias EG, Giles WR, and Chien KR**. Chronic phospholamban-sarcoplasmic reticulum calcium ATPase interaction is the critical calcium cycling defect in dilated cardiomyopathy. *Cell* 99: 313-322, 1999.
238. **Molkentin JD**. A friend within the heart: natriuretic peptide receptor signaling. *J Clin Invest* 111: 1275-1277, 2003.
239. **Moncman CL, and Wang K**. Nebulette: a 107 kD nebulin-like protein in cardiac muscle. *Cell Motil Cytoskeleton* 32: 205-225, 1995.
240. **Morisco C, Sadoshima J, Trimarco B, Arora R, Vatner DE, and Vatner SF**. Is treating cardiac hypertrophy salutary or detrimental: the two faces of Janus. *Am J Physiol Heart Circ Physiol* 284: H1043-H1047, 2003.
241. **Moss RL, and Fitzsimons DP**. Frank-starling relationship: long on importance, short on mechanism. *Circ Res* 90: 11-13, 2002.
242. **Mulieri LA, Hasenfuss G, Leavitt B, Allen PD, and Alpert NR**. Altered myocardial force-frequency

relation in human heart failure. *Circulation* 85: 1743-1750, 1992.

243. **Munch G, Bolck B, Brixius K, Reuter H, Mehlhorn U, Bloch W, and Schwinger RH**. SERCA2a activity correlates with the force-frequency relationship in human myocardium. *Am J Physiol Heart Circ Physiol* 278: H1924-H1932, 2000.
244. **Munch G, Bolck B, Sugaru A, Brixius K, Bloch W, and Schwinger RH**. Increased expression of isoform 1 of the sarcoplasmic reticulum Ca(2+)-release channel in failing human heart. *Circulation* 103: 2739-2744, 2001.
245. **Murad F**. The excitement and rewards of research with our discovery of some of the biological effects of nitric oxide. *Circ Res* 92: 339-341, 2003.
246. **Murphy AM**. Troponin I: in sickness and in health-and normal development. *Circ Res* 91: 449-450, 2002.
247. **Murray DR, and Freeman GL**. Proinflammatory cytokines: predictors of a failing heart? *Circulation* 107: 1460-1462, 2003.
248. **Nadal-Ginard B, Kajstura J, Anversa P, and Leri A**. A matter of life and death: cardiac myocyte apoptosis and regeneration. *J Clin Invest* 111: 1457-1459, 2003.
249. **Nadal-Ginard B, Kajstura J, Leri A, and Anversa P**. Myocyte death, growth, and regeneration in cardiac hypertrophy and failure. *Circ Res* 92: 139-150, 2003.
250. **Nicholls MG, Lainchbury JG, Richards AM, Troughton RW, and Yandle TG**. Brain natriuretic peptide-guided therapy for heart failure. *Ann Med* 33: 422-427, 2001.
251. **Nishikawa N, Yamamoto K, Sakata Y, Mano T, Yoshida J, Miwa T, Takeda H, Hori M, and Masuyama T**. Differential activation of matrix metalloproteinases in heart failure with and without ventricular dilatation. *Cardiovasc Res* 57: 766-774, 2003.
252. **Nobrega ACL, Williamson JW, Garcia JA, and Mitchell JH**. Mechanisms for increasing stroke volume during static exercise with fixed heart rate in humans. *J Appl Physiol* 83: 712-717, 1997.
253. **Ogletree-Hughes ML, Stull LB, Sweet WE, Smedira NG, McCarthy PM, and Moravec CS**. Mechanical unloading restores beta-adrenergic responsiveness and reverses receptor downregulation in the failing human heart. *Circulation* 104: 881-886, 2001.
254. **Ogut O, Hossain MM, and Jin JP**. Interactions between nebulin-like motifs and thin filament regulatory proteins. *J Biol Chem* 278: 3089-3097, 2003.
255. **Olivetti G, Abbi R, Quaini F, Kajstura J, Cheng W, Nitahara JA, Quaini E, Di Loreto C, Beltrami CA, Krajewski S, Reed JC, and Anversa P**. Apoptosis in the failing human heart. *N Engl J Med* 336: 1131-1141, 1997.
256. **Olivetti G, Cigola E, Maestri R, Lagrasta C, Corradi D, and Quaini F**. Recent advances in cardiac hypertrophy. *Cardiovasc Res* 45: 68-75, 2000.
257. **Olson TM, Illenberger S, Kishimoto NY, Huttelmaier S, Keating MT, and Jockusch BM**. Metavinculin mutations alter actin interaction in dilated cardiomyopathy. *Circulation* 105: 431-437, 2002.
258. **Opie, L. H.** Mechanisms of Cardiac Contraction and Relaxation. In Braunwald, E., ed. Heart Disease: A textbook of cardiovascular medicine. Philadelphia, W.B. Saunders Company. 2001, 443-478.
259. **Opie LH, and Sack MN**. Enhanced angiotensin II activity in heart failure: reevaluation of the counterregulatory hypothesis of receptor subtypes. *Circ Res* 88: 654-658, 2001.
260. **Packer M**. Abnormalities of diastolic function as a potential cause of exercise intolerance in chronic heart failure. *Circulation* 81: III78-III86, 1990.
261. **Packer M**. Diastolic function as a target of therapeutic interventions in chronic heart failure. *Eur Heart J* 11 Suppl C: 35-40, 1990.
262. **Pak PH, Maughan L, Baughman KL, and Kass DA**. Marked discordance between dynamic and

passive diastolic pressure-volume relations in idiopathic hypertrophic cardiomyopathy. *Circulation* 94: 52-60, 1996.

263. **Park MH, Scott RL, Uber PA, Harris BC, Chambers R, and Mehra MR**. Usefulness of B-type natriuretic peptide levels in predicting hemodynamic perturbations after heart transplantation despite preserved left ventricular systolic function. *Am J Cardiol* 90: 1326-1329, 2002.
264. **Parmley WW, and Chuck L**. Length-dependent changes in myocardial contractile state. *Am J Physiol* 224: 1195-1199, 1973.
265. **Pasipoularides A, Mirsky I, Hess OM, Grimm J, and Krayenbuehl HP**. Myocardial relaxation and passive diastolic properties in man. *Circulation* 74: 991-1001, 1986.
266. **Patterson SW, and Starling EH** . The regulation of the heart beat. *J Physiol* 48: 465-513, 1914.
267. **Paulus WJ**. Paracrine coronary endothelial modulation of diastolic left ventricular function in man: implications for diastolic heart failure. *J Card Fail* 2: S155-S164, 1996.
268. **Paulus WJ**. How are cytokines activated in heart failure? *Eur J Heart Fail* 1: 309-312, 1999.
269. **Paulus WJ, Grossman W, Serizawa T, Bourdillon PD, Pasipoularides A, and Mirsky I**. Different effects of two types of ischemia on myocardial systolic and diastolic function. *Am J Physiol* 248: H719-H728, 1985.
270. **Paulus WJ, and Shah AM**. NO and cardiac diastolic function. *Cardiovasc Res* 43: 595-606, 1999.
271. **Paulus WJ, Vantrimpont PJ, and Rousseau MF**. Diastolic function of the nonfilling human left ventricle. *J Am Coll Cardiol* 20: 1524-1532, 1992.
272. **Paulus WJ, Vantrimpont PJ, and Shah AM**. Acute effects of nitric oxide on left ventricular relaxation and diastolic distensibility in humans. Assessment by bicoronary sodium nitroprusside infusion. *Circulation* 89: 2070-2078, 1994.
273. **Paulus WJ, Vantrimpont PJ, and Shah AM**. Paracrine coronary endothelial control of left ventricular function in humans. *Circulation* 92: 2119-2126, 1995.
274. **Perez NG, de Hurtado MC, and Cingolani HE**. Reverse mode of the Na+-Ca2+ exchange after myocardial stretch: underlying mechanism of the slow force response. *Circ Res* 88: 376-382, 2001.
275. **Perez NG, Hashimoto K, McCune S, Altschuld RA, and Marban E**. Origin of contractile dysfunction in heart failure - Calcium cycling versus myofilaments. *Circulation* 99: 1077-1083, 1999.
276. **Petrie MC, Caruana L, Berry C, and McMurray JJ**. "Diastolic heart failure" or heart failure caused by subtle left ventricular systolic dysfunction? *Heart* 87: 29-31, 2002.
277. **Petroff MG, Kim SH, Pepe S, Dessy C, Marban E, Balligand JL, and Sollott SJ**. Endogenous nitric oxide mechanisms mediate the stretch dependence of Ca2+ release in cardiomyocytes. *Nat Cell Biol* 3: 867-873, 2001.
278. **Piacentino V, III, Weber CR, Chen X, Weisser-Thomas J, Margulies KB, Bers DM, and Houser SR**. Cellular basis of abnormal calcium transients of failing human ventricular myocytes. *Circ Res* 92: 651-658, 2003.
279. **Piacentino V, III, Weber CR, Gaughan JP, Margulies KB, Bers DM, and Houser SR**. Modulation of contractility in failing human myocytes by reverse-mode Na/Ca exchange. *Ann N Y Acad Sci* 976: 466-471, 2002.
280. **Pieske B, Kretschmann B, Meyer M, Holubarsch C, Weirich J, Posival H, Minami K, Just H, and Hasenfuss G**. Alterations in intracellular calcium handling associated with the inverse force-frequency relation in human dilated cardiomyopathy. *Circulation* 92: 1169-1178, 1995.
281. **Pieske B, Maier LS, Bers DM, and Hasenfuss G**. Ca2+ handling and sarcoplasmic reticulum Ca2+ content in isolated failing and nonfailing human myocardium. *Circ Res* 85: 38-46, 1999.
282. **Pina IL, Apstein CS, Balady GJ, Belardinelli R, Chaitman BR, Duscha BD, Fletcher BJ, Fleg JL,**

Myers JN, and Sullivan MJ. Exercise and heart failure: A statement from the American Heart Association Committee on exercise, rehabilitation, and prevention. *Circulation* 107: 1210-1225, 2003.

283. **Pinsky DJ, Patton S, Mesaros S, Brovkovych V, Kubaszewski E, Grunfeld S, and Malinski T**. Mechanical transduction of nitric oxide synthesis in the beating heart. *Circ Res* 81: 372-379, 1997.
284. **Piuhola J, Szokodi I, Kinnunen P, Ilves M, deChatel R, Vuolteenaho O, and Ruskoaho H**. Endothelin-1 contributes to the Frank-Starling response in hypertrophic rat hearts. *Hypertension* 41: 93-98, 2003.
285. **Plank DM, Yatani A, Ritsu H, Witt S, Glascock B, Lalli MJ, Periasamy M, Fiset C, Benkusky N, Valdivia HH, and Sussman MA**. Calcium Dynamics in the Failing Heart:Restoration by {beta}-Adrenergic Receptor Blockade. *Am J Physiol Heart Circ Physiol* 2003.
286. **Plotnick GD, Becker LC, Fisher ML, Gerstenblith G, Renlund DG, Fleg JL, Weisfeldt ML, and Lakatta EG**. Use of the Frank-Starling mechanism during submaximal versus maximal upright exercise. *Am J Physiol* 251: H1101-H1105, 1986.
287. **Poindexter BJ, Smith JR, Buja LM, and Bick RJ**. Calcium signaling mechanisms in dedifferentiated cardiac myocytes: comparison with neonatal and adult cardiomyocytes. *Cell Calcium* 30: 373-382, 2001.
288. **Pouleur H, Hanet C, Rousseau MF, and van Eyll C**. Relation of diastolic function and exercise capacity in ischemic left ventricular dysfunction. Role of beta-agonists and beta-antagonists. *Circulation* 82: I89-I96, 1990.
289. **Prendergast BD, Sagach VF, and Shah AM**. Basal release of nitric oxide augments the Frank-Starling response in the isolated heart. *Circulation* 96: 1320-1329, 1997.
290. **Prestle J, Quinn FR, and Smith GL**. Ca(2+)-handling proteins and heart failure: novel molecular targets? *Curr Med Chem* 10: 967-981, 2003.
291. **Purcell IF, and Poole-Wilson PA**. Heart failure: why and how to define it? *Eur J Heart Fail* 1: 7-10, 1999.
292. **Quaini F, Urbanek K, Beltrami AP, Finato N, Beltrami CA, Nadal-Ginard B, Kajstura J, Leri A, and Anversa P**. Chimerism of the transplanted heart. *N Engl J Med* 346: 5-15, 2002.
293. **Rademaker MT, Cameron VA, Charles CJ, Lainchbury JG, Nicholls MG, and Richards AM**. Adrenomedullin and heart failure. *Regul Pept* 112: 51-60, 2003.
294. **Reiken S, Wehrens XH, Vest JA, Barbone A, Klotz S, Mancini D, Burkhoff D, and Marks AR**. Beta-blockers restore calcium release channel function and improve cardiac muscle performance in human heart failure. *Circulation* 107: 2459-2466, 2003.
295. **Reuter H, Zobel C, Brixius K, Bolck B, and Schwinger RH**. The force-frequency relationship is dependent on Ca(2+)-influx via L-type- and SR-Ca(2+)-channels in human heart. *Basic Res Cardiol* 94: 159-170, 1999.
296. **Reyes M, Freeman GL, Escobedo D, Lee S, Steinhelper ME, and Feldman MD**. Enhancement of contractility with sustained afterload in the intact murine heart: blunting of length-dependent activation. *Circulation* 107: 2962-2968, 2003.
297. **Richards AM, Lainchbury JG, Nicholls MG, Troughton RW, and Yandle TG**. BNP in hormone-guided treatment of heart failure. *Trends Endocrinol Metab* 13: 151-155, 2002.
298. **Robbins J, and Benson DW**. Structure-function relationships in myosin binding protein-C: taking off the blinders and collaring hypertrophic cardiomyopathy. *Circ Res* 91: 656-658, 2002.
299. **Rockman HA, Koch WJ, and Lefkowitz RJ**. Seven-transmembrane-spanning receptors and heart function. *Nature* 415: 206-212, 2002.
300. **Rosenblueth A, Alanais J, Lopez E, and Rubio R**. The adaptation of ventricular muscle to different circulatory conditions. *Arch Int Physiol Biochim* 67: 358-373, 1959.

301. **Ross J, Jr.** Afterload mismatch in aortic and mitral valve disease: implications for surgical therapy. *J Am Coll Cardiol* 5: 811-826, 1985.
302. **Ross J, Jr.** On variations in the cardiac hypertrophic response to pressure overload. *Circulation* 95: 1349-1351, 1997.
303. **Ross J, Jr.** Adrenergic regulation of the force-frequency effect. *Basic Res Cardiol* 93 Suppl 1: 95-101, 1998.
304. **Ross J, Jr., Miura T, Kambayashi M, Eising GP, and Ryu KH**. Adrenergic control of the force-frequency relation. *Circulation* 92: 2327-2332, 1995.
305. **Ross RS**. The extracellular connections: the role of integrins in myocardial remodeling. *J Card Fail* 8: S326-S331, 2002.
306. **Ross RS, and Borg TK**. Integrins and the myocardium. *Circ Res* 88: 1112-1119, 2001.
307. **Ruegg JC**. Towards a molecular understanding of contractility. *Cardioscience* 1: 163-168, 1990.
308. **Ruwhof C, and van der LA**. Mechanical stress-induced cardiac hypertrophy: mechanisms and signal transduction pathways. *Cardiovasc Res* 47: 23-37, 2000.
309. **Saavedra WF, Tunin RS, Paolocci N, Mishima T, Suzuki G, Emala CW, Chaudhry PA, Anagnostopoulos P, Gupta RC, Sabbah HN, and Kass DA**. Reverse remodeling and enhanced adrenergic reserve from passive external support in experimental dilated heart failure. *J Am Coll Cardiol* 39: 2069-2076, 2002.
310. **Sadoshima J, and Izumo S**. Mechanotransduction in stretch-induced hypertrophy of cardiac myocytes. *J Recept Res* 13: 777-794, 1993.
311. **Sadoshima J, and Izumo S**. The cellular and molecular response of cardiac myocytes to mechanical stress. *Annu Rev Physiol* 59: 551-571, 1997.
312. **Safar ME, Levy BI, and Struijker-Boudier H**. Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. *Circulation* 107: 2864-2869, 2003.
313. **Sakurai S, Adachi H, Hasegawa A, Hoshizaki H, Oshima S, Taniguchi K, and Kurabayashi M**. Brain natriuretic peptide facilitates severity classification of stable chronic heart failure with left ventricular dysfunction. *Heart* 89: 661-662, 2003.
314. **Sarnoff SJ, Mitchell JH, Gilmore JP, and Remensneider JP**. Homeometric autoregulation in the heart. *Circ Res* 8: 1077-1091, 1960.
315. **Schaub MC, Hefti MA, Zuellig RA, and Morano I**. Modulation of contractility in human cardiac hypertrophy by myosin essential light chain isoforms. *Cardiovasc Res* 37: 381-404, 1998.
316. **Scherlag BJ, Bartelstone HJ, Wyte SR, and Hoffman BF**. Variable diastolic ventricular compliance: a general property of mammalian cardiac muscle. *Nature* 209: 1246-1248, 1966.
317. **Schiereck P, Hoefnagel R, De Beer EL, Van Heijst BG, and Mosterd WL**. Direct recording of EDP-EDV relationship in isolated rat left ventricle: effect of diastolic crossbridge formation. *Cardiovasc Res* 28: 715-719, 1994.
318. **Schillinger W, and Kogler H**. Altered phosphorylation and Ca(2+)-sensitivity of myofilaments in human heart failure. *Cardiovasc Res* 57: 5-7, 2003.
319. **Schillinger W, Lehnart SE, Prestle J, Preuss M, Pieske B, Maier LS, Meyer M, Just H, and Hasenfuss G**. Influence of SR Ca(2+)-ATPase and Na(+)-Ca(2+)-exchanger on the force-frequency relation. *Basic Res Cardiol* 93 Suppl 1: 38-45, 1998.
320. **Schluter KD, and Piper HM**. Regulation of growth in the adult cardiomyocytes. *FASEB J* 13 Suppl: S17-S22, 1999.
321. **Schmidt U, Hajjar RJ, Helm PA, Kim CS, Doye AA, and Gwathmey JK**. Contribution of abnormal sarcoplasmic reticulum ATPase activity to systolic and diastolic dysfunction in human heart

failure. *J Mol Cell Cardiol* 30: 1929-1937, 1998.

322. **Schrier RW, and Abraham WT**. Hormones and hemodynamics in heart failure. *N Engl J Med* 341: 577-585, 1999.
323. **Schultz JJ, Witt SA, Glascock BJ, Nieman ML, Reiser PJ, Nix SL, Kimball TR, and Doetschman T**. TGF-beta1 mediates the hypertrophic cardiomyocyte growth induced by angiotensin II. *J Clin Invest* 109: 787-796, 2002.
324. **Schwinger RH, Bohm M, Koch A, Schmidt U, Morano I, Eissner HJ, Uberfuhr P, Reichart B, and Erdmann E**. The failing human heart is unable to use the Frank-Starling mechanism. *Circ Res* 74: 959-969, 1994.
325. **Sears CE, Bryant SM, Ashley EA, Lygate CA, Rakovic S, Wallis HL, Neubauer S, Terrar DA, and Casadei B**. Cardiac neuronal nitric oxide synthase isoform regulates myocardial contraction and calcium handling. *Circ Res* 92: e52-e59, 2003.
326. **Senni M, and Redfield MM**. Heart failure with preserved systolic function. A different natural history? *J Am Coll Cardiol* 38: 1277-1282, 2001.
327. **Shah AM, Andries LJ, Meulemans AL, and Brutsaert DL**. Endocardium modulates myocardial inotropic response to 5-hydroxytryptamine. *Am J Physiol* 257: H1790-H1797, 1989.
328. **Shah AM, Grocott-Mason RM, Pepper CB, Mebazaa A, Henderson AH, Lewis MJ, and Paulus WJ**. The cardiac endothelium: cardioactive mediators. *Prog Cardiovasc Dis* 39: 263-284, 1996.
329. **Shah AM, and Lewis MJ**. Endothelial modulation of myocardial contraction: mechanisms and potential relevance in cardiac disease. *Basic Res Cardiol* 87 Suppl 2: 59-70, 1992.
330. **Shah AM, Mebazaa A, Wetzel RC, and Lakatta EG**. Novel cardiac myofilament desensitizing factor released by endocardial and vascular endothelial cells. *Circulation* 89: 2492-2497, 1994.
331. **Shah AM, Meulemans AL, and Brutsaert DL**. Myocardial inotropic responses to aggregating platelets and modulation by the endocardium. *Circulation* 79: 1315-1323, 1989.
332. **Shah AM, Spurgeon HA, Sollott SJ, Talo A, and Lakatta EG**. 8-bromo-cGMP reduces the myofilament response to Ca2+ in intact cardiac myocytes. *Circ Res* 74: 970-978, 1994.
333. **Shai SY, Harpf AE, Babbitt CJ, Jordan MC, Fishbein MC, Chen J, Omura M, Leil TA, Becker KD, Jiang M, Smith DJ, Cherry SR, Loftus JC, and Ross RS**. Cardiac myocyte-specific excision of the beta1 integrin gene results in myocardial fibrosis and cardiac failure. *Circ Res* 90: 458-464, 2002.
334. **Shannon RP, Komamura K, Stambler BS, Bigaud M, Manders WT, and Vatner SF**. Alterations in myocardial contractility in conscious dogs with dilated cardiomyopathy. *Am J Physiol* 260: H1903-H1911, 1991.
335. **Sharpe, N.** Cardiac remodeling in congestive heart failure. In Hosenpud, J. D. and B. H. Greenberg, eds. Congestive Heart Failure - Pathophysiology, Diagnosis, and Comprehensive Approach to Management. Philadelphia, Lippincott Wiliams & Wilkins. 2000, 67-81.
336. **Shintani, H. and S. A. Glantz.** The left ventricular diastolic pressure-volume relation, relaxation and, filling. In Gaasch, W. H. and M. M. LeWinter, eds. Left ventricular diastolic dysfunction and heart failure. Malvern, Pennsylvania, Lea & Febiger. 1994, 57-88.
337. **Sipido KR, Stankovicova T, Flameng W, Vanhaecke J, and Verdonck F**. Frequency dependence of Ca2+ release from the sarcoplasmic reticulum in human ventricular myocytes from end-stage heart failure. *Cardiovasc Res* 37: 478-488, 1998.
338. **Sjaastad I, Wasserstrom JA, and Sejersted OM**. Heart failure - a challenge to our current concepts of excitation-contraction coupling. *J Physiol* 546: 33-47, 2003.
339. **Smith GL, Masoudi FA, Vaccarino V, Radford MJ, and Krumholz HM**. Outcomes in heart failure patients with preserved ejection fraction. Mortality, readmission, and functional decline. *J Am Coll Cardiol* 41: 1510-1518, 2003.

340. **Smith JA, Shah AM, and Lewis MJ**. Factors released from endocardium of the ferret and pig modulate myocardial contraction. *J Physiol* 439: 1-14, 1991.
341. **Solaro RJ**. Is calcium the 'cure' for dilated cardiomyopathy? *Nat Med* 5: 1353-1354, 1999.
342. **Sollott SJ, Ziman BD, Warshaw DM, Spurgeon HA, and Lakatta EG**. Actomyosin interaction modulates resting length of unstimulated cardiac ventricular cells. *Am J Physiol* 271: H896-H905, 1996.
343. **Somura F, Izawa H, Iwase M, Takeichi Y, Ishiki R, Nishizawa T, Noda A, Nagata K, Yamada Y, and Yokota M**. Reduced myocardial sarcoplasmic reticulum Ca(2+)-ATPase mRNA expression and biphasic force-frequency relations in patients with hypertrophic cardiomyopathy. *Circulation* 104: 658-663, 2001.
344. **Soufer R, Wohlgelernter D, Vita NA, Amuchestegui M, Sostman HD, Berger HJ, and Zaret BL**. Intact systolic left ventricular function in clinical congestive heart failure. *Am J Cardiol* 55: 1032-1036, 1985.
345. **Spinale FG**. Matrix metalloproteinases: regulation and dysregulation in the failing heart. *Circ Res* 90: 520-530, 2002.
346. **Staal EM, Steendijk P, and Baan J**. The trans-cardiac conductance method for on-line measurement of left ventricular volume: assessment of parallel conductance offset volume. *IEEE Trans Biomed Eng* 50: 234-240, 2003.
347. **Sussman MA, McCulloch A, and Borg TK**. Dance band on the Titanic: biomechanical signaling in cardiac hypertrophy. *Circ Res* 91: 888-898, 2002.
348. **Sys SU, De Keulenaer GW, and Brutsaert DL**. Reappraisal of the multicellular preparation for the in vitro physiopharmacological evaluation of myocardial performance. *Adv Exp Med Biol* 453: 441-450, 1998.
349. **Szlachcic J, Massie BM, Kramer BL, Topic N, and Tubau J**. Correlates and prognostic implication of exercise capacity in chronic congestive heart failure. *Am J Cardiol* 55: 1037-1042, 1985.
350. **Tagawa H, Wang N, Narishige T, Ingber DE, Zile MR, and Cooper G**. Cytoskeletal mechanics in pressure-overload cardiac hypertrophy. *Circ Res* 80: 281-289, 1997.
351. **Takeda N**. Cardiomyopathy: Molecular and immunological aspects. *Int J Mol Med* 11: 13-16, 2003.
352. **Takeichi Y, Yokota M, Iwase M, Izawa H, Nishizawa T, Ishiki R, Somura F, Nagata K, Isobe S, and Noda A**. Biphasic changes in left ventricular end-diastolic pressure during dynamic exercise in patients with nonobstructive hypertrophic cardiomyopathy. *J Am Coll Cardiol* 38: 335-343, 2001.
353. **Tanaka R, Barnes MA, Cooper G, and Zile MR**. Effects of anisosmotic stress on cardiac muscle cell length, diameter, area, and sarcomere length. *Am J Physiol* 270: H1414-H1422, 1996.
354. **Tang L, Gao W, and Taylor PB**. Force-frequency response in isoproterenol-induced hypertrophied rat heart. *Eur J Pharmacol* 318: 349-356, 1996.
355. **Tavi P, Han C, and Weckstrom M**. Mechanisms of stretch-induced changes in [Ca2+]i in rat atrial myocytes: role of increased troponin C affinity and stretch-activated ion channels. *Circ Res* 83: 1165-1177, 1998.
356. **Teitel DF, Klautz RJ, Cassidy SC, Steendijk P, van der Velde ET, van Bel F, and Baan J**. The end-systolic pressure-volume relationship in young animals using the conductance technique. *Eur Heart J* 13 Suppl E: 40-46, 1992.
357. **ter Keurs, H. E. and M. I. Noble.** Introduction. In ter Keurs, H. E. and M. I. Noble, eds. Starling's law of the heart revisited. Boston, Kluwer Academic Publishers. 1988, XI-XV.
358. **Thibault G, Amiri F, and Garcia R**. Regulation of natriuretic peptide secretion by the heart. *Annu Rev Physiol* 61: 193-217, 1999.

359. **Tom B, Dendorfer A, and Jan Danser AH**. Bradykinin, angiotensin-(1-7), and ACE inhibitors: how do they interact? *Int J Biochem Cell Biol* 35: 792-801, 2003.
360. **Tomai F, Ciavolella M, Crea F, Gaspardone A, Versaci F, Giannitti C, Scali D, Chiariello L, and Gioffre PA**. Left ventricular volumes during exercise in normal subjects and patients with dilated cardiomyopathy assessed by first-pass radionuclide angiography. *Am J Cardiol* 72: 1167-1171, 1993.
361. **Tomai F, Ciavolella M, Gaspardone A, De Fazio A, Basso EG, Giannitti C, Scali D, Versaci F, Crea F, and Gioffre PA**. Peak exercise left ventricular performance in normal subjects and in athletes assessed by first-pass radionuclide angiography. *Am J Cardiol* 70: 531-535, 1992.
362. **Torre-Amione G, Stetson SJ, Youker KA, Durand JB, Radovancevic B, Delgado RM, Frazier OH, Entman ML, and Noon GP**. Decreased expression of tumor necrosis factor-alpha in failing human myocardium after mechanical circulatory support : A potential mechanism for cardiac recovery. *Circulation* 100: 1189-1193, 1999.
363. **Towbin JA, and Bowles NE**. The failing heart. *Nature* 415: 227-233, 2002.
364. **Tucci PJ, Murad N, Rossi CL, Nogueira RJ, and Santana O, Jr.** Heart rate modulates the slow enhancement of contraction due to sudden left ventricular dilation. *Am J Physiol Heart Circ Physiol* 280: H2136-H2143, 2001.
365. **Ungureanu-Longrois D, Balligand JL, Simmons WW, Okada I, Kobzik L, Lowenstein CJ, Kunkel SL, Michel T, Kelly RA, and Smith TW**. Induction of nitric oxide synthase activity by cytokines in ventricular myocytes is necessary but not sufficient to decrease contractile responsiveness to beta-adrenergic agonists. *Circ Res* 77: 494-502, 1995.
366. **Vahl CF, Timek T, Bonz A, Fuchs H, Dillman R, and Hagl S**. Length dependence of calcium- and force-transients in normal and failing human myocardium. *J Mol Cell Cardiol* 30: 957-966, 1998.
367. **Vahl CF, Timek T, Bonz A, Kochsiek N, Fuchs H, Schaffer L, Rosenberg M, Dillmann R, and Hagl S**. Myocardial length-force relationship in end stage dilated cardiomyopathy and normal human myocardium: analysis of intact and skinned left ventricular trabeculae obtained during 11 heart transplantations. *Basic Res Cardiol* 92: 261-270, 1997.
368. **van der Linden LP**. A critical investigation into the end-systolic pressure-volume relation of the in situ left ventricle (Thesis). Faculty of medicine, Leiden University, the Netherlands, 1995
369. **van der Velden J, Boontje NM, Papp Z, Klein LJ, Visser FC, de Jong JW, Owen VJ, Burton PB, and Stienen GJ**. Calcium sensitivity of force in human ventricular cardiomyocytes from donor and failing hearts. *Basic Res Cardiol* 97 Suppl 1: I118-I126, 2002.
370. **van der Velden J, Klein LJ, Zaremba R, Boontje NM, Huybregts MA, Stooker W, Eijsman L, de Jong JW, Visser CA, Visser FC, and Stienen GJ**. Effects of calcium, inorganic phosphate, and pH on isometric force in single skinned cardiomyocytes from donor and failing human hearts. *Circulation* 104: 1140-1146, 2001.
371. **van der Velden J, Papp Z, Boontje NM, Zaremba R, de Jong JW, Janssen PM, Hasenfuss G, and Stienen GJ**. The effect of myosin light chain 2 dephosphorylation on Ca(2+)-sensitivity of force is enhanced in failing human hearts. *Cardiovasc Res* 57: 505-514, 2003.
372. **van der Velden J, Papp Z, Zaremba R, Boontje NM, de Jong JW, Owen VJ, Burton PB, Goldmann P, Jaquet K, and Stienen GJ**. Increased Ca(2+)-sensitivity of the contractile apparatus in end-stage human heart failure results from altered phosphorylation of contractile proteins. *Cardiovasc Res* 57: 37-47, 2003.
373. **van Wamel AJ, Ruwhof C, van der Valk-Kokshoorn LJ, Schrier PI, and van der LA**. Stretch-induced paracrine hypertrophic stimuli increase TGF-beta1 expression in cardiomyocytes. *Mol Cell Biochem* 236: 147-153, 2002.
374. **Varadarajan P, and Pai RG**. Prognosis of congestive heart failure in patients with normal versus

reduced ejection fractions: Results from a cohort of 2,258 hospitalized patients. *J Card Fail* 9: 107-112, 2003.

375. **Varela-Roman A, Gonzalez-Juanatey JR, Basante P, Trillo R, Garcia-Seara J, Martinez-Sande JL, and Gude F**. Clinical characteristics and prognosis of hospitalised inpatients with heart failure and preserved or reduced left ventricular ejection fraction. *Heart* 88: 249-254, 2002.
376. **Varma N, Morgan JP, and Apstein CS**. Mechanisms underlying ischemic diastolic dysfunction: relation between rigor, calcium homeostasis, and relaxation rate. *Am J Physiol Heart Circ Physiol* 284: H758-H771, 2003.
377. **Vatta M, Stetson SJ, Perez-Verdia A, Entman ML, Noon GP, Torre-Amione G, Bowles NE, and Towbin JA**. Molecular remodelling of dystrophin in patients with end-stage cardiomyopathies and reversal in patients on assistance-device therapy. *Lancet* 359: 936-941, 2002.
378. **Vemuri R, Lankford EB, Poetter K, Hassanzadeh S, Takeda K, Yu ZX, Ferrans VJ, and Epstein ND**. The stretch-activation response may be critical to the proper functioning of the mammalian heart. *Proc Natl Acad Sci U S A* 96: 1048-1053, 1999.
379. **Visse R, and Nagase H**. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res* 92: 827-839, 2003.
380. **von Anrep G**. On the part played by the suprarenals in the normal vascular reaction of the body. *J Physiol* 45: 307-317, 1912.
381. **Wang J, and Morgan JP**. Endothelin reverses the effects of acidosis on the intracellular Ca2+ transient and contractility in ferret myocardium. *Circ Res* 71: 631-639, 1992.
382. **Wang X, Osinska H, Gerdes AM, and Robbins J**. Desmin filaments and cardiac disease: establishing causality. *J Card Fail* 8: S287-S292, 2002.
383. **Watanabe K, Muhle-Goll C, Kellermayer MS, Labeit S, and Granzier H**. Different molecular mechanics displayed by titin's constitutively and differentially expressed tandem Ig segments. *J Struct Biol* 137: 248-258, 2002.
384. **Weber KT**. Targeting pathological remodeling: concepts of cardioprotection and reparation. *Circulation* 102: 1342-1345, 2000.
385. **Weil J, Eschenhagen T, Hirt S, Magnussen O, Mittmann C, Remmers U, and Scholz H**. Preserved Frank-Starling mechanism in human end stage heart failure. *Cardiovasc Res* 37: 541-548, 1998.
386. **Weinberg EO, Shimpo M, Hurwitz S, Tominaga S, Rouleau JL, and Lee RT**. Identification of serum soluble ST2 receptor as a novel heart failure biomarker. *Circulation* 107: 721-726, 2003.
387. **Wencker D, Chandra M, Nguyen K, Miao W, Garantziotis S, Factor SM, Shirani J, Armstrong RC, and Kitsis RN**. A mechanistic role for cardiac myocyte apoptosis in heart failure. *J Clin Invest* 111: 1497-1504, 2003.
388. **Winegrad S**. Endothelial cell regulation of contractility of the heart. *Annu Rev Physiol* 59: 505-525, 1997.
389. **Winegrad S**. Cardiac myosin binding protein C. *Circ Res* 84: 1117-1126, 1999.
390. **Winegrad S**. Myosin binding protein C, a potential regulator of cardiac contractility. *Circ Res* 86: 6-7, 2000.
391. **Woodcock EA, Matkovich SJ, and Binah O**. Ins(1,4,5)P3 and cardiac dysfunction. *Cardiovasc Res* 40: 251-256, 1998.
392. **Woodiwiss AJ, Tsotetsi OJ, Sprott S, Lancaster EJ, Mela T, Chung ES, Meyer TE, and Norton GR**. Reduction in Myocardial Collagen Cross-Linking Parallels Left Ventricular Dilatation in Rat Models of Systolic Chamber Dysfunction. *Circulation* 103: 155-160, 2001.
393. **Wu Y, Bell SP, Trombitas K, Witt CC, Labeit S, LeWinter MM, and Granzier H**. Changes in titin

isoform expression in pacing-induced cardiac failure give rise to increased passive muscle stiffness. *Circulation* 106: 1384-1389, 2002.

394. **Wu Y, Cazorla O, Labeit D, Labeit S, and Granzier H**. Changes in titin and collagen underlie diastolic stiffness diversity of cardiac muscle. *J Mol Cell Cardiol* 32: 2151-2162, 2000.
395. **Wu Y, Labeit S, LeWinter MM, and Granzier H**. Titin: an endosarcomeric protein that modulates myocardial stiffness in DCM. *J Card Fail* 8: S276-S286, 2002.
396. **Wunderlich C, Flogel U, Godecke A, Heger J, and Schrader J**. Acute inhibition of myoglobin impairs contractility and energy state of iNOS-overexpressing hearts. *Circ Res* 92: 1352-1358, 2003.
397. **Wyman RM, Farhi ER, Bing OH, Johnson RG, Weintraub RM, and Grossman W**. Comparative effects of hypoxia and ischemia in the isolated, blood-perfused dog heart: evaluation of left ventricular diastolic chamber distensibility and wall thickness. *Circ Res* 64: 121-128, 1989.
398. **Xiong D, Lee GH, Badorff C, Dorner A, Lee S, Wolf P, and Knowlton KU**. Dystrophin deficiency markedly increases enterovirus-induced cardiomyopathy: a genetic predisposition to viral heart disease. *Nat Med* 8: 872-877, 2002.
399. **Yamada H, Oki T, Tabata T, Manabe K, Fukuda K, Abe M, Iuchi A, and Ito S**. Differences in transmitral flow velocity pattern during increase in preload in patients with abnormal left ventricular relaxation. *Cardiology* 89: 152-158, 1998.
400. **Yamamoto K, Masuyama T, Sakata Y, Nishikawa N, Mano T, Yoshida J, Miwa T, Sugawara M, Yamaguchi Y, Ookawara T, Suzuki K, and Hori M**. Myocardial stiffness is determined by ventricular fibrosis, but not by compensatory or excessive hypertrophy in hypertensive heart. *Cardiovasc Res* 55: 76-82, 2002.
401. **Yamamoto S, Yang G, Zablocki D, Liu J, Hong C, Kim SJ, Soler S, Odashima M, Thaisz J, Yehia G, Molina CA, Yatani A, Vatner DE, Vatner SF, and Sadoshima J**. Activation of Mst1 causes dilated cardiomyopathy by stimulating apoptosis without compensatory ventricular myocyte hypertrophy. *J Clin Invest* 111: 1463-1474, 2003.
402. **Yano M, Kobayashi S, Kohno M, Doi M, Tokuhisa T, Okuda S, Suetsugu M, Hisaoka T, Obayashi M, Ohkusa T, Kohno M, and Matsuzaki M**. FKBP12.6-mediated stabilization of calcium-release channel (ryanodine receptor) as a novel therapeutic strategy against heart failure. *Circulation* 107: 477-484, 2003.
403. **Yip G, Wang M, Zhang Y, Fung JW, Ho PY, and Sanderson JE**. Left ventricular long axis function in diastolic heart failure is reduced in both diastole and systole: time for a redefinition? *Heart* 87: 121-125, 2002.
404. **Yip GW, Zhang Y, Tan PY, Wang M, Ho PY, Brodin LA, and Sanderson JE**. Left ventricular long-axis changes in early diastole and systole: impact of systolic function on diastole. *Clin Sci (Lond)* 102: 515-522, 2002.
405. **Yu CM, Lin H, Yang H, Kong SL, Zhang Q, and Lee SW**. Progression of systolic abnormalities in patients with "isolated" diastolic heart failure and diastolic dysfunction. *Circulation* 105: 1195-1201, 2002.
406. **Zafeiridis A, Jeevanandam V, Houser SR, and Margulies KB**. Regression of cellular hypertrophy after left ventricular assist device support. *Circulation* 98: 656-662, 1998.
407. **Zhu Y, and Nosek TM**. Inositol trisphosphate enhances Ca2+ oscillations but not Ca(2+)- induced Ca2+ release from cardiac sarcoplasmic reticulum. *Pflugers Arch* 418: 1-6, 1991.
408. **Zieman SJ, Gerstenblith G, Lakatta EG, Rosas GO, Vandegaer K, Ricker KM, and Hare JM**. Upregulation of the nitric oxide-cGMP pathway in aged myocardium: physiological response to l-arginine. *Circ Res* 88: 97-102, 2001.
409. **Zile MR**. Heart failure with preserved ejection fraction: is this diastolic heart failure?*. *J Am Coll*

Cardiol 41: 1519-1522, 2003.

410. **Zile MR, and Brutsaert DL**. New concepts in diastolic dysfunction and diastolic heart failure: Part I: diagnosis, prognosis, and measurements of diastolic function. *Circulation* 105: 1387-1393, 2002.
411. **Zile MR, Conrad CH, Gaasch WH, Robinson KG, and Bing OH**. Preload does not affect relaxation rate in normal, hypoxic, or hypertrophic myocardium. *Am J Physiol* 258: H191-H197, 1990.
412. **Zile MR, Green GR, Schuyler GT, Aurigemma GP, Miller DC, and Cooper G**. Cardiocyte cytoskeleton in patients with left ventricular pressure overload hypertrophy. *J Am Coll Cardiol* 37: 1080-1084, 2001.
413. **Zile MR, Koide M, Sato H, Ishiguro Y, Conrad CH, Buckley JM, Morgan JP, and Cooper G**. Role of microtubules in the contractile dysfunction of hypertrophied myocardium. *J Am Coll Cardiol* 33: 250-260, 1999.
414. **Zile MR, Richardson K, Cowles MK, Buckley JM, Koide M, Cowles BA, Gharpuray V, and Cooper G**. Constitutive properties of adult mammalian cardiac muscle cells. *Circulation* 98: 567-579, 1998.
415. **Ziolo MT, and Bers DM**. The real estate of NOS signaling: location, location, location. *Circ Res* 92: 1279-1281, 2003.

Chapter 2

Diastolic left ventricular dysfunction and an upward shift of the left ventricular diastolic pressure-volume relationship

Chapter 2.1

Comparative Effects of Pacing-Induced and Balloon Coronary Occlusion Ischemia on Left Ventricular Diastolic Function in Man

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Comparative Effects of Pacing-Induced and Balloon Coronary Occlusion Ischemia on Left Ventricular Diastolic Function in Man

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Background. **Effects of pacing-induced and coronary occlusion myocardial ischemia on left ventricular (LV) function have been compared only in anesthetized dogs. Diastolic properties of the same LV anterior wall segment were therefore compared in 12 patients with single-vessel proximal left anterior descending coronary artery stenosis at rest, immediately after 7±1.2 minutes of pacing, and at the end of a 1-minute balloon occlusion of coronary angioplasty (CO).**

Methods and Results. **Shifts of the diastolic LV pressure–length relation, derived from simultaneous tip-micromanometer LV pressure recordings and digital subtraction LV angiograms, were used as an index of regional diastolic LV distensibility of the anterior wall segment. Immediately after pacing, LV end-diastolic pressure rose from 13.5±3.5 to 23.8±7.0 mm Hg ($p<0.01$ versus at rest) without a significant change of the LV end-diastolic volume index (83.1±18.9 versus 88.4±16.5 ml/m^2), percentage systolic shortening (%SS) of the ischemic segment fell from 40.1±10.6% to 25.2±8.6% ($p<0.01$), and the diastolic LV pressure–radial length (P–RL) plot of the ischemic segment was shifted upward by 7.1±5.0 mm Hg for portions of the plot that overlapped with the diastolic LV P–RL plot at rest. At the end of CO, LV end-diastolic pressure rose to 20.8±7.8 mm Hg ($p<0.01$ versus at rest) and the LV end-diastolic volume index rose to 95.6±16.3 ml/m^2 ($p<0.05$ versus at rest, $p<0.05$ versus after pacing). Ejection fraction and %SS of the ischemic segment fell respectively from 76.6±6.8% to 46.6±11.4% ($p<0.01$ versus at rest, $p<0.01$ versus after pacing) and from 40.1±10.6% to 6.4±8.6% ($p<0.01$ versus at rest, $p<0.01$ versus after pacing). The diastolic LV P–RL plot of the ischemic segment was shifted upward by 3.1±2.3 mm Hg for portions of the plot that overlapped with the diastolic LV P–RL plot at rest. This upward shift at the end of CO was significantly smaller ($p<0.05$) than that immediately after pacing. At the end of CO, a correlation ($p<0.03$) was observed for the ischemic segment between %SS and upward shift of the diastolic LV P–RL plot.**

Conclusions. **The upward shift of the diastolic LV P–RL plot, which was used as an index of decreased regional diastolic LV distensibility, was larger immediately after pacing than at the end of CO. Persistent systolic shortening of ischemic myocardium seems to be a prerequisite for a decrease in diastolic distensibility of the ischemic segment because of the higher %SS of the ischemic segment immediately after pacing, and because of the correlation at the end of CO between the upward shift of the diastolic LV P–RL plot and %SS of the ischemic segment. (*Circulation* 1991;84:211–222)**

Pacing tachycardia in the presence of coronary stenoses and brief coronary occlusion have opposite effects on diastolic properties of ischemic left ventricular myocardium in anesthetized dogs.[1,2] Increased myocardial oxygen demand caused by pacing tachycardia in the presence of coronary stenoses results in an upward shift of the diastolic pressure–segment length or pressure–wall thickness relations of ischemic myocardium.[1] During brief coronary occlusion, the ischemic region shows no change

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or a shift to the right of these relations.[1,3–7] In contrast to these experimental findings, observations in man reveal pacing-induced angina,[8–12] spontaneous angina,[13] and balloon coronary occlusion[14–17] to have similar effects on left ventricular diastolic properties of ischemic myocardium. Under all these conditions, myocardial ischemia results in an upward shift of the diastolic pressure–volume relation,[8–10,13] of the diastolic pressure–wall thickness relation,[11] and of the diastolic pressure–radial length plot.[12,14–16] No previous study has directly compared regional diastolic pressure–radial length relations in the same patient during both pacing-induced ischemia and balloon occlusion of coronary angioplasty. In the present study we therefore investigated diastolic pressure–radial length relations of the same anterior wall segment in patients with single-vessel proximal tight left anterior descending coronary artery (LAD) stenosis immediately after pacing and during balloon occlusion of coronary angioplasty.

Methods

Patients

Twelve patients (10 men, two women; mean age 53.8 [range 42–77] years) are included in this study. All patients had exercise-induced angina in the absence of angina at rest or previous myocardial infarction as evident from their electrocardiogram at rest and normal wall motion on the left ventricular angiogram. In all patients, diagnostic left heart catheterization and coronary angiography revealed normal left ventricular function at rest and single-vessel coronary disease consisting of a significant (>80%) proximal LAD stenosis. There were no visible collaterals to the distal LAD on contralateral coronary injection before balloon inflation. All positively and negatively inotropic drugs were withheld prior to the study, and only vasodilator medication was continued at the time of the procedure. Premedication consisted of 10 mg diazepam. The study was approved by the ethical committee of St. Antonius Hospital, Nieuwegein, The Netherlands, and of the O.L.V. Hospital, Aalst, Belgium. All patients gave informed consent, and there was no complication related to the procedure or study protocol.

Study Protocol

A 7-French pigtail Sentron tip-micromanometer (Cordis Europe, Rooden, The Netherlands) was advanced from the left femoral artery to the left ventricle. The guiding catheter for the angioplasty procedure was advanced from the right femoral artery. The high-fidelity tip-micromanometer left ventricular pressure signal and a left ventricular angiogram derived by digital subtraction ventriculography were simultaneously recorded in the resting state using angiographic frame markers and an injection marker. The first left ventricular angiogram was performed in the 30° right anterior oblique projection and obtained by the injection of 0.5 ml/kg ioxaglate with a low iodine content (160 mg/ml) and low osmolality. Right ventricular pacing was initiated at a rate of 90 beats/min and was increased in a stepwise manner by 30 beats/min every 2 minutes. Pacing was continued until the appearance of angina. The second left ventricular angiogram was obtained immediately upon cessation of pacing (after 7.0±1.2 minutes) during the first 10 beats of restored sinus rhythm. The third left ventricular angiogram was obtained just prior to balloon deflation at the end of a fourth (patients 1–9) or second (patients 10–12) angioplasty balloon inflation of 60 seconds' duration. End-diastolic pressure had returned to the baseline value prior to the pacing stress test or the angioplasty procedure. All patients had chest pain and ischemic ST segment changes during both the pacing stress test and the balloon occlusion of coronary angioplasty. Coronary angioplasty was successful in all 12 patients, with minimal residual coronary stenosis.

Data Acquisition and Analysis

Left ventricular volumes and pressures were matched using cine frame markers. Left ventricular volumes were calculated according to the area–length method and the regression formula of Kennedy et al.[18] A Philips DVI-CV digital radiographic unit (Eindhoven, The Netherlands) acquired all images during held inspiration at 25 frames/sec in a continuous interlaced mode and stored them on ¾-in. U-Matic videotape. An average mask frame was composed from the cardiac cycle before the appearance of contrast. Frame-by-frame analysis was performed on the third to fourth beat after contrast appearance. All nonsinus rhythm or potentiated beats were excluded from the analysis. All the ventriculograms studied had adequate subtraction and contrast opacification. The Philips DVI-CV unit analyzed regional wall motion using the end-diastolic center of mass as a reference point and 28 sectors emerging from the center of mass. Radial length was calculated for each frame as the distance from the center of mass to the endocardial contour of an ischemic and nonischemic sector.[12,16] Percentage systolic shortening was expressed as the ratio of the difference between the end-diastolic and end-systolic radial lengths divided by the end-diastolic radial length. One unblinded investigator manually traced all the ventricular contours. Each individually reported angiographic value is the mean of three measurements. Intraobserver variabilities for left ventricular volume and radial length measurements were 1.5% and 1.6%, respectively. Two time constants of left ventricular pressure decay were derived from the left ventricular tip-micromanometer pressure signal, which was digitized from the moment of minimum left ventricular dP/dt to a left ventricular pressure that equaled left ventricular end-diastolic pressure plus 5 mm Hg. The time constant T_0 was derived from a monoexponential curve fit to a 0 mm Hg asymptote pressure.[19] The time constant T_{PB} was calculated from a monoexponential curve fit to a nonzero asymptote pressure.[20,21]

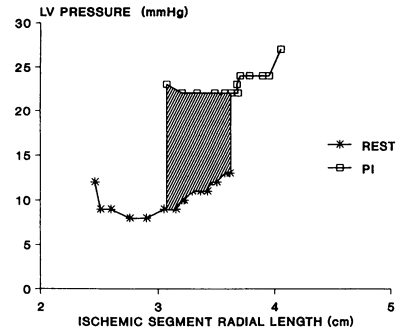

FIGURE 1. *Schematic illustration of quantification of upward shift of diastolic left ventricular (LV) pressure–radial length plot. Planimetry was performed of hatched area enclosed by two LV pressure–radial length plots and by two lines perpendicular to radial length axis at outer borders of radial length zone for which there was overlap between the two LV pressure–radial length plots. This area was subsequently divided by distance between the two perpendicular lines to yield mean pressure value by which overlapping portions of the two diastolic LV pressure–radial length plots had moved upward. This mean pressure value was used to quantify upward shift of diastolic LV pressure–radial length plot during running-induced ischemia (PI) with respect to diastolic LV pressure–radial length plot at rest.*

Diastolic left ventricular pressure–radial length plots of the ischemic segment were constructed by matching corresponding points of left ventricular pressure and radial length of an ischemic sector. Shifts of the diastolic left ventricular pressure–radial length plot during the ischemic episodes were quantified to provide an index of diastolic distensibility of the segment.[22] An upward shift of the diastolic left ventricular pressure–radial length plot was expressed by a mean pressure value over which the overlapping portion of the diastolic left ventricular pressure–radial length plot had moved upward (P_m). P_m was obtained by planimetry of an area enclosed by the two left ventricular pressure–radial length plots and by two lines perpendicular to the radial length axis at the outer borders of a radial length zone for which there was overlap between the two left ventricular pressure–radial length plots (Figure 1). This area was subsequently divided by the distance between the two perpendicular lines to yield P_m.

To assess material properties of the ischemic myocardium, a radial myocardial stiffness modulus for the ischemic segment (E_R) was calculated at rest, during pacing-induced angina, and after 1 minute of balloon coronary occlusion. E_R[1,11,17] was defined as

$$E_R = \Delta\sigma_R/\Delta\epsilon_R = -\Delta P/(\Delta h/h) = -\Delta P/\Delta \ln h$$

The increment in radial stress ($\Delta\sigma_R$) was equal but opposite in sign to the increment in left ventricular pressure at the endocardium, and the increment in radial strain ($\Delta\epsilon_R$) was equal to the increment in wall thickness (Δh) relative to the instantaneous wall thickness (h). Because $\Delta h/h = \Delta \ln h$, E_R was equal to the slope of a P versus ln h plot. The value of h was derived from the instantaneous diastolic ischemic radial length, assuming a constant left ventricular wall mass. E_R values must be compared at a common radial stress or left ventricular pressure level. When comparing E_R at rest with E_R after pacing, a common radial stress level could be defined in six patients. When comparing E_R at rest with E_R at the end of balloon occlusion, a common radial stress level could be defined in four patients. When comparing E_R after pacing with E_R at the end of balloon occlusion, a common radial stress level could be defined in seven patients.

Statistical Analysis

Results ($n=12$) are given as mean±standard deviation. The level of statistical significance was set at $p<0.05$, and the probability value was obtained by Student's t test for paired data and Bonferroni's method of multiple comparison.

Results

Left Ventricular Hemodynamics

Individual values of left ventricular end-diastolic pressure (LVEDP), maximum left ventricular dP/dt, left ventricular end-diastolic volume index (LVEDVI), ejection fraction (EF), heart rate, and left ventricular peak systolic pressure obtained at rest, upon cessation of pacing, and at the end of balloon coronary occlusion just prior to balloon deflation are shown in Table 1.

LVEDP rose from 13.5±3.5 mm Hg at rest to 23.8±7.0 mm Hg immediately following pacing ($p<0.01$) and to 20.8±7.8 mm Hg ($p<0.01$) at the end of balloon coronary occlusion. LVEDVI rose from 83.1±18.9 ml/m^2 at rest to 88.4±16.5 ml/m^2 (difference not significant) immediately following pacing and to 95.6±16.3 ml/m^2 ($p<0.05$ versus at rest and after pacing) at the end of balloon coronary occlusion. EF decreased from 76.7±6.8% at rest to 70.8±8.9% immediately following pacing (difference not significant) and to 46.6±11.4% at the end of coronary occlusion ($p<0.01$ versus at rest and after pacing).

Left Ventricular Hemodynamic Relaxation Indices and Global Diastolic Left Ventricular Properties

Hemodynamic left ventricular relaxation indices measured at rest, immediately following pacing, and at the end of balloon coronary occlusion are shown in Table 2.

LVEDP rose to comparable levels at the end of balloon coronary occlusion and immediately following pacing, but LVEDVI was significantly greater at the end of balloon coronary occlusion than following pacing ($p<0.05$) or at rest ($p<0.05$) (Table 1). These findings are consistent with pacing-induced ischemia causing an upward shift of the end-diastolic left ventricular pressure–volume relation and balloon coronary occlusion ischemia causing an upward and rightward shift of the relation. Two examples (pa-

TABLE 1. Effects of Pacing-Induced Ischemia and of Balloon Coronary Occlusion on Left Ventricular Hemodynamics

	LVEDP (mm Hg)			dP/dt_{max} (mm Hg/sec)			LVEDVI (ml/m^2)		
Pt	Rest	PI	CO	Rest	PI	CO	Rest	PI	CO
1	8	14	14	850	1,100	540	56	67	69
2	12	18	22	1,020	1,120	1,050	102	96	97
3	18	26	20	1,150	1,700	925	75	76	80
4	12	15	26	1,900	2,100	1,400	86	87	103
5	17	23	42	1,700	1,750	1,400	72	103	116
6	15	25	16	1,100	1,300	1,100	61	92	90
7	19	34	26	1,400	1,550	1,300	80	82	88
8	14	14	18	1,700	1,900	1,200	92	79	97
9	9	30	14	2,000	2,000	1,100	122	126	125
10	10	31	19	1,600	1,615	1,200	78	80	86
11	15	27	16	1,150	1,100	1,000	102	102	113
12	13	28	17	1,760	1,760	1,760	71	71	83
Mean±SD	13.5±3.5	23.8±7.0*	20.8±7.8*	1,444.2±381.2	1,582.9±353.9†	1,164.6±298.8†‡	83.1±18.9	88.4±16.5	95.6±16.3†§

	EF (%)			HR (beats/min)			LVPSP (mm Hg)		
Pt	Rest	PI	CO	Rest	PI	CO	Rest	PI	CO
1	83	70	51	66	85	71	75	100	75
2	84	67	50	60	66	70	96	106	103
3	62	72	48	70	80	80	115	124	95
4	83	75	37	60	97	80	150	140	160
5	80	80	44	60	65	65	145	150	130
6	74	77	34	60	75	75	115	130	110
7	71	55	30	67	70	83	150	150	160
8	72	70	59	76	103	103	100	110	125
9	84	87	73	75	83	88	110	140	108
10	78	64	45	78	88	94	156	152	123
11	71	59	43	78	88	89	162	172	165
12	78	74	45	79	89	94	133	167	175
Mean±SD	76.7±6.8	70.8±8.9	46.6±11.4*‡	69.1±7.9	82.4±11.8*	82.7±11.4*	125.6±27.7	136.8±23.3†	127.4±31.5

Pt, patient number; PI, pacing-induced ischemia; CO, coronary occlusion; LVEDP, left ventricular end-diastolic pressure; LVEDVI, left ventricular end-diastolic volume index; EF, ejection fraction; HR, heart rate; LVPSP, left ventricular peak systolic pressure.
*†$p<0.01$, 0.05, respectively, different from rest.
‡§$p<0.01$, 0.05, respectively, different from PI.

tients 9 and 12) of the diastolic left ventricular pressure–volume relation are shown in Figure 2.

Regional Left Ventricular Function

Regional wall motion data of ischemic and nonischemic left ventricular segments at rest, following pacing, and at the end of balloon coronary occlusion are shown in Table 3.

Figures 3, 4, and 5 show diastolic left ventricular pressure–radial length plots of the ischemic segment observed in all 12 patients. Pacing-induced ischemia caused an upward shift of the diastolic left ventricular pressure–radial length relation of the ischemic segment during all of diastole in six patients (Figure 3: patient 1, Figure 4: patient 7, and Figure 5: patients 9–12) and during early-to-mid diastole in four patients (Figure 3: patients 2 and 3 and Figure 4: patients 6 and 8). There was no shift of the diastolic left ventricular pressure–radial length relation in two patients (Figure 3: patient 4 and Figure 4: patient 5). Pacing-induced ischemia caused large reductions (68% and 58%) of segmental shortening in patients 4 and 5, respectively. At the end of balloon coronary occlusion, the diastolic left ventricular pressure–radial length plot was superimposable on the plot at rest in six patients (Figure 3: patient 2; Figure 4: patients 6 and 8; and Figure 5: patients 10, 11, and 12). In six patients (Figure 3: patients 1, 3, and 4; Figure 4: patients 5 and 7; and Figure 5: patient 9), the diastolic left ventricular pressure–radial length plot was shifted upward at the end of balloon coronary occlusion.

The upward shift of the diastolic left ventricular pressure–radial length plot was quantified by P_m. The shift of the diastolic left ventricular pressure–radial length plot was used as an index of regional diastolic distensibility. The higher P_m, the lower regional diastolic distensibility. Following pacing P_m equaled 7.1±5.0 mm Hg, and at the end of balloon coronary occlusion it equaled 3.1±2.3 mm Hg ($p<0.05$). At the

TABLE 2. **Effects of Pacing-Induced Ischemia and of Balloon Coronary Occlusion on Left Ventricular Relaxation Indices**

	dP/dt_{min} (mm Hg/sec)			T_0 (msec)			T_{PB} (msec)		
Pt	Rest	PI	CO	Rest	PI	CO	Rest	PI	CO
1	−900	−950	−630	48	41	58	58	69	117
2	−1,200	−1,060	−1,200	29	41	48	36	46	81
3	−1,200	−1,180	−850	41	56	57	31	44	39
4	−1,750	−1,900	−1,325	28	30	48	16	59	88
5	−1,675	−1,600	−900	49	48	87	40	72	47
6	−950	−1,275	−1,025	52	62	48	63	65	56
7	−1,500	−1,400	−1,250	43	54	86	56	80	121
8	−1,100	−1,450	−1,100	37	33	57	28	26	80
9	−1,600	−1,800	−1,200	34	51	48	39	30	56
10	−2,200	−1,440	−1,500	25	52	68	48	80	106
11	−1,080	−900	−1,150	34	45	52	62	58	59
12	−1,440	−1,440	−1,440	24	24	58	32	38	76
Mean±SD	−1,382.9±382.7	−1,366.3±312.5	−1,130.8±250.2*	37.4±9.8	45.1±11.3	59.8±13.9†‡	42.7±15.0	56.0±18.6*	77.6±27.1†‡

Pt, patient number; PI, pacing-induced ischemia; CO, coronary occlusion; T_0, time constant of left ventricular pressure decay with 0 mm Hg asymptote pressure; T_{PB}, time constant of left ventricular pressure decay with nonzero asymptote pressure.
*†$p<0.05$, 0.01, respectively, different from rest.
‡$p<0.05$ different from PI.

end of balloon coronary occlusion, a correlation was observed between the upward shift of the diastolic left ventricular pressure–radial length plot of the ischemic segment, quantified by P_m, and segmental shortening of the ischemic segment, expressed as a fraction of the value at rest (fSS) ($P_m=7.6\times fSS+1.8$; $r=0.64$, $p<0.03$).

Values for E_R were calculated at rest, following pacing, and at the end of balloon coronary occlusion. For each patient, E_R at rest, E_R following pacing, and E_R at the end of balloon coronary occlusion can be compared only at similar diastolic left ventricular pressures. At a diastolic left ventricular pressure of 10.8±3.0 mm Hg ($n=6$), E_R was comparable at rest (68±55 mm Hg) and following pacing (51±56 mm Hg). At a diastolic left ventricular pressure of 10.0±3.6 mm Hg ($n=4$), E_R was similar at rest (115±81 mm Hg) and at the end of a 1-minute balloon coronary occlusion (121±98 mm Hg). At a diastolic left ventricular pressure of 14.6±5.6 mm Hg ($n=7$), E_R showed a nonsignificant change from 61±50 mm Hg following pacing to 106±113 mm Hg at the end of balloon coronary occlusion.

Discussion

Diastolic Distensibility of Ischemic Myocardium

The initial effect of ischemia on diastolic distensibility of the left ventricle has been the subject of intense research in patients with coronary artery

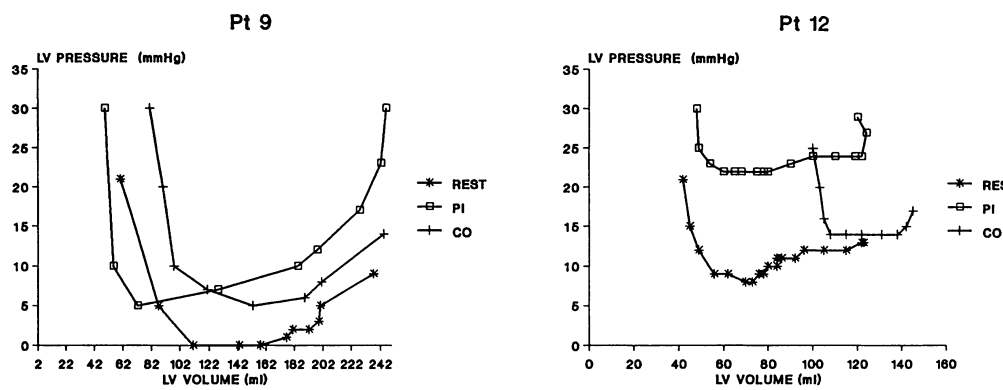

FIGURE 2. *Diastolic left ventricular (LV) pressure–volume relations at rest, during pacing-induced ischemia (PI), and at end of balloon coronary occlusion (CO) in patients 9* (left) *and 12* (right), *representing extremes of responses to CO. In patient 9, systolic shortening was preserved at end of CO; both PI and CO caused diastolic LV pressure–volume relation to shift upward. In patient 12, ejection fraction was greatly reduced at end of CO. Diastolic LV pressure–volume relation during PI was shifted upward, and relation at end of CO was superimposable on terminal portion of diastolic LV pressure–volume relation at rest.*

TABLE 3. Effects of Pacing-Induced Ischemia and of Balloon Coronary Occlusion on Left Ventricular Regional Function

	Ischemic segment						Nonischemic segment					
	EDRL (cm)			%SS			EDRL (cm)			%SS		
Pt	Rest	PI	CO	Rest	PI	CO	Rest	PI	CO	Rest	PI	CO
1	4.4	4.0	4.3	36	28	11	2.3	2.7	2.5	55	59	45
2	4.5	4.7	4.9	25	21	5	2.9	3.1	3.4	58	57	51
3	4.9	5.2	5.2	36	33	4	2.7	2.8	2.1	50	52	47
4	5.9	5.5	5.7	47	15	5	2.5	2.9	2.9	48	66	38
5	4.7	5.2	5.2	57	24	13	3.0	3.0	3.4	70	70	47
6	4.9	5.1	4.8	32	29	12	2.0	2.6	2.7	45	57	38
7	5.6	5.2	5.5	39	25	7	2.7	2.9	2.9	50	46	46
8	4.7	4.7	4.6	29	29	5	2.6	2.5	2.5	46	55	32
9	5.7	5.8	5.5	49	42	26	3.3	3.3	3.4	52	52	60
10	4.0	4.4	4.2	49	28	−3	3.1	3.2	3.0	48	47	35
11	4.9	4.7	4.9	29	9	−2	2.9	3.0	3.2	42	36	40
12	3.7	4.0	4.1	53	19	−6	3.0	2.9	3.0	37	46	34
Mean±SD	4.8±0.7	4.9±0.6	4.9±0.5	40.1±10.6	25.2±8.6*	6.4±8.6*†	2.7±0.4	2.9±0.2‡	2.9±0.4	50.1±8.4	53.6±9.3	42.8±8.1‡§

Pt, patient number; PI, pacing-induced ischemia; CO, coronary occlusion; EDRL, end-diastolic radial length; %SS, percentage systolic shortening.

*‡$p<0.01$, 0.05, respectively, different from rest.

§$p<0.01$, 0.05, respectively, different from PI.

disease. In patients with triple-vessel coronary disease, pacing angina caused an upward shift of the diastolic left ventricular pressure–volume relation[8–10] and an upward shift of the diastolic left ventricular pressure–radial length[12] or diastolic left ventricular pressure–wall thickness[11] relations of the ischemic myocardium. Coronary occlusion during transluminal angioplasty caused a comparable decrease in diastolic distensibility of the ischemic region.[15] Hence, in humans the effects of pacing angina and of brief coronary occlusion on regional diastolic distensibility appear to be similar. No study so far has analyzed diastolic distensibility of the same ischemic region during both pacing angina and balloon occlusion of coronary angioplasty. In the present study, therefore, we compared in the same patient diastolic distensibility of the same anterior wall segment during both pacing-induced ischemia and balloon occlusion of a proximal LAD stenosis. A shift of the diastolic left ventricular pressure–radial length plot was used as an index of regional left ventricular distensibility.

In the present study, LVEDP rose to similar levels during pacing-induced ischemia and at the end of balloon coronary occlusion. LVEDVI remained comparable to resting values during pacing-induced ischemia but rose significantly at the end of a 1-minute balloon coronary occlusion. These findings are consistent with an upward shift of the end-diastolic left ventricular pressure–volume relation during pacing-induced ischemia and an upward but rightward shift of the same relation after 1 minute of balloon coronary occlusion. Regional left ventricular diastolic distensibility of the ischemic segment followed a similar trend when considering pooled patient data: overlapping portions of the diastolic left ventricular pressure–radial length plot shifted upward to a greater extent during pacing-induced ischemia (7.1±5.0 mm Hg) than at the end of a 1-minute balloon coronary occlusion (3.1±2.3 mm Hg, $p<0.05$).

When considering individual patient data (Figures 3, 4, and 5) on diastolic distensibility of the ischemic segment during pacing-induced ischemia and at the end of a 1-minute balloon coronary occlusion, a variable response becomes evident. Pacing-induced ischemia caused an upward shift of the diastolic left ventricular pressure–radial length relation of the ischemic region during all of diastole in six patients and during early-to-mid diastole in four. There was no shift of the diastolic left ventricular pressure–radial length relation in two patients. Following pacing, there was a 34±26% reduction of systolic shortening of the ischemic region. In contrast to previous studies on regional left ventricular diastolic distensibility,[11] the upward shift of the diastolic left ventricular pressure–radial length relation observed in the present study was limited to early and mid diastole in four of 12 patients. Previous reports examined the effects of pacing angina on diastolic left ventricular function in patients with triple-vessel coronary disease,[9–11] whereas we studied patients with single-vessel coronary disease. In patients with triple-vessel coronary disease, pacing-induced angina resulted in an upward shift of the entire diastolic left ventricular pressure–volume or pressure–wall thickness relations. The smaller amount of myocardium at risk[23] in single-vessel coronary disease could explain the limitation to early and mid diastole of the upward shift of the diastolic left ventricular pressure–radial length relation observed in some patients of the present study. In a recent study[24] on conscious dogs with a single-vessel coronary stenosis of the left circumflex artery, a similar upward shift limited to the early portion of the left ventricular diastolic pressure–volume relation was observed during exercise. In two patients (Figure 3:

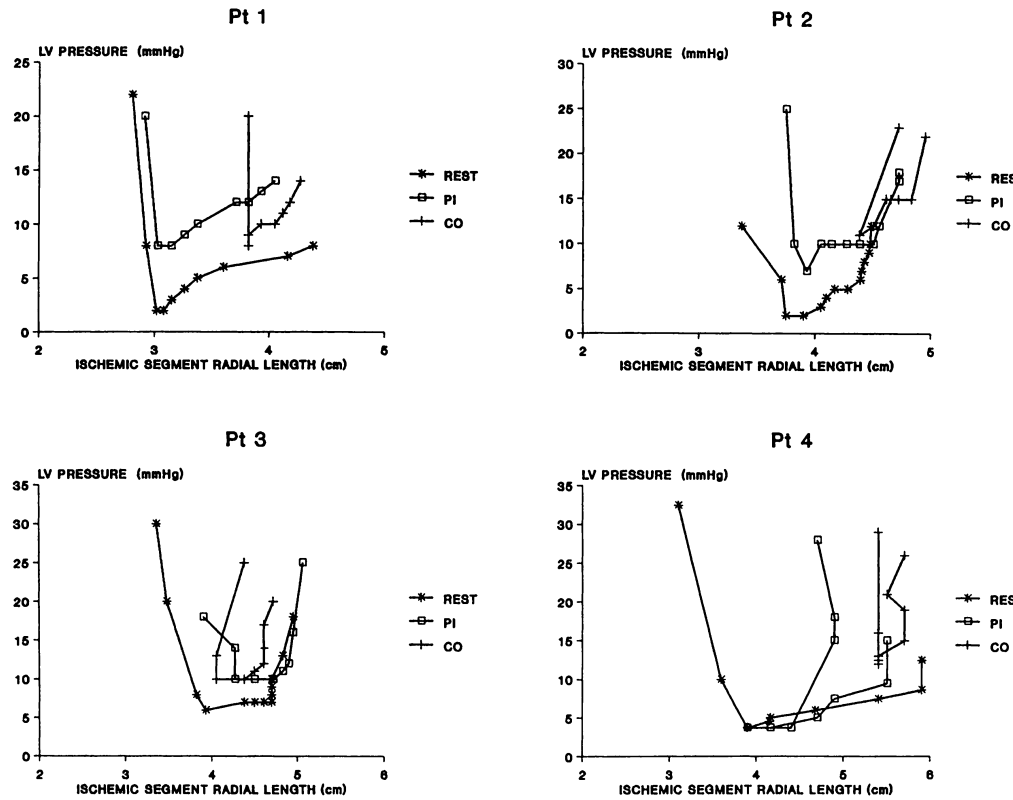

FIGURE 3. *Diastolic left ventricular (LV) pressure–radial length plots at rest, following pacing-induced ischemia (PI), and at end of balloon coronary occlusion (CO) in patients 1–4.*

patient 4 and Figure 4: patient 5) pacing-induced ischemia resulted in a profound depression of systolic performance and no change in regional diastolic distensibility of the ischemic myocardium. One of these patients had an electrocardiographically and scintigraphically strongly positive exercise stress test in the absence of anginal symptoms. A high threshold for anginal pain perception explained the patient's tolerance to prolonged pacing in the present study. In these two patients, in whom systolic performance was severely depressed following pacing, the diastolic left ventricular pressure–radial length plot remained unaltered. In previous experiments on pacing tachycardia in anesthetized dogs with coronary stenoses, a similar relation between systolic performance and diastolic distensibility was observed; when systolic performance was severely depressed the diastolic pressure–radial length relation failed to change.[1]

At the end of balloon coronary occlusion, we observed no change of the diastolic left ventricular pressure–radial length plot of the ischemic segment in six of 12 patients; in the other six patients the diastolic left ventricular pressure–radial length plot was shifted upward. At the end of balloon coronary occlusion, a correlation ($p<0.03$) was observed between the upward shift of the diastolic left ventricular pressure–radial length plot and segmental shortening expressed as a fraction of the value at rest for the ischemic segment. This correlation reconciles some of the contradictory findings on the initial effect of balloon coronary occlusion on diastolic properties of ischemic myocardium. Bertrand et al[17] observed a marked decrease in EF from 72±6% to 46±10% and no significant change in left ventricular diastolic properties during balloon coronary occlusion. In contrast, Wijns et al[15] and Kass et al[25] found smaller depressions of systolic performance during balloon coronary occlusion (a 20% decrease in systolic segmental shortening and a fall in EF from 69±8% to 54±12%, respectively) and reported an upward shift of the diastolic left ventricular pressure–radial length plot of the ischemic zone and an upward shift of the diastolic left ventricular pressure–volume relation. These different responses to balloon coronary occlu-

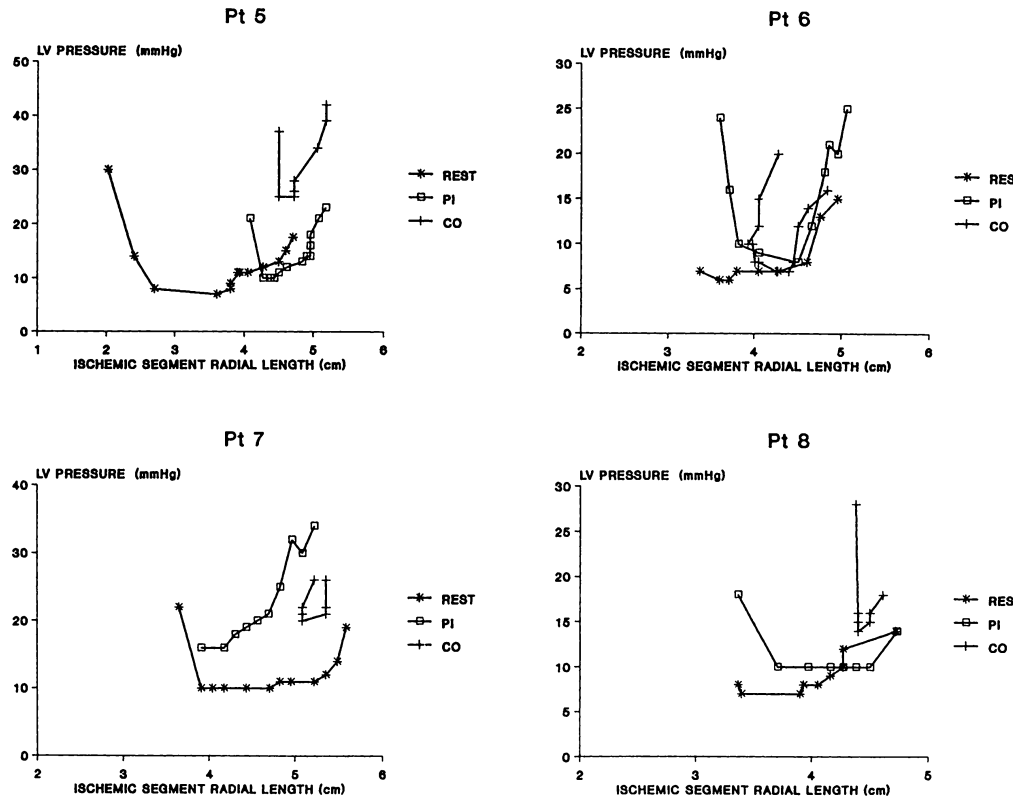

FIGURE 4. *Diastolic left ventricular (LV) pressure–radial length plots at rest, during pacing-induced ischemia (PI), and at end of balloon coronary occlusion (CO) in patients 5–8.*

sion are probably related to the presence of clinical and objective evidence of myocardial ischemia during the balloon inflations. In the present study and in the study by Bertrand et al,[17] all patients had angina and significant ischemic ST segment changes at the time of the measurements. In the present study the balloon inflation time (60 seconds) was longer than in the study by Wijns et al[15] (20 or 50 seconds). In the present study the fourth or second balloon inflation was used, whereas in the study by Wijns et al[15] the balloon inflation during which the left ventricular angiogram was obtained varied from the third to the tenth. Bertrand et al[17] consistently used the first balloon inflation, and despite an inflation time shorter than that in the present study, they observed a similar depression of left ventricular systolic function. A recent study on the reproducibility of sequential balloon inflations,[26] however, found the duration of the first inflation to be unreliable because of a variable degree of occlusion by the deflated balloon preceding the actual first inflation.

Pathophysiological Mechanisms

In the present study, pacing-induced ischemia caused a smaller reduction in systolic left ventricular performance and a larger decrease in global and regional diastolic left ventricular distensibility than 1 minute of balloon coronary occlusion. Several pathophysiological mechanisms could contribute to the difference in regional diastolic function of these two interventions.

Following pacing-induced ischemia, diastolic left ventricular function is assessed during initial relief of the ischemic stress (i.e., upon cessation of pacing) in contrast to following coronary occlusion, when function is assessed at the nadir of the ischemic stress. During experimental exercise or isoproterenol-induced ischemia,[27,28] there is subendocardial hypoperfusion followed by increased blood flow to the ischemic region after the ischemic stress episode, even when residual stenosis permits no change in total coronary blood flow. During the postpacing episode, a similar reactive hyperemia in the ischemic region could contribute to the observed decrease in diastolic

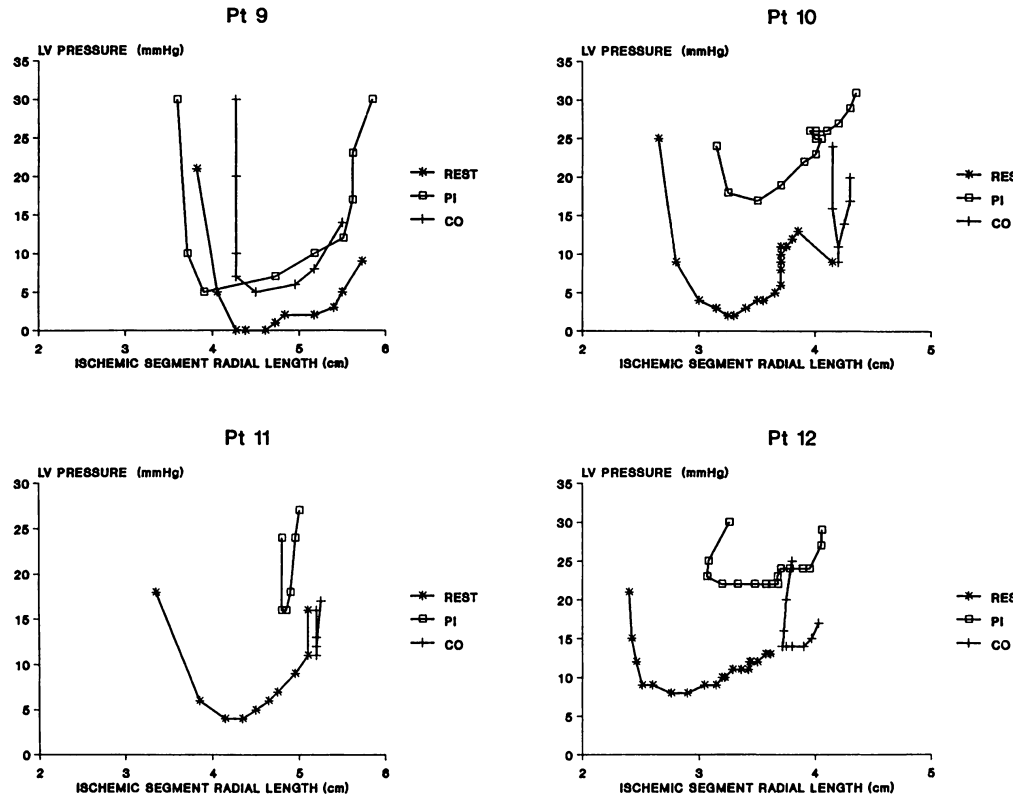

FIGURE 5. *Diastolic left ventricular (LV) pressure–radial length plots at rest, during pacing-induced ischemia (PI), and at end of balloon coronary occlusion (CO) in patients 9–12.*

left ventricular distensibility because of a reperfusion effect.[29] This explanation implies that the decrease of diastolic distensibility of the ischemic region starts immediately upon cessation of pacing and is absent during the pacing episode itself. Studies on left ventricular pressure–volume relations during pacing tachycardia in patients with coronary artery disease, however, revealed a progressive upward shift of the relation that started during the pacing episode.[30]

Absence or presence of tissue metabolites during the initial stages of myocardial ischemia could also modulate the mechanical response of ischemic myocardium.[31,32] Washout of tissue metabolites is more likely to occur during pacing-induced ischemia than during brief coronary occlusion.

Different intensities and durations of ischemia during pacing-induced angina and balloon coronary occlusion could have influenced the different responses of the global left ventricle and of the ischemic segment to both interventions. In a recent study by Applegate et al,[33] postpacing and coronary occlusion ischemia produced similar changes in systolic and diastolic function of the ischemic region when both ischemic stresses resulted in a comparable hemodynamic end point (i.e., LVEDP). The present study did not achieve in each individual patient such equal hemodynamic end points, so unequal degrees of ischemia as a result of the two ischemic stress episodes cannot be excluded. Nevertheless, for the pooled patient data the finding of widely different decreases in left ventricular EF but similar elevations of left ventricular filling pressure is incompatible with unequal severity of ischemia modulating the same type of left ventricular failure and is more likely explained by different types of left ventricular failure during the two interventions. Determination of the adenosine triphosphate and creatine phosphate contents of the ischemic myocardium, such as performed in dogs under similar conditions by Momomura et al,[2] could resolve the issue of unequal intensities of myocardial ischemia during the two interventions.

Study Limitations

Left ventricular volumes and radial lengths of ischemic and nonischemic segments were derived

from single-plane right anterior oblique ventriculograms and a moving reference point angiographic method. These methodological limitations influence regional wall motion measurements during the ischemic episode because of absent visualization of the interventricular septum and because of motion of the center of mass toward the area of akinesia. The latter artifact leads to overestimation of systolic shortening of the ischemic segment, underestimation of systolic shortening of the nonischemic segment, and reduction of an eventual rightward shift of the diastolic left ventricular pressure–radial length relation. This motion of the center of mass at the end of the balloon coronary occlusion probably accounts for the decrease in percentage systolic shortening of the nonischemic segment and for the absence of a rightward shift of the diastolic left ventricular pressure–radial length relations as previously described in animal studies with somewhat longer periods of coronary occlusion.[3–7]

In the present study, diastolic properties of ischemic myocardium were characterized by shifts of the diastolic left ventricular pressure–radial length plot and by E_R. Such a shift of the diastolic left ventricular pressure–radial length relation of the ischemic segment was quantified by P_m, which was no measure of myocardial stiffness or of the slope of the diastolic left ventricular pressure–radial length relation. Material properties of ischemic myocardium were analyzed by E_R, which was compared at common radial stress or left ventricular pressure levels.[1,11,17] There was a trend, although statistically nonsignificant, for a higher E_R at the end of balloon coronary occlusion than at rest and during pacing-induced ischemia. Using the same method a similar trend, which also failed to reach statistical significance, was observed by Bertrand et al[17] during balloon coronary occlusion. In contrast to a previous study by Bourdillon et al,[11] E_R remained unaltered during pacing-induced ischemia. In the present study, however, E_R was derived from instantaneous diastolic left ventricular pressure and not (as in the previous study) from passive diastolic left ventricular pressure. Passive diastolic left ventricular pressure equaled the difference between the instantaneous diastolic left ventricular pressure and the active diastolic left ventricular relaxation pressure, which was extrapolated from the time constant of isovolumic left ventricular pressure decay. Such extrapolation seems questionable because of a recently demonstrated interaction between myocardial reextension, as occurs during left ventricular filling, and residual cardiac muscle force development.[34] A decrease in diastolic distensibility of the myocardium without an increase in E_R, as observed in the present study during pacing-induced ischemia, resembles the increase in cardiac muscle resting tension during reoxygenation contracture.[35] Such an increase in cardiac muscle resting tension occurred without a change in muscle stiffness and was explained by reversible diastolic cross-bridge cycling in the absence of rigor bonds.

In the present study, right ventricular diastolic pressure was not measured. During pacing-induced ischemia and balloon coronary occlusion, left ventricular filling pressures rose to similar values. Reactive pulmonary hypertension and altered right ventricular loading were therefore probably comparable during the two interventions. Eventual biventricular interaction because of pericardial constraint should have had a similar effect on the left ventricular diastolic pressure–volume relations and therefore does not explain the divergent mechanical responses.

In the present study, regional diastolic distensibility during pacing-induced ischemia was investigated in patients with single-vessel LAD coronary stenosis. Most previous studies on left ventricular performance during pacing-induced angina analyzed left ventricular wall motion and hemodynamics in patients with triple-vessel coronary disease. Recently, a study by Dawson and Gibson[36] included patients with single- and double-vessel coronary disease, and another recent study by Yamanishi et al[37] comprised only patients with stable effort angina related to either single-vessel LAD stenosis or single-vessel right coronary artery stenosis. The present results on the effects of pacing-induced ischemia on left ventricular performance are similar to the last two studies and differ from previous studies on patients with multivessel involvement[9–11] insofar that the present study failed to observe significant decreases in global left ventricular systolic performance indices such as maximum left ventricular dP/dt and left ventricular EF during pacing ischemia. In the study by Dawson and Gibson[36] maximum left ventricular dP/dt was also unaltered during pacing ischemia. In their study left ventricular EF changed from 73±7% to 68±8%, which equals the 5% decrease in left ventricular EF observed in the present study. During pacing-induced ischemia in the present study, percentage systolic shortening of the ischemic segment fell from 40.1±10.6% to 25.2±8.6% ($p<0.01$). The decrease in percentage systolic shortening of the ischemic segment observed in the present study exceeded that previously reported during pacing angina in patients with single-vessel LAD stenosis (from 36±6% to 24±8%)[37] and the decrease in anterior wall segmental shortening (from 16.7±2.6% to 12.7±1.5%) observed during pacing ischemia in a two-vessel coronary stenosis animal model.[1,2,38] In the present study, systolic bulging was observed in the three patients (10–12) in whom measurements were obtained at the end of the second balloon coronary occlusion and not in the nine patients (1–9) in whom measurements were obtained at the end of the fourth balloon coronary occlusion. A recent study on the reproducibility of sequential balloon inflations[26] found all inflations except the first one to cause similar ischemic stresses. The observation that systolic performance at the end of the fourth balloon inflation was better preserved than at the end of the second balloon inflation despite similar ischemic stresses could therefore be consistent with myocar-

dial preconditioning to ischemic stress by previous balloon inflations.[39]

Coronary occlusion depressed systolic shortening of the ischemic segment more profoundly than pacing-induced ischemia, even leading in some patients (Table 3, patients 10–12) to systolic bulging and early diastolic recoil. This segmental asynchrony[40,41] observed in patients 10–12 at the end of balloon coronary occlusion could contribute to the greater prolongation of the time constant of left ventricular pressure decay during balloon coronary occlusion than during pacing-induced ischemia.

Coronary occlusion resulted in a variable depression of segmental shortening, probably because of unequal recruitment of collateral flow. Such a recruitment occurred despite the absence of visible collaterals on contralateral coronary injection at the time of diagnostic coronary angiography. This illustrates the need for assessment of collateralization by a quantitative measure such as coronary wedge pressure.[42,43] Such measurement would have facilitated interpretation of regional left ventricular performance during both pacing-induced angina and balloon occlusion of coronary angioplasty. Similarly, measurement of coronary sinus washout of hydrogen and other metabolites could confirm the potential role of tissue metabolites as determinants of systolic performance and diastolic distensibility during the initial stages of myocardial ischemia. Future studies are therefore needed to correlate regional performance of ischemic myocardium with simultaneous measurements of coronary wedge pressure and coronary sinus concentrations of hydrogen and tissue metabolites.

Acknowledgments

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References

1. Paulus WJ, Grossman W, Serizawa T, Bourdillon PD, Pasipoularides A, Mirsky I: Different effects of two types of ischemia on myocardial systolic and diastolic function. *Am J Physiol* 1985;248:H719–H728
2. Momomura SI, Ingwall J, Parker JA, Sahagian P, Ferguson JJ, Grossman W: The relationships of high energy phosphates, tissue pH and regional blood flow to diastolic distensibility in the ischemic dog myocardium. *Circ Res* 1985;57:822–835
3. Theroux P, Franklin D, Ross J Jr, Kemper WS: Regional myocardial function during acute coronary artery occlusion and its modification by pharmacologic agents in the dog. *Circ Res* 1974;35:896–908
4. Tyberg JV, Forrester JS, Wyatt HL, Goldner S, Parmley WW, Swan HJC: An analysis of segmental ischemic dysfunction utilizing the pressure–length loop. *Circulation* 1974;49: 748–754
5. Theroux P, Ross J Jr, Franklin D, Kemper WS, Sasayama S: Regional myocardial function in the conscious dog during acute coronary occlusion and responses to morphine, propranolol, nitroglycerin, and lidocaine. *Circulation* 1976;53:302–314
6. Wong BYS, Toyama M, Reis RL, Doodyer AVN: Sequential changes in left ventricular compliance during acute coronary occlusion in the isovolumic working canine heart. *Circ Res* 1978;43:274–286
7. Hess OM, Osakada G, Lavelle JF, Gallagher KP, Kemper WS, Ross J Jr: Diastolic myocardial wall stiffness and ventricular relaxation during partial and complete coronary occlusions in the conscious dog. *Circ Res* 1983;52:387–400
8. Dwyer EM Jr: Left ventricular pressure–volume alterations and regional disorders of contraction during myocardial ischemia induced by atrial pacing. *Circulation* 1970;42:1111–1122
9. Barry WH, Brooker JZ, Alderman EL, Harrison DC: Changes in diastolic stiffness and tone of the left ventricle during angina pectoris. *Circulation* 1974;49:255–263
10. Mann T, Brodie BR, Grossman W, McLaurin LP: Effect of angina on the left ventricular diastolic pressure–volume relationship. *Circulation* 1977;55:761–766
11. Bourdillon PD, Lorell BH, Mirsky I, Paulus WJ, Wynne J, Grossman W: Increased regional myocardial stiffness of the left ventricle during pacing-induced angina in man. *Circulation* 1983;67:316–323
12. Sasayama S, Nonogi H, Miyazaki S, Sakurai T, Kawai C, Eiho S, Kuwahara M: Changes in diastolic properties of the regional myocardium during pacing-induced ischemia in human subjects. *J Am Coll Cardiol* 1985;5:599–606
13. Sharma B, Behrens TW, Erlein D, Hodges M, Asinger RW, Francis GS: Left ventricular diastolic properties and filling characteristics during spontaneous angina pectoris at rest. *Am J Cardiol* 1983;52:704–709
14. Serruys PW, Wijns W, Van den Brand M, Meij S, Slager C, Schuurbier CH, Hugenholtz P, Brower RW: Left ventricular performance, regional blood flow, wall motion and lactate metabolism during transluminal angioplasty. *Circulation* 1984; 70:25–36
15. Wijns W, Serruys PW, Slager CJ, Grimm J, Krayenbuehl HS, Hugenholtz P, Hess OM: Effect of coronary occlusion during percutaneous transluminal angioplasty in humans on left ventricular chamber stiffness and regional diastolic pressure–radius relations. *J Am Coll Cardiol* 1986;7:455–463
16. Carlson EB, Hinohara T, Morris KG: Recovery of systolic and diastolic left ventricular function after a 60-second coronary arterial occlusion during percutaneous transluminal coronary angioplasty for angina pectoris. *Am J Cardiol* 1987;60:460–466
17. Bertrand ME, Lablanche JM, Fourrier JL, Traisnel G, Mirsky I: Left ventricular systolic and diastolic function during acute coronary artery balloon occlusion in humans. *J Am Coll Cardiol* 1988;12:341–347
18. Kennedy JW, Trenholme SE, Kasser IS: Left ventricular volume and mass from single-plane cineangiocardiogram: A comparison of anteroposterior and right anterior oblique methods. *Am Heart J* 1970;80:343–352
19. Weiss JL, Frederiksen JW, Weisfeldt ML: Hemodynamic determinants of the time course of fall in canine left ventricular pressure. *J Clin Invest* 1970;58:751–760
20. Caroll JD, Hess OM, Hirzel HO, Krayenbuehl HP: Exercise-induced ischemia: The influence of altered relaxation on early diastolic pressures. *Circulation* 1983;67:521–528
21. Mirsky I: Assessment of diastolic function: Suggested methods and future considerations. *Circulation* 1984;69:836–841
22. Grossman W: Relaxation and diastolic distensibility of the regionally ischemic left ventricle, in Grossman W, Lorell BH (eds): *Diastolic Relaxation of the Heart.* Boston, Martinus Nijhoff Publishing, 1988, pp 193–203
23. McKay RG, Aroesty JM, Heller GV, Silverman KJ, Parker AJ, Als AV, Come PC, Kolodny GM, Grossman W: The pacing stress reexamined: Correlation of pacing-induced hemodynamic changes with the amount of myocardium at risk. *J Am Coll Cardiol* 1984;3:1469–1481
24. Miyazaki S, Guth BD, Miura T, Indolfi C, Schulz R, Ross J Jr: Changes of left ventricular diastolic function in exercising dogs without and with ischemia. *Circulation* 1990;81:1058–1070
25. Kass DA, Midei M, Brinker J, Maughan WL: Influence of coronary occlusion during PTCA on end-systolic and end-

diastolic pressure–volume relations in humans. *Circulation* 1990;81:447–460
26. Perry RA, Seth A, Hunt A, Smith SCH, Westwood E, Woolgar N, Shiu MF: Balloon occlusion during coronary angioplasty as a model of myocardial ischemia: Reproducibility of sequential inflations. *Eur Heart J* 1989;10:791–800
27. Gallagher KP, Osakada G, Matsuzaki M, Kemper WS, Ross J Jr: Myocardial blood flow and function with critical coronary stenosis in exercising dogs. *Am J Physiol* 1982;243:H698–H707
28. Gallagher KP, Kumada T, Battler A, Kemper WS, Ross J Jr: Isoproterenol-induced myocardial dysfunction in dogs with coronary stenoses. *Am J Physiol* 1982;242:H260–H267
29. Ross J Jr: Is there a true increase in myocardial stiffness with acute ischemia? *Am J Cardiol* 1989;63:87E–91E
30. Aroesty JM, McKay RG, Heller GV, Royal HD, Als AV, Grossman W: Simultaneous assessment of left ventricular systolic and diastolic dysfunction during pacing-induced ischemia. *Circulation* 1985;71:889–900
31. Apstein CS, Grossman W: Opposite initial effects of supply and demand ischemia on left ventricular diastolic compliance: The ischemia-diastolic paradox. *J Mol Cell Cardiol* 1987;19: 119–128
32. Paulus WJ: Upward shift and outward bulge: The divergent myocardial effects of pacing angina and of brief coronary occlusion. *Circulation* 1990;81:1436–1439
33. Applegate RJ, Walsh RA, O'Rourke RA: Comparative effects of pacing-induced and flow limited ischemia on left ventricular function. *Circulation* 1990;81:1380–1392
34. Sys SU, Paulus WJ, Claes VA, Brutsaert DL: Post-reextension force decay of relaxing cardiac muscle. *Am J Physiol* 1987;253: H256–H261
35. Lewis MJ, Housmans PR, Claes VA, Brutsaert DL, Henderson AH: Myocardial stiffness during hypoxic and reoxygenation contracture. *Cardiovasc Res* 1980;14:339–344
36. Dawson JR, Gibson DG: Left ventricular filling and early diastolic function at rest and during angina in patients with coronary artery disease. *Br Heart J* 1989;61:248–257
37. Yamanishi K, Fujita M, Sasayama S, Nakajima H, Asanoi H, Ohno A: Functional characteristics of nonischemic region during pacing-induced myocardial ischemia in angina pectoris. *Am J Cardiol* 1988;61:1214–1218
38. Serizawa T, Carabello BA, Grossman W: Effect of pacing-induced ischemia on left ventricular diastolic pressure–volume relations in dogs with coronary stenoses. *Circ Res* 1980;46: 430–439
39. Deutsch E, Berger M, Kussmaul WG, Hirschfeld JW, Herrmann HC, Laskey WK: Adaptation to ischemia during percutaneous transluminal coronary angioplasty. *Circulation* 1990; 82:2044–2051
40. Kumada T, Karliner JS, Pouleur H, Gallagher KP, Shirato K, Ross J Jr: Effects of coronary occlusion on early ventricular diastolic events in conscious dogs. *Am J Physiol* 1979;237: 542–549
41. Aoyagi T, Lizuka M, Takahashi T, Ohya T, Serizawa T, Momomura S, Sato H, Mochizuki T, Matsui H, Ikenouchi H, Ma Y, Sugimoto T: Wall motion asynchrony prolongs time constant of left ventricular relaxation. *Am J Physiol* 1989;257: H883–H890
42. Meier B, Leuthy P, Finci L, Steffinino GD, Rutishauser W: Coronary wedge pressure in relation to spontaneously visible and recruitable collaterals. *Circulation* 1987;75:906–913
43. De Bruyne B, Meier B, Finci L, Urban P, Rutishauser W: Potential protective effect of high coronary wedge pressure on left ventricular function after coronary occlusion. *Circulation* 1988;78:566–572

KEY WORDS • coronary angioplasty • myocardial ischemia • pacing angina • diastolic function

Chapter 2.2

Comparative Effects of Ischemia and Hypoxemia on Left Ventricular Systolic and Diastolic Function in Humans

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Comparative Effects of Ischemia and Hypoxemia on Left Ventricular Systolic and Diastolic Function in Humans

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Background. **During the initial phase of an ischemic insult, left ventricular (LV) performance depends on the complex interaction between oxygen deprivation, vascular turgor, and accumulation of metabolites. In experimental preparations, low-flow ischemia decreases systolic shortening and increases diastolic LV distensibility, whereas pacing-induced ischemia or hypoxic perfusion produces smaller decreases in systolic shortening but decreases LV diastolic distensibility. The purpose of this study was to investigate the different effects of low-flow ischemia, pacing-induced ischemia, and hypoxemic perfusion on LV performance in humans.**

Methods and Results. **In 20 patients with a significant stenosis in the left anterior descending coronary artery, micromanometer-tip LV pressure recordings (n=20), LV angiography (n=18), and coronary sinus blood sampling (n=11) were obtained at rest and during the following conditions: pacing-induced ischemia (PI) (n=11), low-flow ischemia of balloon coronary occlusion (CO) (n=20), and hypoxemia induced by balloon coronary occlusion with hypoxemic perfusion distal to the occlusion (CO+P) (n=11). LV stroke work index fell from 75±17 g · m at rest to 43±14 g · m at the end of CO (n=18; $P<.001$). In addition, LV stroke work index was lower at the end of CO than during PI (50±11 vs 77±15 g · m; n=11; $P<.002$) and was lower at the end of CO than at the end of CO+P (35±7 vs 46±9 g · m; n=9; $P<.02$). LV end-diastolic pressure rose from 16±5 mm Hg at rest to 23±6 mm Hg at the end of CO (n=20; $P<.001$). However, LV end-diastolic pressure was lower at the end of CO than during PI (20±5 vs 30±5 mm Hg; n=11; $P<.002$) and was lower at the end of CO than at the end of CO+P (26±5 vs 34±7 mm Hg; n=11; $P<.01$). LV end-diastolic volume index increased from 75±14 mL/m^2 at rest to 79±15 mL/m^2 at the end of CO (n=18; $P<.05$). Left ventricular end-diastolic volume index increased to values similar to those for CO during PI (79±13 mL/m^2; n=11; P=NS) and at the end of CO+P (78±14 mL/m^2; n=9; P=NS). Higher values of LV end-diastolic pressure and unchanged values of LV end-diastolic volume index for PI and CO+P, compared with CO, suggested a lower end-diastolic LV distensibility during PI and during hypoxemia, as compared with low-flow ischemia. Upward shifts of individual diastolic LV pressure-volume curves during PI (9 of 11 patients) and at the end of CO+P (7 of 9 patients), compared with CO, were also consistent with lower LV diastolic distensibility during pacing-induced ischemia and during hypoxemia, compared with low-flow ischemia. Coronary sinus lactate, H^+, and K^+ levels increased after balloon deflation (CO and CO+P) and during pacing (PI).**

Conclusions. **Thus, during low-flow ischemia, LV systolic performance was lower and LV diastolic distensibility larger than during pacing-induced ischemia or hypoxemia. The variable response of the human myocardium to different types of ischemia was probably related to the degree of vascular turgor and accumulation of tissue metabolites.** *Circulation* **1993;88:461-471)**

KEY WORDS • ischemia • hypoxia • angioplasty • diastole

Left ventricular performance during the initial phase of an ischemic insult results from a complex interaction between oxygen deprivation, accumulation of metabolites, and vascular turgor. These interactions have been investigated primarily in isolated, isovolumically beating, and retrogradely perfused guinea pig, rat, and rabbit hearts.[1] In the buffer-perfused guinea pig and rabbit heart,[2,3] a switch from aerobic to hypoxic perfusate induced a rise in resting tension and a progressive decline in developed tension. In the blood-perfused rabbit heart, a reduction in coronary perfusion pressure caused a rise in resting tension when pacing tachycardia was superimposed on the reduction in coronary perfusion pressure.[4] In the rat heart, a complete interruption of coronary flow produced a fall in resting tension and a faster loss in developed tension than during hypoxic perfusion.[5]

To investigate in humans these disparate initial left ventricular effects of different types of ischemia, the present study compared, in the same patients, left

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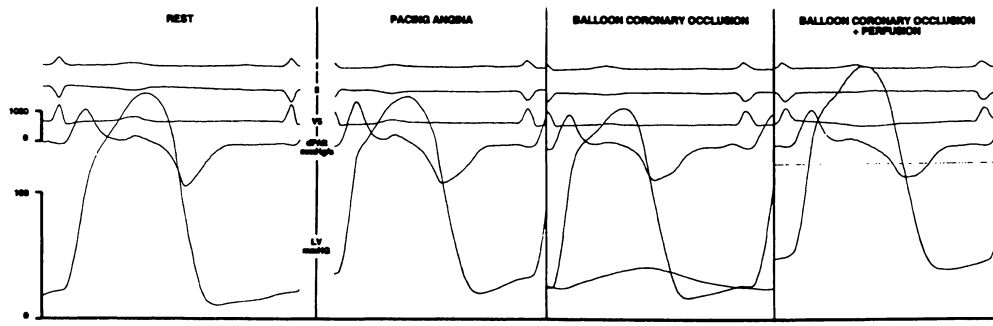

FIG 1. *Tracings of (from left to right) two surface leads (I and II) and one precordial lead (V_5) ECG, the left ventricular dP/dt signal, and the left ventricular tip-micromanometer pressure recording at rest, at cessation of pacing during pacing-induced angina, at the end of a regular balloon coronary occlusion, and at the end of an equally long balloon coronary occlusion with distal hypoxic perfusion (Tables 1 and 2, patient 10). Left ventricular diastolic pressures were higher during pacing angina and at the end of balloon coronary occlusion with distal hypoxic perfusion than at the end of the regular balloon coronary occlusion.*

ventricular performance during balloon coronary occlusion (low-flow ischemia), during pacing-induced angina (low-flow, high-demand ischemia), and/or during balloon coronary occlusion with maintained hypoxemic perfusion distal to the balloon occlusion (hypoxemia).

Methods

Patients

Twenty patients (15 men, 5 women; mean age, 57 years; range, 42 to 72 years) were included in this study. All patients had exercise-induced angina and a clinically and electrocardiographically positive exercise stress test. There was no evidence of previous myocardial infarction on the ECG at rest or on the baseline left ventricular angiogram. Diagnostic left heart catheterization and coronary angiography revealed normal baseline global and regional left ventricular function and single-vessel coronary artery disease consisting of a significant proximal left anterior descending coronary artery (LAD) stenosis. Percent diameter and percent area of the proximal LAD stenosis, calculated by electronic caliper technique, were 67±8% and 88±5%, respectively. There were no visible collaterals to the distal LAD on contralateral coronary injection. Long-acting nitrates, β-blockers, and calcium entry blockers were withheld at least 24 hours before the procedure except in two patients who had experienced angina during mild exercise during the 48-hour period preceding hospital admission. Premedication consisted of 10 mg diazepam. The study protocol was approved by the ethical committee of the O.L.V. Ziekenhuis, Aalst (Belgium). All patients gave informed consent, and there was no complication related to procedure or study protocol.

Catheterization Protocol

In all patients, a 7F pigtail Sentron tip-micromanometer (Cordis Europe, Rooden, The Netherlands) was advanced from the left femoral artery to the left ventricle, and an 8F angioplasty guiding catheter was advanced from the right femoral artery. All pressures were referenced to atmospheric pressure at the level of the midchest. The tip-micromanometer catheter was calibrated externally against a mercury reference and matched against luminal pressure. A left ventricular dP/dt signal was derived from the high-fidelity left ventricular pressure signal with an electronic differentiator. The pressure signals, the left ventricular dP/dt signal, and three leads of the ECG were recorded on a Gould ES 1000 multichannel recorder (Fig 1). In patients 1 through 11, a 7F NIH catheter was positioned in the coronary sinus from a left antecubital vein or from the right femoral vein, and its position was confirmed by injection of contrast agent. During angioplasty balloon inflation, the coronary wedge pressure, which was measured through the fluid-filled lumen of the balloon catheter,[6,7] was 29±11 mm Hg (range, 11 to 45 mm Hg), and the mean pressure difference between coronary wedge pressure and left ventricular end-diastolic pressure at the end of the balloon coronary occlusion was 8±6 mm Hg (range, 3 to 15 mm Hg). All left ventricular angiograms were performed on conventional cine film at 50 or 25 frames per second in 30° right anterior oblique projection by injection of 45 mL ioxaglate over a period of 3 seconds. An angiographic frame marker was used to match the high-fidelity left ventricular pressure recording and the left ventricular angiogram.

Comparative effects of pacing ischemia and balloon coronary occlusion. In 11 patients (Tables 1 and 2, patients 1 through 11), the effects of pacing-induced ischemia and balloon coronary occlusion ischemia on left ventricular function and washout of tissue metabolites were investigated. After baseline left ventricular angiogram and simultaneous left ventricular tip-micromanometer pressure recordings, left ventricular filling pressures were allowed to return to control level. Subsequently, right ventricular pacing was initiated at a rate of 120 beats per minute and was increased stepwise by 20 beats per minute every 2 minutes. Pacing was continued for a total pacing period of 6 minutes and stopped prematurely only if the patient experienced severe angina. In two patients, pacing was continued for more than 6 minutes and maintained at a rate of 160 beats per minute for an additional 3- or 4-minute period until appearance of chest pain. Immediately upon cessation of pacing, a second left ventricular angiogram

TABLE 1. Effects of Pacing-Induced Angina, Balloon Coronary Occlusion, and Balloon Coronary Occlusion With Distal Perfusion on Left Ventricular Systolic Function

	LVPSP (mm Hg)				LVEF (%)				LV dP/dt_{max} (mm Hg/s)				LVSWI (g·m)			
Patient	R	PI	CO	CO+P	R	PI	CO	CO+P	R	PI	CO	CO+P	R	PI	CO	CO+P
1	156	152	123	...	78	64	45	...	1600	1615	1200	...	87	75	50	...
2	162	172	165	...	71	59	43	...	1150	1180	1000	...	114	104	75	...
3	133	167	175	...	78	74	45	...	1760	1760	1600	...	73	79	59	...
4	197	225	161	...	68	65	44	...	1480	1520	1200	...	77	87	38	...
5	175	158	160	...	61	57	53	...	1120	1100	1100	...	54	48	44	...
6	152	155	159	...	72	54	51	...	1400	1600	1300	...	68	59	58	...
7	140	162	150	...	77	71	45	...	1200	1200	900	...	76	92	51	...
8	153	173	156	...	69	59	41	...	1880	2200	1440	...	76	80	56	...
9	138	177	99	...	57	47	39	...	1100	1400	800	...	60	61	34	...
10	187	183	173	190	59	47	29	27	1500	1700	1250	1350	102	79	43	54
11	162	195	155	142	61	57	34	42	1100	1150	850	900	83	85	43	52
12	126	...	132	150	...	...	...	...	925	...	950	1050	...	...	...	...
13	162	...	138	162	63	...	21	31	1700	...	1250	1250	84	...	24	46
14	134	...	152	134	65	...	29	50	1500	...	1250	1100	44	...	24	41
15	142	...	145	192	73	...	40	39	1400	...	1400	1750	66	...	39	45
16	150	...	160	178	72	...	36	37	1600	...	1840	2040	74	...	39	56
17	152	...	135	145	67	...	38	27	1350	...	1150	1050	69	...	30	25
18	176	...	161	130	60	...	35	46	1600	...	1450	1250	54	...	31	45
19	150	...	117	165	...	...	...	...	1200	...	900	1300	...	...	...	...
20	157	...	152	176	67	...	25	29	1100	...	1000	900	90	...	31	51
Mean ±SD	155 ±18	174 ±20	148 ±19	160 ±21	68 ±7	59 ±9*†	39 ±9†	36 ±8†	1383 ±263	1493 ±317*	1191 ±268†	1267 ±335	75 ±17	77 ±15*	43 ±14†	46 ±9†‡

LVPSP, left ventricular peak systolic pressure; LVEF, left ventricular ejection fraction; LV dP/dt_{max}, maximum left ventricular dP/dt; LVSWI, left ventricular stroke work index; R, rest; PI, pacing-induced ischemia; CO, balloon coronary occlusion ischemia; CO+P, balloon coronary occlusion ischemia with distal perfusion.

*$P<.01$ vs CO; †$P<.01$ vs R; ‡$P<.02$ vs CO.

and simultaneous left ventricular tip-micromanometer pressure recordings were obtained. To allow diastolic left ventricular pressures to return to control level, a 15-minute time interval separated the end of the pacing run from the beginning of the angioplasty procedure. A third left ventricular angiogram and simultaneous left ventricular tip-micromanometer pressure recordings were obtained at the end of the second angioplasty balloon inflation of 60-second duration. All patients experienced chest pain and ischemic ST segment changes at the end of the balloon coronary occlusion. Coronary angioplasty was successful in all patients with minimal residual coronary stenosis.

Comparative effects of pacing ischemia, balloon coronary occlusion, and balloon coronary occlusion with distal perfusion. In two patients (Tables 1 and 2, patients 10 and 11), the effects of pacing-induced ischemia, balloon coronary occlusion, and balloon coronary occlusion with distal perfusion on left ventricular function and washout of tissue metabolites were investigated. After the pacing stress test, the first two balloon coronary occlusions and return of left ventricular filling pressures to control level, a third balloon coronary occlusion of the same duration as the second balloon coronary occlusion (60 seconds) was performed. During this third balloon coronary occlusion, saline was perfused through the distal lumen of the balloon catheter. To mimic resting LAD coronary artery flow, the flow rate was set at 1 mL/s. This value was based on previous measurements in humans of great cardiac vein flow (44±4 mL/min[8]; 69±17 mL/min[9]) or of LAD graft flow in the presence of an occluded anterior descending artery (49±5 mL/min[8]). As the saline solution equilibrated with room air in the perfusion pump, the amount of oxygen dissolved in the saline solution was calculated to equal ±5 mL/L, or approximately 38 times less than the amount of oxygen contained in arterial blood at 95% saturation. Hence, a saline infusion at 1 mL/s corresponded to hypoxemic conditions as it approximated normal LAD flow and as it resulted in an oxygen delivery 38 times lower than normal. At the end of this third balloon coronary occlusion, a left ventricular angiogram and simultaneous left ventricular pressure recordings were obtained. Both patients experienced more severe chest pain during the balloon occlusion with distal perfusion than during the regular first two balloon occlusions.

Comparative effects of balloon coronary occlusion and balloon coronary occlusion with distal perfusion. In nine patients (Tables 1 and 2, patients 12 through 20), the effects of balloon coronary occlusion and balloon coronary occlusion with distal perfusion on left ventricular function were investigated. After baseline recordings and a first angioplasty balloon inflation, a second angioplasty balloon inflation with distal perfusion was performed. At the time of chest pain, a second left ventricular angiogram and simultaneous left ventricular pressure recordings were obtained. After return of left ventricular pressure to baseline value, a regular third angioplasty balloon inflation of the same duration as the

TABLE 2. Effects of Pacing-Induced Angina, Balloon Coronary Occlusion, and Balloon Coronary Occlusion With Distal Perfusion on Left Ventricular Diastolic Function

	LVEDP (mm Hg)				LVMDP (mm Hg)				LVEDVI (mL/m^2)				LV dP/dt$_{min}$ (mm Hg/s)				T_0 (ms)			
Patient	R	PI	CO	CO+P	R	PI	CO	CO+P	R	PI	CO	CO+P	R	PI	CO	CO+P	R	PI	CO	CO+P
1	10	31	19	...	1	16	8	...	78	80	86	...	2200	1440	1500	...	25	52	68	...
2	15	27	16	...	3	14	10	...	102	102	113	...	1080	900	1150	...	34	45	52	...
3	13	28	17	...	9	21	14	...	71	71	83	...	1440	1440	1440	...	24	24	58	...
4	22	37	19	...	10	19	9	...	63	64	63	...	1760	1600	1500	...	46	59	64	...
5	9	33	13	...	2	21	10	...	58	55	59	...	1500	1400	1440	...	49	63	50	...
6	17	20	21	...	8	12	15	...	67	74	75	...	1850	1800	1800	...	39	40	43	...
7	11	23	14	...	5	9	7	...	73	80	76	...	1220	1400	1200	...	42	42	52	...
8	22	31	26	...	6	17	8	...	80	80	85	...	1920	2400	1600	...	35	34	62	...
9	16	28	17	...	2	11	8	...	76	75	82	...	1150	1200	800	...	45	47	58	...
10	23	35	29	45	12	22	19	42	99	96	94	96	2000	1600	1200	1200	48	56	53	82
11	16	35	24	30	7	24	13	21	88	86	94	84	1600	1350	950	1000	42	63	69	70
12	12	...	26	41	3	...	15	32	...	...	...	...	1300	...	900	775	27	...	75	79
13	15	...	25	36	2	...	14	24	85	...	81	87	1950	...	1375	1375	40	...	46	79
14	17	...	38	36	8	...	20	19	59	...	59	62	1475	...	1475	1150	39	...	55	69
15	17	...	23	34	5	...	13	23	65	...	67	62	1700	...	1650	1650	42	...	46	57
16	25	...	32	38	10	...	21	28	71	...	80	83	1600	...	1640	1640	56	...	64	73
17	10	...	17	17	3	...	10	15	70	...	62	67	1750	...	1250	1000	40	...	61	60
18	18	...	24	29	9	...	12	22	53	...	65	64	2500	...	1600	1350	30	...	44	69
19	10	...	24	34	7	...	15	27	...	...	...	...	1450	...	1000	1250	44	...	57	80
20	24	...	25	37	15	...	18	33	91	...	97	95	1850	...	1500	1600	65	...	82	89
Mean	16	30	23	34	6	17	13	26	75	79	79	78	1665	1503	1349	1272	41	48	58	73
±SD	±5	±5*†	±6†	±7*†	±4	±5*†	±4†	±8*†	±14	±13	±15‡	±14	±358	±377	±280†	±286†	±10	±12	±10†	±10†§

LVEDP, left ventricular end-diastolic pressure, LVMDP, left ventricular minimum diastolic pressure; LVEDVI; left ventricular end-diastolic volume index; LV dP/dt$_{min}$, minimum left ventricular dP/dt; T_0, time constant of left ventricular pressure decay with zero asymptote; R, rest; PI, pacing-induced ischemia; CO, balloon coronary occlusion ischemia; CO+P, balloon coronary occlusion ischemia with distal perfusion.

*$P<.01$ vs CO; †$P<.01$ vs R; ‡$P<.05$ vs R; §$P<.02$ vs CO.

second inflation was performed. At the end of this third angioplasty balloon inflation, a third left ventricular angiogram and simultaneous tip-micromanometer left ventricular pressure recordings were obtained. Patients 10 and 11 had experienced the most severe chest pain during the balloon occlusion with distal perfusion. To minimize discomfort for patients 12 through 20, the balloon occlusion with distal perfusion preceded the regular balloon occlusion during which left ventricular function was measured, and instead of having a preset 60-second duration of the balloon occlusion with distal perfusion, its duration was made variable and lasted until the occurrence of chest pain (after 51±12 seconds). The regular balloon occlusion, during which left ventricular function was measured, followed the balloon occlusion with distal perfusion and lasted equally long (51±12 seconds).

Data Analysis

Hemodynamic data. All hemodynamic data (Tables 1 and 2) were averaged throughout a complete respiratory cycle. The time constants of left ventricular pressure decay (T_0 and T_{PB}) were derived from exponential curve fits with zero and variable (PB) asymptote pressures to the digitized left ventricular pressure data points of isovolumic left ventricular relaxation.[10] Pressure data points were obtained at 3-milliseconds intervals by digitizing the left ventricular pressure signal from the moment of left ventricular dP/dt$_{min}$ to a time at which left ventricular pressure equaled left ventricular end-diastolic pressure plus 5 mm Hg.

Angiographic data. Left ventricular volumes were calculated from single-plane left ventricular cineangiograms performed in 30° right anterior oblique projection using the area-length method and a regression equation.[11] Frame-by-frame analysis was performed on the third to fourth beat after contrast appearance, and nonsinus and potentiated beats were excluded from the analysis. In two patients (Tables 1 and 2, patients 12 and 19), no volumetric data were reported because of ventricular premature beats on one of the left ventricular angiograms. Left ventricular pressure-volume plots were constructed by matching corresponding points of left ventricular pressure and volume using the cine frame marker. Left ventricular stroke work index (Table 1) was calculated as the product of stroke volume index and mean systolic left ventricular pressure. Each individually reported angiographic value (Tables 1 and 2) is the average of three measurements (intraobserver variability for left ventricular volume, 1.5%).

Metabolic data. Blood samples were obtained from the coronary sinus and from the femoral artery before pacing, during the last 15 seconds of each pacing step, and 10, 30, and 120 seconds after cessation of pacing (Tables 1 and 2, patients 1 through 11). Similar samples were obtained before balloon inflation, at the end of the balloon inflation period, and 10, 30, and 120 seconds after deflation of the balloon (Tables 1 and 2, patients 1

through 11) and before balloon inflation with distal perfusion, at the end of the balloon inflation period with distal perfusion, and 10, 30, and 120 seconds after deflation of the balloon and cessation of the distal perfusion (Tables 1 and 2, patients 10 and 11). The timing of sampling was based on previously reported K^+ concentration measured by catheter electrode in the coronary sinus during and after balloon inflations of coronary angioplasty.[12] On each blood sample, pH and K^+ and lactate concentrations were determined. For determination of lactates, blood was rapidly centrifuged, and the supernatant fluid was stored at −20°C. Lactate in the supernatant was analyzed by oxidase-catalyzed conversion of lactate to pyruvate and hydrogen peroxide in the presence of oxygen. Potassium concentration was assessed by indirect potentiometry, and pH was determined by an ABL 30 blood gas analyzer.

Statistical analysis. Results are given as mean±SD. Differences were considered significant when $P<.05$. A paired t test was used for single comparisons and a Bonferroni method for multiple comparisons.

Results

Effects on Left Ventricular Systolic Function

Table 1 shows individual values of indices of left ventricular systolic function, and Fig 1 shows a set of recordings obtained in a representative patient. At the end of balloon coronary occlusion, heart rate was significantly higher than at rest (79±13 vs 74±12 beats per minute; $P<.01$; n=20). In the patients in whom left ventricular function was compared at rest, at cessation of pacing, and at the end of balloon coronary occlusion, heart rate during pacing ischemia was significantly higher than at rest (85±12 vs 78±11 beats per minute; $P<.01$; n=11) and comparable to heart rate at the end of balloon coronary occlusion (85±12 vs 83±13 beats per minute; NS; n=11). In the patients in whom left ventricular function was compared at rest, at the end of balloon coronary occlusion, and at the end of balloon coronary occlusion with distal perfusion, heart rate at the end of balloon coronary occlusion with distal perfusion was higher than at rest (78±11 vs 69±13 beats per minute; $P<.01$; n=11) and comparable to heart rate at the end of balloon coronary occlusion (78±11 vs 76±13 beats per minute; NS; n=11). At the end of balloon coronary occlusion, left ventricular ejection fraction (LVEF) was significantly lower than at rest (39±9% vs 68±7%; $P<.001$; n=18). In the patients in whom left ventricular function was compared at rest, at cessation of pacing, and at the end of balloon coronary occlusion, LVEF during pacing ischemia was lower than at rest (59±9% vs 68±7%; $P<.002$; n=11) but higher than at the end of balloon coronary occlusion (59±9% vs 43±7%; $P<.001$; n=11). In the patients in whom left ventricular function was compared at rest, at the end of balloon coronary occlusion, and at the end of balloon coronary occlusion with distal perfusion, LVEF at the end of balloon coronary occlusion with distal perfusion was lower than at rest (36±8% vs 65±5%; $P<.001$; n=9) and comparable to LVEF at the end of the regular balloon coronary occlusion (36±8% vs 32±6%; NS; n=9). Left ventricular stroke work index (LVSWI) at the end of balloon coronary occlusion was significantly lower than at rest (43±14 vs 75±17 g·m; $P<.001$; n=18). In the patients in whom left ventricular function was compared at rest, upon cessation of pacing, and at the end of balloon coronary occlusion, LVSWI during pacing ischemia was higher than at the end of balloon coronary occlusion (77±15 vs 50±11 g·m; $P<.002$; n=11). In the patients in whom left ventricular function was compared at rest, at the end of balloon coronary occlusion, and at the end of balloon coronary occlusion with distal perfusion, LVSWI at the end of balloon coronary occlusion with distal perfusion was lower than at rest (46±9 vs 74±17 g·m; $P<.01$; n=9) but higher than at the end of the regular balloon coronary occlusion (46±9 vs 35±7 g·m; $P<.02$; n=9).

Effects on Left Ventricular Diastolic Function

Table 2 shows individual values of indices of left ventricular diastolic function. Left ventricular end-diastolic pressure (LVEDP) was significantly higher at the end of balloon coronary occlusion than at rest (23±6 vs 16±5 mm Hg; $P<.001$; n=20). In the patients in whom left ventricular function was compared at rest, at cessation of pacing, and at the end of balloon coronary occlusion, LVEDP during pacing ischemia was significantly higher than at rest (30±5 vs 16±5 mm Hg; $P<.001$; n=11) and than at the end of balloon coronary occlusion (30±5 vs 20±5 mm Hg; $P<.002$; n=11). In the patients in whom left ventricular function was compared at rest, at the end of balloon coronary occlusion, and at the end of balloon coronary occlusion with distal perfusion, LVEDP at the end of balloon coronary occlusion with distal perfusion was significantly higher than at rest (34±7 vs 17±5 mm Hg; $P<.001$; n=11) and than at the end of the regular balloon coronary occlusion (34±7 vs 26±5 mm Hg; $P<.01$; n=11). As evident from Table 2, the results for left ventricular minimum diastolic pressure were similar to those for LVEDP. The time constant of left ventricular pressure decay with zero asymptote pressure (T_0) was significantly larger at the end of balloon coronary occlusion than at rest (58±10 vs 41±10 milliseconds; $P<.001$; n=20). During pacing-induced ischemia, T_0 was significantly larger than at rest on single-comparison analysis but not on multiple-comparison analysis (48±12 vs 39±8 milliseconds; NS; n=11). At the end of balloon coronary occlusion with distal perfusion, T_0 was significantly larger than at rest (73±10 vs 43±11 milliseconds; $P<.001$; n=11) and than at the end of the regular balloon coronary occlusion (73±10 vs 59±12 milliseconds; $P<.02$; n=11). The time constant of left ventricular pressure decay with variable asymptote pressure (T_{PB}) was significantly larger than at rest (67±24 milliseconds), at the end of balloon coronary occlusion (112±57 milliseconds; $P<.05$; n=20), during pacing-induced ischemia (98±33 milliseconds; $P<.02$; n=11), and at the end of balloon coronary occlusion with distal perfusion (112±59 milliseconds; $P<.02$; n=11). At the end of balloon coronary occlusion, left ventricular end-diastolic volume index (LVEDVI) was significantly larger than at rest (79±15 vs 75±14 mL/m^2; $P<.05$; n=18). During pacing ischemia and at the end of balloon coronary occlusion with distal perfusion, LVEDVI was comparable with LVEDVI at the end of the regular balloon coronary occlusion. The higher LVEDP observed during pacing ischemia and at the end of balloon coronary occlusion with distal perfusion, therefore, indicated lower left ventricular end-diastolic distensibility than at the end of the regular balloon coronary occlusion.

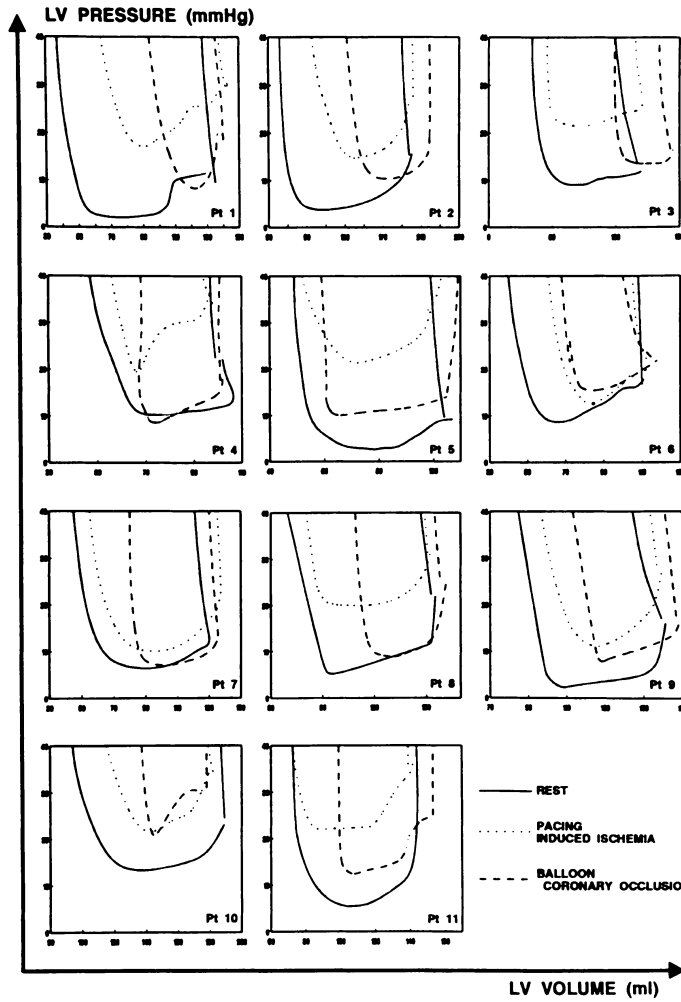

FIG 2. *Individual diastolic left ventricular (LV) pressure-volume relations at rest, during pacing-induced angina, and at the end of balloon coronary occlusion. In 9 of the 11 patients (1 through 5, 7 through 9, 11), the diastolic LV pressure-volume relation during pacing-induced ischemia was shifted upward compared with both the diastolic left ventricular pressure-volume relation at rest and at the end of balloon coronary occlusion.*

Fig 2 shows individual diastolic left ventricular pressure-volume relations at rest, during pacing-induced ischemia at cessation of pacing, and at the end of balloon coronary occlusion. In 9 of the 11 patients, the diastolic left ventricular pressure-volume relation during pacing-induced ischemia was shifted upward compared with both the diastolic left ventricular pressure-volume relations at rest and at the end of balloon coronary occlusion. In one patient (patient 10), both the diastolic left ventricular pressure-volume relation during pacing-induced ischemia and at the end of balloon coronary occlusion shifted upward compared with the diastolic left ventricular pressure-volume relation observed at rest. In one patient (patient 6), both the diastolic left ventricular pressure-volume relation during pacing-induced ischemia and at the end of balloon coronary occlusion fell on the relation observed at rest. Fig 3 shows individual diastolic left ventricular pressure-volume relations at rest, at the end of balloon coronary occlusion, and at the end of an equally long balloon coronary occlusion with distal perfusion. In seven of the nine patients, the diastolic left ventricular pressure-volume relation at the end of balloon coronary occlusion with distal perfusion was shifted upward compared with both the diastolic left ventricular pressure-volume relations observed at rest and at the end of the regular balloon coronary occlusion. In two patients (patients 14 and 17), the diastolic left ventricular pressure-volume relations at the end of balloon coronary occlusion and at the end of balloon coronary occlusion with distal perfusion showed a similar upward shift compared with the relation at rest.

Coronary Sinus Washout of Lactate, H^+, and K^+

Fig 4 shows coronary sinus lactate concentrations measured in patient 10 of Tables 1 and 2. During pacing, there was a progressive rise of coronary sinus lactate, and before

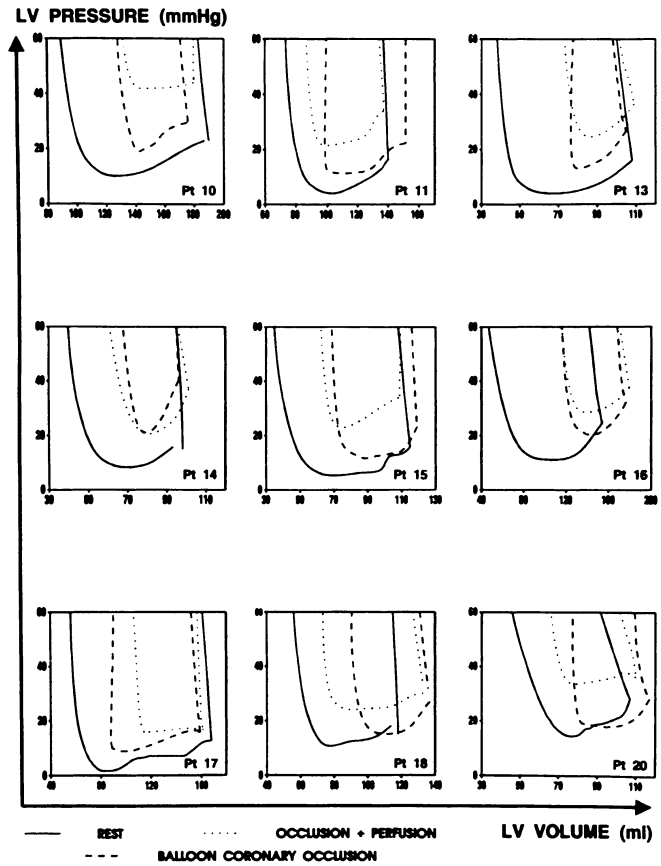

FIG 3. *Individual diastolic left ventricular pressure-volume relations at rest, at the end of balloon coronary occlusion, and at the end of an equally long balloon coronary occlusion with distal perfusion. In seven of the nine patients (patients 10, 11, 13, 15, 16, 18, and 20), the diastolic left ventricular pressure-volume relation at the end of balloon coronary occlusion with distal perfusion was shifted upward compared with the diastolic left ventricular pressure-volume relation both at rest and at the end of balloon coronary occlusion.*

cessation of pacing, coronary sinus lactate concentration was significantly different from the baseline value (8.8±3.8 vs 6.5±3.8 mmol/L; $P<.05$). Ten seconds after cessation of pacing (8.7±3.8 mmol/L; $P<.05$) and 30 seconds after cessation of pacing (8.4±3.7 mmol/L; $P<.05$), there was persistent elevation of coronary sinus lactate concentration. During balloon coronary occlusion, coronary sinus lactate concentration remained unaltered, but a significant rise in coronary sinus lactate concentration was observed 10 seconds after deflation of the balloon (16.5±6.7 mmol/L; $P<.05$), 30 seconds after deflation of the balloon (14.2±4.3 mmol/L; $P<.05$), and 120 seconds after deflation of the balloon (9.5±3.8 mmol/L; $P<.05$). During balloon coronary occlusion with distal perfusion, coronary sinus lactate concentration followed a pattern similar to the regular balloon coronary occlusion.

A significant rise in coronary sinus K^+ concentration was observed with respect to baseline value (3.8±0.4 mEq/L) at the end of the first pacing step (4.0±0.1 mEq/L; $P<.01$), at the end of the second pacing step (4.0±0.1 mEq/L; $P<.01$), and at the end of the third pacing step (4.0±0.1 mEq/L; $P<.01$). Upon cessation of pacing, coronary sinus K^+ concentration dropped below baseline value. A significant rise in serum K^+ was observed after release of the balloon (4.4±0.6 mEq/L; $P<.01$) but not during the balloon occlusion periods.

No significant change in coronary sinus pH was observed during pacing, after pacing, during balloon coronary occlusion, and during balloon coronary occlusion with distal perfusion. With respect to baseline value (7.38±0.04), a significant drop in coronary sinus pH was observed 10 seconds (7.26±0.06; $P<.01$) and 30 seconds (7.34±0.07; $P<.01$) after release of the balloon. Arterial lactate and K^+ concentrations and pH remained unaltered throughout the study.

Discussion

Experimental and Clinical Evidence on Left Ventricular Performance in Different Types of Ischemia

In the guinea pig left ventricle, hypoxic buffer perfusion induced, within 5 minutes, a decline in developed tension and a rise in resting tension[2] consistent with a drop in left ventricular distensibility. In a similar rabbit heart preparation, the acute effects of hypoxia were compared with low-flow ischemia.[3] Hypoxia caused a

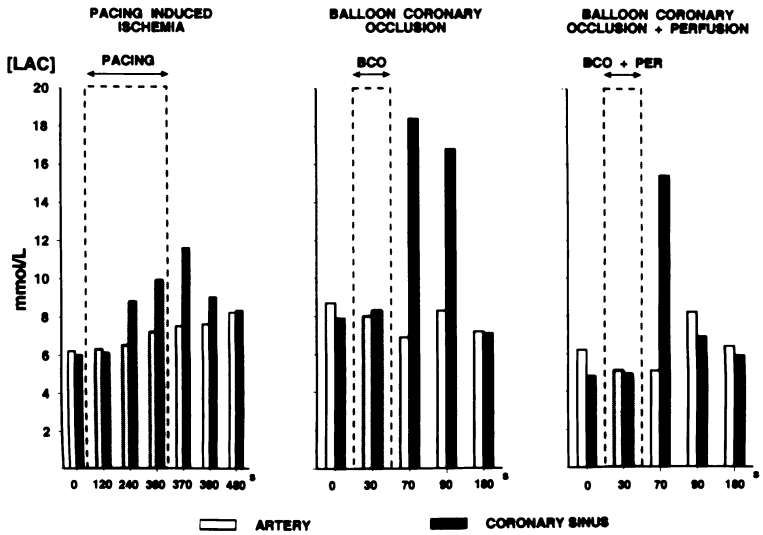

FIG 4. *Bar graphs showing coronary sinus and arterial lactate (LAC) concentrations measured in patient 10 (Tables 1 and 2) before pacing, during the last 15 seconds of each pacing step, 10, 30, and 120 seconds after cessation of pacing, before balloon coronary occlusion, during balloon coronary occlusion, 10, 30, and 120 seconds after release of the balloon, before balloon coronary occlusion with distal perfusion, during balloon coronary occlusion with distal perfusion, and 10, 30, and 120 seconds after release of the balloon and cessation of distal perfusion. During pacing-induced ischemia, the rise in coronary sinus lactate concentration started during the pacing episode and continued after cessation of pacing. During balloon coronary occlusion (BCO) and balloon coronary occlusion with distal perfusion (BCO+PER), there was no rise in coronary sinus lactate concentration during the inflation period, and the rise in coronary sinus lactate concentration occurred after balloon deflation.*

rise and low-flow ischemia an initial fall in left ventricular filling pressures, and hypoxia resulted in a slower decline of left ventricular developed pressure. Replacement of buffer by blood perfusion induced a rise of left ventricular filling pressures, when pacing tachycardia was superimposed on low-flow ischemia.[4]

During brief single-vessel coronary occlusion in anesthetized or conscious dogs,[13-21] myocardial shortening of the affected segment was replaced by passive bulging, and the diastolic pressure–segment length relation showed a rightward shift.[13,15,18,21] In conscious dogs with a single-vessel coronary stenosis, exercise resulted in an upward shift of the early portion of the left ventricular diastolic pressure–volume relation,[20] and in anesthetized pigs with single-vessel coronary stenosis,[22] pacing resulted in an upward shift of the entire left ventricular diastolic pressure–segment length relation. In anesthetized dogs with two-vessel coronary stenoses,[17-19] pacing resulted in subendocardial ischemia, a drop in left ventricular systolic performance, and an upward shift of the diastolic left ventricular pressure–volume relation. When pacing tachycardia in the presence of two-vessel coronary stenoses resulted in transmural myocardial ischemia,[21] there was more profound impairment of left ventricular systolic performance with occasional bulging and unaltered diastolic left ventricular distensibility. In isovolumic dog hearts, global ischemia increased left ventricular diastolic distensibility[23] even in the presence of pacing tachycardia,[24] but a hypoxic perfusate of methemoglobin-containing red blood cells[25] decreased left ventricular diastolic distensibility.

Previous studies in humans on the initial effects of ischemia on left ventricular performance investigated a single type of ischemic insult such as pacing-induced ischemia,[26-28] exercise-induced ischemia,[29] spontaneous coronary spasm,[30] and balloon coronary occlusion ischemia[31-34] except for the study of Bronzwaer et al,[35] who compared left ventricular performance during both pacing-induced and balloon coronary occlusion ischemia in the same patient. The present study confirmed the findings of Bronzwaer et al and was the first to compare, in the same patient, left ventricular function at the end of a regular balloon coronary occlusion and at the end of an equally long balloon coronary occlusion during which saline was perfused through the distal lumen of the balloon catheter (hypoxemia). The present comparison of the left ventricular effects of balloon occlusion ischemia, pacing-induced ischemia, and hypoxemia revealed pacing-induced ischemia and hypoxemia to cause less depression of left ventricular systolic performance and a larger reduction of left ventricular diastolic distensibility than balloon occlusion ischemia. Pathophysiological mechanisms, which could contribute to the variable response of human myocardium subjected to different types of ischemia include (1) vascular turgor during the ischemic episode or during the hyperemic phase after the ischemic episode, (2) buildup or washout of tissue metabolites during the ischemic episode, and (3) unequal intensity of the ischemic stress episodes.

Vascular Turgor During Ischemic Episode and Hyperemic Phase

Coronary perfusion pressure, coronary flow, or both influence left ventricular systolic performance (Gregg phenomenon)[36] and left ventricular diastolic distensibility (Salisbury effect).[37] The Gregg phenomenon was recently reappraised by the slower decline of left ventricular developed pressure in the microembolized heart without coronary depressurization than after interruption of coronary flow.[38] The slower loss of left ventricular systolic performance in the microembolized heart was attributed to persistent vascular turgor, which stretched cardiac sarcomeres by mechanical coupling of the vascular network and the myocardium. The better preservation of left ventricular stroke work during balloon coronary occlusion with distal perfusion, as observed in the present study, was consistent with a similar interaction between coronary vascular turgor and initial ischemic contractile failure. Recent insights on how cardiac muscle stretch affects cardiac muscle performance suggested an intrinsic molecular property of troponin-C to mediate the rising limb of the cardiac muscle length–active tension relation.[39] Hence, vascular turgor could affect cardiac muscle performance by a mechanism similar to tissue metabolites, namely, modulation of myofilamentary calcium sensitivity. Moreover, the vascular network could also affect myofilamentary calcium sensitivity through release of substances from the coronary endothelium.[40-41] In this respect, patients experienced more severe chest pain during the balloon inflation with distal perfusion. This could be consistent with a larger production of bradykinin, which is known to stimulate cardiac afferent nerve endings or nociceptors and to trigger a release of substances from endothelial cells. The increased severity of chest pain during the balloon inflation with distal perfusion could have resulted in increased cardiac sympathetic stimulation. The comparable heart rate at the end of both balloon inflations and the larger prolongation of the time constant of left ventricular pressure decay (T_0) during the balloon inflation with distal perfusion, however, argue against unequal sympathetic stimulation.

Coronary perfusion influences left ventricular diastolic distensibility by changing coronary vascular engorgement. An increase in LVEDP at increased coronary perfusion pressure was first observed in an isovolumic canine left ventricle (Salisbury effect)[37] and confirmed by other investigators, who observed larger changes in diastolic left ventricular wall stiffness when coronary perfusion pressure was altered in a failing left ventricle.[42] During balloon occlusion ischemia, the drop of coronary perfusion pressure could increase diastolic left ventricular distensibility. A rightward and downward shift of the diastolic left ventricular pressure–volume relation was indeed observed in the present study during balloon coronary occlusion in 5 of the 20 patients (Figs 2 and 3, patients 2, 7, 16, 18, and 20). Even when the presence of a critical coronary stenosis permits no change in total coronary blood flow, a reactive hyperemic response to the ischemic subendocardium after the pacing stress episode could contribute to the observed decrease in diastolic left ventricular distensibility.[43] During balloon occlusion with distal saline perfusion, coronary perfusion pressure was preserved and probably contributed in the present study to the larger decrease in diastolic left ventricular distensibility compared with regular balloon coronary occlusion. Hypoxic whole blood perfusion instead of saline perfusion could have resulted in an even larger reduction in left ventricular diastolic distensibility because in an isovolumic rabbit heart, replacement of buffer by blood perfusion was a prerequisite for a consistent fall in left ventricular diastolic distensibility during low-flow, high-demand ischemia.[4] These experiments favored the notion of higher mechanical vascular support of surrounding tissues during whole-blood perfusion.

Buildup or Washout of Tissue Metabolites

Coronary occlusion ischemia, pacing-induced ischemia, and hypoxemia exert unequal effects on buildup of tissue metabolites, such as H^+ and inorganic phosphate. These tissue metabolites, especially inorganic phosphate,[44-46] induce contractile failure by reducing calcium sensitivity of myofilaments despite increased amplitude of the calcium transient and myoplasmic calcium overload. In the present studies, coronary sinus lactate, H^+, and K^+ concentrations were measured at rest, during and after the pacing-stress episode, during and after the regular balloon coronary occlusion, and during and after the balloon coronary occlusion with distal coronary perfusion (see Fig 4). In the absence of simultaneous coronary sinus flow measurements, the coronary sinus lactate, H^+, and K^+ concentrations provided information on the time course of metabolite handling but not on metabolite production. During pacing-induced ischemia, washout of tissue metabolites started during the pacing-stress episode and continued after pacing. Since left ventricular function was measured immediately at cessation of pacing, the better preservation of left ventricular systolic performance could be related to the ongoing washout of tissue metabolites. Balloon coronary occlusion ischemia caused metabolites to appear in the coronary sinus at a different time than pacing-induced ischemia. During the actual balloon coronary occlusion, there was no rise of coronary sinus lactate concentration (Fig 4), the peak of which was observed after release of the angioplasty balloon during the hyperemic phase. At the end of the balloon inflation, when left ventricular function was measured, buildup of tissue metabolites could explain the larger decrease of LVSWI compared with pacing-induced ischemia. During balloon coronary occlusion with distal perfusion, the time pattern of coronary sinus lactate concentration was comparable to the regular balloon coronary occlusion. This observation, however, did not exclude unequal intracellular accumulation of metabolites during both coronary occlusions because of failure to measure inorganic phosphate concentrations and because of failure to perform great cardiac vein sampling to selectively assess LAD drainage.

During balloon inflation with distal perfusion, the perfusate was a mixture of blood obtained from coronary collaterals (mean coronary wedge pressure, 29 ± 11 mm Hg) and regular saline (pH, 7.0) infused through the distal lumen of the balloon catheter. The saline infusion rate was set at a value of 1 mL/s to mimic resting LAD flow.[8,9] The perfusate was of lower pH, hypokalemic, and hypocalcemic relative to whole-blood perfusion. In isolated cardiac muscle preparations, both

acidosis and combined hypokalemia-hypocalcemia exerted a negative inotropic effect.[47] The higher LVSWI observed during balloon inflation with distal perfusion compared with the regular balloon inflation was therefore unrelated to the altered composition of the perfusate relative to whole blood. The lower pH of the perfusate relative to whole blood could have reduced diffusion of hydrogen ions out of the myocardium, and hypoxic whole-blood perfusion could probably have resulted in even better preservation of LVSWI.

Unequal Intensity of the Ischemic Stress Episodes

Unequal intensity of the ischemic stress of pacing-induced ischemia, of balloon coronary occlusion, and of hypoxemia could have influenced left ventricular performance in the three conditions. In a study on anesthetized open-chest, open-pericardium dogs, pacing-induced ischemia and coronary occlusion ischemia produced similar changes in contractile function of the ischemic region, when both ischemic stress episodes achieved the same elevation of LVEDP, which was used as a marker of the intensity of the ischemic stress.[21] In the present study, LVEDP was lowest at the end of balloon coronary occlusion. Despite lowest LVEDP at the end of balloon coronary occlusion, LVSWI showed the largest reduction. Hence, when elevation of LVEDP is used as a marker of the intensity of the ischemic stress episode, the reduction of left ventricular systolic performance appears to be larger at the end of the least severe ischemic stress episode. This implies that factors other than the intensity of the ischemic stress episode modulate left ventricular performance during the initial stages of ischemia.

During balloon occlusion with distal perfusion, myocardial oxygen delivery could have been higher than during regular balloon occlusion because of oxygen dissolved in the perfusate. Because of the use of blood-free perfusate, the amount of oxygen dissolved was minimal (±5 mL/L) and approximately 38 times less than in arterial blood. Moreover, during balloon occlusion with distal perfusion, oxygen delivery to the ischemic region from collateral flow must have been less than during regular balloon inflation because of higher intravascular pressure created by the perfusion pump in the epicardial coronary arteries distal to the balloon occlusion.

Conclusions

Left ventricular function was compared in the same patient with single-vessel LAD coronary stenosis at the end of balloon coronary occlusion, immediately after a pacing stress test, and/or at the end of balloon coronary occlusion with hypoxic perfusion distal to the balloon occlusion (hypoxemia). At the end of balloon coronary occlusion, left ventricular systolic performance was lower and left ventricular diastolic distensibility larger than during pacing-induced ischemia or hypoxemia. Degree of vascular turgor and unequal accumulation of tissue metabolites could explain the variable response of human myocardium to different types of ischemia.

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References

1. Apstein CS, Grossman W. Opposite initial effects of supply and demand ischemia on left ventricular diastolic compliance: the ischemia-diastolic paradox. *J Mol Cell Cardiol.* 1987;19:119-128.
2. Nayler WG, Yepez CE, Poole-Wilson PA. The effect of β-adrenoreceptor and Ca^{2+} antagonist drugs on the hypoxia-induced increase in resting tension. *Cardiovasc Res.* 1978;12:666-674.
3. Serizawa T, Vogel WM, Apstein CS, Grossman W. Comparison of acute alterations in left ventricular relaxation and diastolic chamber stiffness induced by hypoxia and ischemia. *J Clin Invest.* 1981; 64:91-102.
4. Isoyama S, Apstein CS, Wexler LF, Grice WN, Lorell BH. Acute decrease in left ventricular diastolic chamber distensibility during simulated angina in isolated hearts. *Circ Res.* 1987;61:925-933.
5. Wexler LF, Weinberg EO, Ingwall JS, Apstein CS. Acute alterations in diastolic left ventricular chamber distensibility: mechanistic differences between hypoxemia and ischemia in isolated perfused rabbit and rat hearts. *Circ Res.* 1986;59:515-528.
6. Meier B, Luethy P, Finci L, Steffenino GD, Rutishauser W. Coronary wedge pressure in relation to spontaneously visible and recruitable collaterals. *Circulation.* 1987;75:906-913.
7. De Bruyne B, Meier B, Finci L, Urban P, Rutishauser W. Potential protective effect of high coronary wedge pressure on left ventricular function after coronary occlusion. *Circulation.* 1988;78:566-572.
8. Pepine CJ, Mehta J, Webster WW, Nichols WW. In vivo validation of a thermodilution method to determine regional left ventricular blood flow in patients with coronary disease. *Circulation.* 1978;58: 795-802.
9. Serruys PW, Wijns W, van den Brand M, Meij S, Slager C, Schuurbiers JCH, Hugenholtz PG, Brower RW. Left ventricular performance, regional blood flow, wall motion, and lactate metabolism during transluminal angioplasty. *Circulation.* 1984;70:25-36.
10. Paulus WJ, Vantrimpont PJ, Rousseau MF. Diastolic function of the non-filling human left ventricle. *J Am Coll Cardiol.* 1992;20: 1524-1532.
11. Fifer MA, Grossman W. Measurement of ventricular volumes, ejection fraction, mass and wall stress. In: Grossman W, Baim DS, eds. *Cardiac Catheterization, Angiography, and Intervention.* 4th ed. Philadelphia, Pa. Lea & Febiger; 1991:300-318.
12. Webb SC, Rickards AF, Poole-Wilson PA. Coronary sinus potassium concentration recorded during coronary angioplasty. *Br Heart J.* 1983;50:146-148.
13. Tyberg JV, Forrester JS, Wyatt HL, Goldner SJ, Parmley WW, Swan HJC. An analysis of segmental ischemic dysfunction utilizing the pressure-length loop. *Circulation.* 1974;49:748-754.
14. Theroux P, Franklin D, Ross J Jr, Kemper WS. Regional myocardial function during acute coronary artery occlusion and its modification by pharmacological agents in the dog. *Circ Res.* 1974;35: 896-908.
15. Hess OM, Osakada G, Lavelle JF, Gallagher KP, Kemper WS, Ross J Jr. Diastolic myocardial wall stiffness and ventricular relaxation during partial and complete coronary occlusions in the conscious dog. *Circ Res.* 1983;52:387-400.
16. Tomoike H, Franklin D, Mc Kown D, Kemper WS, Guberek M, Ross J Jr. Regional myocardial dysfunction and hemodynamic abnormalities during strenuous exercise in dogs with limited coronary flow. *Circ Res.* 1978;42:487-496.
17. Serizawa T, Carabello BA, Grossman W. Effect of pacing induced ischemia on left ventricular diastolic pressure-volume relations in dogs with coronary stenoses. *Circ Res.* 1980;46:430-439.
18. Paulus WJ , Grossman W, Serizawa T, Bourdillon PD, Pasipoularides A, Mirsky I. Different effects of two types of ischemia on myocardial systolic and diastolic function. *Am J Physiol.* 1985;248: H719-H728.
19. Momomura SI, Bradley AB, Grossman W. Left ventricular diastolic pressure-segment length relations and end-diastolic distensibility in dogs with coronary stenoses: an angina physiology model. *Circ Res.* 1984;55:203-214.
20. Miyazaki S, Guth BD, Miura T, Indolfi C, Schulz R, Ross J Jr. Changes of left ventricular diastolic function in exercising dogs without and with ischemia. *Circulation.* 1990;81:1058-1070.
21. Applegate RJ, Walsh RA, O'Rourke RA. Comparative effects of pacing-induced and flow-limited ischemia on left ventricular function. *Circulation.* 1990;81:1380-1392.
22. Takahashi T, Levine MJ, Grossman W. Regional diastolic mechanics of ischemic and nonischemic myocardium in the pig heart. *J Am Coll Cardiol.* 1991;17:1203-1213.

23. Palacios I, Newell JB, Powell WJ Jr. Left ventricular end-diastolic pressure-volume relationships with experimental acute global ischemia. *Circulation.* 1976;39:735-744.
24. Lorell BH, Palacios I, Daggett WM, Jacobs ML, Fowler BN, Newell JB. Right ventricular distension and left ventricular compliance. *Am J Physiol.* 1981;240:H87-H98.
25. Wyman RM, Farhi ER, Bing OHL, Johnson RG, Weintraub RM, Grossman W. Comparative effects of hypoxia and ischemia in the isolated, blood-perfused dog heart: evaluation of left ventricular diastolic chamber distensibility and wall thickness. *Circ Res.* 1989; 64:121-128.
26. Barry WH, Brooker JZ, Alderman EL, Harrison DC. Changes in diastolic stiffness and tone of the left ventricle during angina pectoris. *Circulation.* 1974;49:255-263.
27. Mann T, Goldberg S, Mudge GH, Grossman W. Factors contributing to altered left ventricular diastolic properties during angina pectoris. *Circulation.* 1979;59:14-20.
28. Sasayama S, Nonogi H, Miyazaki S, Sakurai T, Kawai C, Eiho S, Kuwahara M. Changes in diastolic properties of the regional myocardium during pacing-induced ischemia in human subjects. *J Am Coll Cardiol.* 1985;5:599-606.
29. Carroll JD, Hess OM, Hirzel HO, Krayenbuehl HP. Exercise-induced ischemia: the influence of altered relaxation on early diastolic pressures. *Circulation.* 1983;67:521-528.
30. Sharma B, Behrens TW, Erlein D, Hodges M, Asinger RW, Francis GS. Left ventricular diastolic properties and filling characteristics during spontaneous angina pectoris at rest. *Am J Cardiol.* 1983;52:704-709.
31. Wijns W, Serruys PW, Slager CJ, Grimm J, Krayenbuehl HS, Hugenholtz P, Hess OM. Effect of coronary occlusion during percutaneous transluminal angioplasty in humans on left ventricular chamber stiffness and regional diastolic pressure-radius relations. *J Am Coll Cardiol.* 1986;7:455-463.
32. Bertrand ME, Lablanche JM, Fourrier JL, Traisnel G, Mirsky I. Left ventricular systolic and diastolic function during acute coronary artery balloon occlusion in humans. *J Am Coll Cardiol.* 1988; 12:341-347.
33. Kern MJ, Deligonul U, Labovitz A. Influence of drug therapy on the ischemic response to acute coronary occlusion in man: supply-side economics. *Am Heart J.* 1989;118:361-380.
34. Kass DA, Midei M, Brinker J, Maughan WL. Influence of coronary occlusion during PTCA on end-systolic and end-diastolic pressure-volume relations in humans. *Circulation.* 1990;81:447-460.
35. Bronzwaer JGF, De Bruyne B, Ascoop CAPL, Paulus WJ. Comparative effects of pacing-induced and balloon coronary occlusion ischemia on left ventricular diastolic function in man. *Circulation.* 1991;84:211-222.
36. Gregg DE. Effect of coronary perfusion pressure or coronary flow on oxygen usage of the myocardium. *Circ Res.* 1963;13:497-500.
37. Salisbury PF, Cross CE, Rieben PA. Influence of coronary pressure on myocardial elasticity. *Circ Res.* 1960;8:794-800.
38. Koretsune Y, Corretti MC, Kusuoka H, Marban E. Mechanism of early ischemic contractile failure: inexcitability, metabolite accumulation or vascular collapse? *Circ Res.* 1991;68:255-262.
39. Babu A, Sonnenblick E, Gulati J. Molecular basis for the influence of muscle length on myocardial performance. *Science.* 1988;240:74-76.
40. Brutsaert DL, Meulemans AL, Sipido KR, Sys SU. Effects of damaging the endocardial surface on the mechanical performance of isolated cardiac muscle. *Circ Res.* 1988;62:358-366.
41. McClellan G, Weisberg A, Kato NS, Ramaciotti C, Sharkey A, Winegrad S. Contractile proteins in myocardial cells are regulated by factor(s) released by blood vessels. *Circ Res.* 1992;70:787-803.
42. Gaasch WH, Bing OHL, Franklin A, Rhodes D, Bernard SA, Weintraub RM. The influence of acute alterations in coronary blood flow on left ventricular diastolic compliance and wall thickness. *Eur J Cardiol.* 1978;7(suppl):147-161.
43. Ross J Jr. Is there a true increase in myocardial stiffness with acute ischemia? *Am J Cardiol.* 1989;63:87E-91E.
44. Momomura SI, Ingwall J, Parker JA, Sahagian P, Ferguson JJ, Grossman W. The relationships of high energy phosphates, tissue pH, and regional blood flow to diastolic distensibility in the ischemic dog myocardium. *Circ Res.* 1985;57:822-835.
45. Koretsune Y, Marban E. Relative roles of Ca^{2+}-dependent and Ca^{2+}-independent mechanisms in hypoxic contractile dysfunction. *Circulation.* 1990;82:528-535.
46. Arai AE, Pantely GA, Thoma WJ, Anselone CG, Bristow JD. Energy metabolism and contractile function after 15 beats of moderate myocardial ischemia. *Circ Res.* 1992;70:1137-1145.
47. Ruigrok TJC, Slade AM, Poole-Wilson PA. The calcium paradox in isolated frog heart: Ringer revisited. *Eur Heart J.* 1983;4(suppl H): 89-96.

Chapter 2.3

Comparative Effects of Ischemia and Hypoxemia on Left Ventricular Diastolic Function in Humans

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31. COMPARATIVE EFFECTS OF ISCHEMIA AND HYPOXEMIA ON LEFT VENTRICULAR DIASTOLIC FUNCTION IN HUMANS

Walter J. Paulus, Bernard De Bruyne, and Jean G.F. Bronzwaer

Different types of ischemia have unequal effects on initial left ventricular mechanical dysfunction because of a complex interaction between oxygen deprivation, accumulation of tissue metabolites, and vascular turgor [1]. Initial left ventricular mechanical dysfunction was first compared during different types of ischemia in an isolated, isovolumically beating, and retrogradely perfused rabbit heart [2] and later in an anesthetized open-chest, open pericardium dog heart [3,4]. In the buffer-perfused rabbit heart [2,5], hypoxic coronary perfusion induced a rise in resting tension, in contrast to reduced coronary perfusion, which produced a fall in resting tension. Moreover, the decline in developed tension progressed faster during reduced than during hypoxic perfusion. In anesthetized dogs, pacing tachycardia in the presence of coronary stenoses and brief coronary occlusion had opposite effects on global and regional diastolic left ventricular distensibility [3,4]. Pacing tachycardia in the presence of coronary stenoses resulted in an upward shift of the global diastolic left ventricular pressure-volume and of the regional diastolic pressure-segment length and pressure wall-thickness relations of the ischemic myocardium. During brief coronary occlusion, the ischemic region showed no change or a shift to the right of these relations [6–8].

In contrast to these experimental findings, clinical studies in patients with coronary disease described the initial left ventricular mechanical dysfunction of only a single type of ischemic insult. During pacing-induced [9–11] or spontaneous angina [12], an upward shift of the global diastolic left ventricular pressure-volume relation and of the regional diastolic pressure-radial length [13] and pressure-wall thickness [14] relations of the ischemic myocardium was observed. Since the advent of coronary angioplasty, the myocardial effects of a brief reduction of coronary blood flow could easily be investigated in humans during the angioplasty balloon coronary occlusion. Initial studies appreciated the mechanical left ventricular dysfunction of balloon coronary occlusion by loss of global and regional systolic performance [15], and subsequently by altered diastolic left ventricular performance [16–19], which appeared to be an earlier and more sensitive marker of left ventricular mechanical dysfunction during ischemia of balloon coronary occlusion. The mechanical dysfunction resulting from balloon coronary occlusion ischemia was further characterized by studies of pharmacological pretreatment [20] and by comparing the effects of sequential balloon coronary occlusions [21–23].

In contrast to the experimental data, no clinical study had directly compared initial left ventricular mechanical dysfunction resulting from different types of ischemia in the same patient. To investigate the initial left ventricular effects of different types of ischemia in the same patient, the present studies [24,25] compared left ventricular performance in patients with single-vessel left anterior descending coronary disease during balloon coronary occlusion, during pacing-induced angina, and during balloon coronary occlusion with maintained hypoxic perfusion distal to the balloon occlusion.

Materials and Methods

PATIENTS

Twelve patients (10 men, 2 women; mean age 54 years; age range 42–77 years) were included in the

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comparative analysis of pacing-induced and balloon coronary occlusion ischemia [24]. Eleven patients (8 men, 3 women; mean age 57 years; age range 42–71 years) were included in the comparative analysis of balloon coronary occlusion ischemia and hypoxemia [25]. All patients suffered from exercise-induced angina. The exercise stress test was both clinically and electrocardiographically positive. There was no history of angina at rest or of previous myocardial infarction. There was no evidence of previous myocardial infarction on the electrocardiogram, and there was normal wall motion on the baseline left ventricular angiogram. Diagnostic left heart catheterization and coronary angiography showed normal global and regional left ventricular function and single-vessel coronary artery disease, consisting of a significant (>80%) proximal left anterior descending stenosis. Contralateral coronary injection before balloon dilatation revealed no visible collaterals to the distal left anterior descending coronary artery. β-blockers and calcium entry blockers were withheld at least 24 hours prior to the study, except in two patients, who had experienced angina during mild exercise during the 48 hour period preceding hospital admission. Premedication consisted of 10 mg diazepam. All patients gave informed consent, the study protocol was approved by the hospital ethical committee, and there were no complications related to the angioplasty procedure or the study protocol.

STUDY PROTOCOL

A 7Fr pigtail Sentron tip-micromanometer catheter (Cordis Europe, Rooden, the Netherlands) was advanced from the left femoral artery to the left ventricle and an 8Fr angioplasty guiding catheter was advanced from the right femoral artery. The tip-micromanometer catheter was calibrated externally against a mercury reference and matched against luminal pressure. All pressures were referenced to atmospheric pressure at the level of the midchest. The pressure signals, the left ventricular dP/dt signal, which was derived from the left ventricular pressure signal by an electronic differentiator, and leads of the electrocardiogram were recorded on a Gould ES 1000 multichannel recorder. A 7Fr NIH catheter was positioned in the coronary sinus from a left antecubital vein or from the right femoral vein to obtain coronary sinus blood samples during the different interventions. Simplus balloon catheters (USCI) were used to perform the coronary angioplasty, and during angioplasty balloon inflation the coronary wedge pressure was measured through the fluid filled lumen of the balloon catheter, which was connected to a pressure transducer. The use of coronary wedge pressure as an index of coronary collateral recruitment was demonstrated in previous studies [26,27]. Mean coronary wedge pressure during balloon inflation was 29 ± 11 mmHg (range 11–45 mmHg), and the mean pressure difference between coronary wedge pressure and left ventricular end-diastolic pressure at the end of the balloon coronary occlusion was 8 ± 6 mmHg (range 3–15 mmHg). Left ventricular angiograms were performed in 30° right anterior oblique projection and matched with the left ventricular tip-micromanometer pressure recording using an angiographic frame marker.

Comparative Effects of Pacing-Induced and Balloon Coronary Occlusion Ischemia on Left Ventricular Function. Following baseline left ventricular angiography and left ventricular tip-micromanometer pressure recordings, left ventricular filling pressures were allowed to return to control level. Right ventricular pacing was initiated at a rate of 90 beats/min and was stepwise increased every 2 minutes by 30 beats/min. Pacing was continued until the appearance of angina. Immediately upon cessation of pacing, a second left ventricular angiogram and simultaneous tip-micromanometer left ventricular pressure recordings were obtained. To allow diastolic left ventricular pressures to return to baseline, a 15 minute interval separated the end of the pacing run from the start of the angioplasty procedure. A third left ventricular angiogram and simultaneous left ventricular tip-micromanometer pressure recordings were obtained at the end of the fourth (patients 1–9) or second (patients 10–12) angioplasty balloon inflation of 60 seconds duration. All patients experienced chest pain and ischemic ST-segment changes at the end of the balloon coronary occlusion. Coronary angioplasty was successful in all patients with minimal residual coronary stenosis.

Comparative Effects of Balloon Coronary Occlusion and Balloon Coronary Occlusion with Distal Perfusion on Left Ventricular Function. After baseline angiography and left ventricular pressure recordings, left ventricular filling pressures were allowed to return to baseline value. A second left ventricular angiogram was obtained at the end of the second balloon coronary occlusion. After return of left ventricular filling pressures to baseline value, a third angioplasty balloon inflation of equal duration to the second angioplasty balloon inflation was performed. During this third angioplasty balloon inflation, saline was perfused through the distal lumen of the balloon catheter at a flow rate that equaled the normal left anterior descending flow in humans (1 ml/sec)

[15,28]. At the end of this third balloon inflation, a left ventricular angiogram and simultaneous left ventricular pressure recordings were obtained.

DATA ANALYSIS

Hemodynamic Data. All hemodynamic data were averaged over a complete respiratory cycle. The time constant of left ventricular pressure decay was derived from an exponential curve fit with zero asymptote pressure to the digitized left ventricular pressure data points, which were obtained at 3 msec intervals by digitizing the left ventricular pressure signal from the moment of left ventricular dP/dt min to a time at which left ventricular pressure equaled left ventricular end-diastolic pressure plus 5 mmHg [29].

Angiographic Data. Left ventricular volumes were calculated from single plane left ventricular cineangiograms performed in 30° right anterior oblique projection using the area length method and a regression equation [30]. Nonsinus and potentiated beats were excluded from analysis, which was performed on the third to fourth beat after contrast appearance. Left ventricular pressure-volume plots were constructed by matching corresponding points of left ventricular pressure and volume using the cineframe marker. Left ventricular stroke work index was calculated as the product of stroke volume index and mean systolic left ventricular pressure. Regional wall motion was analyzed using the end-diastolic center of mass and 28 sectors emerging from the center of mass. Radial length was calculated for each frame as the distance from the center of mass to the endocardial contour of an ischemic and nonischemic sector. Percentage systolic shortening was the ratio of the difference between end-diastolic and end-systolic radial lengths divided by the end-diastolic radial length. Each individual angiographic value was the average of three measurements. Intraobserver variability for left ventricular volume and radial length measurements was 1.5% and 1.6%, respectively.

Metabolic Data. Blood samples were repetitively obtained from the coronary sinus and from femoral artery before pacing, during the last 15 sec of each pacing step, and 10, 30, and 120 seconds after cessation of pacing. The same samples were drawn before balloon inflation; at the end of the balloon inflation period; and 10, 30, and 120 seconds after deflation of the balloon; before balloon inflation with distal perfusion; at the end of the balloon inflation period with distal perfusion; and 10, 30, and 120 seconds after deflation of the balloon and cessation of the distal perfusion. The timing of sampling was based on the previously reported K^+ concentration measured by catheter electrode in the coronary sinus during and following balloon inflations of coronary angioplasty [31]. On each blood sample, pH, K^+, and lactate concentrations were determined. For determination of lactates, blood was rapidly centrifuged and the supernatant fluid was stored at $-20°C$. Lactate in the supernatant was analyzed by oxidase catalyzed conversion of lactate to pyruvate and hydrogen peroxide in the presence of oxygen. Potassium concentration was assessed by indirect potentiometry; pH was determined by an ABL 30 blood gas analyzer.

Statistical Analysis. Results are given as mean ± standard deviation. Statistical significance was set at $p < 0.05$ and was obtained by Student's t test for paired data and by Bonferroni's method for multiple comparisons.

Results

COMPARATIVE EFFECTS OF PACING-INDUCED AND BALLOON CORONARY OCCLUSION ISCHEMIA ON LEFT VENTRICULAR FUNCTION IN HUMANS

Figure 31-1 shows two surface lead electrocardiograms (I–II), one precordial lead electrocardiogram, the left ventricular dP/dt signal, the left ventricular tip-micromanometer pressure recording, and the end-diastolic and end-systolic frames of a left ventricular cineangiogram obtained at rest, upon cessation of pacing during an episode of pacing-induced angina, and at the end of a balloon coronary occlusion in a representative patient out of a group of 12 patients subjected to this study protocol [24]. Left ventricular end-diastolic pressure rose from 14 ± 4 mmHg at rest to 24 ± 7 mmHg ($p < 0.01$) during pacing-induced ischemia and to 21 ± 8 mmHg ($p < 0.01$) at the end of balloon coronary occlusion. Left ventricular end-diastolic volume index rose from 83 ± 19 ml/m^2 at rest (R) to 88 ± 17 ml/m^2 (NS) during pacing-induced ischemia (PI), and to 96 ± 16 ml/m^2 ($p < 0.05$ vs. R and PI) at the end of balloon coronary occlusion. The similar rise of left ventricular end-diastolic pressure during pacing-induced ischemia and at the end of balloon coronary occlusion and the significant increase in left ventricular end-diastolic volume index at the end of balloon coronary occlusion were consistent with an upward shift of the left ventricular end-diastolic pressure-volume relation during pacing-induced ischemia and

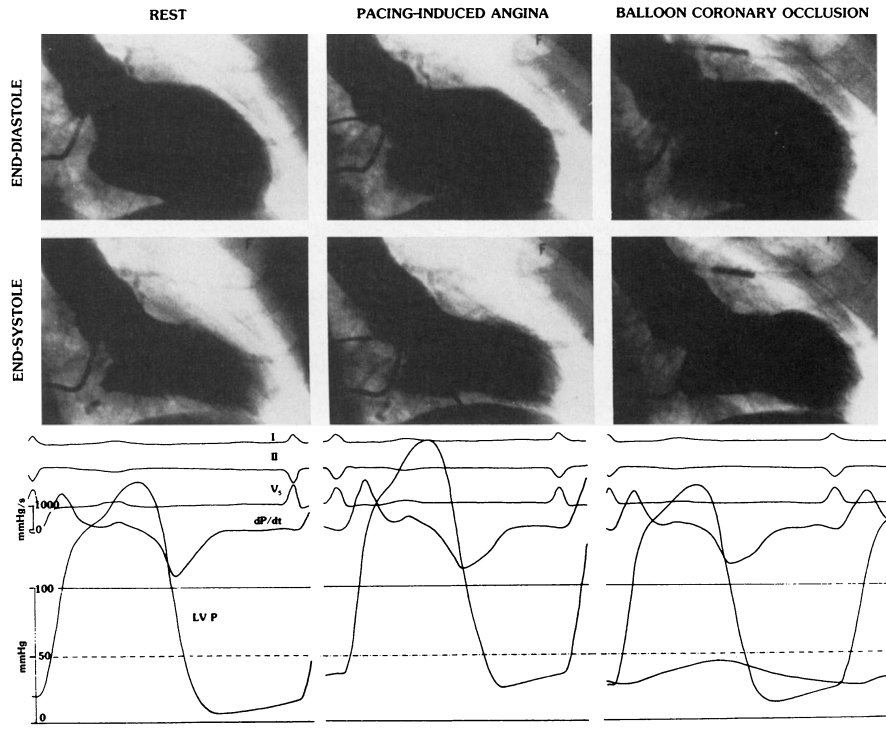

FIGURE 31-1. Two surface lead electrocardiograms (I–II), one precordial lead electrocardiogram, the left ventricular dP/dt signal, the left ventricular tip-micromanometer pressure recording, and the end-diastolic and end-systolic frames of a left ventricular cineangiogram obtained in a representative patient at rest, upon cessation of pacing during an episode of pacing-induced angina, and at the end of a balloon coronary occlusion.

an upward and rightward shift of the same relation at the end of balloon coronary occlusion. Figure 31-2 shows a representative example of diastolic left ventricular pressure-volume relations at rest, during pacing-induced ischemia, and at the end of balloon coronary occlusion. The diastolic left ventricular pressure-volume relation during pacing-induced ischemia was shifted upward, and the diastolic left ventricular pressure-volume relation at the end of balloon coronary occlusion was shifted rightward as compared to the relation at rest. Left ventricular ejection fraction fell from $77 \pm 7\%$ at rest (R) to $71 \pm 9\%$ during pacing induced ischemia (PI; NS) and to $47 \pm 11\%$ at the end of balloon coronary occlusion ($p < 0.01$ vs. R and PI). Left ventricular peak systolic pressure rose from 126 ± 28 mmHg at rest to 137 ± 23 mmHg during pacing-induced angina ($p < 0.05$) and was unaltered at the end of balloon coronary occlusion (127 ± 32 mmHg). Left ventricular stroke work index was significantly different from rest (R) (75 ± 17 g.m) at the end of balloon coronary occlusion (43 ± 14 g.m; $p <$ 0.01 vs. R and PI) but not during pacing-inducing ischemia (PI; 77 ± 15 g.m; Figure 31-3). Heart rate showed a similar increase from 69.1 ± 7.9 beats/min at rest to 82.4 ± 11.8 beats/min during pacing-induced ischemia ($p < 0.01$) and to 82.7 ± 11.4 beats/min at the end of balloon coronary occlusion ($p < 0.01$).

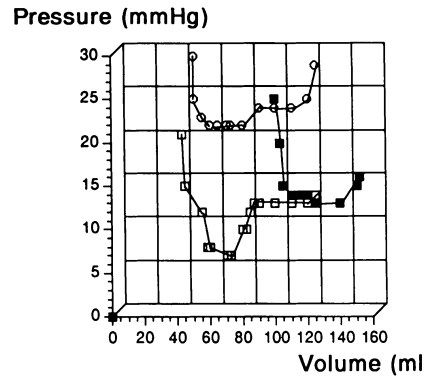

FIGURE 31-2. Diastolic left ventricular pressure-volume relations obtained in a representative patient at rest (□), upon cessation of pacing during an episode of pacing-induced angina (○), and at the end of a balloon coronary occlusion (■). The diastolic left ventricular pressure-volume relation during pacing-induced ischemia was shifted upward and the diastolic left ventricular pressure-volume relation at the end of a balloon coronary occlusion was shifted rightward as compared to the diastolic left ventricular pressure-volume relation at rest.

Regional wall motion data of ischemic and nonischemic segments showed a significant drop in percent systolic shortening of the ischemic segment from $40 \pm 11\%$ at rest (R) to $25 \pm 9\%$ during pacing-induced ischemia (PI; $p < 0.01$ vs. R) and to $6 \pm 9\%$ at the end of balloon coronary occlusion ($p < 0.01$ vs. R and PI). The nonischemic segment showed no change in percent systolic shortening during pacing-induced ischemia (PI) and a decrease in percent systolic shortening from $50 \pm 8\%$ at rest (R) to $43 \pm 8\%$ at the end of balloon coronary occlusion ($p < 0.05$ vs. R and PI). The change in percent systolic shortening of the nonischemic segment was explained by motion of the center of mass towards the area of akinesia, which led to underestimation of systolic shortening of the nonischemic segment at the end of balloon coronary occlusion. The upward shift of the diastolic left ventricular pressure-radial length relation was quantified by P_m. P_m was obtained by planimetry of an area enclosed by the two left ventricular pressure-radial length plots and by two lines perpendicular to the radial length axis at the outer borders of a radial length zone for which there was overlap between the two left ventricular pressure-radial length plots and by division of this area by the distance between the two perpendicular lines [24]. P_m was inversely related to regional left ventricular distensibility. P_m

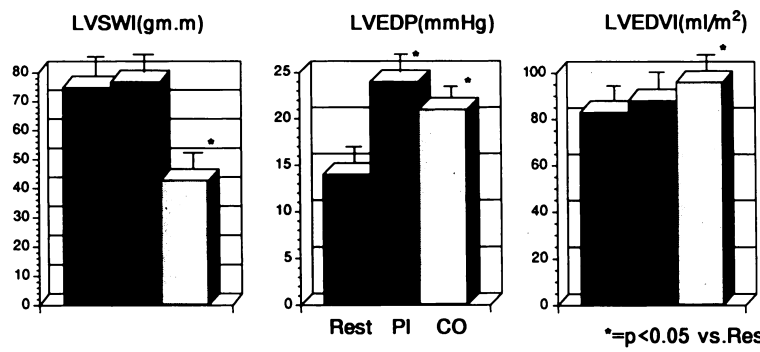

FIGURE 31-3. Bar graphs showing left ventricular stroke work index (LVSWI), left ventricular end-diastolic pressure (LVEDP), and left ventricular end-diastolic volume index (LVEDVI) observed at rest, during pacing-induced ischemia (PI), and at the end of balloon coronary occlusion (CO).

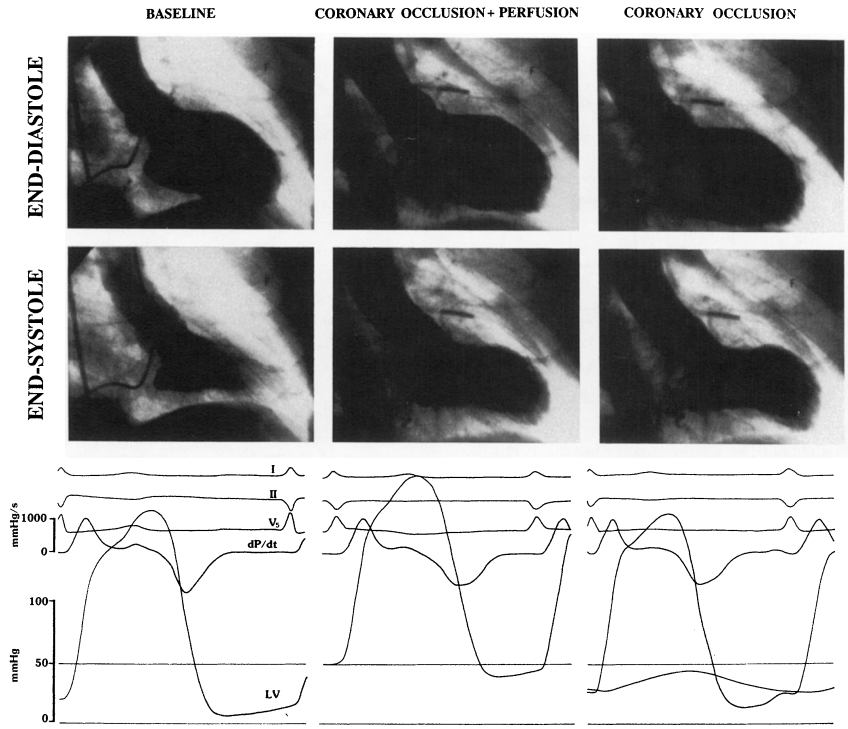

FIGURE 31-4. Two surface lead electrocardiograms (I–II), one precordial lead electrocardiogram, the left ventricular dP/dt signal, the left ventricular tip-micromanometer pressure recording, and the end-diastolic frame of a left ventricular cineangiogram obtained in a representative patient at rest, at the end of a balloon coronary occlusion with distal saline perfusion (hypoxemia), and at the end of an equally long balloon coronary occlusion.

was larger following pacing (7.1 ± 5.0 mmHg) than at the end of balloon coronary occlusion (3.1 ± 2.3 mmHg; $p < 0.05$). At the end of balloon coronary occlusion, a correlation was observed between P_m and segmental shortening expressed as a fraction of the value at rest (fSS; $P_m = 7.6 \times fSS + 1.8$; $r = 0.64$; $p < 0.03$).

COMPARATIVE EFFECTS OF BALLOON CORONARY OCCLUSION ISCHEMIA AND HYPOXEMIA ON LEFT VENTRICULAR FUNCTION IN HUMANS

Figure 31-4 shows two surface lead electrocardiograms (I–II), one precordial lead electrocardiogram, the left ventricular dP/dt signal, the left ventricular tip-micromanometer pressure recording, and the end-diastolic and end-systolic frames of a left ventricular cineangiogram obtained at rest, at the end of a balloon coronary occlusion, and at the end of a balloon coronary occlusion with distal perfusion (hypoxemia) in a representative patient out of a group of 11 patients subjected to this comparative study protocol [25].

Left ventricular end-diastolic pressure at the end of balloon coronary occlusion with distal perfusion was significantly higher than at rest (34 ± 7 vs. 17 ± 5 mmHg; $p < 0.001$) and than at the end of the regular balloon coronary occlusion (26 ± 5 mmHg; $p < 0.01$). Left ventricular minimum diastolic pres-

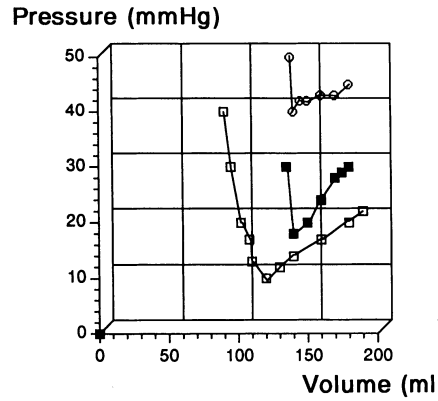

FIGURE 31-5. Diastolic left ventricular pressure-volume relations obtained in a representative patient at rest (□), at the end of a balloon coronary occlusion (■), and at the end of an equally long balloon coronary occlusion with distal saline perfusion (hypoxemia) (○). The diastolic left ventricular pressure-volume relation during hypoxemia was shifted upward compared to the diastolic left ventricular pressure-volume relation at rest and at the end of balloon coronary occlusion.

sure at the end of balloon coronary occlusion with distal perfusion was significantly higher than at rest (26 ± 8 vs. 6 ± 4 mmHg; $p < 0.01$) and than at the end of the regular balloon coronary occlusion (13 ± 4 mmHg; $p < 0.01$). At the end of balloon coronary occlusion, left ventricular end-diastolic volume index was significantly larger than at rest (79 ± 15 vs. 75 ± 14 ml/m^2; $p < 0.05$), whereas left ventricular end-diastolic volume index at the end of balloon coronary occlusion with distal perfusion was comparable to the value at rest. Because of unaltered left ventricular end-diastolic volume index, the higher left ventricular end-diastolic pressure observed at the end of balloon coronary occlusion with distal perfusion was consistent with a decrease in left ventricular end-diastolic distensibility compared to the resting value and the regular balloon coronary occlusion.

Figure 31-5 shows diastolic left ventricular pressure-volume relations at rest, at the end of balloon coronary occlusion, and at the end of balloon coronary occlusion with distal perfusion in a representative patient. The diastolic left ventricular pressure-volume relation at the end of balloon coronary occlusion with distal perfusion was shifted upward as compared to both the diastolic left ventricular pressure-volume relations at rest and at the end of the regular balloon coronary occlusion. Left ventricular ejection fraction was significantly lower at the end of balloon coronary occlusion with distal perfusion than at rest (36 ± 8 vs. $65 \pm 5\%$; $p < 0.001$) and was comparable to left ventricular ejection fraction at the end of the regular balloon coronary occlusion ($32 \pm 6\%$; NS). Left ventricular peak systolic pressure was comparable at rest (155 ± 18 mmHg), at the end of balloon coronary occlusion (148 ± 19 mmHg), and at the end of balloon coronary occlusion with distal perfusion (160 ± 21 mmHg). Left ventricular stroke work index at the end of the regular balloon coronary occlusion (35 ± 7 g.m) was lower than at rest (74 ± 17 g.m; $p < 0.01$) and than at the end of the balloon coronary occlusion with distal perfusion (46 ± 9 g.m; $p < 0.02$; Figure 31-6). Heart rate showed a similar increase from 69 ± 13 beats/min at rest to 76 ± 13 beats/min ($p < 0.01$) at the end of the regular balloon coronary occlusion and to 78 ± 11 beats/min ($p < 0.01$) at the end of the regular balloon coronary occlusion with distal perfusion.

CORONARY SINUS WASHOUT OF LACTATE, H^+, AND K^+

Figure 31-7 shows a representative example of coronary sinus concentrations of lactate and K^+, and coronary sinus pH measurements before pacing (t0), during the last 15 seconds of each pacing step (t1, t2, t3), immediately upon cessation of pacing (t4), 30 and 120 seconds following pacing (t5, t6), before balloon coronary occlusion (t'0), during balloon coronary occlusion (t'1), immediately upon release of the balloon (t'2), and 30 and 120 seconds following release of the balloon (t'3, t'4). During pacing there was a progressive rise in coronary sinus lactate and K^+ concentrations, which were significantly different from arterial concentrations at, respectively, the third and the first pacing step. Upon cessation of pacing there was an immediate fall in coronary sinus K^+ concentration. Coronary sinus lactate concentration remained elevated with respect to arterial concentration for 30 seconds following pacing. During balloon coronary occlusion, coronary sinus lactate and K^+ concentrations remained unaltered during the balloon inflation period but rose significantly upon release of the angioplasty balloon. A significant drop in coronary sinus pH occurred following release of the angioplasty balloon. During balloon coronary occlusion with distal perfusion, the pattern of coronary sinus lactate, K^+ concentrations, and pH were similar to the regular balloon coronary occlusion.

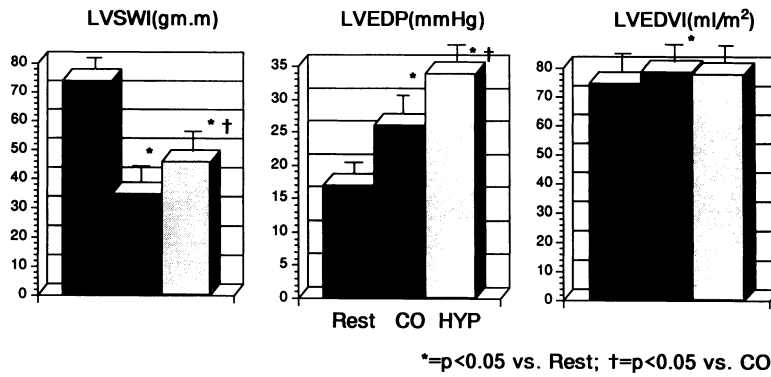

FIGURE 31-6. Bar graphs showing left ventricular stroke work index (LVSWI), left ventricular end-diastolic pressure (LVEDP), and left ventricular end-diastolic volume index (LVEDVI) observed at rest, at the end of a balloon coronary occlusion (CO), and at the end of an equally long balloon coronary occlusion with distal saline perfusion (hypoxemia; HYP).

Arterial lactate, K^+ concentrations, and pH remained unaltered throughout the study.

Discussion

LEFT VENTRICULAR DIASTOLIC DYSFUNCTION DURING DIFFERENT TYPES OF ISCHEMIA: EXPERIMENTAL EVIDENCE

The complex and often opposite interactions on left ventricular performance of lack of oxygen supply, of accumulation of tissue metabolites, and of vascular turgor were first investigated during the early phase of an ischemic insult in isolated, isovolumically beating and retrogradely Krebs-perfused rodent hearts (Figure 31-8). In the guinea-pig left ventricle, a switch from aerobic to hypoxic perfusion at constant coronary perfusion pressure induced within a 5-minute period a fall in developed tension and a rise in resting tension [32]. Because of the isovolumic contraction mode, this rise in resting tension was consistent with a drop in left ventricular distensibility. Increasing heart rate during the hypoxic perfusion period promoted the rise in resting tension. In a similar type of isolated, buffer-perfused rabbit heart with constant-volume left ventricular balloon, the acute effects of hypoxia with and without pacing tachycardia were compared to low-flow ischemia with and without pacing tachycardia [2]. Hypoxia caused a faster rise in left ventricular filling pressures and a slower decline of left ventricular developed pressure than low-flow ischemia, which resulted in an initial fall of left ventricular filling pressures. Pacing tachycardia superimposed on hypoxia accelerated the rise in left ventricular filling pressures, whereas pacing tachycardia superimposed on low-flow ischemia resulted in a rise of left ventricular filling pressures in only 2 of the 14 experiments.

Replacement of buffer perfusion by blood perfusion in the same isolated, isovolumically beating and retrogradely perfused rabbit left ventricle resulted in a consistent elevation of left ventricular filling pressures or a drop in left ventricular distensibility when pacing tachycardia was superimposed on global low-flow ischemia [33]. Hence, in the isovolumic rodent heart the initial left ventricular effects of an ischemic insult can be summarized as follows: (1) Low-flow ischemia results in a loss of left ventricular systolic performance and an increase in left ventricular chamber distensibility; (2) pacing tachycardia superimposed on low-flow ischemia results in a faster loss of left ventricular systolic performance and a decrease in left ventricular diastolic distensibility in blood but not in buffer-perfused preparations; (3) hypoxia results in a slower loss of left ventricular systolic performance and a decrease in left ventricular chamber distensibility, which is accelerated by superimposition of pacing tachycardia.

In anesthetized or conscious dogs, numerous

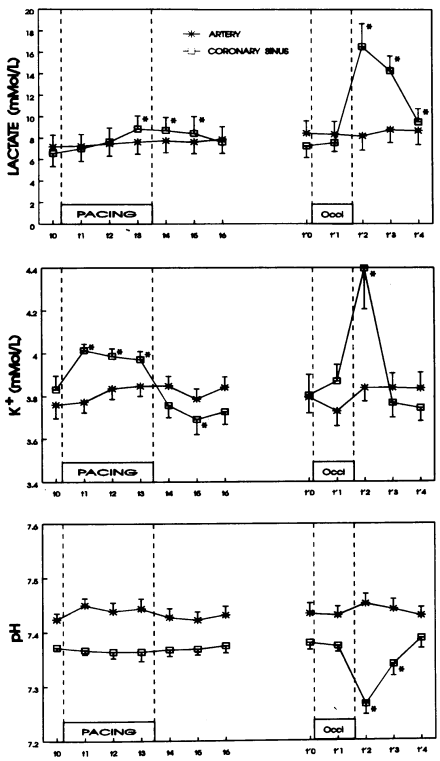

FIGURE 31-7. Coronary sinus and arterial lactate, K^+ concentrations and pH measured in a representative patient before pacing (t0); during pacing at 1 minute intervals (t1, t2, t3); 10, 30, and 120 seconds following cessation of pacing (t4, t5, t6); before balloon coronary occlusion (t'0); during balloon coronary occlusion (t'1); and 10, 30, and 120 seconds following release of the balloon (t'2, t'3, t'4). The rise in coronary sinus lactate and K^+ concentration started during the pacing episode, and coronary sinus lactate concentration remained elevated after cessation of pacing. There was no rise in coronary sinus lactate and K^+ concentration during the balloon occlusion (Occl) period. The rise in coronary sinus lactate and K^+ concentration and the drop in coronary sinus pH occurred after balloon deflation.

studies investigated left ventricular performance during brief episodes of single-vessel coronary occlusion and during pacing or exercise stress superimposed on single- or two-vessel coronary stenoses [6,8,34–39] (see Figure 31-8). During brief episodes of single-vessel coronary occlusion (=low-flow ischemia), myocardial shortening of the ischemic segment was replaced by passive bulging, and the diastolic pressure-segment length relation showed a rightward shift, suggestive of increased myocardial distensibility [3,6,8,39]. In conscious dogs with a single-vessel coronary stenosis, exercise (=limited flow-high demand ischemia) resulted in an upward shift of the early portion of the left ventricular diastolic pressure-volume relation [38], and a recent study in anesthetized pigs [40], which have less collateral perfusion than dogs, reported an upward shift of the entire left ventricular diastolic pressure-segment length relation after pacing in the presence of a single-vessel coronary stenosis. In anesthetized dogs, a drop in left ventricular systolic performance and an upward shift of the diastolic left ventricular pressure-volume relation was observed when pacing tachycardia superimposed on two-vessel coronary stenoses resulted in subendocardial ischemia and a large amount of myocardium at risk [35–37]. When pacing tachycardia superimposed on two-vessel coronary stenoses resulted in transmural myocardial ischemia, there was more profound impairment of left ventricular systolic performance with occasional bulging and unaltered diastolic left ventricular distensibility [39]. In isolated isovolumic dog hearts, global low-flow ischemia resulted in an increase in left ventricular diastolic distensibility [41], even in the presence of pacing tachycardia [42], but in the same preparation [43] a hypoxic perfusate of methemoglobin-containing red blood cells resulted in a significant decrease in left ventricular diastolic distensibility.

These experiments in dogs and pigs lead to the following conclusions on the early left ventricular effects of an ischemic insult: (1) Coronary occlusion (low-flow ischemia) results in a prompt reversal of systolic shortening into holosystolic bulging and an increase in myocardial diastolic left ventricular distensibility; (2) increased myocardial oxygen demand through pacing or exercise in the presence of reduced coronary flow (limited flow–high demand ischemia) causes reduced systolic shortening and a decrease in myocardial diastolic distensibility, provided the presence of sufficient amount of myocardium at risk and subendocardial ischemia; (3) hypoxia induces a prompt decrease in left ventricular diastolic distensibility.

	Rodent: Low flow ischemia	Rodent: Low flow, high demand ischemia	Rodent: Hypoxia	Dog: Low flow ischemia	Dog: Low flow, high demand ischemia	Dog : Hypoxia
Fall in LV distensibility		Serizawa JCI 1981 Isoyama Circ Res 1987	Nayler Cardiovasc Res 1978 Serizawa JCI 1981 Wexler Circ Res 1986		Serizawa Circ Res 1980 Momomura Circ Res 1984 Paulus AJP 1985	Wyman Circ Res 1989
Rise or no change in LV distensibility	Serizawa JCI 1981 Wexler Circ Res 1986			Tyberg Circ 1974 Palacios Circ 1976 Hess Circ Res 1983	Lorell AJP 1981 Applegate Circ 1990	

FIGURE 31-8. Overview of experimental evidence on left ventricular diastolic dysfunction during different types of ischemia.

	Man: Balloon Occlusion Ischemia	Man: Spontaneous Coronary Spasm	Man: Pacing -induced Ischemia	Man: Exercise -induced Ischemia	Man: Hypoxia
Fall in LV distensibility	Wijns JACC 1986 Carlson AJC 1987 Kass Circ 1990	Sharma AJC 1983	Barry Circ 1974 Bourdillon Circ 1983 Sasayama JACC 1985 Bronzwaer Circ 1991	Caroli Circ 1983 Nonogi JACC 1989	De Bruyne Circ 1991
Rise or no change in LV distensibility	Bertrand JACC 1988 Bronzwaer Circ 1991				

FIGURE 31-9. Overview of clinical evidence on left ventricular diastolic dysfunction during different types of ischemia.

COMPARISON OF INITIAL LEFT VENTRICULAR DYSFUNCTION DURING DIFFERENT TYPES OF ISCHEMIA: CLINICAL EVIDENCE

Previous studies on the initial effects of ischemia on global and regional left ventricular function in humans looked at a single type of ischemic insult (Figure 31-9): pacing-induced ischemia [9–11,13,14], exercise-induced ischemia [44,45], spontaneous coronary spasm [12], or balloon coronary occlusion ischemia [15–23]. Pacing tachycardia in the presence of triple-vessel coronary disease [9–11] and spontaneous angina [12] resulted in an upward shift of the global diastolic left ventricular pressure-volume relation, of the diastolic left ventricular pressure-radial length relation [13], and of the diastolic left ventricular pressure-wall thickness relation [14] of the ischemic myocardium. Exercise resulted in similar changes, but at end diastole there was a trend for the diastolic left ventricular pressure-radial length relation to converge towards the resting curve [45]. A similar finding was recently reported

during exercise after heart transplantation [46] and was explained by a blunted lusitropic left ventricular response to catecholamines because of simultaneous use during exercise of left ventricular preload reserve. At the end of balloon coronary occlusion, most of the previous studies [16,17,19], except one [18], observed an upward shift of the global diastolic left ventricular pressure-volume relation and the regional diastolic left ventricular pressure-radial length relation of the ischemic segment. The present studies [24,25] were the first to compare in the same patient the initial left ventricular effects of different types of ischemia: low-flow ischemia of balloon coronary occlusion, limited flow-high demand ischemia of pacing-induced angina, and/or hypoxemia induced by balloon coronary occlusion with maintained hypoxic perfusion distal to the balloon occlusion.

When comparing pacing-induced ischemia to balloon coronary occlusion ischemia, the following conclusions were reached [24]: (1) During pacing-induced ischemia, left ventricular ejection fraction was larger than at the end of balloon coronary occlusion. (2) During both interventions, left ventricular end-diastolic pressure rose but left ventricular end-diastolic volume index was significantly larger than the control value only at the end of balloon coronary occlusion. This was consistent with an upward shift of the end-diastolic pressure volume relation during pacing-induced ischemia and a more rightward shift of the end-diastolic pressure volume relation at the end of balloon coronary occlusion. (3) The upward shift of the diastolic left ventricular pressure-radial length plot of the ischemic segment was larger during pacing-induced ischemia than at the end of balloon coronary occlusion. (4) At the end of balloon coronary occlusion, a correlation was observed for the ischemic segment between systolic shortening and the upward shift of the diastolic left ventricular pressure-radial length plot.

This correlation observed at the end of balloon coronary occlusion between systolic shortening of the ischemic segment and the upward shift of the diastolic left ventricular pressure-radial length plot reconciles the contradictory results between the present and some of the previous studies on diastolic left ventricular distensibility changes at the end of balloon coronary occlusion. The studies by Wijns et al. [16] and by Kass et al. [19] observed a 20% decrease in systolic segmental shortening, a fall in ejection fraction from $69 \pm 8\%$ to $54 \pm 12\%$, and a decrease in global or regional left ventricular diastolic distensibility, as evident from an upward shift of the diastolic pressure-radial length or pressure-volume relations. The present study observed a 34% decrease in systolic segmental shortening and a fall in ejection fraction from $77 \pm 7\%$ to $47 \pm 11\%$, which was comparable to the fall in ejection fraction observed by Bertrand et al. [18] (from $72 \pm 6\%$ to $46 \pm 10\%$). Both the present study and the study by Bertrand et al. observed, respectively, an upward and rightward shift of the diastolic pressure-radial length relation and no significant change in the radial stiffness modulus. Hence, an interstudy comparison reveals an interaction at the end of balloon coronary occlusion between systolic left ventricular performance and diastolic left ventricular distensibility. This interaction was similar to the correlation observed in the present study [24] at the end of balloon coronary occlusion between individual data on systolic performance and diastolic distensibility.

The variability in depression of systolic performance at the end of balloon coronary occlusion, both individually and among different studies, probably relates to the presence or absence of objective evidence of myocardial ischemia at the end of the balloon occlusion episode, to differences in balloon inflation time, to variable recruitment of collaterals, and to procurement of data during either first or subsequent balloon inflations. In the study of Wijns et al. [16], balloon inflation time (± 30 seconds) was shorter than in the present study (± 60 seconds), and the balloon inflation used for study of left ventricular performance varied from a third to a tenth balloon inflation, whereas in the present study [24] the balloon inflation varied from a second to a fourth balloon inflation. In the present study all patients had angina and ST-segment changes at the end of balloon coronary occlusion. Moreover, the present study documented the absence of significant coronary collateralization by coronary wedge pressure measurement during balloon coronary occlusion.

Previous studies on the effects of pacing stress on left ventricular performance examined patients with triple-vessel coronary disease and observed an upward shift of the entire diastolic left ventricular pressure-volume [9,10] or diastolic pressure-wall thickness relation [14]. The present study [24] examined patients with single-vessel coronary disease, and the smaller amount of myocardium at risk in these patients could explain the occasional limitation of the upward shift of the diastolic left ventricular pressure-volume or of the diastolic pressure-radial length relation to early and mid-diastole. A similar upward shift limited to the initial portion of the diastolic left ventricular pressure-segment length relation was recently also reported in conscious dogs with a single-vessel coronary stenosis of the left circumflex coronary artery [38]. Despite the presence of single-vessel

coronary disease, some patients experienced profound depression of left ventricular systolic performance during pacing-induced ischemia. In these patients the diastolic left ventricular pressure-volume and the diastolic pressure-radial length relations failed to change. A similar relation between depressed systolic performance and unaltered diastolic distensibility during pacing-induced ischemia has been reported by other investigators in humans [13] and in anesthetized dogs with two-vessel coronary stenoses [3]. Hence, a drastic reduction of left ventricular systolic performance during either pacing-induced or balloon occlusion ischemia precludes in humans reductions in global or regional diastolic left ventricular distensibility.

The present studies [25] also compared left ventricular diastolic distensibility at the end of a balloon coronary occlusion and at the end of an equally long balloon coronary occlusion during which saline was perfused through the distal lumen of the balloon catheter at a flow rate (=1 ml/sec) that equalled resting left anterior descending flow. The latter intervention resulted in a myocardial oxygen delivery that was 38 times lower than normal and therefore mimicked hypoxemic conditions. During balloon occlusion with distal perfusion, myocardial oxygen delivery was probably comparable to regular balloon occlusion because the minimal amount of oxygen dissolved in the perfusate was offset by reduced oxygen delivery from collateral flow. During balloon inflation with distal perfusion, collateral flow was less than during regular balloon inflation because of higher intravascular pressure created by the perfusion pump in the epicardial coronary arteries distal to the balloon occlusion. Continuous saline perfusion during balloon coronary occlusion caused no change in left ventricular end-diastolic volume at the end of the occlusion episode but a marked elevation of left ventricular minimum and end-diastolic pressures, as compared to regular balloon coronary occlusion. This profound decrease in left ventricular diastolic distensibility at the end of balloon coronary occlusion with distal perfusion was accompanied by better preservation of left ventricular systolic performance, as evident from the higher left ventricular stroke work index. Hence, the effects of hypoxemia on left ventricular performance in humans resemble the effects of hypoxia in isolated rodent or dog preparations by the smaller depression of left ventricular systolic function and by the larger decrease of left ventricular diastolic distensibility.

Several pathophysiological mechanisms could contribute to the unequal effect of different types of ischemia on the human myocardium; (1) variable vascular turgor and myocardial stretch during the ischemic episode or during the hyperemic phase following the ischemic episode, (2) buildup or washout of tissue metabolites during the ischemic episode, (3) unequal intensity of the ischemic stress episodes, and (4) regional dyssynchrony and biventricular interaction.

VASCULAR TURGOR AND MYOCARDIAL STRETCH DURING ISCHEMIA AND HYPEREMIA

Coronary perfusion pressure, coronary flow, or both influence left ventricular systolic performance (Gregg phenomenon) [47] and left ventricular diastolic distensibility (Salisbury effect) [48]. The Gregg phenomenon received renewed attention as the initial mediator of the loss of left ventricular systolic function [49,50]. In the isovolumic rodent heart, a Gregg phenomenon was demonstrated during the initial stages of no-flow global myocardial ischemia by the slower decline of left ventricular developed pressure in the microembolized heart without coronary depressurization than after simple interruption of coronary flow [49]. Only in the microembolized heart did the time course of left ventricular pressure decline correspond to the buildup of tissue metabolites such as phosphate or H^+, which are known to deactivate cardiac muscle through desensitization of myofilaments. The earlier loss of left ventricular systolic function during interruption of coronary flow was therefore attributed to loss of vascular stretch on adjacent sarcomeres. Vascular turgor could affect muscle sarcomere stretch through mechanical coupling of the vascular network and the myocardium. Recent experiments in pig hearts, however, correlated the initial loss of myocardial function during moderate ischemia of single-vessel coronary stenosis with the fall in high energy phosphates and not with the decrease in muscle preload [50].

The present observations confirm in humans the importance of coronary vascular turgor as a mediator of the loss in left ventricular systolic performance during low-flow ischemia of balloon coronary occlusion. When coronary vascular turgor was maintained during balloon coronary occlusion by distal perfusion, there was better preservation of left ventricular stroke work at the end of an equally long balloon coronary occlusion. This preservation of left ventricular stroke work could also result from unequal buildup of tissue metabolites during regular balloon coronary occlusion and balloon coronary occlusion with distal perfusion. Coronary sinus lactate concentration showed, however, a similar time course in both the regular balloon coronary occlusion and the balloon

coronary occlusion with distal perfusion. In both interventions, coronary sinus lactate concentration during the actual balloon inflation period was comparable to arterial lactate concentration, and peak coronary sinus lactate concentration occurred at a comparable moment after release of the angioplasty balloon. This finding suggests the better preservation of systolic performance at the end of balloon coronary occlusion with distal perfusion to be related more to vascular turgor affecting cardiac muscle stretch than to unequal tissue levels of metabolites. Recent insights on how cardiac muscle stretch affects muscle performance suggest an intrinsic molecular property of troponin-C to mediate the rising limb of the cardiac muscle length-active tension relation [51]. This implies that vascular turgor affects cardiac muscle performance by a mechanism similar to tissue metabolites, namely, modulation of myofilamentary calcium sensitivity, and that the relative contributions of vascular turgor and of tissue metabolites on the maintenance of systolic function during the initial stages of ischemia are hard to separate. Moreover, vascular stretch could affect adjacent cardiac muscle sarcomeres, not only mechanically, but also through release from the coronary endothelium of substances, which have recently been shown to alter cardiac muscle performance, also through modulation of myofilamentary calcium sensitivity [52,53].

To measure coronary depressurization during balloon coronary occlusion, we recently obtained high-fidelity intracoronary pressure recordings using a 0.018 angioplasty guidewire with a micromanometer pressure transducer mounted on the guidewire tip. Figure 31-10 shows high-fidelity recordings of intracoronary pressure distal to the occluded balloon and of left ventricular pressure. During balloon

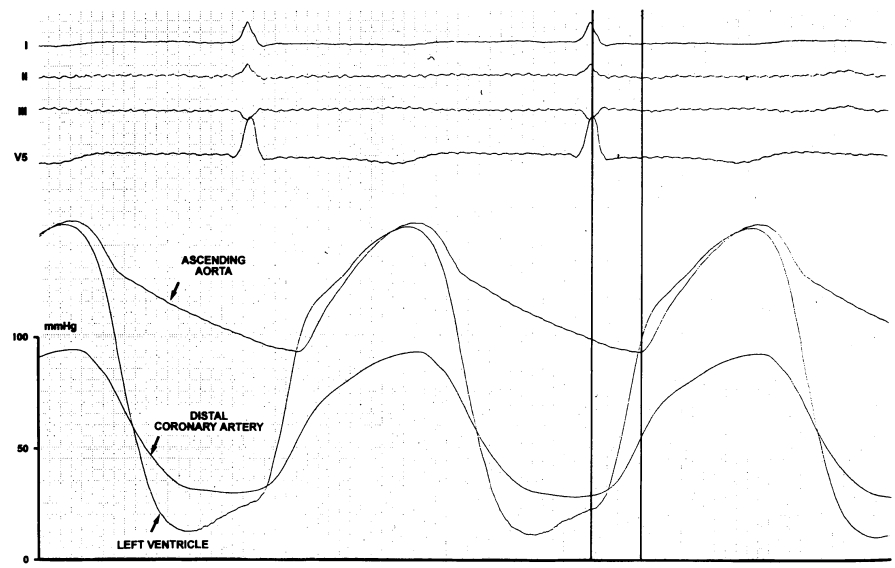

FIGURE 31-10. From *top to bottom*: Three surface lead electrocardiograms (I, II, III), one precordial lead electrocardiogram, a high-fidelity micromanometer aortic pressure recording, a high-fidelity micromanometer left ventricular pressure recording, and a high-fidelity micromanometer intracoronary pressure recording obtained distal to an inflated angioplasty balloon and derived from a micromanometer pressure transducer mounted on an 0.018 angioplasty guidewire. During balloon occlusion ischemia there is reduced diastolic intracoronary pressure. During systole, however, there is immediate buildup of intracoronary pressure, probably because of distention of the balloon occluded epicardial coronary compartment by blood squeezed out of the adjacent and normally contracting left ventricular segments. This buildup of systolic intracoronary pressure argues against the existence of total vascular collapse during balloon occlusion ischemia in humans.

occlusion, there is indeed reduced diastolic intracoronary pressure. During systole, however, there is immediate buildup of intracoronary pressure, probably because of squeezing of blood from adjacent normally contracting zones into the balloon occluded epicardial coronary compartment. This immediate buildup of intracoronary pressure during systole argues against the existence of total vascular collapse during balloon occlusion ischemia in humans and confirms the results from recent experiments in pigs, which correlated the initial loss of myocardial function during moderate ischemia of single-vessel coronary stenosis with the fall in high energy phosphates, and not with the decrease in muscle preload [50].

Salisbury et al. were the first to observe in an isovolumic canine left ventricle an increase in left ventricular end-diastolic pressure at increased coronary perfusion pressure [48]. Alterations in left ventricular diastolic distensibility after changes in coronary vascular engorgment and coronary perfusion have subsequently been confirmed by other investigators, who observed larger changes in diastolic left ventricular wall stiffness when coronary perfusion pressure was altered in a failing left ventricle than in a normal left ventricle [54]. The relative importance of coronary perfusion pressure vs. coronary flow as determinants of myocardial wall stiffness was elucidated by Olsen et al. [55], who observed a significant effect of coronary perfusion pressure and not of coronary flow on left ventricular compliance and who favored direct sarcomere stretching by the coronary vascular tree [56] as the mechanism underlying erectile stiffening of the left ventricular myocardium. During balloon occlusion ischemia (low-flow ischemia), the drop in coronary perfusion pressure distal to the balloon occlusion could increase diastolic left ventricular distensibility. A rightward and downward shift of the diastolic left ventricular pressure-volume relation was indeed observed in the present studies in some patients at the end of balloon coronary occlusion (see Figure 31-2). During pacing-induced ischemia (limited flow-high demand ischemia), coronary perfusion pressure distal to the coronary stenosis falls. Based on the Salisbury effect, this drop in coronary perfusion pressure distal to the coronary stenosis would tend to counteract the decrease in left ventricular diastolic distensibility observed during pacing-induced ischemia. On the other hand, during pacing-induced ischemia left ventricular function is assessed during initial relief of the ischemic stress episode, in contrast to balloon coronary occlusion ischemia, in which left ventricular function is assessed at the nadir of the ischemic stress episode. Even when a critical coronary stenosis permits no change in total coronary blood flow, a reactive hyperemic response to the ischemic subendocardium following the pacing stress episode could contribute to the observed decrease in diastolic left ventricular distensibility [57], as previously observed during exercise [58] or isoproterenol infusion [59] in dogs with critical coronary stenosis. During balloon coronary occlusion with distal perfusion (hypoxemia), coronary perfusion pressure was preserved and probably contributed to the larger decrease in diastolic left ventricular distensibility as compared to regular balloon coronary occlusion.

BUILDUP OR WASHOUT OF TISSUE METABOLITES

Low-flow ischemia of balloon coronary occlusion, limited flow-high demand ischemia of pacing-induced angina, and hypoxemia exert different effects on buildup of tissue metabolies, such as H^+ and inorganic phosphate. Acidosis as a result of buildup of tissue metabolites drives the creatine-kinase reaction, which replenishes ATP, depletes creatine phosphate, and produces inorganic phosphate [4]. Despite increased amplitude of the calcium transient and myoplasmic calcium overload, this rise in inorganic phosphate [50] induces contractile failure by reducing the calcium sensitivity of myofilaments. The present studies measured coronary sinus lactate, H^+, and K^+ concentrations at rest, during, and following the pacing-stress episode; during and following the regular balloon coronary occlusion; and during and following an equally long balloon coronary occlusion with distal perfusion (see Figure 31-7). Because no simultaneous coronary sinus flow measurements were performed, the coronary sinus lactate, H^+, and K^+ concentrations provide no quantitative information on myocardial metabolite production rate but allow assessment of the time course of metabolite handling during the different types of ischemic insult. During pacing-induced ischemia, metabolites started to appear in the coronary sinus during the pacing-stress episode before cessation of pacing and continued to be washed out after the pacing-stress episode.

The better preservation of left ventricular systolic performance and the larger decrease of left ventricular diastolic distensibility, as compared to balloon occlusion ischemia, could well have been influenced by the ongoing washout of tissue metabolites. Partial removal of inorganic phosphate and of H^+ preserved myofilamentary calcium sensitivity and could explain the better preservation of left ventricular systolic performance. Partial removal of metabolites could also explain the larger decrease in diastolic left ventricular distensibility, because preserved myofila-

mentary calcium sensitivity could allow for diastolic crossbridge cycling in the presence of a simultaneous myoplasmic calcium overload.

During the low-flow ischemia of balloon coronary occlusion, the time course of coronary sinus lactate, H^+, and K^+ concentrations was different from the time course during the limited flow-high demand ischemia of pacing-induced ischemia. During the ischemic stress episode of balloon coronary occlusion, there was no rise of coronary sinus lactate concentration (see Figure 31-7), the peak of which was observed after deflation of the angioplasty balloon during the hyperemic phase. At the end of the balloon inflation, when left ventricular function was measured, buildup of tissue metabolites and the ensuing decrease in myofilamentary calcium sensitivity could explain the significantly larger decrease in left ventricular stroke work index at the end of balloon coronary ischemia than during pacing-induced ischemia. The time pattern of coronary sinus lactate concentration during balloon occlusion with distal perfusion was comparable to the regular balloon occlusion. Because of the similar time pattern of coronary sinus lactate concentration in both balloon occlusions, the better preservation of left ventricular systolic performance, and the decrease in left ventricular diastolic distensibility observed during balloon inflation with distal perfusion seemed more likely to be related, respectively, to differences of vascular stretch (Gregg phenomenon) and to differences in vascular engorgement (Salisbury effect) than to unequal myocardial buildup of tissue metabolites. Because of the relative insensitivity of coronary sinus sampling to detect local washout of metabolites and because of the failure to perform great cardiac vein sampling to assess selectively left anterior descending drainage, unequal intracellular accumulation of metabolites during coronary occlusion and during coronary occlusion with distal perfusion was not excluded, however.

The perfusate used during the balloon inflations with distal perfusion was a mixture of regular saline (pH = 7.0) infused through the distal lumen of the balloon catheter at a constant flow rate of 1 ml/sec and blood coming from coronary collaterals (mean coronary wedge pressure = 29 ± 11 mmHg). Compared to whole blood perfusion, this perfusate was of lower pH, hypokalemic, and hypocalcemic. The higher left ventricular systolic performance observed in the present study during balloon inflation with distal perfusion compared with the regular balloon inflation is, however, unrelated to the altered composition of the perfusate relative to whole blood because in isolated cardiac muscle preparations both acidosis and combined hypokalemia-hypocalcemia exerted a negative inotropic effect [60]. Hypoxic whole-blood perfusion could probably have resulted in even better preservation of left ventricular systolic performance and in even larger reductions in left ventricular diastolic distensibility, because the lower pH of the perfusate as compared to whole blood perfusion could have resulted in reduced diffusion of hydrogen ions out of the myocardium and because in isovolumic rabbit hearts replacement of buffer perfusion by blood perfusion caused a consistent elevation of left ventricular filling pressures and a consistent fall in left ventricular diastolic distensibility during low-flow–high-demand ischemia [33]. Whole blood perfusion was, therefore, considered to provide higher mechanical vascular support of surrounding myocardium. Moreover, as compared to saline perfusion, whole blood perfusion could modify the release from vascular endothelial cells of factors that influence myofilamentary calcium sensitivity [52,53]. Patients experienced more severe chest pain during the balloon inflation with distal perfusion, probably consistent with a larger production of bradykinin, which triggers cardiac nociceptors and paracrine secretions from endothelial cells. These factors released from vascular endothelial cells could increase myofilamentary calcium sensitivity and contribute to the better preservation of left ventricular stroke work index observed during the balloon inflation with distal perfusion. Increased cardiac sympathetic stimulation could have resulted from the increased severity of chest pain during the balloon inflation with distal perfusion. At the end of both balloon inflations heart rates were, however, comparable, and at the end of the balloon inflation with distal perfusion, the prolongation of the time constant of left ventricular pressure decay was larger. The latter cannot be reconciled with a larger lusitropic effect of more intense sympathetic stimulation.

UNEQUAL INTENSITY OF THE ISCHEMIC STRESS EPISODES

Unequal intensity of ischemia could have interfered with global and regional left ventricular function during the different ischemic stress episodes. In a study on anesthetized dogs, pacing-induced ischemia in the presence of coronary stenoses and coronary occlusion ischemia produced similar changes in systolic and diastolic function of the ischemic left ventricular region if left ventricular end-diastolic pressure was matched in each experiment during both ischemic stress episodes [39]. In the present clinical studies, comparable levels of left ventricular end-diastolic pressure could not be achieved in each patient, but

the pooled patient data comparing pacing-induced and balloon occlusion ischemia revealed similar left ventricular end-diastolic pressures as a result of both ischemic stress episodes. Despite these similar elevations in left ventricular filling pressures, left ventricular ejection fractions and left ventricular end-diastolic volume indices were significantly different. When comparing balloon occlusion ischemia to hypoxemia, the pooled patient data showed the highest left ventricular end-diastolic pressure during hypoxemia, when the left ventricular stroke work index showed the smallest reduction. These observations cannot be reconciled with the left ventricle operating on a single diastolic left ventricular compliance curve during the different ischemic stress episodes and with unequal severity of ischemia of the different ischemic stress episodes inducing different degrees of left ventricular failure.

During balloon occlusion ischemia with distal perfusion, more oxygen could be delivered to the myocardium because of oxygen dissolved in the perfusate. The amount of oxygen dissolved in the perfusate was, however, minimal (±5 ml/l) and approximately 38 times less than in arterial blood. This additional amount of oxygen delivered to the ischemic region during balloon inflation with perfusion was probably offset by reduced oxygen delivery to the ischemic region from collateral flow because of higher intravascular pressure created by the perfusion pump in the epicardial coronary arteries distal to the balloon occlusion.

The most accurate assessment of the severity of ischemia in these different types of intervention is measurement of myocardial adenosine triphosphate and creatine phosphate contents in the ischemic myocardium as performed in dogs using a transmural biopsy drill by Momomura et al. [4] during pacing-induced and coronary occlusion ischemia and as performed noninvasively in humans during handgrip-induced angina using nuclear-magnetic-resonance spectroscopy [61].

REGIONAL DYSSYNCHRONY AND BIVENTRICULAR INTERACTION

Slower left ventricular isovolumic relaxation and filling can result from loss of synchronicity of contraction and relaxation of different left ventricular segments [62,63]. As evident from clinical observations in patients with coronary artery disease [64] and from experimental findings in a single-vessel coronary stenosis model in pigs [40], synchronicity of diastolic wall motion of ischemic and nonischemic left ventricular segments is drastically affected by brief coronary occlusion and only slightly affected by pacing-induced ischemia in the presence of coronary stenoses.

In a patient who had received radioopaque markers in the left ventricular myocardium at the time of coronary bypass surgery, regional wall motion could be accurately followed during balloon occlusion of a saphenous vein bypass graft [64]. During the balloon occlusion, the segmental shortening pattern evolved from systolic shortening to midsystolic bulging and to holosystolic bulging with early diastolic recoil. The dyssynchronous early diastolic recoil slowed isovolumic left ventricular relaxation but did not alter regional diastolic distensibility of the ischemic segment, as evident from the unchanged diastolic pressure-segment length relation of the ischemic segment. This confirmed in humans the findings of numerous animal experiments investigating the regional left ventricular effect of brief coronary artery ligation [6,8].

A similar loss of synchronous early diastolic filling has also been implicated as the cause of the upward shift of the diastolic left ventricular pressure-volume relation in humans during pacing-induced angina [65]. During pacing-induced angina in humans, dyssynchrony between ischemic and nonischemic segments is, however, much smaller than during coronary occlusion, as evident from the ±50 msec reduction in time to peak posterior wall thickness [14] and the ±50 msec reduction of time to peak segment lengthening [66]. Moreover, the decrease in diastolic left ventricular distensibility during pacing-induced angina was larger when the amount of myocardium at risk assessed by a simultaneously performed thallium scan was larger [67]. If dyssynchony between ischemic and nonischemic left ventricular segments was the mechanism for the decrease in diastolic left ventricular distensibility, an equal magnitude of ischemic and nonischemic areas would tend to cause the largest shifts in diastolic left ventricular distensibility. A similar argument against regional dyssynchrony as the mechanism for the decrease in diastolic left ventricular distensibility during pacing-induced ischemia is provided by patients with aortic stenosis, in whom pacing results in an upward shift of the diastolic left ventricular pressure-volume relation despite uniform distribution of subendocardial ischemia [68].

During pacing-induced and balloon coronary occlusion ischemia, left ventricular end-diastolic pressure rose to a similar level. Reactive pulmonary hypertension and right ventricular loading, although not directly measured, were therefore probably comparable in both interventions. Eventual right-ventricular interaction through the shared interventricular

septum must have been similar and fails to explain the unequal changes in diastolic left ventricular distensibility in both interventions.

Conclusions

Diastolic left ventricular pressure-volume and diastolic left ventricular pressure-radial length relations were compared in patients with significant left anterior descending coronary stenosis during pacing-induced ischemia (low-flow–high-demand ischemia), during balloon coronary occlusion (low-flow ischemia), and during balloon coronary occlusion with preserved hypoxic perfusion distal to the balloon occlusion (hypoxemia). During pacing-induced ischemia and at the end of balloon coronary occlusion with distal perfusion, left ventricular systolic performance showed a smaller decrease and left ventricular diastolic distensibility a larger decrease than at the end of balloon coronary occlusion. Better preservation of myofilamentary calcium sensitivity during pacing-induced ischemia as a result of washout of tissue metabolites, of mechanical stretch of maintained coronary pressurization, and of endothelial factors released by continuing coronary flow could explain not only the smaller decrease in left ventricular systolic performance but also the larger decrease in left ventricular diastolic distensibility because of diastolic crossbridge cycling in the presence of a simultaneous myoplasmic calcium overload.

References

1. Apstein CS, Grossman W (1987). Opposite initial effects of supply and demand ischemia on left ventricular diastolic compliance: The ischemia-diastolic paradox. J Mol Cell Cardiol 19:119–128.
2. Serizawa T, Vogel WM, Apstein CS, Grossman W (1981). Comparison of acute alterations in left ventricular relaxation and diastolic chamber stiffness induced by hypoxia and ischemia. J Clin Invest 64:91–102.
3. Paulus WJ, Grossman W, Serizawa T, Bourdillon PD, Pasipoularides A, Mirsky I (1985). Different effects of two types of ischemia on myocardial systolic and diastolic function. Am J Physiol 248:H719–H728.
4. Momomura SI, Ingwall J, Parker JA, Sahagian P, Ferguson JJ, Grossman W (1985). The relationships of high energy phosphates, tissue pH, and regional blood flow to diastolic distensibility in the ischemic dog myocardium. Circ Res 57:822–835.
5. Wexler LF, Weinberg EO, Ingwall JS, Apstein CS (1986). Acute alterations in diastolic left ventricular chamber distensibility: Mechanistic differences between hypoxemia and ischemia in isolated perfused rabbit and rat hearts. Circ Res 59:515–528.
6. Tyberg JV, Forrester JS, Wyatt HL, Goldner SJ, Parmley WW, Swann HJC (1974). An analysis of segmental ischemic dysfunction utilizing the pressure-length loop. Circulation 49:748–754.
7. Wong BYS, Toyama M, Reis RL, Goodayer AVN (1978). Sequential changes in left ventricular compliance during acute coronary occlusion in the isovolumic working canine heart. Circ Res 43:274–286.
8. Hess OM, Osakada G, Lavelle JF, Gallagher KP, Kemper WS, Ross J Jr (1983). Diastolic myocardial wall stiffness and ventricular relaxation during partial and complete coronary occlusions in the conscious dog. Circ Res 52:387–400.
9. Dwyer EM Jr (1970). Left ventricular pressure-volume alterations and regional disorders of contraction during myocardial ischemia induced by atrial pacing. Circulation 42:1111–1122.
10. Barry WH, Brooker JZ, Alderman EL, Harrison DC (1974). Changes in diastolic stiffness and tone of the left ventricle during angina pectoris. Circulation 49: 255–263.
11. Mann T, Goldberg S, Mudge GH, Grossman W (1979). Factors contributing to altered left ventricular diastolic properties during angina pectoris. Circulation 59:14–20.
12. Sharma B, Behrens TW, Erlein D, Hodges M, Asinger RW, Francis GS (1983). Left ventricular diastolic properties and filling characteristics during spontaneous angina pectoris at rest. Am J Cardiol 52:704–709.
13. Sasayama S, Nonogi H, Miyazaki S, Sakurai T, Kawai C, Eiho S, Kuwahara M (1985). Changes in diastolic properties of the regional myocardium during pacing-induced ischemia in human subjects. J Am Coll Cardiol 5:599–606.
14. Bourdillon PD, Lorell BH, Mirsky I, Paulus WJ, Wynne J, Grossman W (1983). Increased regional myocardial stiffness of the left ventricle during pacing induced angina in man. Circulation 67:316–323.
15. Serruys PW, Wijns W, van den Brand M, Meij S, Slager C, Schuurbiers JCH, Hugenholtz PG, Brower RW (1984). Left ventricular performance, regional blood flow, wall motion, and lactate metabolism during transluminal angioplasty. Circulation 70:25–36.
16. Wijns W, Serruys PW, Slager CJ, Grimm J, Krayenbuehl HS, Hugenholtz P, Hess OM (1986). Effect of coronary occlusion during percutaneous transluminal angioplasty in humans on left ventricular chamber stiffness and regional diastolic pressure-radius relations. J Am Coll Cardiol 7:455–463.
17. Carlson EB, Hinohara T, Morris KG (1987). Recovery of systolic and diastolic left ventricular function after a 60-second coronary arterial occlusion during percutaneous transluminal coronary angioplasty for angina pectoris. Am J Cardiol 60:460–466.
18. Bertrand ME, Lablanche JM, Fourrier JL, Traisnel G, Mirsky I (1988). Left ventricular systolic and diastolic function during acute coronary artery balloon occlusion in humans. J Am Coll Cardiol 12:341–347.
19. Kass DA, Midei M, Brinker J, Maughan WL (1990).

Influence of coronary occlusion during PTCA on end-systolic and end-diastolic pressure-volume relations in humans. Circulation 81:447–460.
20. Kern MJ, Deligonul U, Labovitz A (1989). Influence of drug therapy on the ischemic response to acute coronary occlusion in man: Supply-side economics. Am Heart J 118:361–380.
21. Deutsch E, Berger M, Kussmaul WG, Hirshfeld JW Jr, Herrmann HC, Laskey WK (1990). Adaptation to ischemia during percutaneous transluminal coronary angioplasty. Circulation 82:2044–2051.
22. Cribier A, Korsatz L, Koning R, Rath P, Gamra H, Stix G, Merchant S, Chan C, Letac B (1992). Improved myocardial ischemic response and enhanced collateral circulation with long repetitive coronary occlusion during angioplasty: A prospective study. J Am Coll Cardiol 20:578–586.
23. De Bruyne B, Vantrimpont PJ, Goethals M, Stockbroeckx J, Paulus WJ (1992). Can coronary angioplasty serve as a model of myocardial preconditioning (abstr)? Circulation 86:I-378.
24. Bronzwaer JGF, De Bruyne B, Ascoop CAPL, Paulus WJ (1991). Comparative effects of pacing-induced and balloon coronary occlusion ischemia on left ventricular diastolic function in man. Circulation 84: 211–222
25. De Bruyne B, Bronzwaer JGF, Heyndrickx GR, Paulus WJ (1993). Comparative effects of ischemia and hypoxemia on left ventricular systolic and diastolic function in man. Circulation 88:461–471.
26. Meier B, Luethy P, Finci L, Steffenino GD, Rutishauser W (1987). Coronary wedge pressure in relation to spontaneously visible and recruitable collaterals. Circulation 75:906–913.
27. De Bruyne B, Meier B, Finci L, Urban P, Rutishauser W (1988). Potential protective effect of high coronary wedge pressure on left ventricular function after coronary occlusion. Circulation 78:566–572.
28. Pepine CJ, Mehta J, Webster WW, Nichols WW (1978). In vivo validation of a thermodilution method to determine regional left ventricular blood flow in patients with coronary disease. Circulation 58:795–802.
29. Paulus WJ, Vantrimpont PJ, Rousseau MF (1992). Diastolic function of the non-filling human left ventricle. J Am Coll Cardiol 20:1524–1532.
30. Fifer MA, Grossman W (1991). Measurement of ventricular volumes, ejection fraction, mass and wall stress. In Grossman W, Baim DS (eds): *Cardiac Catheterization, Angiography, and Intervention*, 4th ed. Philadelphia: Lea & Febiger, pp 300–318.
31. Webb SC, Rickards AF, Poole-Wilson PA (1983). Coronary sinus potassium concentration recorded during coronary angioplasty. Br Heart J 50:146–148.
32. Nayler WG, Yepez CE, Poole-Wilson PA (1978). The effect of β-adrenoreceptor and Ca^{2+} antagonist drugs on the hypoxia-induced increase in resting tension. Cardiovasc Res 12:666–674.
33. Isoyama S, Apstein CS, Wexler LF, Grice WN, Lorell BH (1987). Acute decrease in left ventricular diastolic chamber distensibility during simulated angina in isolated hearts. Circ Res 61:925–933.
34. Tomoike H, Franklin D, Mc Kown D, Kemper WS, Guberek M, Ross J Jr (1978). Regional myocardial dysfunction and hemodynamic abnormalities during strenuous exercise in dogs with limited coronary flow. Circ Res 42:487–496.
35. Serizawa T, Carabello BA, Grossman W (1980). Effect of pacing induced ischemia on left ventricular diastolic pressure-volume relations in dogs with coronary stenoses. Circ Res 46:430–439.
36. Paulus WJ, Serizawa T, Grossman W (1982). Altered left ventricular diastolic properties during pacing-induced ischemia in dogs with coronary stenoses: Potentiation by caffeine. Circ Res 50:218–227.
37. Momomura SI, Bradley AB, Grossman W (1984). Left ventricular diastolic pressure-segment length relations and end-diastolic distensibility in dogs with coronary stenoses: An angina physiology model. Circ Res 55:203–214.
38. Miyazaki S, Guth BD, Miura T, Indolfi C, Schulz R, Ross J Jr (1990). Changes of left ventricular diastolic function in exercising dogs without and with ischemia. Circulation 81:1058–1070.
39. Applegate RJ, Walsh RA, O'Rourke RA (1990). Comparative effects of pacing-induced and flow-limited ischemia on left ventricular function. Circulation 81:1380–1392.
40. Takahashi T, Levine MJ, Grossman W (1991). Regional diastolic mechanics of ischemic and nonischemic myocardium in the pig heart. J Am Coll Cardiol 17:1203–1213.
41. Palacios I, Newell JB, Powell WJ Jr (1976). Left ventricular end-diastolic pressure-volume relationships with experimental acute global ischemia. Circulation 39:735–744.
42. Lorell BH, Palacios I, Daggett WM, Jacobs ML, Fowler BN, Newell JB (1981). Right ventricular distension and left ventricular compliance. Am J Physiol 240:H87–H98.
43. Wyman RM, Farhi ER, Bing OHL, Johnson RG, Weintraub RM, Grossman W (1989). Comparative effects of hypoxia and ischemia in the isolated, blood-perfused dog heart: Evaluation of left ventricular diastolic chamber distensibility and wall thickness. Circ Res 64:121–128.
44. Carroll JD, Hess OM, Hirzel HO, Krayenbuehl HP (1983). Exercise-induced ischemia: The influence of altered relaxation on early diastolic pressures. Circulation 67:521–528.
45. Nonogi H, Hess OM, Bortone AS, Ritter M, Carroll JD, Krayenbuehl HP (1989). Left ventricular pressure-length relation during exercise-induced ischemia. J Am Coll Cardiol 13:1062–1070.
46. Paulus WJ, Bronzwaer JGF, Felice H, Kishan N, Wellens F (1992). Deficient acceleration of left ventricular relaxation during exercise after heart transplantation. Circulation 86:1175–1185.
47. Gregg DE (1963). Effect of coronary perfusion pres-

sure or coronary flow on oxygen usage of the myocardium. Circ Res 13:497–500.
48. Salisbury RF, Cross CE, Rieben PA (1960). Influence of coronary pressure on myocardial elasticity. Circ Res 8:794–800.
49. Koretsune Y, Corretti MC, Kusuoka H, Marban E (1991). Mechanism of early ischemic contractile failure. Inexcitability, metabolite accumulation or vascular collapse? Circ Res 68:255–262.
50. Arai AE, Pantely GA, Thoma WJ, Anselone CG, Bristow JD (1992). Energy metabolism and contractile function after 15 beats of moderate myocardial ischemia. Circ Res 70:1137–1145.
51. Babu A, Sonnenblick E, Gulati J (1988). Molecular basis for the influence of muscle length on myocardial performance. Science 240:74–76.
52. Brutsaert DL, Meulemans AL, Sipido KR, Sys SU (1988). Effects of damaging the endocardial surface on the mechanical performance of isolated cardiac muscle. Circ Res 62:358–366.
53. McClellan G, Weisberg A, Kato NS, Ramaciotti C, Sharkey A, Winegrad S (1992). Contractile proteins in myocardial cells are regulated by factor(s) released by blood vessels. Circ Res 70:787–803.
54. Gaasch WH, Bing OHL, Franklin A, Rhodes D, Bernard SA, Weintraub RM (1978). The influence of acute alterations in coronary blood flow on left ventricular diastolic compliance and wall thickness. Eur J Cardiol 7(Suppl):147–161.
55. Olsen CO, Attarian DE, Jones RN, Hill RC, Sink JD, Lee KL, Wechsler AS (1981). The coronary pressure-flow determinants of left ventricular compliance in dogs. Circ Res 49:856–865.
56. Poche R, Arnold G, Gahlen D (1971). Uber den Einfluss des Perfusionsdruckes im Coronarsystem des stillgestellten, aerob perfundierten, isolierten Meerschweinchenherzens auf Stoffwechsel und Feinstruktur des Herzmuskels. Virchows Arch (Cell Pathol) 8: 252–266.
57. Ross J Jr (1989). Is there a true increase in myocardial stiffness with acute ischemia? Am J Cardiol 63:87E–91E.
58. Gallagher KP, Osakada G, Matsuzaki M, Kemper WS, Ross J Jr (1982). Myocardial blood flow and function with critical coronary stenosis in exercising dogs. Am J Physiol 243:H698–H707.
59. Gallagher KP, Kumada T, Battler A, Kemper WS, Ross J Jr (1982). Isoproterenol-induced myocardial dysfunction in dogs with coronary stenoses. Am J Physiol 242:H260–H267.
60. Ruigrok TJC, Slade AM, Poole-Wilson PA (1983). The calcium paradox in isolated frog heart: Ringer revisited. Eur Heart J 4(Suppl H):89–96.
61. Weiss RG, Bottomley PA, Hardy CJ, Gerstenblith G (1990). Regional myocardial metabolism of high-energy phosphates during isometric exercise in patients with coronary artery disease. N Engl J Med 323: 1593–1600.
62. Brutsaert DL, Rademakers FE, Sys SU (1984). Triple control of relaxation: Implications in cardiac disease. Circulation 69:190–196.
63. Heyndrickx GR, Paulus WJ (1990). Effect of asynchrony on left ventricular relaxation. Circulation 81(Suppl III):41–47.
64. Jaski BE, Serruys PW, ten Katen H, Meij S (1985). Epicardial wall motion and left ventricular function during coronary graft angioplasty in humans. J Am Coll Cardiol 6:695–700.
65. Dawson JR, Gibson DG (1989). Left ventricular filling and early diastolic function at rest and during angina in patients with coronary artery disease. Br Heart J 61:248–257.
66. Nakamura Y, Sasayama S, Nonogi H, Miyazaki S, Fujita M, Kihara Y, Konishi T, Kawai C (1987). Effects of pacing-induced ischemia on early left ventricular filling and regional myocardial dynamics and their modification by nifedipine. Circulation 76: 1232–1244.
67. Mc Kay RG, Aroesty JM, Heller GV, Silverman KJ, Parker AJ, Als AV, Come PC, Kolodny GM, Grossman W (1984). The pacing stress reexamined: Correlation of pacing-induced hemodynamic changes with the amount of myocardium at risk. J Am Coll Cardiol 3:1469–1481.
68. Fifer MA, Bourdillon PD, Lorell BH (1984). Altered left ventricular diastolic properties during pacing-induced angina in patients with aortic stenosis. Circulation 74:675–683.

Chapter 2.4

Deficient Acceleration of Left Ventricular Relaxation During Exercise After Heart Transplantation

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Deficient Acceleration of Left Ventricular Relaxation During Exercise After Heart Transplantation

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Background. **The exercise-induced rise in left ventricular filling pressures after cardiac transplantation is considered to be the result of a blunted heart rate response, of elevated venous return, and of unfavorable passive late-diastolic properties of the cardiac allograft. In contrast to passive late-diastolic left ventricular properties, the effect of left ventricular relaxation on the exercise-induced rise in left ventricular filling pressures of the cardiac allograft has not yet been studied. In the present study, the response of left ventricular relaxation to exercise was investigated in transplant recipients and compared with left ventricular relaxation observed in normal control subjects exercised to the same heart rate. Moreover, the response of left ventricular relaxation of the cardiac allograft to β-adrenoreceptor stimulation, to reduced left ventricular afterload, and to increased myocardial activator calcium was investigated by infusion of dobutamine and of nitroprusside and by postextrasystolic potentiation.**

Methods and Results. **Twenty-seven transplant recipients were studied 1 year ($n=17$), 2 years ($n=7$), 3 years ($n=2$), and 4 years ($n=1$) after transplantation. All patients were free of rejection and of significant graft atherosclerosis at the time of study. Tip-micromanometer left ventricular pressure recordings and cardiac hemodynamics were obtained at rest, during supine bicycle exercise stress testing ($n=27$), during dobutamine infusion at a heart rate matching the heart rate at peak exercise ($n=8$), during nitroprusside infusion ($n=9$), and after postextrasystolic potentiation ($n=10$). Tip-micromanometer left ventricular pressure recordings were also obtained in a normal control group ($n=9$) at rest and during supine bicycle exercise stress testing to a heart rate, which matched the heart rate of the transplant recipient group at peak exercise. Left ventricular relaxation rate was measured by calculation of a time constant of left ventricular pressure decay (T) derived from an exponential curve fit to the digitized tip-micromanometer left ventricular pressure signal. In the transplant recipients, exercise abbreviated T from 43±6 to 40±8 msec ($p<0.01$) and caused a rise of left ventricular minimum diastolic pressure (LVMDP) from 5±2 to 9±6 mm Hg ($p<0.001$). In normal control subjects, exercise induced a 2.5 times larger abbreviation of T (from 42±7 to 34±6 msec; $p<0.001$) and a small drop in LVMDP from 5±2 to 4±3 mm Hg ($p<0.05$). In the transplant recipients, the change in T (ΔT) from rest to exercise was variable ranging from an abbreviation, as observed in normal controls, to a prolongation and was significantly correlated with the change in RR interval (ΔRR) and the change in left ventricular end-diastolic pressure (ΔLVEDP) ($\Delta T=0.068\Delta RR+0.58\Delta LVEDP-2.2$; $r=0.76$; $p<0.001$). In a first subset of transplant recipients ($n=8$), dobutamine infusion resulted in a heart rate equal to the heart rate at peak exercise, a left ventricular end-diastolic pressure (8±7 mm Hg) lower than at peak exercise (22±6 mm Hg; $p<0.05$) and a T value (32±9 msec), which was shorter than both resting value (44±5 msec; $p<0.005$) and value observed at peak exercise (40±8 msec; $p<0.01$). In a second subset of transplant recipients ($n=9$), nitroprusside infusion and postextrasystolic potentiation resulted in a significant prolongation of T from 41±7 to 56±10 msec ($p<0.05$) and a characteristic negative dP/dt upstroke pattern with downward convexity as previously observed in left ventricular hypertrophy.**

Conclusions. **Exercise after cardiac transplantation resulted in a smaller acceleration of left ventricular relaxation than in a normal control group exercised to the same heart rate. These transplant recipients, who made the largest use of left ventricular preload reserve during exercise, showed least acceleration of left ventricular relaxation. This association between a rise of left ventricular end-diastolic pressure and slower left ventricular isovolumic relaxation was also evident in the individual transplant recipient from the slower isovolumic relaxation during exercise than during dobutamine infusion despite equal heart rates. After postextrasystolic potentiation during nitroprusside infusion, a slow left ventricular relaxation with downward convexity of the dP/dt signal was observed in the cardiac allograft. This finding suggests depressed function of the sarcoplasmic reticulum in left ventricular myocardium after transplantation, which could be related either to decreased adrenergic tone or to foregoing ischemic injury during organ retrieval or to hypertrophy caused by cyclosporine induced arterial hypertension. (*Circulation* 1992;86:1175–1185)**

KEY WORDS • heart transplantation • hemodynamics • diastolic function • exercise

Exercise elevates left ventricular filling pressures in the cardiac allograft after orthotopic heart transplantation.[1-6] This rise in left ventricular filling pressures, which decreases exercise tolerance after heart transplantation,[7] has been related to an inadequate heart rate response[6] and therefore an excessive dependence on preload reserve to raise cardiac output. The use of preload reserve induces a prompt rise in left ventricular filling pressures because of a diastolic left ventricular pressure-volume relation, which is steeper than normal and shifted to the left. This altered diastolic left ventricular pressure-volume relation of the allograft has been variably ascribed to a mismatch of donor-recipient heart size, to cyclosporine-induced arterial hypertension, to intervening episodes of allograft rejection, or to ischemic injury incurred during organ retrieval or caused by graft vascular disease. The exercise-induced rise in left ventricular filling pressures after cardiac transplantation is therefore considered to be the result of unfavorable passive late-diastolic left ventricular properties of the allograft[6] and of a mismatch between venous return and heart rate.

In contrast to passive late-diastolic left ventricular properties, the effect of left ventricular relaxation on the exercise-induced rise of left ventricular filling pressures after heart transplantation has not yet been investigated. After heart transplantation, denervation or a nonuniform and limited degree of reinnervation[8] could lead to a blunted response of left ventricular relaxation to exercise, which could especially affect early diastolic left ventricular pressures. The important effect during exercise of left ventricular pressure decay on early diastolic left ventricular pressures is evident from previous observations in patients with coronary artery disease. When exercise induces ischemia in patients with coronary artery disease, left ventricular pressure decay is slower than normal and markedly elevates the early diastolic left ventricular pressure nadir.[9,10]

In the present study, the response of left ventricular relaxation to exercise was investigated by obtaining tip-micromanometer left ventricular pressure recordings during supine bicycle exercise stress testing in transplant recipients and in a normal control group of patients, which was exercised to the same heart rate as the transplant recipients. To elucidate whether the abnormal response to exercise of left ventricular relaxation of the cardiac allograft could be attributed to a decreased responsiveness of left ventricular relaxation to β_1-adrenoreceptor stimulation, dobutamine was infused after the exercise stress test in a subset of transplant recipients to achieve a heart rate, which matched the heart rate at peak exercise. In another subset of transplant recipients, the effects on left ventricular relaxation of increased myocardial activator calcium and of left ventricular afterload were investigated by postextrasystolic potentiation and by administration of nitroprusside.

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Methods

Patients

Control patients. The control study group comprised nine patients (four women, five men; ages, 36-66 years; mean age, 53 years) referred for evaluation of chest pain. There was no clinical or echocardiographic evidence of congenital, valvular, or cardiomyopathic heart disease. Left ventricular and coronary angiography revealed normal left ventricular volumes, normal ejection fraction, and absence of coronary artery disease. At the time of study, no patient was taking positive or negative inotropic drugs.

Transplant recipients. Twenty-seven patients (18 men, nine women; mean age, 50 years; age range, 24-66 years) were studied after orthotopic heart transplantation. Seventeen patients were studied 1 year after transplantation, seven patients 2 years after transplantation, two patients 3 years after transplantation, and one patient 4 years after transplantation. Patients were treated with cyclosporine, prednisone, and azathioprine immunosuppression. At the time of study, no patient had biopsy evidence of rejection requiring therapy. Eleven patients had experienced previous episodes ($\leq$ two episodes) of moderate to severe allograft rejection, as assessed by serial endomyocardial biopsies and clinical course. Eighteen patients received treatment for arterial hypertension, which consisted of calcium channel blockers in 13 patients, of ACE inhibitors in two patients, and of prazosine in three patients. Routine annual postoperative left ventricular and coronary angiography revealed normal left ventricular function in all patients (ejection fraction, 72±10%; left ventricular end-diastolic volume index, 56±17 ml/m^2) and angiographically normal coronary arteries in the absence of accelerated graft atherosclerosis. Left ventricular end-diastolic volume index and ejection fraction were calculated from single-plane left ventricular cineangiograms performed in 30° right anterior oblique projection using the area-length method and a regression equation.[11] At the time of study, no patient received digitalis, β-blockers, or calcium channel blockers. The study protocol was approved by the local ethical committee. All patients gave informed consent, and there was no complication related to the procedure or study protocol.

Hemodynamic Studies

Catheterization protocol. Transplant recipients (n=27) underwent left-right heart catheterization, left ventricular angiography, and coronary angiography as part of their routine annual postoperative clinical evaluation using right femoral artery and vein. Control patients (n=9) underwent left heart catheterization, left ventricular angiography, and coronary angiography. All pressures were referenced to atmospheric pressure at the level of the midchest. Left ventricular pressure was measured with a high-fidelity tip-micromanometer catheter calibrated externally against a mercury reference and matched against luminal pressure. Pressure

TABLE 1. Exercise Hemodynamics of Cardiac Allograft (Systolic Function)

	Heart rate (bpm)		LVPSP (mm Hg)		LV dP/dt_{max} (mm Hg/sec)		Cardiac output (l/min)	
Patient	Rest	Exercise	Rest	Exercise	Rest	Exercise	Rest	Exercise
1	90	109	163	155	1,480	1,640	6.3	9.0
2	96	112	158	180	1,780	2,300	5.6	9.0
3	74	84	168	194	1,260	1,380	4.6	7.0
4	85	93	162	165	1,820	2,300	6.2	6.9
5	92	122	131	143	1,794	2,816	6.7	8.4
6	86	93	146	188	3,174	3,850	5.3	5.4
7	99	103	156	158	2,665	2,619	7.5	9.0
8	74	100	146	140	1,890	2,940	6.5	10.2
9	79	103	149	163	1,860	2,520	4.1	7.3
10	78	88	143	163	1,280	1,880	5.3	7.4
11	72	94	123	195	1,020	1,800	6.5	10.4
12	79	94	154	180	1,360	1,600	4.7	6.8
13	73	96	150	197	1,340	1,920	3.1	5.3
14	86	113	125	167	1,080	1,960	5.1	8.9
15	70	79	162	166	1,440	1,660	5.3	7.0
16	90	96	142	180	1,213	1,493	4.3	7.3
17	85	94	141	148	1,600	1,720	6.0	7.9
18	94	105	144	149	1,340	1,600	6.6	9.1
19	84	102	177	170	1,240	1,280	5.3	8.5
20	81	114	142	177	1,400	2,013	7.3	10.1
21	91	110	149	178	1,480	2,173	6.1	8.7
22	95	111	116	120	960	1,293	5.7	9.5
23	94	106	177	174	1,760	2,200	7.1	10.3
24	77	84	180	182	1,320	1,460	4.3	5.4
25	99	120	198	252	1,280	1,900	8.9	13.1
26	100	111	136	149	1,360	1,680	5.1	7.4
27	92	107	155	179	1,400	1,540	5.5	8.4
Mean±SD	85	100*	152	171*	1,541	1,983*	5.7	8.3*
	9	12	19	25	476	583	1.2	1.7

LVPSP, left ventricular peak systolic pressure; LV dP/dt_{max}, left ventricular maximum rate of pressure development.
*$p<0.001$.

signals and a bipolar standard lead of the electrocardiogram were recorded on a Gould ES 1000 multichannel recorder. Pressure signals were digitized on line with a Hewlett Packard 9836 computer and averaged throughout a complete respiratory cycle.

Bicycle exercise stress testing. Left ventricular pressure and left ventricular dP/dt were recorded before and after the patient's feet were attached to the pedals of the bicycle and subsequently at one-minute intervals during supine bicycle exercise, which was performed at a constant submaximal workload for 6 minutes (Tables 1–3).[12] In transplant recipients, cardiac output measurements ($n=27$) and right atrial pressure recordings ($n=5$) were obtained before exercise and during the last minute of exercise. The exercise factor was calculated as the ratio of the exercise-induced increment of cardiac output to the increment of oxygen consumption (normal value, 6.0).[12] In six transplant recipients, a second left ventricular angiogram was obtained in the last minute of exercise.

Effects of β_1-adrenoreceptor stimulation. To investigate responsiveness of the cardiac allograft to β_1-adrenoreceptor stimulation in a subset of transplant recipients ($n=8$), the effect of dobutamine on left ventricular pressure decay was investigated after the exercise stress test after return of hemodynamics to baseline conditions (Table 4). Dobutamine infusion rate was adjusted to achieve a heart rate response equal to the maximal heart rate observed during exercise. Dobutamine was administered intravenously at an infusion rate of 2.5 μg/kg per minute in six patients, of 3.75 μg/kg per minute in one patient, and of 5 μg/kg per minute in one patient.

Effects of postextrasystolic potentiation and arterial vasodilation. The effects on left ventricular relaxation of increased myocardial activator calcium and reduced left ventricular afterload were investigated by postextrasystolic potentiation and by administration of nitroprusside in a second subset of transplant recipients ($n=10$), who underwent bicycle exercise stress testing but no dobutamine infusion. The effects of postextrasystolic potentiation on left ventricular pressure decay were investigated by premature ventricular beats, which were induced at minimum coupling interval by a right ventricular pacing catheter (Table 5, postextrasystolic potentiation). Subsequently, in nine of the 10 patients, in

TABLE 2. Exercise Hemodynamics of Cardiac Allograft (Diastolic Function)

	LVMDP (mm Hg)		LVEDP (mm Hg)		LV dP/dt_{min} (mm Hg/sec)		T (msec)	
Patient	Rest	Exercise	Rest	Exercise	Rest	Exercise	Rest	Exercise
1	3	10	14	27	1,880	2,160	45	47
2	5	9	11	23	1,848	2,320	41	38
3	3	28	10	40	1,620	1,820	45	60
4	3	3	12	13	2,510	2,725	40	35
5	4	8	14	22	1,725	2,162	42	26
6	6	10	12	20	2,277	3,740	30	30
7	4	12	16	25	2,757	2,849	31	34
8	4	2	18	28	2,730	2,665	44	26
9	4	10	15	23	2,460	2,680	50	43
10	3	5	18	33	1,640	1,980	45	45
11	3	10	16	31	1,200	2,120	51	38
12	2	13	12	27	1,568	1,820	45	48
13	6	10	14	27	1,620	2,360	47	41
14	5	3	15	16	1,710	2,480	42	30
15	7	9	15	21	1,600	1,660	50	50
16	5	9	13	20	1,867	2,280	44	38
17	−1	−1	10	18	2,040	2,080	47	48
18	5	8	11	22	1,660	1,880	41	40
19	9	15	15	30	1,720	1,800	46	47
20	9	15	20	30	1,460	2,080	46	41
21	−1	−2	10	16	1,800	2,560	33	27
22	5	7	21	20	960	1,113	45	43
23	7	12	15	22	1,800	2,200	45	39
24	7	11	15	22	1,580	1,732	49	46
25	6	14	25	44	1,220	1,520	44	43
26	2	3	10	22	1,760	2,080	40	34
27	7	10	12	22	1,680	1,980	47	40
Mean±SD	5	9*	14	25*	1,803	2,179*	43	40†
	2	6	4	7	431	504	6	8

LVMDP, left ventricular minimum diastolic pressure; LVEDP, left ventricular end-diastolic pressure; LV dP/dt_{min}, left ventricular minimum rate of pressure development; T, time constant of left ventricular pressure decay.
*$p<0.001$; †$p<0.01$.

whom extrasystoles were administered, a nitroprusside infusion was started at an infusion rate of 0.5 μg/kg per minute and was increased by 0.5 μg/kg per minute every 3 minutes until mean aortic pressure had fallen by 20–30 mm Hg as compared with baseline measurements (Table 5, nitroprusside infusion), and premature ventricular beats at an identical coupling interval were again induced (Table 5, nitroprusside+postextrasystolic potentiation). The postextrasystolic data (Table 5) were the average of three postextrasystolic beats at an identical minimum coupling interval in each patient.

Data Analysis

The time constant of left ventricular pressure decay (T) was derived from the digitized pressure data points of isovolumic left ventricular relaxation using an exponential curve fit with zero asymptote pressure. Pressure data points were obtained at 3-msec intervals by digitizing the left ventricular pressure signal from the moment of left ventricular dP/dt_{min} to a time at which left ventricular pressure equaled left ventricular end-diastolic pressure plus 5 mm Hg. When T values were compared with each another (Tables 2–5), the reported T values were derived for each patient from curve fits with identical starting point (the lowest pressure at which left ventricular dP/dt_{min} occurred) and end point (the pressure that equaled the highest left ventricular end-diastolic pressure plus 5 mm Hg). This avoids erroneous changes in T induced by a shift of the starting or end point of the time constant analysis.[13–15] The correlation coefficient for the exponential curve fits of the time constant analysis always exceeded 0.98.

Phase plane plots of the left ventricular pressure signal during isovolumic relaxation were constructed by matching corresponding left ventricular pressure and left ventricular dP/dt data points (Figures 4 and 6).[14]

All data were reported as mean±SD. Statistical significance was set at $p<0.05$ and was obtained by Bonferroni method for a multiple comparison analysis and by Student's *t* test for paired data.

Results

Exercise Hemodynamics of Cardiac Allograft

The effects of supine bicycle exercise stress testing on systolic and diastolic left ventricular function of th

TABLE 3. Left Ventricular Function of Cardiac Allograft and Normal Heart During Exercise at Matching Heart Rates

	Transplant recipients (n=27)	Controls (n=9)
Heart rate (bpm)	100±12	99±10
LVPSP (mm Hg)	171±25	150±21*
LVMDP (mm Hg)	9±6	4±3*
LVEDP (mm Hg)	25±7	17±5*
LV dP/dt_{max} (mm Hg/sec)	1,983±583	1,656±251
LV dP/dt_{min} (mm Hg/sec)	2,179±504	2,041±424
T (msec)	40±8	34±6*

LVPSP, left ventricular peak systolic pressure; LVMDP, left ventricular minimum diastolic pressure; LVEDP, left ventricular end-diastolic pressure; LV dP/dt_{max}, left ventricular maximum rate of pressure development; LV dP/dt_{min}, left ventricular minimum rate of pressure development; T, time constant of left ventricular pressure decay.

*$p<0.05$ versus transplant recipients.

TABLE 4. Left Ventricular Function of Cardiac Allograft During Exercise and Dobutamine Infusion at Matching Heart Rates (n=8)

	Rest	Exercise	Dobutamine
Heart rate (bpm)	85±9	102±14*	102±10*
LVPSP (mm Hg)	145±19	159±19	140±21
LVMDP (mm Hg)	5±4	7±6	3±6
LVEDP (mm Hg)	15±4	22±6†	8±7‡
LV dP/dt_{max} (mm Hg/sec)	1,318±213	1,712±326†	1,908±345*
LV dP/dt_{min} (mm Hg/sec)	1,619±314	1,957±463	1,703±324
T (msec)	44±5	40±8	32±9*§

LVPSP, left ventricular peak systolic pressure; LVMDP, left ventricular minimum diastolic pressure; LVEDP, left ventricular end-diastolic pressure; LV dP/dt_{max}, left ventricular maximum rate of pressure development; LV dP/dt_{min}, left ventricular minimum rate of pressure development; T, time constant of left ventricular pressure decay.

*$p<0.005$ versus rest; †$p<0.05$ versus rest; ‡$p<0.05$ versus exercise; §$p<0.01$ versus exercise.

cardiac allograft are summarized in Tables 1 and 2 and compared with left ventricular function of a normal control group of patients exercised to the same heart rate in Table 3. In the normal control group of patients, exercise induced a rise in LVPSP from 125±19 to 150±21 mm Hg ($p<0.01$), in left ventricular dP/dt_{max} from 1,331±264 to 1,656±251 mm Hg/second ($p<0.05$), and in left ventricular dP/dt_{min} from 1,547±276 to 2,041±424 mm Hg/second ($p<0.001$). Exercise induced a rise in heart rate from 72±9 to 99±10 beats per minute ($p<0.001$). Left ventricular minimum diastolic pressure fell from 5±2 to 4±3 mm Hg ($p<0.05$), whereas left ventricular end-diastolic pressure (LVEDP) remained unaltered (17±5 mm Hg). In the transplant recipients, exercise induced a significant rise in left ventricular peak systolic pressure (LVPSP), left ventricular dP/dt_{max}, and left ventricular dP/dt_{min} (Tables 1 and 2). In contrast to the control group, left ventricular minimum diastolic pressure (LVMDP) and LVEDP rose during exercise after transplantation (Table 2). In the transplant recipients, the exercise factor (exercise factor=ratio of the increase in cardiac output divided by the corresponding increase in oxygen consumption) equaled 6.7±2.3 (normal value=6.0).[12] In these transplant recipients (n=5), in whom right atrial pressure was measured at rest and at peak exercise, right atrial pressure rose from 4±2 to 8±3 mm Hg ($p<0.01$). In these transplant recipients (n=6), in whom a second left ventricular angiogram was performed at peak exercise, left ventricular end-diastolic volume index rose from 60±8 to 75±10 ml/m^2 ($p<0.01$). Left ventricular end-systolic volume index (LVESVI) and left ventricular ejection fraction (LVEF) remained unaltered (LVESVI rest, 20±6 ml/m^2; LVESVI exercise, 20±9 ml/m^2, p=NS; LVEF rest, 66±9%; LVEF exercise, 72±13%, p=NS). Diastolic left ventricular pressure-volume relations at rest and during exercise obtained in a single patient, representative of the transplant recipient group, are shown in Figure 1.

Acceleration of Left Ventricular Relaxation During Exercise

The control group of patients showed a consistent and significant abbreviation of the time constant of left ventricular pressure decay from 42±7 to 34±6 msec ($p<0.001$), when exercised to a heart rate, which matched the heart rate at peak exercise in the transplant recipient group. In the transplant recipients, the time constant of left ventricular pressure decay shortened slightly at peak exercise from 43±6 to 40±8 msec ($p<0.01$), but the response of left ventricular relaxation was highly variable (Table 2), ranging from an abbreviation (Figure 2) as observed in the normal control group to an unchanged value (Figure 3) or even a prolongation. Despite matching heart rates at peak exercise, the abbreviation of the time constant of left ventricular pressure decay was 2.5 times larger in the normal control group than in the transplant recipient group (Table 3). On multiple regression analysis for the pooled transplant recipient data (n=27), the change in the time constant of left ventricular relaxation from rest to exercise (ΔT) was significantly correlated with the change in RR interval (ΔRR) and with the change in LVEDP ($\Delta LVEDP$) ($\Delta T=0.068\,\Delta RR+0.58\,\Delta LVEDP-2.2$; $r=0.76$; $p<0.001$). The partial regressions for each independent variable were statistically significant (for ΔRR, $p<0.001$; for $\Delta LVEDP$, $p<0.001$) and both ΔRR and $\Delta LVEDP$ were mutually independent as evident from the ab-

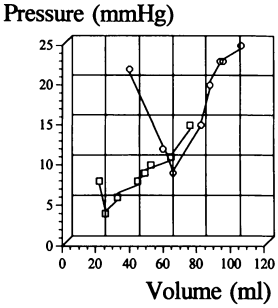

FIGURE 1. *Graph showing diastolic left ventricular pressure-volume relations at rest* (□) *and at peak exercise* (○) *observed in a single patient, which is representative of the transplant recipient group.*

TABLE 5. Effects of Nitroprusside Infusion and Postextrasystolic Potentiation on Diastolic Function of Cardiac Allograft

	Rest (n=10)	PESP (n=10)	NIT (n=9)	NIT+PESP (n=9)
Heart rate (bpm)	88±9	. . .	89±12	. . .
LVPSP (mm Hg)	158±27	158±37	113±16†	108±19‡§
LVMDP (mm Hg)	6±5	5±4	2±2	2±3
LVEDP (mm Hg)	16±7	15±4	10±3	10±3
LV dP/dt_{max} (mm Hg/sec)	1,888±595	2,328±586*	1,878±599	2,253±683‡‖
LV dP/dt_{min} (mm Hg/sec)	2,098±435	1,959±384	1,518±300†	1,068±343‡§‖
T (msec)	41±7	44±6	46±7	56±10‡§‖

PESP, postextrasystolic potentiation; NIT, nitroprusside infusion; LVPSP, left ventricular peak systolic pressure; LVMDP, left ventricular minimum diastolic pressure; LVEDP, left ventricular end-diastolic pressure; LV dP/dt_{max}, left ventricular maximum rate of pressure development; LV dP/dt_{min}, left ventricular minimum rate of pressure development; T, time constant of left ventricular pressure decay.
*p<0.05 rest versus PESP; †p<0.05 rest versus NIT; ‡p<0.05 rest versus NIT+PESP; §p<0.05 PESP versus NIT+PESP; ‖p<0.05 NIT versus NIT+PESP.

sence of correlation between ΔRR and ΔLVEDP. Therefore, during exercise after transplantation, the increase in heart rate accelerates left ventricular relaxation as evident from the correlation of ΔT with ΔRR and the use of left ventricular preload reserve slows left ventricular relaxation as evident from the correlation of ΔT with ΔLVEDP.

Comparative Effects of Exercise and Dobutamine on Left Ventricular Relaxation of Cardiac Allograft

In a subgroup of eight transplant recipients, left ventricular function was compared at peak exercise and during infusion of dobutamine at matching heart rates (Table 4, Figure 3). LVPSP was not significantly different at peak exercise and during dobutamine infusion, but LVEDP was significantly higher during exercise than during dobutamine infusion. Despite use of left ventricular preload reserve, as evident from the rise in LVEDP, left ventricular dP/dt_{max} was comparable during exercise and during dobutamine infusion. Dobutamine infusion induced an abbreviation of the time constant of left ventricular relaxation (T), which was significant both with respect to resting value and value observed at peak exercise. Superimposed left ventricu-

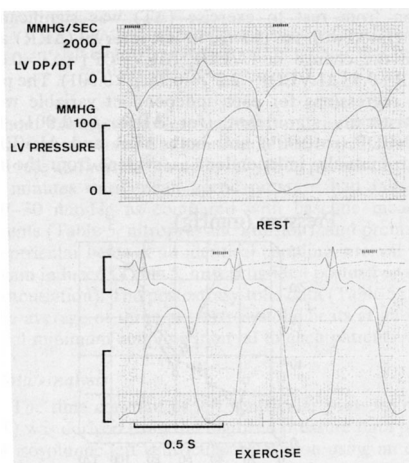

FIGURE 2. *Single-lead ECG, left ventricular (LV) dP/dt, and LV pressure recordings at rest and at peak exercise in a patient who showed adequate acceleration of LV relaxation during exercise and lower LV minimum diastolic pressure during exercise.*

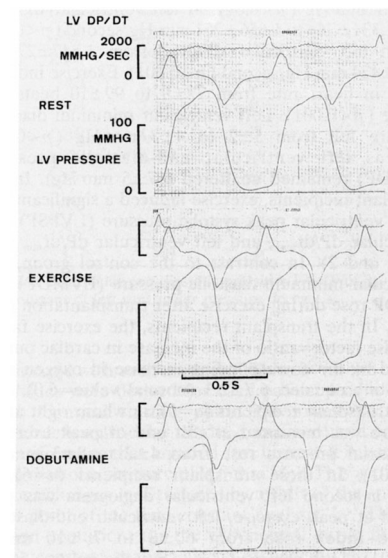

FIGURE 3. *Single-lead ECG, left ventricular (LV) dP/dt, and LV pressure recordings at rest, at peak exercise, and during dobutamine infusion in a patient who used LV preload reserve during exercise, as evident from the elevated LV end-diastolic pressure during exercise. In this patient, isovolumic LV relaxation rate failed to improve during exercise with a concomitant increase of LV minimum diastolic pressure. During dobutamine infusion, heart rate response equaled heart rate response at peak exercise. Despite equal heart rate response and comparable LV peak systolic pressure, isovolumic LV relaxation rate was faster during dobutamine infusion than at peak exercise.*

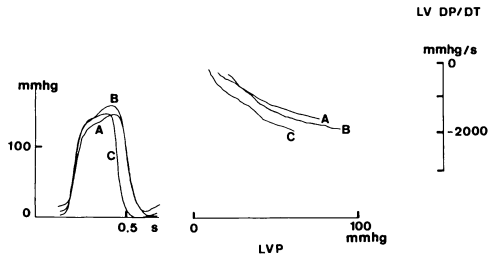

FIGURE 4. Left panel: *Superimposed averaged left ventricular pressure (LVP) recordings obtained in the patient shown in Figure 3 at rest (A), at peak exercise (B), and during dobutamine infusion (C). Dobutamine infusion resulted in an earlier onset and a faster course of isovolumic left ventricular relaxation compared with both rest and peak exercise recordings.* Right panel: *Set of phase-plane plots (LV dP/dt versus LVP) of left ventricular isovolumic relaxation pressure obtained in the patient shown in Figure 3 at rest (A), at peak exercise (B), and during dobutamine infusion (C). For a given LVP value, corresponding LVdP/dt value was more negative for curve C and, therefore, the rate of change was higher for curve C.*

lar pressure recordings and corresponding phase-plane plots (left ventricular pressure versus left ventricular dP/dt) of isovolumic left ventricular relaxation were constructed at rest, at peak exercise, and during dobutamine infusion (Figure 4).

Effects of Nitroprusside Infusion and Postextrasystolic Potentiation on Left Ventricular Relaxation of the Cardiac Allograft

To investigate the effect of left ventricular afterload, left ventricular relaxation rate was measured in a subset of transplant recipients after nitroprusside infusion, which lowered left ventricular peak systolic pressure from 158±27 to 113±16 mm Hg (Table 5). Lowering of left ventricular peak systolic pressure prolonged the time constant of isovolumic left ventricular relaxation from 41±7 to 46±7 msec. This prolongation failed to reach statistical significance on the multicomparison analysis of Table 5 but reached statistical significance ($p=0.045$) on single comparison analysis between resting and nitroprusside values. The effect of increased myocardial activator calcium on isovolumic left ventricular relaxation rate was investigated in the cardiac allograft in potentiated beats preceded by a single ventricular extrasystole at minimum coupling interval. At rest, postextrasystolic potentiation did not alter isovolumic left ventricular relaxation rate, but during nitroprusside infusion, the same intervention resulted in a significantly slower left ventricular relaxation rate, as evident from the prolongation of the time constant of left ventricular relaxation (T) from 46±7 to 56±10 msec. In eight of the nine patients subjected to this protocol, this prolongation was accompanied by a negative dP/dt upstroke pattern with downward convexity, as evident from the recordings shown in Figure 5. Superimposed left ventricular pressure recordings and corresponding phase-plane plots (left ventricular pressure versus left ventricular dP/dt) of isovolumic left

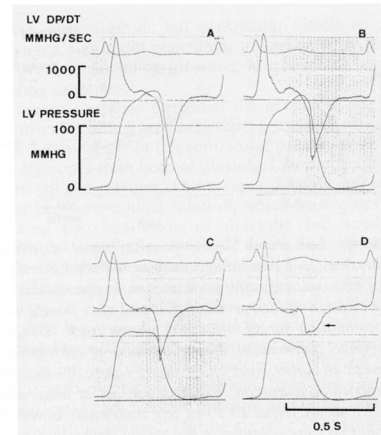

FIGURE 5. *Single-lead ECG, left ventricular (LV) dP/dt, and LV pressure recordings at rest (A), after postextrasystolic potentiation (B), during infusion of nitroprusside (C), and after postextrasystolic potentiation during infusion of nitroprusside (D). Postextrasystolic potentiation during infusion of nitroprusside (D) slows isovolumic LV relaxation and induces a negative dP/dt upstroke pattern with downward convexity (see arrow).*

ventricular relaxation were constructed at rest, after postextrasystolic potentiation, during infusion of nitroprusside, and after postextrasystolic potentiation during infusion of nitroprusside (Figure 6).

Discussion

An increase in left ventricular filling pressures with exercise has been repeatedly observed in cardiac transplant recipients and contributes to the lower than normal exercise tolerance after orthotopic heart transplantation.[7] This increase in left ventricular filling pressures has mainly been attributed to the use of preload reserve during exercise because of the blunted heart rate response and a steeper than normal diastolic left ventricular pressure-volume relation.[5,6] Elevated left ventricular filling pressures during exercise could result not only from abnormal passive diastolic left ventricular properties but also from altered left ventricular relaxation kinetics. Left ventricular relaxation kinetics of the cardiac allograft have so far only been investigated at rest[16] and not during exercise.

Effect of Exercise on Left Ventricular Relaxation

In the normal control group, submaximal supine bicycle exercise induced an acceleration of left ventricular isovolumic relaxation, which significantly exceeded the acceleration of left ventricular relaxation in the transplant recipients despite matching heart rates at peak exercise. Similar improvements in left ventricular relaxation rate during exercise were reported in normal subjects by other investigators.[17,18] This faster isovolumic left ventricular relaxation of the normal left ventricle during exercise probably contributed to the significant fall in left ventricular minimum diastolic pressure

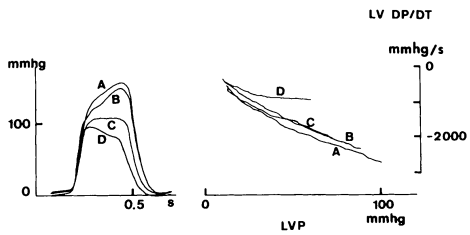

FIGURE 6. Left panel: *Superimposed averaged left ventricular pressure (LVP) recordings obtained in the patient shown in Figure 5 at rest (A), after postextrasystolic potentiation (B), during infusion of nitroprusside (C), and after postextrasystolic potentiation during infusion of nitroprusside (D). Postextrasystolic potentiation during infusion of nitroprusside resulted in an earlier onset and a slower course of isovolumic left ventricular relaxation.* Right panel: *Set of phase-plane plots (LV dP/dt versus LVP) of left ventricular isovolumic relaxation pressure obtained in the patient shown in Figure 5 at rest (A), after postextrasystolic potentiation (B), during infusion of nitroprusside (C), and after postextrasystolic potentiation during infusion of nitroprusside (D). For a given LVP value, corresponding LVdP/dt value was less negative for curve D and, therefore, the rate of change was lower for curve D.*

observed in the present and previous studies.[17] In patients with coronary disease and exercise-induced ischemia,[9] the exercise related acceleration of left ventricular relaxation was smaller than in normal subjects and was accompanied by a significant rise of left ventricular minimum diastolic pressure, as observed in the present study in the transplant recipient group. A depressed acceleration of left ventricular relaxation during exercise was observed also in patients with hypertrophic cardiomyopathy[18] and could have contributed to the exercise-induced elevation of left ventricular filling pressures observed in these patients.[19]

Deficient Acceleration of Left Ventricular Relaxation During Exercise After Heart Transplantation

In the present study, the acceleration of left ventricular relaxation during exercise was investigated in transplant recipients. For the entire study group, exercise induced a small (<10%) acceleration of left ventricular relaxation. Individual patient response was variable, which ranged from an almost normal response to paradoxical slowing of isovolumic relaxation. On multiple regression analysis, the acceleration of left ventricular relaxation during exercise was correlated with the increase in heart rate and the increase in left ventricular filling pressure. As evident from the correlation of ΔT with $\Delta LVEDP$, the use of left ventricular preload reserve was associated with slower left ventricular isovolumic relaxation. As evident from the correlation of ΔT with ΔRR, the increase in heart rate was associated with accelerated left ventricular isovolumic relaxation. This acceleration could be the result of a direct heart rate dependent effect (Bowditch phenomenon) or adrenergic stimulation. In conscious dogs,[20] pacing tachycardia from 100 to 200 beats per minute resulted in no change of the time constant of left ventricular pressure decay. When heart rate was held constant at 200 beats per minute, exercise produced a fall in the time constant of left ventricular pressure decay to a value, which equaled the value observed during unpaced exercise at the same heart rate. From these observations, it appears that the acceleration of left ventricular relaxation is mediated through adrenergic stimulation and not through a direct heart rate dependent effect.

The effects of use of left ventricular preload reserve on isovolumic left ventricular relaxation rate are complex. Animal studies[21,22] showed slowing of left ventricular relaxation at higher left ventricular end-diastolic volumes, but if left ventricular systolic pressure was kept constant after diastolic left ventricular volume infusion, the time constant of isovolumic left ventricular relaxation remained unaltered.[23] Similar conclusions were reached when left ventricular preload was reduced, as reported in normal control subjects early after inferior vena cava occlusion.[24] In contrast to these studies, the relation between use of left ventricular preload reserve and isovolumic left ventricular relaxation was observed in the present study during exercise. Increased responsiveness of contractile proteins to calcium because of increased muscle preload[25] could possibly counteract decreased responsiveness of contractile proteins by β_1-receptor stimulation and explain the relation between use of left ventricular preload reserve and slower isovolumic left ventricular relaxation observed in the present study during exercise after transplantation.

In the present study, left ventricular isovolumic relaxation rate during exercise was significantly slower than during dobutamine infusion at rest despite similar β-adrenoreceptor stimulation, as evident from the equal heart rate responses during both interventions. Because of significantly higher left ventricular end-diastolic pressure during exercise than during dobutamine infusion, slower left ventricular isovolumic relaxation rate during exercise confirmed in the individual transplant recipient the association between use of left ventricular preload reserve and impairment of left ventricular isovolumic relaxation during exercise. This association was already evident from the multiple regression analysis on the pooled transplant group data, which revealed during exercise a similar inverse correlation between the acceleration of left ventricular relaxation and the rise of left ventricular end-diastolic pressure. Slower isovolumic left ventricular relaxation during exercise than during dobutamine infusion despite equal heart rate response could result from different actions of humorally administered and neurally released β-mimetics. Because of its humoral administration route, dobutamine infusion causes uniform stimulation of the left ventricle. Exercise after transplantation results not only in elevation of circulating catecholamines but also probably in some neural release of catecholamines because of recently demonstrated partial reinnervation.[8] As previously observed during intracoronary isoproterenol infusion,[22] a spatially heterogeneous release of catecholamines, as occurs during partial reinnervation, could contribute to slower left ventricular relaxation kinetics during exercise than during dobutamine infusion.

During dobutamine infusion, the time constant of left ventricular pressure decay of the cardiac allograft was significantly smaller than at rest (32 msec versus 44 msec). This finding is consistent with preserved respon-

siveness of left ventricular relaxation of the cardiac allograft to β_1-adrenoreceptor stimulation. This response of left ventricular relaxation in transplant recipients even exceeds the response in normal subjects as evident from a recent study,[26] which observed during dobutamine infusion (5 μg/kg per minute) an 8-msec decrease in the time constant of left ventricular pressure decay, which was, however, accompanied by a larger increase (95%) in left ventricular dP/dt_{max} than the currently observed increase (30%) in the cardiac allograft. Similar discrepancies between contraction and relaxation phase responses to β-adrenoreceptor stimulation were also observed in the failing human left ventricle and could be consistent with differentially mediated effects of β-adrenoreceptor stimulation on voltage dependent calcium channel and on sarcoplasmic reticular calcium reuptake.[26,27]

Elevated Left Ventricular Filling Pressures During Exercise After Heart Transplantation

In the transplant recipients, exercise induced a significant rise in left ventricular minimum and end-diastolic pressures, whereas in the normal control group, exercise to a similar heart rate induced a small but significant drop in left ventricular minimum diastolic pressure and no change in left ventricular end-diastolic pressure. This elevation of left ventricular filling pressures in the cardiac allograft during exercise could be the result of slower early diastolic left ventricular pressure decay, of altered elastic left ventricular recoil, of altered late diastolic properties, or of ventricular interaction related to elevated right atrial pressures.

After mitral valve opening, the decay of contractile activity has been estimated from an extrapolation of isovolumic left ventricular pressure decay.[28,29] Such an extrapolation revealed a substantial contribution of residual contractile activity to early diastolic left ventricular pressures. Slower isovolumic left ventricular pressure decay during exercise in the transplant recipients than in the control group could, therefore, explain the higher left ventricular minimum diastolic pressure during exercise after transplantation. The precise interaction between decay of contractile activity, early diastolic left ventricular pressures, and left ventricular filling was investigated in anesthetized dogs[30] and in isolated papillary muscles.[31] In the canine left ventricle, an earlier onset of left ventricular filling blunts the rate of left ventricular pressure decay,[30] and in the isolated papillary muscle, isometric force in the postreextension phase is larger when reextension occurs earlier. During exercise after transplantation, there is an important rise in left ventricular filling pressures and, therefore, also in mitral valve opening pressure with a concomitant earlier onset of mitral inflow, which, in turn, could lead to further slowing of left ventricular pressure decay after mitral valve opening and to further elevation of early diastolic left ventricular pressures.

In conscious dogs,[20] left ventricular end-systolic volume during exercise was unaltered. In human control subjects,[17] left ventricular end-systolic volume index fell slightly at peak exercise. In the present study, left ventricular angiograms obtained at peak exercise revealed an unchanged left ventricular end-systolic volume in the transplant recipients. An unchanged left ventricular end-systolic volume at peak exercise would leave early diastolic left ventricular elastic recoil unchanged and could contribute to higher early diastolic left ventricular filling pressures in transplant recipients during exercise.

Previous studies explained the abnormal rise in left ventricular filling pressures during exercise after transplantation by use of left ventricular preload reserve and by a steeper than normal diastolic left ventricular pressure-volume relation. This steeper diastolic left ventricular pressure-volume relation, which was recently confirmed by calculation of diastolic left ventricular stiffness moduli at rest,[16] could be the consequence of a mismatch between donor and recipient heart size,[5] of ischemic injury incurred at the time of graft retrieval,[16,32] of repetitive episodes of rejection,[33–35] or of cardiac hypertrophy triggered by cyclosporine-induced arterial hypertension.[36] The present study confirmed the use of left ventricular preload reserve during exercise after transplantation as obvious from the rise in left ventricular end-diastolic pressure and in left ventricular end-diastolic volume. During exercise after transplantation, the initial portion of the diastolic left ventricular pressure-volume relation was shifted upward and the mid to terminal portion of the diastolic left ventricular pressure-volume relation coincided with the rest curve (Figure 1). In conscious dogs[20,37] and in human control subjects,[17] exercise induced a downward shift of the diastolic left ventricular pressure-volume relation, especially in its initial portion. An upward shift of the initial portion of the diastolic left ventricular pressure-volume relation, as observed during exercise after transplantation, was also reported in conscious dogs during exercise after β-blockade[20] and related to inappropriate sympathetic stimulation, which affects early diastolic contractile tension decay.

During exercise after transplantation, a significant rise in right atrial pressures was observed in the present study. Because of pericardial constraints or ventricular interaction, a rise in right atrial or diastolic right ventricular pressures could shift the diastolic left ventricular pressure-volume relation upward. Such an upward shift would, however, not be limited to the initial portion of the diastolic left ventricular pressure volume relation, as observed in the present study but would affect the entire diastolic left ventricular pressure-volume relation.

Left Ventricular Relaxation of Cardiac Allograft: Effects of Afterload and Activator Calcium

The effects of reduced left ventricular afterload and of increased myocardial activator calcium on left ventricular relaxation were investigated in transplant recipients by administration of nitroprusside and by postextrasystolic potentiation. As evident from the superimposed left ventricular pressure recordings of Figure 6, administration of sodium nitroprusside was accompanied by an earlier onset of left ventricular isovolumic relaxation. This earlier onset of left ventricular isovolumic relaxation resulted not only from arterial vasodilation but also probably from myocardial deactivation because of a nitroprusside-induced elevation of myocardial cyclic guanosine monophosphate level.[38] In contrast to the normal left ventricle,[39] lowering of left ventricular peak systolic pressure by nitroprusside infusion induced prolongation of the time constant of left ventricular pressure decay from 41 to 46 msec. This

prolongation failed to reach statistical significance on multicomparison analysis but reached statistical significance ($p=0.045$) on single comparison analysis between resting and nitroprusside values. This trend in the cardiac allograft for slower left ventricular relaxation at lower arterial load argues against the rise of left ventricular peak systolic pressure during exercise as the cause of the deficient acceleration of left ventricular relaxation.

After postextrasystolic potentiation, there was no change in the time constant of left ventricular pressure decay at rest, as previously reported in normal subjects.[40] During nitroprusside infusion, however, postextrasystolic potentiation resulted in a marked prolongation of the time constant of left ventricular pressure decay to 56 msec. This prolongation was accompanied by a negative dP/dt upstroke pattern with a downward convexity (Figure 5). This negative dP/dt upstroke pattern has previously been reported in acute coronary occlusion[41] and in the hypertrophied left ventricle of aortic stenosis after drastic left ventricular unloading by combined aortic valvuloplasty-nitroprusside infusion.[14] The induction of this slow left ventricular relaxation pattern in the cardiac allograft by unloading and postextrasystolic potentiation could suggest delayed myoplasmic calcium removal in left ventricular myocardium after transplantation similar to the delayed myoplasmic calcium removal previously observed in hypertrophied myocardium. This could be the result of a depressed function of the sarcoplasmic reticulum either because of decreased adrenergic tone caused by denervation or limited reinnervation or because of ischemic injury at the time of organ retrieval or because of hypertrophy related to cyclosporine-induced arterial hypertension.

Conclusion

Exercise after orthotopic heart transplantation resulted in an acceleration of left ventricular relaxation, which was 2.5 times smaller than in a normal control group exercised to the same heart rate. The individual response of left ventricular relaxation to exercise was variable, which ranged from normal acceleration of isovolumic relaxation to paradoxical slowing of isovolumic relaxation. Those patients, who had the largest elevation of left ventricular end-diastolic pressure during exercise, showed least acceleration of isovolumic relaxation rate. This association between a rise of left ventricular end-diastolic pressure and slower left ventricular isovolumic relaxation was also evident in the individual transplant recipient from the slower isovolumic left ventricular relaxation during exercise than during dobutamine infusion at equal heart rates. After postextrasystolic potentiation during nitroprusside infusion, a slow relaxation with downward convexity of the dP/dt signal was observed in the cardiac allograft. This finding suggests depressed function of the sarcoplasmic reticulum in left ventricular myocardium after transplantation, which could be related either to decreased adrenergic tone or to foregoing ischemic injury during organ removal or to hypertrophy caused by cyclosporine induced arterial hypertension.

References

1. Campeau L, Pospisil L, Grondin P, Dyrda I, LePage G: Cardiac catheterization findings at rest and after exercise in patients following cardiac transplantation. *Am J Cardiol* 1970;25:523–528
2. Pope SE, Stinson EB, Daughters GT, Schroeder JS, Ingels NB, Alderman EL: Exercise response of the denervated heart in long-term cardiac transplant recipients. *Am J Cardiol* 1980;46:213–218
3. Pflugfelder PW, Purves PD, Mc Kenzie FN, Kostuk WJ: Cardiac hemodynamics during supine exercise in cyclosporine-treated orthotopic heart transplant recipients: Assessment by radionuclide angiography. *J Am Coll Cardiol* 1987;10:336–341
4. Pflugfelder PW, Mc Kenzie FN, Kostuk WJ: Hemodynamic profiles at rest and during supine exercise after orthotopic cardiac transplantation. *Am J Cardiol* 1988;61:1328–1333
5. Hosenpud JD, Morton MJ, Wilson RA, Pantely GA, Norman DJ, Cobanoglu MA, Starr A: Abnormal exercise hemodynamics in cardiac allograft recipients 1 year after cardiac transplantation. *Circulation* 1989;80:525–532
6. Rudas L, Pflugfelder PW, Kostuk WJ: Comparison of hemodynamic responses during dynamic exercise in the upright and supine postures after orthotopic cardiac transplantation. *J Am Coll Cardiol* 1990;16:1367–1373
7. Stevenson LW, Sietsema K, Tillisch JH, Lem V, Walden J, Kobashigawa JA, Moriguchi J: Exercise capacity for survivors of cardiac transplantation or sustained medical therapy for stable heart failure. *Circulation* 1990;81:78–85
8. Wilson RF, Christensen BV, Olivari MT, Simon A, White CW, Laxson DD: Evidence for structural sympathetic reinnervation after orthotopic cardiac transplantation in humans. *Circulation* 1991;83:1210–1220
9. Carroll JD, Hess OM, Hirzel HO, Krayenbuehl HP: Exercise-induced ischemia: The influence of altered relaxation on early diastolic pressures. *Circulation* 1983;67:521–528
10. Nonogi H, Hess OM, Bortone AS, Ritter M, Carroll JD, Krayenbuehl HP: Left ventricular pressure-length relation during exercise-induced ischemia. *J Am Coll Cardiol* 1989;13:1062–1070
11. Fifer MA, Grossman W: Measurement of ventricular volumes, ejection fraction, mass and wall stress, in Grossman W, Baim DS (eds): *Cardiac Catheterization, Angiography, and Intervention.* 4th ed. Philadelphia, Pa., Lea & Febiger, 1991, pp 300–318
12. Lorell BH, Grossman W: Dynamic and isometric exercise during cardiac catheterization, in, Grossman W, Baim DS (eds): *Cardiac Catheterization, Angiography, and Intervention.* 4th ed. Philadelphia, Pa., Lea & Febiger, 1991, pp 267–282
13. Martin G, Gimeno JV, Cosin J, Guillem MI: Time constant of isovolumic pressure fall: New numerical approaches and significance. *Am J Physiol* 1984;247:H283–H294
14. Paulus WJ, Heyndrickx GR, Buyl P, Goethals MA, Andries E: Wide range load shifts of combined aortic valvuloplasty-arterial vasodilation slow isovolumic relaxation of the hypertrophied left ventricle. *Circulation* 1990;81:886–898
15. Paulus WJ, Nellens P, Heyndrickx GR, Andries E: Can time constants of left ventricular pressure decay be correctly compared, when asymptote pressures are unequal? (abstract) *Eur Heart J* 1988;9(suppl 1):305
16. Hausdorf G, Banner NR, Mitchell A, Khaghani A, Martin M, Yacoub M: Diastolic function after cardiac and heart-lung transplantation. *Br Heart J* 1989;62:123–132
17. Nonogi H, Hess OM, Ritter M, Krayenbuehl HP: Diastolic properties of the normal left ventricle during supine exercise. *Br Heart J* 1988;60:30–38
18. Murgo JP, Craig WE, Pasipoularides A: Evaluation of time course of left ventricular isovolumic relaxation in man, in, Grossman W, Lorell BH (eds): *Diastolic Relaxation of the Heart,* Martinus Nijhoff Publishing, Boston, Mass, 1988, pp 125–132
19. Paulus WJ, Nellens P, Heyndrickx GR, Andries E: Effects of long-term treatment with amiodarone on exercise hemodynamics and left ventricular relaxation in patients with hypertrophic cardiomyopathy. *Circulation* 1986;74:544–554
20. Cheng CP, Igarashi Y, Little WC: Mechanism of augmented rate of left ventricular filling during exercise. *Circ Res* 1992;70:9–19
21. Raff GL, Glantz SA: Volume loading slows left ventricular isovolumic relaxation rate: Evidence of load-dependent relaxation in the intact dog heart. *Circ Res* 1981;48:813–824
22. Gillebert TC, Lew WYW: Nonuniformity and volume loading independently influence isovolumic relaxation rates. *Am J Physiol* 1989;257:H1927–H1935
23. Gaasch WH, Carroll JD, Blaustein AS, Bing OHL: Myocardial relaxation: Effects of preload on the time course of isovolumetric relaxation. *Circulation* 1986;73:1037–1041
24. Varma SK, Owen RM, Smucker ML, Feldman MD: Is Tau a preload-independent measure of isometric relaxation? *Circulation* 1989;80:1757–1765

25. Babu A, Sonnenblick E, Gulati J: Molecular basis for the influence of muscle length on myocardial performance. *Science* 1988;240: 74–76
26. Parker JD, Landzberg JS, Bittl JA, Mirsky I, Colucci WS: Effects of β-adrenergic stimulation with dobutamine on isovolumic relaxation in the normal and failing human left ventricle. *Circulation* 1991;84:1040–1048
27. Colucci WS: In vivo studies of myocardial β-adrenergic receptor pharmacology in patients with congestive heart failure. *Circulation* 1990;82:I-44–I-51
28. Pasipoularides A, Mirsky I, Hess OM, Krayenbuehl HP: Myocardial relaxation and passive diastolic properties in man. *Circulation* 1986;74:991–1001
29. Bourdillon PD, Lorell BH, Mirsky I, Paulus WJ, Wynne J, Grossman W: Increased regional myocardial stiffness of the left ventricle during pacing-induced angina in man. *Circulation* 1983;67:316–323
30. Nikolic S, Yellin EL, Tamura K, Vetter H, Tamura T, Meisner JS, Frater RWM: Passive properties of canine left ventricle: Diastolic stiffness and restoring forces. *Circ Res* 1988;62:1210–1222
31. Sys SU, Paulus WJ, Claes VA, Brutsaert DL: Postreextension force decay of relaxing cardiac muscle. *Am J Physiol* 1987;253: H256–H261
32. Pickering JG, Boughner DR: Fibrosis in the transplanted heart and its relation to donor ischemic time. *Circulation* 1990;81: 949–958
33. Valentine HA, Appleton CP, Hatle LK, Hunt SA, Billingham ME, Shumway NE, Stinson EB, Popp RL: A hemodynamic and Doppler echocardiographic study of ventricular function in long-term cardiac allograft recipients. *Circulation* 1989;79:66–75
34. Amende I, Simon R, Seegers A, Daniel W, Heublein B, Hetzer R, Haverich A, Hood WP, Lichtlen PR, Schutzenmeister R, Wenzlaff P: Diastolic dysfunction during acute cardiac allograft rejection. *Circulation* 1990;81(suppl III):III-60–III-70
35. Seacord LM, Miller LW, Pennington DG, McBride LR, Kern MJ: Reversal of constrictive/restrictive physiology with treatment of allograft rejection. *Am Heart J* 1990;120:455–459
36. Lorell BH, Grossman W: Cardiac hypertrophy: The consequences for diastole. *J Am Coll Cardiol* 1987;9:1189–1193
37. Miyazaki S, Guth BD, Miura T, Indolfi C, Schulz R, Ross J Jr: Changes of left ventricular diastolic function in exercising dogs without and with ischemia. *Circulation* 1990;81:1058–1070
38. Smith JA, Shah AM, Lewis MJ: Factors released from endocardium of the ferret and pig modulate myocardial contraction. *J Physiol* (Lond) 1991;439:1–14
39. Starling MR, Montgomery DG, Mancini GBJ, Walsh RA: Load independence of the rate of isovolumic relaxation in man. *Circulation* 1987;76:1274–1281
40. Paulus WJ, Sys SU, Nellens P, Heyndrickx GR, Andries E: Post-extrasystolic potentiation worsens fast filling of the hypertrophied left ventricle in aortic stenosis and hypertrophic cardiomyopathy. *Circulation* 1988;78:928–940
41. Kumada T, Karliner JS, Pouleur H, Gallagher KP, Shirato K, Ross J Jr: Effects of coronary occlusion on early ventricular diastolic events in conscious dogs. *Am J Physiol* 1979;237: H542–H549

Chapter 3

Diastolic left ventricular dysfunction and a lack of rightward shift of the left ventricular diastolic pressure-volume relationship

Chapter 3.1

Endomyocardial Nitric Oxide Synthase and Left Ventricular Preload Reserve in Dilated Cardiomyopathy

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Endomyocardial Nitric Oxide Synthase and Left Ventricular Preload Reserve in Dilated Cardiomyopathy

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Background—Patients with heart failure have modified myocardial expression of nitric oxide synthase (NOS), as is evident from induction of calcium-insensitive NOS isoforms. The functional significance of this modified NOS gene expression for left ventricular (LV) contractile performance was investigated in patients with dilated nonischemic cardiomyopathy.

Methods and Results—In patients with dilated, nonischemic cardiomyopathy, invasive measures of LV contractile performance were derived from LV microtip pressure recordings and angiograms and correlated with intensity of gene expression of inducible (NOS2) and constitutive (NOS3) NOS isoforms in simultaneously procured LV endomyocardial biopsies (n=20). LV endomyocardial expression of NOS2 was linearly correlated with LV stroke volume ($P=0.001$; $r=0.66$), LV ejection fraction ($P=0.007$; $r=0.58$), and LV stroke work ($P=0.003$; $r=0.62$). In patients with elevated LV end-diastolic pressure (>16 mm Hg), a closer correlation was observed between endomyocardial expression of NOS2 and LV stroke volume ($P=0.001$; $r=0.74$), LV ejection fraction ($P=0.0007$; $r=0.77$), and LV stroke work ($r=0.82$; $P=0.0002$). LV endomyocardial expression of NOS3 was linearly correlated with LV stroke volume ($P=0.01$; $r=0.53$) and LV stroke work ($P=0.01$; $r=0.52$). To establish the role of nitric oxide (NO) as a mediator of the observed correlations, substance P (which causes endothelial release of NO) was infused intracoronarily (n=12). In patients with elevated LV end-diastolic pressure, an intracoronary infusion of substance P increased LV stroke volume from 72±13 to 91±16 mL ($P=0.06$) and LV stroke work from 67±11 to 90±15 g · m ($P=0.03$) and shifted the LV end-diastolic pressure–volume relation to the right.

Conclusions—In patients with dilated cardiomyopathy, an increase in endomyocardial NOS2 or NOS3 gene expression augments LV stroke volume and LV stroke work because of a NO-mediated rightward shift of the diastolic LV pressure-volume relation and a concomitant increase in LV preload reserve. **(*Circulation*. 1999;99:3009-3016.)**

Key Words: nitric oxide ■ cardiomyopathy ■ ventricles ■ diastole

In patients with heart failure, the functional significance for left ventricular (LV) performance of modified myocardial expression of nitric oxide synthase (NOS) remains unclear.[1] Expression of cytokine-inducible, calcium-insensitive NOS isoform (NOS2) was first reported in the ventricular myocardium of patients with dilated cardiomyopathy after myocarditis[2] and, subsequently, in the myocardium of failing hearts, regardless of the underlying cause.[3] In the latter study, NOS2 gene expression was more frequent in NYHA class II patients than in class IV patients, but in a subsequent study,[4] high NOS2 gene expression was associated not with functional class but with low LV ejection fraction. In allograft recipients, a Doppler echocardiographic study[5] showed that NOS2 gene expression correlated with LV dysfunction, but an invasive assessment of LV performance[6] failed to demonstrate any effects of high myocardial NOS2 expression on LV ejection fraction or on the peak rate of rise of LV pressure ($LV\ dP/dt_{max}$). In the normal human heart and in allograft recipients, intracoronary infusions of NO donors or of substance P, which releases nitric oxide (NO) from the coronary endothelium, also failed to alter LV ejection fraction or LV dP/dt_{max}; instead, they hastened LV relaxation and increased LV diastolic distensibility.[7,8] These effects of NO on diastolic LV function were recently corroborated by experimental observations. In isolated guinea pig hearts, administration of a NOS inhibitor reduced LV diastolic distensibility,[9] and in the beating rabbit heart, a porphyrinic microsensor in the LV wall revealed a prominent diastolic peak of myocardial NO concentration.[10]

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In end-stage explanted human cardiomyopathic hearts, expression and activity of endothelial constitutive NOS isoform (NOS3) was reduced, but expression and activity of

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NOS2 were enhanced.[11] In trabeculae isolated from these hearts, NOS2 activity hastened isometric tension decline and blunted the β-agonist–induced increase in twitch tension. A blunted myocardial contractile response to β-agonists because of NOS activity was also observed in adult rat cardiomyocytes,[1] in dogs with pacing-induced heart failure,[12] in patients with LV dysfunction,[13,14] and in the human allograft.[6,13]

To investigate the in vivo functional significance of modified myocardial NOS expression for LV contractile performance in patients with nonischemic, dilated cardiomyopathy, invasive measures of LV function were derived from LV angiograms and high-fidelity tip-micromanometer LV pressure recordings; they were then correlated with the intensity of myocardial NOS2 and NOS3 gene expression in simultaneously procured LV endomyocardial biopsies. To confirm the role of NO as a mediator of the observed correlation between LV stroke volume or LV stroke work and myocardial NOS2 or NOS3 activity, repeat LV angiograms and LV pressure recordings were obtained during intracoronary substance P infusion, which causes receptor-mediated release of NO from the coronary endothelium.[8,15]

Methods

Patients

A total of 32 patients with nonischemic, dilated cardiomyopathy underwent diagnostic cardiac catheterization and coronary angiography. The group consisted of 9 women and 23 men (mean age, 51 years; range, 23 to 75 years). For ethical reasons, heart failure therapy was maintained; it consisted of angiotensin-converting enzyme (ACE) inhibitors (n=31), diuretics (n=31), digitalis (n=14), and β-blockers (n=2; Table; patients 11 and 31). LV angiography revealed a LV end-diastolic volume index of 148±37 mL/m^2 and a LV ejection fraction of 29±10%. A LV end-diastolic volume index >102 mL/m^2 (normal average value +2SD) and an LV ejection fraction ≤45% were used as cutoff values for dilated cardiomyopathy. Informed consent was obtained from all patients, and the study was approved by the local review boards. There were no complications.

Study Protocol

LV pressure was measured by using a high-fidelity tip-micromanometer catheter, and right heart pressures were measured by using a Swan-Ganz catheter. Left ventricular endomyocardial biopsies were obtained in 20 patients (Table; patients 1 through 15 and 28 through 32) using a long bioptome-guiding sheath and a disposable transfemoral bioptome (Cordis Corp). Baseline LV function (Table) of the 20 patients in whom LV endomyocardial biopsies were obtained was comparable to the baseline LV function of the entire patient cohort.

After diagnostic cardiac catheterization, an intracoronary infusion of substance P (20 pmol/min for a 5-minute period)[8,13] was performed in 12 patients (Table; patients 16 through 27). At the end of the infusion period, hemodynamic measures were repeated. In 11 patients (Table; patients 1, 2, 4 through 9, 12, 13, and 15), an intravenous infusion of dobutamine[6,13] was administered; it was progressively titrated upward to a dose of 11±2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ to increase resting heart rate by 20 bpm. The infusion protocols for substance P and for dobutamine were extensively described in a previous study.[13] Data from 3 patients who were part of this previous study were included in the present study.

Reverse Transcription–Polymerase Chain Reaction for NOS2 and NOS3 mRNA

Biopsy Procurement

Biopsy samples used for the subsequent quantification of NOS2 and NOS3 mRNA by reverse transcription–polymerase chain reaction (RT-PCR) were immediately frozen in liquid nitrogen and stored at −80°C. In 15 patients (Table; patients 1 through 15), 1 biopsy sample was used, and in 5 patients (Table; patients 28 through 32), 2 biopsy samples from different sites of the LV cavity were used. In these 5 patients, the variability of the NOS2 and NOS3 mRNA concentrations was 7±2% and 15±3%, respectively.

RNA Extraction

Total RNA from 25 LV endomyocardial biopsies was extracted according to Trizol reagent protocol (Life Technologies). Purified RNA was dissolved in water, and the concentration was measured by absorbance at 260 nm. Quantitative RT-PCR for NOS2 and NOS3 was then performed in the presence of a defined amount of specific RNA mutant as an internal standard.

Internal Standard Preparation

The NOS2 and NOS3 internal standards were subcloned into a pSP(64)poly(A) vector (Promega). cRNA was then synthesized in vitro as a sense probe from 10 μg of the PvuI-linearized plasmid using SP6 RNA polymerase in the presence of 10 μCi of α-^{32}P-UTP. The concentration of the transcript was determined after measuring the radioactivity incorporated into the RNA product.

Oligonucleotides Used for RT-PCR

For NOS2, the primers chosen were 5′-AAGACCCAGTGCCC-TGCTTT-3′ for sense and 5′-CGCAAACATAGAGGTGGCC-3′ for antisense; they allowed the distinct amplification of NOS2 mRNA (388 bp) and of the internal standard (452 bp). For NOS3, the primers chosen were 5′-TGCCTGCCCCACTGCTCCTC-3′ for sense and 5′-TGCACGGTCTGCAGGACGTTGGT-3′ for antisense, thus amplifying a DNA fragment of 616 bp for NOS3 and of 680 bp for the internal standard. These primers were chosen to encompass several introns to avoid amplification of contaminating genomic DNA.

Quantitative RT-PCR Protocol

Total RNA was reverse-transcribed with a fixed amount of the specific synthetic RNA and 200 U of Moloney murine leukemia virus reverse transcriptase (Life Technologies). Single-strand cDNA synthesis was performed in 20 μL of reaction buffer (in mmol/L): Tris-HCl 20 (pH 8.3), KCl 50, $MgCl_2$ 4, dNTP 1, and DTT 10 and 0.2 μmol/L oligo-p(dT). The reaction mixture was incubated for 10 minutes at 25°C and then for 60 minutes at 37°C. The resultant cDNA was amplified using 2.5 U of Taq DNA polymerase (Boehringer) and 0.5 μmol/L sense and antisense primers in 50 μL of (in mmol/L) KCl 50, Tris-HCl 10 (pH 8.3), $MgCl_2$ 4, and dNTP 1 and 0.01% gelatin. A total of 28 amplification cycles were undertaken as follows: denaturation at 94°C for 1 minute, annealing at 62°C for NOS2 and at 63°C for NOS3 for 1 minute, and extension at 72°C for 1 minute. The final extension was performed for 10 minutes. To quantify NOS isoform mRNA levels, a trace amount of [^{32}P]-dCTP was included in the PCR reaction. After PCR amplification, the PCR products were separated on a 5% polyacrylamide gel, and radioactive signals were analyzed using a computer-based imaging system (Fuji Bas 1000, Fuji Medical Systems).

Data Analysis

LV volumes were derived from single-plane LV angiograms by using the area-length method and a regression equation. The duration of LV electromechanical systole (LVEST), which indicates the time to onset of LV relaxation, was measured as the interval from the Q wave on the ECG to the moment of the peak rate of fall of LV pressure (LV dP/dt_{min}). LV stroke work was derived from the area within the LV pressure-volume diagram. Single comparison data were analyzed with a Student *t* test for paired data.

Results

NOS Gene Expression and LV Function at Rest

The Table summarizes indices of baseline LV function in the dilated, nonischemic cardiomyopathy study population. Fig-

Baseline LV Function of the Dilated Cardiomyopathy Study Group

Patient	HR, bpm	LVPSP, mm Hg	LVEDP, mm Hg	LV dP/dt_{max}, mm Hg/s	LVEDVI, mL/m^2	LVEF, %
1	78	108	14	1120	108	32
2	79	103	30	550	216	14
3	92	79	35	450	181	8
4	88	157	31	1450	142	20
5	78	100	36	560	178	44
6	88	91	24	510	162	33
7	77	137	21	1240	106	39
8	82	80	24	550	240	30
9	85	150	29	1010	207	39
10	92	107	7	1050	105	39
11	55	126	12	1250	143	39
12	82	127	27	1230	146	35
13	85	112	20	630	142	27
14	89	110	38	700	214	8
15	78	95	20	720	160	17
16	88	108	22	750	162	28
17	95	100	29	750	168	22
18	82	144	36	920	170	32
19	94	84	27	640	133	21
20	91	165	17	1300	117	32
21	62	96	14	900	113	42
22	90	140	34	800	114	29
23	107	137	30	1400	120	45
24	104	121	25	1150	178	25
25	81	107	14	1250	108	45
26	88	150	7	650	174	21
27	115	95	31	840	134	23
28	70	195	28	1150	129	31
29	90	139	18	1200	107	25
30	67	115	9	950	105	45
31	88	108	40	700	106	38
32	72	144	14	1000	142	27
Mean±SD	85±12	120±27	24±9	917±288	148±37	30±10

HR indicates heart rate; LVPSP, left ventricular peak systolic pressure; LVEDVI, left ventricular end-diastolic volume index; and LVEF, left ventricular ejection fraction. Other abbreviations are as in text.

ure 1 shows representative RT-PCR amplification products of NOS2 and NOS3 mRNA derived from LV endomyocardial biopsies obtained from 4 patients (Table; patients 1, 2, 6, 8). In the subgroup of patients (n=20) in whom LV endomyocardial biopsies were obtained, a significant linear correlation was observed between intensity of NOS2 mRNA expression and LV stroke volume ($P=0.001$; $r=0.66$), LV ejection fraction ($P=0.007$; $r=0.58$), or LV stroke work ($P<0.003$; $r=0.62$) (Figure 2). In the same group of patients, a significant linear correlation was also observed between intensity of NOS3 mRNA expression and LV stroke volume ($P=0.01$; $r=0.53$) or LV stroke work ($P=0.01$; $r=0.52$) (Figure 3). There were no significant correlations between intensity of NOS2 or NOS3 mRNA expression and other hemodynamic indices, such as LV end-diastolic volume index, LV end-systolic volume index, or LV dP/dt_{max}.

A closer correlation was observed between intensity of NOS2 mRNA expression and LV stroke volume ($P=0.001$; $r=0.74$), LV ejection fraction ($P=0.0007$; $r=0.77$), or LV stroke work ($P=0.0002$; $r=0.82$) when the analysis was limited to those patients who, at the time of the study, were using LV preload reserve, as evident from elevated LV end-diastolic pressure (LVEDP>16 mm Hg) (Figure 4). Limiting the analysis to patients who were using LV preload reserve did not improve the correlation between intensity of NOS3 mRNA expression and LV stroke volume or LV stroke work.

LV Preload Reserve and Coronary Endothelial NO

To investigate the myocardial effects of NO on LV preload reserve, an intracoronary infusion of substance P was performed

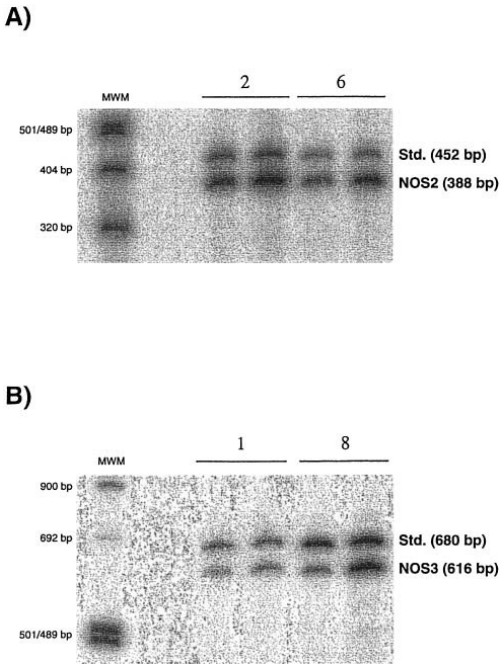

Figure 1. Representative RT-PCR amplification products of NOS2 mRNA (A) and NOS3 mRNA (B) derived from LV endomyocardial biopsies obtained in 4 patients (Table; patients 1, 2, 6, and 8). For each patient, 25 and 50 ng of total RNA were reverse-transcribed in presence of fixed amount of internal standard (Std.; 2000 molecules for patients 1 and 6, 10 000 molecules for patients 2 and 8).

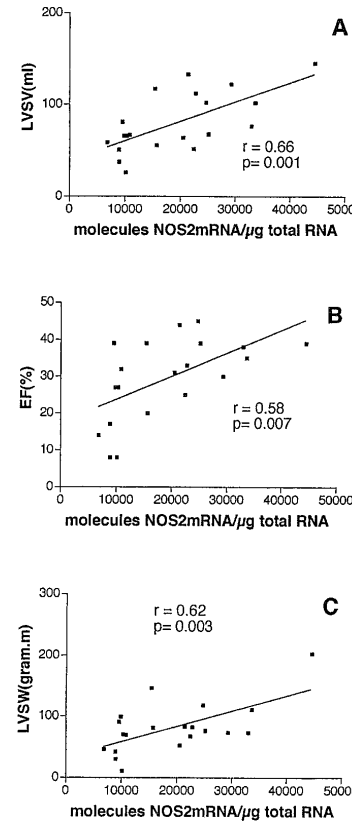

Figure 2. Linear correlations between LV stroke volume (LVSV) (A), LV ejection fraction (EF) (B), and LV stroke work (LVSW) (C) and intensity of NOS2 mRNA expression.

in 12 patients. This resulted in a significant fall in LV peak systolic pressure from 121±8 to 111±7 mm Hg (P=0.0009), in LV end-systolic pressure from 64±6 to 58±6 mm Hg (P=0.01) and in LVEDP from 25±3 to 18±2 mm Hg (P<0.0001) and in LVEST from 410±17 to 393±17 ms (P=0.04). There were no significant changes in LV dP/dt_{max}, heart rate, LV end-diastolic volume, LV ejection fraction, LV stroke volume, or LV stroke work, except in those patients who, at the time of the study, had elevated LV filling pressures (LVEDP >16 mm Hg). In these patients, intracoronary substance P significantly increased LV end-diastolic volume from 220±26 to 240±29 mL (P=0.04), LV stroke volume from 72±13 to 91±16 mL (P=0.06), and LV stroke work from 67±11 to 90±15 g · m (P=0.03) (Figure 5).

NOS Gene Expression and LV Response to β-Adrenoreceptor Stimulation

Intravenous infusion of dobutamine caused a significant increase in heart rate from 82±1 to 100±3 bpm (P=0.0001) and in LV dP/dt_{max} from 924±120 to 1356±175 mm Hg/s (P<0.0001), a significant decrease in LVEST from 430±19 to 328±16 ms (P=0.0002), and no change in LV peak or end-systolic pressures. LV endomyocardial NOS2 mRNA expression was inversely correlated with the dobutamine-induced decrease in LVEST (P=0.04; r=0.63) and the ratio of the dobutamine-induced decrease in LVEST divided by the dobutamine-induced increase in LV dP/dt_{max} (expressed as a fraction of baseline LV dP/dt_{max} value) (P=0.004; r=0.78) (Figure 6). The dobutamine-induced changes in heart rate and in LV dP/dt_{max} were unrelated to LV endomyocardial NOS2 mRNA. The dobutamine-induced changes in heart rate, LV dP/dt_{max}, LVEST, and the ratio of ΔLVEST/ΔLV dP/dt_{max} were also unrelated to LV endomyocardial NOS3 mRNA expression.

Discussion

The present study provides the first evidence that LV endomyocardial NOS2 and NOS3 gene expression varies with the severity of LV dysfunction in patients with dilated, nonischemic cardiomyopathy. In these patients, higher LV endomyocardial NOS2 and NOS3 gene expression was accompanied by higher LV stroke volume and higher LV stroke work.

Myocardial NOS Gene Expression in Heart Failure

Experimental evidence on endomyocardial NOS3 expression and activity in heart failure provides support for the idea that

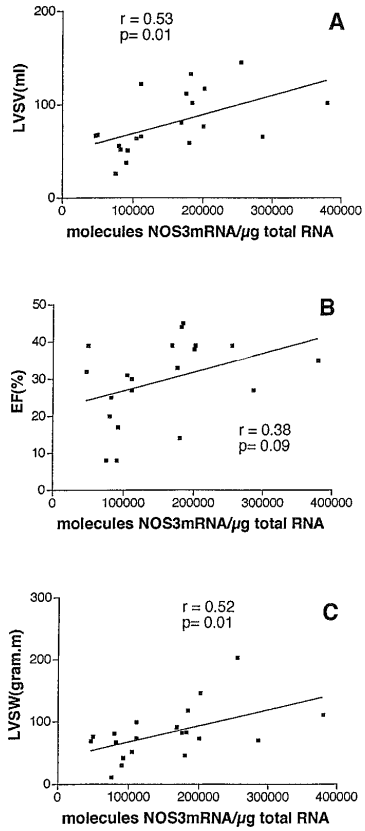

Figure 3. Linear correlations between LV stroke volume (LVSV) (A), LV ejection fraction (EF) (B), and LV stroke work (LVSW) (C) and intensity of NOS3 mRNA expression.

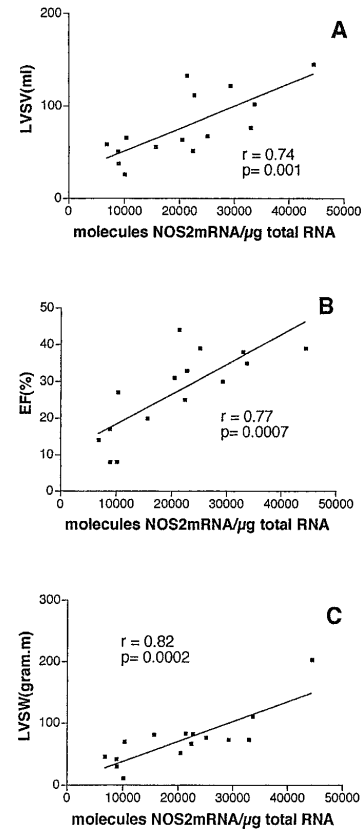

Figure 4. Linear correlations between LV stroke volume (LVSV) (A), LV ejection fraction (EF) (B), and LV stroke work (LVSW) (C) and intensity of NOS2 mRNA expression in patients with elevated LV filling pressures (LVEDP>16 mm Hg).

intensity of NOS3 gene expression varies with the severity of LV dysfunction. In a pacing-induced heart failure dog model, NOS3 activity increased after 2 weeks of pacing, as evident from enhanced endothelium-dependent relaxation of isolated coronary artery rings.[16] This increase in NOS3 activity could have resulted from higher coronary blood flow because of increased myocardial metabolic demand[17] or from higher mechanical stress because of LV cavity dilatation.[10] In the same pacing-induced heart failure dog model, cardiac NO production was reduced after 4 weeks of pacing, and this reduction was accompanied by a switch in myocardial substrate use, a rise in LV end-diastolic pressure, and a fall in LV stroke work.[18]

Clinical studies reporting on myocardial NOS3 gene expression and activity in patients with dilated cardiomyopathy used right ventricular tissue obtained by transvascular biopsy,[2–4] right ventricular tissue excised during cardiopulmonary bypass,[3] or LV tissue from explanted hearts at the time of cardiac transplantation.[11,19,20] Detection of upregulation of myocardial NOS3 as a result of mechanical stress applied to the left ventricle requires LV and not right ventricular tissue, as illustrated in spontaneously hypertensive rats in which NOS3 activity was upregulated in LV but not right ventricular tissue.[21] Both low[11] and high[19,20] intensities of NOS3 gene expression have been observed in the LV tissue of end-stage explanted cardiomyopathic human hearts. These conflicting results are explained by the present study, which observed that the intensity of NOS3 gene expression varied with the severity of LV dysfunction. The present study determined NOS2 and NOS3 gene expression in LV tissue samples procured by LV transvascular biopsy. The use of LV transvascular biopsies allowed LV endomyocardial NOS gene expression to be assessed not only in patients with end-stage heart failure but also in patients with compensated heart failure. In the present study, most patients were on ACE inhibitor therapy. ACE inhibitor therapy could have augmented NOS3 expression, as evident from the Trial on Reversing Endothelial Dysfunction (TREND), which showed improved coronary endothelial-dependent vasodilator responses during chronic quinapril therapy.[22] The 2 patients in the present study who were on β-blocker therapy had high-

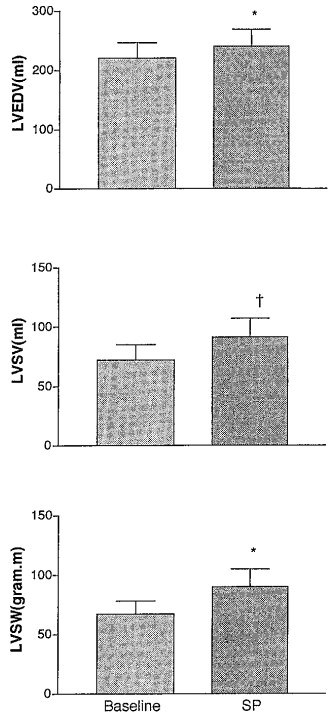

Figure 5. Effects of intracoronary infusion of substance P (SP) on LV end-diastolic volume (LVEDV) (A), LV stroke volume (LVSV) (B), and LV stroke work (LVSW) (C) in patients with elevated LV filling pressures (LVEDP>16 mm Hg). *$P<0.05$; †$P=0.06$.

intensity NOS3 gene expression. In failing human myocardium, NOS3 expression is more abundant in patients on β-blocker therapy,[19] probably because of increased transcription and subcellular targeting to plasmalemmal caveolae of NOS3 as a result of reduced myocardial cAMP content.[23,24]

In patients with dilated cardiomyopathy, upregulated myocardial NOS2 activity or gene expression was reported by most[2–4,11,19] but not all[20] investigators. In a previous study,[3] upregulated myocardial NOS2 gene expression was more frequently observed in patients in NYHA class II than in NYHA class IV. This finding corresponds with the present observation of higher NOS2 gene expression in patients with moderate LV dysfunction. The concordance between both studies, despite the use of LV tissue in the present study and right ventricular tissue in the previous one, supports regulation of myocardial NOS2 expression not by local mechanical stresses but by humoral factors, such as cytokines and neurohormones. No differences in the intensity of NOS2 gene expression were observed in patients receiving digitalis.

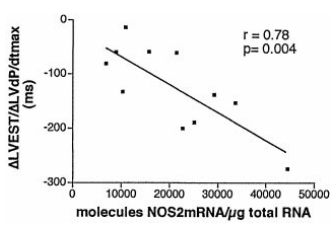

Figure 6. Inverse correlation between intensity of NOS2 mRNA expression and dobutamine-induced abbreviation of LV electromechanical systole time (ΔLVEST) divided by dobutamine-induced increase in LV dP/dt_{max}. When endomyocardial NOS2 mRNA was higher, the dobutamine-induced abbreviation of LV contraction was larger for similar dobutamine-induced rise in LV dP/dt_{max}.

Low Myocardial NOS Gene Expression in Severe LV Dysfunction

In the present study, both NOS2 and NOS3 gene expression were lower in patients with low LV stroke volume and low LV stroke work. The parallel reduction of NOS2 and NOS3 mRNA in patients with severe LV dysfunction could have resulted from faster degradation of mRNA in biopsies from this patient group. The procedure of biopsy procurement was, however, identical for all patients and, therefore, not responsible for the observed difference. In cardiac myocytes, regulators of gene expression of NOS isoenzymes usually produce opposite effects on NOS2 and NOS3 mRNA. Cytokines increase NOS2 mRNA, and interferon-γ and interleukin-1β decrease NOS3 mRNA.[1] cAMP stimulates NOS2 mRNA stability[25] and downregulates transcription of NOS3 mRNA.[23] These regulators are, therefore, probably not involved in the observed parallel reduction of gene expression of NOS isoenzymes in patients with low LV stroke work. A possible explanation for the parallel reduction of gene expression of NOS isoenzymes in severe LV dysfunction could be depletion of the myocyte population because of apoptotic cell death triggered by high myocardial concentrations of NO or catecholamines.[26] Such a depletion of the viable myocyte population could explain both the parallel reduction of gene expression of NOS isoenzymes and the impairment of LV contractile performance.

Myocardial NOS Gene Expression and LV Preload Reserve

In the present study, linear correlations were observed between LV stroke volume or LV stroke work and intensity of myocardial NOS2 or NOS3 gene expression. Patients with dilated cardiomyopathy are highly dependent on preload recrutable LV stroke work to compensate for reduced inotropic reserve.[27] This enhancement of preload recrutable LV stroke work results from a rightward displacement of the diastolic LV pressure-volume relation. In the present study, an intracoronary infusion of substance P, which releases NO from the coronary endothelium,[15] induced an acute rightward displacement of the LV end-diastolic pressure-volume relation and an increase in LV stroke volume and LV stroke work when LV filling pressures were elevated. This short-term, NO-induced increase in LV diastolic distensibility, LV stroke volume, and LV stroke work supports a myocardial action of NO to mediate the observed correlations between myocardial NOS gene expression and LV stroke volume or LV stroke work. A similar myocardial action of NO on LV preload

reserve was recently observed in isolated guinea pig hearts.[9] In this preparation, adding N^G-monomethyl-L-arginine, a specific inhibitor of NOS, to the coronary perfusate resulted in a leftward displacement of the diastolic LV pressure-volume relation and a reduction of LV stroke volume. Similar actions of NO or its second messenger, cGMP, on diastolic myocardial properties have been observed in isolated rat cardiomyocytes[28,29] and in isolated rabbit cardiomyocytes.[30] In isolated rat cardiomyocytes, an increase in diastolic cell length was observed after exposure to a cGMP analogue[28] or a NO donor.[29] In isolated rabbit cardiomyocytes, exposure to lipopolysaccharides[30] altered cell volume through an NO- and cGMP-mediated mechanism. Beneficial effects of NO on diastolic LV properties could result not only from short-term cGMP-mediated myocardial actions but also from long-term effects on the LV interstitium because of altered extracellular matrix metalloproteinase activity and collagen turnover.[31]

Myocardial NOS Gene Expression and β-Adrenoceptor Stimulation

The present study also demonstrated a significant correlation between the dobutamine-induced abbreviation of LV contraction and NOS2 mRNA expression. In a previous study using isolated muscle strips from explanted human cardiomyopathic hearts, a similar correlation was observed between NOS2 activity measured by citrulline formation and β-agonist–induced changes in the timing of isometric force decline.[11] In the present study population, no relation was found at baseline between the duration of LV contraction and myocardial NOS mRNA expression, despite the elevated adrenergic drive and the presence of such a relation during intravenous infusion of dobutamine. This suggests that the myocardial effects of elevated plasma catecholamines in heart failure may be offset by a simultaneous reduction in myocardial β1-receptors and/or a simultaneous increase in myocardial G_i-proteins.

Study Limitations

The myocardial presence of NOS was established in the present study by demonstrating NOS mRNA by quantitative RT-PCR and not by directly demonstrating NO or cGMP in the myocardium. This would provide more definite proof of NOS presence and activity because of earlier reports of posttranscriptional and posttranslational modification of NOS.[24] In the present study, the effects of NO on LV stroke volume or on LV stroke work were reproduced during intracoronary infusion of substance P, which releases NO from the coronary endothelium. Intracoronary infusion of specific NOS antagonists, which was not performed in the present study, could provide further evidence for NO contributing to LV preload reserve in these cardiomyopathic hearts. To address the issue of homogeneous endomyocardial expression of NOS isoenzymes, multiple biopsies were obtained from different LV sites in 5 patients. In these patients, the variability of NOS2 and NOS3 mRNA concentrations in the different biopsy samples was, respectively, 7±2% and 15±3%.

Conclusions

In the present study, which was the first to analyze gene expression of NOS isoenzymes in LV transvascular biopsies from dilated cardiomyopathy patients, the intensity of NOS2 and NOS3 gene expression was linearly correlated with LV stroke volume and LV stroke work. Intracoronary infusions of substance P, which releases NO from the coronary endothelium, revealed that these correlations could have resulted from a NO-mediated rightward shift of the LV end-diastolic pressure-volume relation and a concomitant increase in LV preload reserve. Future studies should be directed at identifying the mechanisms responsible for the fall in gene expression of NOS isoenzymes in patients with severe cardiomyopathic LV dysfunction, because the present study suggests NO exerts a beneficial hemodynamic effect in the failing human heart through maintenance of the Frank-Starling response.

Acknowledgment

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References

1. Kelly RA, Balligand JL, Smith TW. Nitric oxide and cardiac function. *Circ Res*. 1996;79:363–380.
2. de Belder AJ, Radomski M, Why H, Richardson PJ, Bucknall CA, Salas E, Martin JF. Nitric oxide synthase activities in human myocardium. *Lancet*. 1993;341:84–85.
3. Haywood GA, Tsao PS, von der Leyen HE, Mann MJ, Keeling PJ, Trindade PT, Lewis NP, Byrne CD, Rickenbacher PR, Bishopric NH, Cooke JP, McKenna WJ, Fowler MB. Expression of inducible nitric oxide synthase in human heart failure. *Circulation*. 1996;93:1087–1094.
4. Satoh M, Nakamura M, Tamura G, Makita S, Segawa I, Tashiro A, Satodate R, Hiramori K. Inducible nitric oxide synthase and tumor necrosis factor-α in myocardium in human dilated cardiomyopathy. *J Am Coll Cardiol*. 1997;29:716–724.
5. Lewis NP, Tsao PS, Rickenbacher PR, Xue C, Johns RA, Haywood GA, von der Leyen H, Trindade PT, Cooke JP, Hunt SA, Billingham ME, Valantine HA, Fowler MB. Induction of nitric oxide synthase in the human allograft is associated with contractile dysfunction of the left ventricle. *Circulation*. 1996;93:720–729.
6. Paulus WJ, Kästner S, Pujadas P, Shah AM, Drexler H, Vanderheyden M. Left ventricular contractile effects of inducible nitric oxide synthase in the human allograft. *Circulation*. 1997;96:3436–3442.
7. Paulus WJ, Vantrimpont PJ, Shah AM. Acute effects of nitric oxide on left ventricular relaxation and diastolic distensibility in humans. *Circulation*. 1994;89:2070–2078.
8. Paulus WJ, Vantrimpont PJ, Shah AM. Paracrine coronary endothelial control of left ventricular function in humans. *Circulation*. 1995;92:2119–2126.
9. Prendergast BD, Sagach VF, Shah AM. Basal release of nitric oxide augments the Frank-Starling response in the isolated heart. *Circulation*. 1997;96:1320–1329.
10. Pinsky DJ, Patton S, Mesaros S, Brovkovych V, Kubaszewski E, Grunfeld S, Malinski T. Mechanical transduction of nitric oxide synthesis in the beating heart. *Circ Res*. 1997;81:372–379.
11. Drexler H, Kästner S, Strobel A, Studer R, Brodde OE, Hasenfuss G. Expression, activity and functional significance of inducible nitric oxide synthase in the failing human heart. *J Am Coll Cardiol*. 1998;32:955–963.
12. Yamamoto S, Tsutsui H, Tagawa H, Saito K, Takahashi M, Tada H, Yamamoto M, Katoh M, Egashira K, Takeshita A. Role of myocyte nitric oxide in β-adrenergic hyporesponsiveness in heart failure. *Circulation*. 1997;95:1111–1114.
13. Bartunek J, Shah AM, Vanderheyden M, Paulus WJ. Dobutamine enhances cardiodepressant effects of receptor-mediated coronary endothelial stimulation. *Circulation*. 1997;95:90–96.

14. Hare JM, Givertz MM, Creager MA, Colucci WS. Increased sensitivity to nitric oxide synthase inhibition in patients with heart failure: potentiation of β-adrenergic inotropic responsiveness. *Circulation*. 1998;97:161–166.
15. Quyyumi AA, Mulcahy D, Andrews NP, Husain S, Panza JA, Cannon RO. Coronary vascular nitric oxide activity in hypertension and hypercholesterolemia: comparison of acetylcholine and substance P. *Circulation*. 1997;95:104–110.
16. O'Murchu B, Miller VM, Perrella MA, Burnett JC Jr. Increased production of nitric oxide in coronary arteries during congestive heart failure. *J Clin Invest*. 1994;93:165–171.
17. Sessa WC, Pritchard K, Seyedi N, Wang J, Hintze TH. Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. *Circ Res*. 1994;74: 349–353.
18. Recchia FA, McConnell PL, Bernstein RD, Vogel TR, Xu X, Hintze TH. Reduced nitric oxide production and altered myocardial metabolism during the decompensation of pacing-induced heart failure in the conscious dog. *Circ Res*. 1998;83:969–979.
19. Fukuchi M, Hussain SNA, Giaid A. Heterogeneous expression and activity of endothelial and inducible nitric oxide synthases in end-stage human heart failure: their relation to lesion site and β-adrenergic receptor therapy. *Circulation*. 1998;98:132–139.
20. Stein B, Eschenhagen T, Rüdiger J, Scholz H, Förstermann U, Gath I. Increased expression of constitutive nitric oxide synthase III, but not inducible nitric oxide synthase II, in human heart failure. *J Am Coll Cardiol*. 1998;32:1179–1186.
21. Nava E, Noll G, Lüscher TF. Increased activity of constitutive nitric oxide synthase in cardiac endothelium in spontaneous hypertension. *Circulation*. 1995;91:2310–2313.
22. Mancini GBJ, Henry GC, Macaya C, O'Neill BJ, Pucillo AL, Carere RG, Wargovich TJ, Mudra H, Lüscher TF, Klibaner MI, Haber HE, Uprichard ACG, Pepine CJ, Pitt B. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease: the TREND (trial on reversing endothelial dysfunction) study. *Circulation*. 1996;94:258–265.
23. Belhassen L, Kelly RA, Smith TW, Balligand JL. Nitric oxide synthase (NOS3) and contractile responsiveness to adrenergic and cholinergic agonists in the heart. *J Clin Invest*. 1996;97:1908–1915.
24. Belhassen L, Feron O, Kaye DM, Michel T, Kelly RA. Regulation by cAMP of post-translational processing and subcellular targeting of endothelial nitric-oxide synthase (Type 3) in cardiac myocytes. *J Biol Chem*. 1997;272:11198–11204.
25. Oddis CV, Simmons RL, Hattler BG, Finkel MS. cAMP enhances inducible nitric oxide synthase mRNA stability in cardiac myocytes. *Am J Physiol*. 1995;269:H2044–H2050.
26. Communal C, Singh K, Pimentel DR, Colucci WS. Norepinephrine stimulates apoptosis in adult rat ventricular myocytes by activation of the β-adrenergic pathway. *Circulation*. 1998;98:1329–1334.
27. Higginbotham MB, Sullivan MJ, Coleman RE, Cobb FR. Regulation of stroke volume during exercise in patients with severe left ventricular dysfunction: importance of the Starling mechanism. *J Am Coll Cardiol*. 1987;9:58–66.
28. Shah AM, Spurgeon HA, Sollott SJ, Talo A, Lakatta EG. 8-Bromo-cGMP reduces the myofilament response to Ca^{2+} in intact cardiac myocytes. *Circ Res*. 1994;74:970–978.
29. Ito N, Bartunek J, Spitzer KW, Lorell BH. Effects of the nitric oxide donor sodium nitroprusside on intercellular pH and contraction in hypertrophied myocytes. *Circulation*. 1997;95:2303–2311.
30. Lew WYW, Ryan J, Yasuda S. Lipopolysaccharide induces cell shrinkage in rabbit ventricular cardiac myocytes. *Am J Physiol*. 1997;272: H2989–H2993.
31. Weber KT. Extracellular matrix remodeling in heart failure. A role for de novo angiotensin II generation. *Circulation*. 1997;96:4065–4082.

Chapter 3.2

Endomyocardial Nitric Oxide Synthase and the Hemodynamic Phenotypes of Human Dilated Cardiomyopathy and of Athlete's Heart

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Endomyocardial nitric oxide synthase and the hemodynamic phenotypes of human dilated cardiomyopathy and of athlete's heart

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Abstract

Objective: In dilated cardiomyopathy and in athlete's heart, progressive LV dilatation is accompanied by rightward displacement of the diastolic LV pressure–volume relation. In dilated cardiomyopathy, an increase in diastolic LV stiffness can limit this rightward displacement thereby decreasing LV systolic performance. Because nitric oxide (NO) reduces diastolic LV stiffness, the present study relates diastolic LV stiffness and LV systolic performance to intensity of endomyocardial NO synthase (NOS) gene expression in dilated cardiomyopathy and in athlete's heart. **Methods:** Microtip LV pressures, conductance-catheter or angiographic LV volumes, echocardiographic LV wall thicknesses and snap-frozen LV endomyocardial biopsies were obtained in 33 patients with dilated cardiomyopathy and in three professional cyclists referred for sustained ventricular tachycardia. Intensity of LV endomyocardial inducible NOS (NOS2) and constitutive NOS (NOS3) gene expression was determined using quantitative reverse transcription–polymerase chain reaction (RT–PCR). **Results:** Dilated cardiomyopathy patients with higher diastolic LV stiffness-modulus and lower LV stroke work had lower NOS2 and NOS3 gene expression at any given level of LV end-diastolic wall stress. The intensity of NOS2 and NOS3 gene expression observed in athlete's heart was similar to dilated cardiomyopathy with low LV diastolic stiffness-modulus and preserved LV stroke work. **Conclusions:** High LV endomyocardial NOS gene expression is observed in athlete's heart and in dilated cardiomyopathy with low diastolic LV stiffness and preserved LV stroke work. Favourable effects on the hemodynamic phenotype of high LV endomyocardial NOS gene expression could result from a NO-mediated decrease in diastolic LV stiffness and a concomitant rise in LV preload reserve.

Keywords: Cardiomyopathy; Gene expression; Hemodynamics; Nitric oxide; Ventricular function

Abbreviations: ACE, angiotensin converting enzyme; CVF, collagen volume fraction; HR, heart rate; IL-1β, interleukin-1β; IL-6, interleukin 6; *k*, left ventricular chamber stiffness constant; LV, left ventricular; LV dP/dt_{max}, peak rate of left ventricular pressure rise; LVEDP, left ventricular end-diastolic pressure; LVEDV, left ventricular end-diastolic volume; LVEDVI, left ventricular end-diastolic volume index; LVEDWS, left ventricular end-diastolic wall stress; LVEF, left ventricular ejection fraction; LVPSP, left ventricular peak systolic pressure; LVSW, left ventricular stroke work; NO, nitric oxide; NOS, nitric oxide synthase; NOS2, inducible nitric oxide synthase; NOS3, constitutive nitric oxide synthase; RT–PCR, reverse transcription–polymerase chain reaction; Stiffness-Mod, radial myocardial left ventricular stiffness modulus; TNFα, tumor necrosis factor α

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This article is referred to in the Editorial by J.T. Stark et al. (pages 225–228) in this issue.

1. Introduction

Rightward displacement of the diastolic LV pressure–volume relation underlies progressive LV dilatation and remodeling in congestive cardiomyopathy [1]. Limitation of this rightward displacement leads to a hemodynamic phenotype with elevated LV diastolic stiffness, poor LV

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systolic performance and poor prognosis [2,3]. Because of nitric oxide (NO)'s ability to shift the diastolic LV pressure–volume relationship rightward in patients with dilated cardiomyopathy [4,5], a low intensity of LV endomyocardial NOS gene expression could underlie the development of this hemodynamic phenotype. The objective of the present study therefore was to relate intensity of LV endomyocardial NOS gene expression to LV diastolic stiffness and to LV systolic performance in patients with congestive cardiomyopathy. A rightward displacement of the diastolic LV pressure–volume relation is also observed in athlete's heart [6]. We therefore investigated the same relationships in professional cyclists referred for malignant ventricular arrhythmias.

Elevated LV diastolic wall stress has previously been shown to be associated with myocardial gene expression and production of neurohormones [7], natriuretic peptides [8], cytokines [9], stress proteins [10] and growth factors [11]. To detect effects of LV diastolic wall stress on endomyocardial NOS gene expression, the present study also related intensity of LV endomyocardial NOS gene expression to prevailing LV diastolic wall stress. At the time of cardiac catheterization, measurements of LV diastolic stiffness, LV systolic performance and LV wall stress were derived from microtip LV pressures, conductance-catheter or angiographic LV volumes and echocardiographic LV wall thickness. Intensity of endomyocardial inducible NOS (NOS2) and constitutive NOS (NOS3) gene expression was determined on simultaneously procured snap-frozen LV endomyocardial biopsies using quantitative reverse transcription–polymerase chain reaction (RT–PCR).

2. Methods

2.1. Patients

2.1.1. Dilated cardiomyopathy

A total of 33 patients with nonischemic dilated cardiomyopathy underwent diagnostic cardiac catheterization, coronary angiography and LV biopsy procurement. The

Table 1
Baseline LV function and NOS2–NOS3 gene expression expressed as molecules-mRNA/μg-total RNA

Patient	HR (beats/min)	LVPSP (mmHg)	LVEDP (mmHg)	LV dP/dt_{max} (mmHg/s)	LVEDV (ml)	LVEF (%)	Stiffness-Mod (mmHg)	NOS2	NOS3
1	78	108	14	1350	206	32	93	10787	46453
2	79	103	25	525	411	14	500	6829	179459
3	92	79	33	425	325	8	660	10093	75252
4	88	157	35	1403	283	20	500	15635	79571
5	78	100	35	650	302	44	184	21344	181374
6	88	91	24	500	341	33	160	22670	175135
7	77	137	18	1525	181	39	95	25094	49143
8	82	80	22	450	408	30	183	29260	110451
9	85	150	21	1000	372	39	124	44471	254794
10	92	107	5	840	209	39	33	9476	167944
11	55	126	9	1275	301	32	69	15335	201364
12	82	127	26	1225	293	35	163	33666	379246
13	85	112	20	575	256	27	181	10256	285683
14	63	90	15	450	567	8	500	7664	139508
15	78	95	20	850	304	17	333	8855	91863
16	70	195	29	1125	207	31	242	20452	104400
17	90	139	19	1225	204	25	190	22336	81751
18	67	115	8	900	214	45	36	24657	183253
19	88	108	42	725	201	38	263	32989	199892
20	72	144	14	1000	244	34	127	9809	110481
21	95	97	33	672	141	24	367	8388	223331
22	105	102	28	1021	230	10	700	4898	174558
23	75	108	14	785	686	10	350	24701	200915
24	96	137	25	1230	273	19	416	20386	364629
25	74	115	27	900	292	20	338	19330	257533
26	78	89	4	743	262	40	43	37365	295265
27	87	121	14	1150	189	45	67	8708	142205
28	71	128	21	600	194	35	140	26559	316238
29	57	151	15	850	236	33	107	34280	179913
30	105	129	25	1500	269	16	500	6280	116058
31	56	92	9	950	270	36	64	63763	305238
32	73	90	29	525	211	15	483	16831	197612
33	69	97	28	550	499	7	933	7812	203562
Mean	80	116	21	894	290	26	277	20030	184100
±S.D.	13	26	9	326	116	12	221	13000	87280

group consisted of 10 women and 23 men (mean age: 50 years; range 23–76). Individual and mean baseline hemodynamic data are listed in Table 1. At the time of study, heart failure therapy was maintained for ethical reasons and consisted of angiotensin-converting-enzyme (ACE) inhibitors ($n=33$), diuretics ($n=31$), digitalis ($n=15$) and β-blockers ($n=3$; Table 1: patients 10, 19, 24). No patient was using NO-donors. Patients 1–13, 15 (Table 1) were also part of a previous study [4]. A LV end-diastolic volume index (LVEDVI)>102 ml/m^2 and a LV ejection fraction (LVEF)≤45% were used as cutoff values for dilated cardiomyopathy (normal values: LVEDVI=72±15 ml/m^2; LVEF=72±8%). LV endomyocardial biopsies were obtained using a long bioptome-guiding sheath and a disposable transfemoral bioptome (Cordis Corp). Histological examination of the endomyocardial biopsies revealed no evidence of myocarditis.

2.1.2. Athlete's heart

Three professional cyclists (mean age: 31 years) underwent diagnostic cardiac catheterization, coronary angiography and LV biopsy procurement. They were referred for sustained exercise-induced ventricular tachycardia. Their hemodynamic and echocardiographic studies were consistent with the diagnosis of athlete's heart as evident from a large LVEDVI (129±11 ml/m^2), a normal LVEF (67±9%), a low resting heart rate (55±3 beats/min) and a slightly elevated echocardiographic posterior LV wall thickness (10.3±0.3 mm) (normal value: 9.1±0.5 mm). They took no medications at the time of study. LV endomyocardial biopsies were obtained using a long bioptome-guiding sheath and a disposable transfemoral bioptome (Cordis Corp). Histological examination of the endomyocardial biopsies revealed no evidence of myocarditis.

2.2. Study protocol

Right heart pressures and cardiac outputs were measured using a Swan-Ganz thermodilution catheter. LV pressure and LV dP/dt were derived from a high-fidelity tip-micromanometer catheter ($n=33$). In eight patients, a conductance pressure–volume catheter (Leycom Sigma, Zoetermeer, The Netherlands) was inserted into the left ventricle for continuous and on-line LV volume and pressure measurement. In these eight patients, a 8F balloon catheter was introduced from the right femoral vein into the right atrium. Transient pullback of the inflated balloon into the inferior vena cava, allowed for assessment of diastolic LV function using end-diastolic pressure–volume points of multiple, variably preloaded beats [12] (Fig. 1). The study protocol was approved by the local review board (VU University Medical Center, Amsterdam) and conformed with the principles outlined in the declaration of Helsinki. Written informed consent was obtained for the study protocol and there were no complications related to procedure or study protocol.

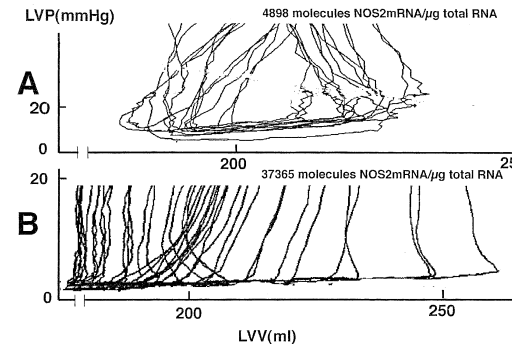

Fig. 1. Diastolic portions of LV pressure–volume loops recorded by a LV micromanometer conductance catheter during transient pullback of an inflated balloon into the inferior vena cava in a patient with low NOS2 gene expression (panel A) and in a patient with high NOS2 gene expression (panel B).

2.3. Reverse transcription–polymerase chain reaction

Biopsy procurement, RNA extraction, internal standard preparation, oligonucleotides used for RT–PCR and quantitative RT–PCR protocol were extensively described previously [4]. NOS2 and NOS3 mRNA's were determined in all patients. Tumor necrosis factor α (TNF α), Interleukin-1β (IL-1β) and Interleukin 6 (IL-6) were determined in a subgroup of 10 patients.

2.4. Quantitative morphometry

In a subgroup of patients ($n=20$), collagen volume fraction (CVF) was determined on EVG (elastica von Gieson) stained sections of biopsies placed in 5% formalin using an automated image analyzer (Prodit).

2.5. Data analysis

LV volumes were derived from single-plane LV angiograms by use of the area–length method and a regression equation. LV stroke work was derived from the area within the LV pressure–volume diagram. Echocardiographic LV posterior wall thickness (h) measurements were derived from a sector scanner (HP Sonos 5500) using a 3 Mhz transducer. Circumferential LV end-diastolic wall stress (EDWS) was calculated from microtip LV pressure (P), angiographic LV end-diastolic major (a) and minor (b) semi-axes and h using the formula [13]

$$\mathrm{LVEDWS} = (Pb/h)(1 - h/2b - b^2/2a^2)$$

To assess diastolic LV myocardial material properties, a

radial myocardial LV stiffness modulus (Stiffness-Mod) was calculated from the microtip LV pressure and h using the formula [14,15]

$$\text{Stiffness-Mod} = \Delta\sigma_R/\Delta\varepsilon_R = -\Delta P/(\Delta h/h) = -\Delta P/\Delta \ln h$$

and assuming the increment in radial stress ($\Delta\sigma_R$) to be equal but opposite in sign to the increment in LV pressure (P) at the endocardium and the increment in radial strain ($\Delta\varepsilon_R$) to be equal to the increment in wall thickness (Δh) relative to the instantaneous wall thickness. Because $\Delta h/h = \Delta \ln h$, Stiffness-Mod was equal to the slope of a P vs. $\ln h$ plot.

In eight patients, in whom a conductance catheter was inserted into the LV and in whom a transient caval balloon occlusion was performed, a LV chamber stiffness constant (k) was calculated from an exponential curve fit to the multiple LV end-diastolic pressure–volume points of the variably preloaded beats during caval balloon occlusion using the formula [16]

$$P = b\, e^{kV}$$

where P is the LV end-diastolic pressure, V the LV end-diastolic volume and b the curve-fitting parameter. For these eight patients, ranking of k and Stiffness-Mod yielded an identical order, thereby validating the use of Stiffness-Mod as a measure of LV stiffness.

All results are given as mean±standard deviation and the level of statistical significance was set at $P<0.05$.

3. Results

3.1. Endomyocardial NOS 2 gene expression and the hemodynamic phenotype of dilated cardiomyopathy

Table 1 summarizes indices of LV systolic and diastolic function observed in the dilated cardiomyopathy study population. Fig. 1 shows representative examples of diastolic portions of LV pressure–volume loops recorded by a LV micromanometer conductance catheter during transient pullback of an inflated balloon into the inferior vena cava. For the dilated cardiomyopathy study population, a significant linear correlation was observed between intensity of NOS2 gene expression and LV diastolic stiffness modulus (Stiffness-Mod) ($P=0.003$; $r=0.50$), LV stroke work (SW) ($P=0.0008$; $r=0.56$) and LV ejection fraction (EF) ($P=0.004$; $r=0.49$) (Fig. 2). In the group of patients in whom a balloon caval occlusion was performed, a significant correlation was also observed between intensity of NOS2 gene expression and LV chamber stiffness constant (k) ($P=0.01$; $r=0.52$). In these groups of patients, in whom myocardial collagen volume fraction (CVF) or intensity of myocardial proinflammatory cytokine (TNFα; IL-1β; IL-6) gene expression was determined, no correlation was observed between intensity of NOS2 gene expression and CVF or cytokine gene expression. In Fig. 2, most

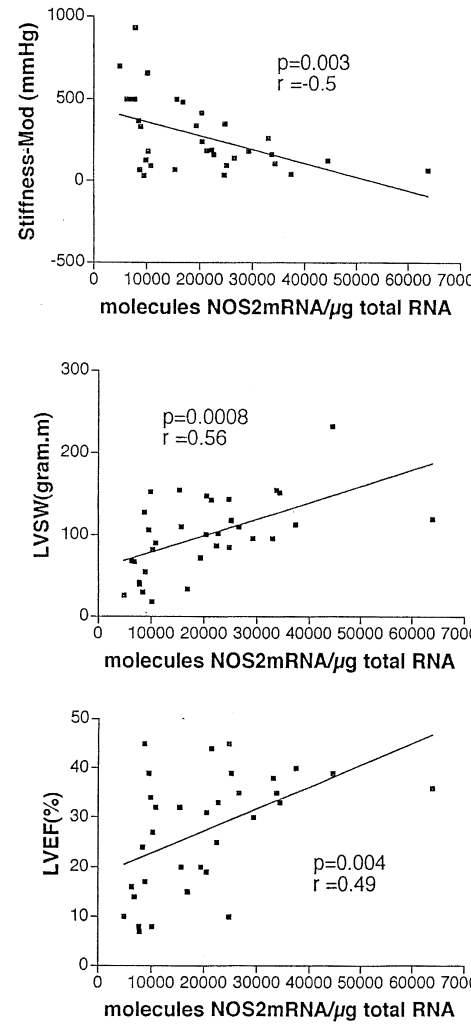

Fig. 2. Linear correlations between intensity of endomyocardial NOS2 gene expression and LV diastolic stiffness modulus (Mod), LV stroke work (SW) and LV ejection fraction (EF).

of the data scatter arose from patients wih low NOS2 gene expression. In patients with low NOS2 gene expression, the broad range of LV diastolic stiffness-Mod, LVSW and LVEF corresponded with a broad range of LV end-diastolic wall stress (LVEDWS) as shown in Figs. 3 and 4, which plot NOS2 gene expression in function of LVEDWS for different levels of LV diastolic stiffness-Mod or LVEF.

In both Figs. 3 and 4, patients with high LV diastolic stiffness-Mod (>300 kdyne/cm^2) or low LVEF ($<20\%$) clustered in the upper left hand corner of the graph, patients with intermediate values occupied the middle

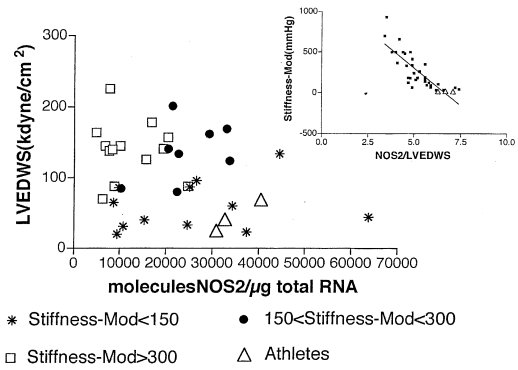

Fig. 3. Intensity of endomyocardial NOS2 gene expression plotted against LV end-diastolic wall stress (EDWS) for dilated cardiomyopathy patients with different levels of LV diastolic stiffness modulus (Mod) and for the professional cyclists. The professional cyclists coincided with dilated cardiomyopathy patients with low LV diastolic stiffness-Mod. For the individual data points of the cardiomyopathy patients, the slope of the LVEDWS-NOS2 gene expression relation varies with the different levels of LV diastolic stiffness-Mod. This is also evident from the insert which plots the NOS2/LVEDWS ratio as a function of LV diastolic stiffness-Mod. In the insert, the professional cyclists coincide with the bottom part of the relation between NOS2/LVEDWS ratio and LV diastolic stiffness-Mod observed in cardiomyopathy patients.

portion of the graph and patients with low diastolic stiffness-Mod ($<$150 kdyne/cm^2) or high LVEF ($>$30%) clustered in the lower right hand corner of the graph. For the individual data points of Figs. 3 and 4, the slope of a line connecting the data point to the origin of the graph, appeared to correlate closely with the different levels of LV diastolic stiffness-Mod or LVEF. The inserts of Figs. 3 and 4 confirmed this impression and showed the correlations between NOS2/LVEDWS ratio and LV diastolic stiffness-Mod ($P<0.0001$; $r=0.80$) or LVEF ($P<0.0001$; $r=0.69$) to be superior to the correlations shown in Fig. 2. The NOS2/LVEDWS ratio was also more closely correlated with LVSW ($P=0.0002$ and $r=0.61$).

3.2. Endomyocardial NOS3 gene expression and the hemodynamic phenotype of dilated cardiomyopathy

For the dilated cardiomyopathy study population, no significant correlations were observed between intensity of NOS3 gene expression and LV diastolic stiffness-Mod, LVSW, LVEF, CVF or intensity of cytokine gene expression. Fig. 5 plots intensity of NOS3 gene expression in function of LVEDWS for different levels of LV diastolic stiffness-Mod. Patients with high ($>$300 kdyne/cm^2) and intermediate LV diastolic stiffness-Mod clustered in the middle portion of the graph and patients with low LV diastolic stiffness-Mod ($<$150 kdyne/cm^2) in the lower portion of the graph. As evident from the insert of Fig. 5, the NOS3/LVEDWS ratio was significantly correlated with the LV diastolic stiffness-Mod ($P=0.0001$; $r=0.58$). The slope of this linear relation was less steep than the slope of the linear relation between NOS2/LVEDWS versus LV diastolic stiffness-Mod (Fig. 3), consistent with less depression of NOS3 gene expression than of NOS2 gene expression in advanced LV dysfunction. The NOS3/LVEDWS ratio was also significantly correlated with LVEF ($P=0.003$; $r=0.51$) and with LVSW ($P=0.02$; $r=0.39$).

3.3. Endomyocardial NOS gene expression and the hemodynamic phenotype of athlete's heart

All three athletes had slightly elevated LVEDWS because of high LV end-diastolic volume and had low LV

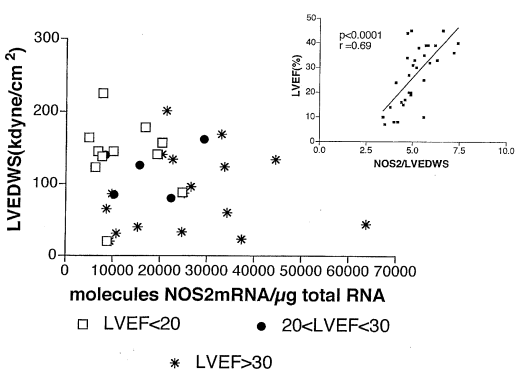

Fig. 4. Intensity of endomyocardial NOS2 gene expression plotted against LV end-diastolic wall stress (EDWS) for different levels of LV ejection fraction (EF). For the individual data points, the slope of the LVEDWS–NOS2 gene expression relation varies with the different levels of LVEF. This is also evident from the insert which plots the NOS2/LVEDWS ratio as a function of LVEF.

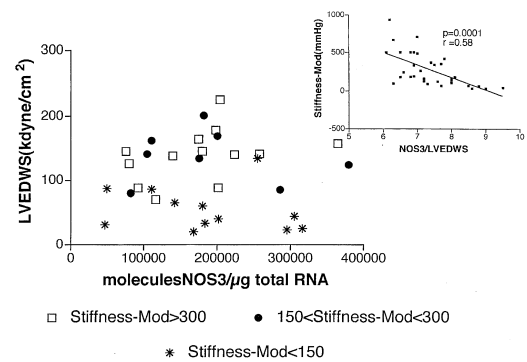

Fig. 5. Intensity of endomyocardial NOS3 gene expression plotted against LV end-diastolic wall stress (EDWS) for different levels of LV diastolic stiffness modulus (Mod). For the individual data points, the slope of the LVEDWS–NOS3 gene expression relation is higher for high and intermediate levels of LV diastolic stiffness-Mod. This is also evident from the insert which plots the NOS3/LVEDWS ratio as a function of LV diastolic stiffness-Mod.

diastolic stiffness-Mod. Fig. 3 also shows the data points of the cyclists: the relation between NOS2 gene expression and LVEDWS observed in the athletes coincided with the relation observed in dilated cardiomyopathy patients with low LV diastolic stiffness-Mod and in the insert the athletes fell on the bottom portion of the relation between NOS2/LVEDWS ratio and LV diastolic stiffness-Mod observed in the cardiomyopathy population. The relation between NOS3 gene expression and LVEDWS observed in the athletes also coincided with the relation observed in dilated cardiomyopathy patients with low LV diastolic stiffness-Mod.

4. Discussion

4.1. Endomyocardial NOS and the hemodynamic phenotype of dilated cardiomyopathy

The present study demonstrates lower LV endomyocardial NOS gene expression at a given level of LVEDWS to correspond with a worse hemodynamic phenotype of dilated cardiomyopathy characterized by higher LV stiffness, lower LVSW and lower LVEF. A similar finding was reported by Haywood et al., who found lower endomyocardial NOS2 gene expression in NYHA class IV heart failure than in NYHA class II [17]. Selective downregulation of myocardial gene expression in more advanced stages of LV dysfunction has also been reported for other genes. Lower levels of protoöncogenes were observed in peroperative biopsy samples of patients with aortic regurgitation and major LV dysfunction [18] and a fall in expression of gp130 and of interleukin-6 related cardiotrophin-1 was proposed to trigger transition to a maladaptive LV response to hemodynamic overload [19].

Downregulation of endomyocardial NOS gene expression in patients with the worst hemodynamic phenotype could have resulted from altered myocardial expression of proinflammatory cytokines, from reduction of the cardiomyocyte population because of apoptotic cell death, from altered transmission of wall stress to the cardiomyocytes or from altered presence of certain growth factors.

In experimental preparations, proinflammatory cytokines are potent stimulators of myocardial NOS2 gene expression and in patients with dilated nonischemic cardiomyopathy, increased myocardial expression of proinflammatory cytokines has been observed [20]. In a subset of patients, intensity of myocardial gene expression of proinflammatory cytokines was therefore determined. The lack of correlation in these patients between proinflammatory cytokines and NOS2, supports additional control of myocardial NOS2 expression by other mechanisms such as elevation of LV wall stress. Because of important proapoptotic effects of proinflammatory cytokines [20], the lack of correlation between cytokines and NOS2 also argues against apoptotic cell death as the cause of the downregulated NOS2 in patients with the worst hemodynamic phenotype. An increase in apoptotic cell death should however be ruled out by looking at specific markers of apoptosis in the biopsy samples, which was not performed in the present study.

The remodeling process of progressive LV cardiomyopathic dysfunction involves increased extracellular matrix degradation and turnover because of upregulation of certain matrix metalloproteinases and downregulation of tissue inhibitors of metalloproteinases [21,22]. This leads to disruption of the collagen weave around individual myocytes and to malalignment or slippage of myocytes [23]. Disruption of the collagen weave could alter transmission of LV wall stress to the cardiomyocytes and disturb wall stress-induced gene expression because of an altered cascade of signals originating from the collagen fibers and descending to sarcolemmal integrins, to cytoskeletal proteins and to nuclear membranes [1]. The lack of correlation in the present study between CVF and intensity of NOS2 gene expression, argues against myocardial fibrosis as the cause of the observed downregulation of NOS2 in patients with the worst hemodynamic phenotype.

Previous studies demonstrated abundant myocardial expression of TGFβ in patients with dilated cardiomyopathy [24]. TGFβ is known to reduce mRNA stability of NOS2 [25] and a high myocardial level of TGFβ is therefore a potential mechanism for the fall in NOS2 mRNA observed in patients with the worst hemodynamic phenotype. Variability in the relative increase of myocardial NOS and TGFβ expression after an elevation of LVEDWS could contribute to the variability of the hemodynamic phenotype of patients with dilated cardiomyopathy in analogy to the variable response of TGFβ to experimental LV pressure or volume overload [26].

In the present study, the intensity of endomyocardial NOS gene expression was higher in cardiomyopathy patients with preserved LVSW and low diastolic LV Stiffness-Mod. Patients with this hemodynamic phenotype had an intensity of endomyocardial NOS gene expression comparable to professional athletes. This finding confirmed recent observations in dilated cardiomyopathy patients which failed to demonstrate cardiodepressant effects related to myocardial NO production or to the presence of NOS2 gene expression [27]. Absence of a NO- or NOS2-induced cardiodepressant effect [28,29] was also observed in isolated muscle strips of explanted cardiomyopathic human hearts. In these muscle strips, a NOS inhibitor left baseline isometric tension development unaltered [30]. High myocardial concentrations of NO could also favorably affect the hemodynamic phenotype by blunting growth promoting effects of neurohormones on cardiomyocytes [31] and by preventing expression of a phenotype with depressed Ca^{++} ATPase activity and LV diastolic dysfunction [32].

4.2. Endomyocardial NOS and the hemodynamic phenotype of athlete's heart

The present study observed similar levels of endomyocardial NOS expression in patients with mild LV dysfunction and in athletes. In dogs, exercise is known to augment activity of coronary endothelial NOS3 both acutely [33] and chronically as a result of increased NOS3 gene expression [34]. Endomyocardial expression of NOS2 has not been reported previously in athletes or in exercising animals but endomyocardial expression of heat shock proteins and of atrial natriuretic peptide was observed in rats subjected to repetitive daily episodes of treadmill running [35,36]. The observed upregulation of endomyocardial NOS2 gene expression in athletes could therefore have resulted from a myocardial stress response to repetitive exercise-related mechanical stretching. In arterial smooth muscle, cyclic stretch was recently reported to enhance expression of NOS2 and of insuline-like growth factor I (IGF-I) [37]. Transcardiac IGF-I production is also increased in physiological hypertrophy of athlete's heart [38] and the observed upregulation of NOS2 could counteract mitogenic effects of this enhanced IGF-I production.

The upregulation of endomyocardial NOS2 gene expression in athletes challenges exclusive control of NOS2 gene expression by immune mediators [39,40] and supports a 'constitutive' expression of NOS2 [41] susceptible to upregulation by mechanical stress. The abundant myocardial expression of NOS2 in the fetus [42], in hypertrophied [43] and in senescent [44] myocardium also supports the notion of a 'constitutive' myocardial expression of NOS2, which gets upregulated during adult life as part of a stress-induced reexpression of the fetal gene programme. An identical myocardial response to exercise- and overload-related stretch was recently also demonstrated by the different ACE genotypes, which induced a similar distribution of LV hypertrophy in response to training and to arterial hypertension [45].

5. Study limitations

The present study correlated the hemodynamic phenotype with endomyocardial NOS mRNA and not with endomyocardial NOS protein, myocardial cGMP concentration or transcardiac nitrite/nitrate production, which would have provided more definite proof of endomyocardial NO activity because of posttranscriptional and posttranslational modification of NOS [39]. A previous study in transplant recipients however showed elevated myocardial cGMP and NOS2 protein immunostaining to correlate with NOS2 mRNA expression [46] and in the same patient population higher transcardiac nitrite/nitrate production was recently demonstrated in the presence of upregulated NOS2 gene expression [47]. The limited procurement of endomyocardial tissue by transvascular biopsy did not allow for simultaneous NOS2 immunostaining and determination of myocardial cGMP concentration. The use of transvascular biopsies however provided the advantage of obtaining endomyocardial tissue also from patients with mild LV dysfunction and not only from patients with the worst hemodynamic phenotype referred for cardiac transplantation. It was precisely in the group of patients with mild LV dysfunction that the level of endomyocardial NOS gene expression corresponded to the level observed in athletes.

LV endomyocardial NOS gene expression was derived from a single biopsy sample, which therefore did not take into account spatial heterogeneity of gene expression [41]. In a previous study however, multiple biopsies from different LV sites were obtained in the same patient and in this study [4] the variability of endomyocardial NOS2 and NOS3 gene expression was low (7 and 15%). Spatial nonuniformity of LV wall thickness changes could have influenced Stiffness-Mod calculations but in the subgroup of patients, in whom balloon caval occlusions were obtained, Stiffness-Mod correlated closely with k, an index of global diastolic LV function. The concurrent use of certain medications could also have influenced intensity of endomyocardial NOS gene expression. All cardiomyopathy patients were on ACE inhibitor therapy, which is known to upregulate coronary endothelial NOS3 expression [48] and some patients were on β-blocker therapy, which also upregulates endomyocardial NOS3 [49]. ACE inhibitor therapy could explain the better maintenance of NOS3 than of NOS2 gene expression in the patients with the worst hemodynamic phenotype.

The number of athletes investigated in the present study was limited ($n=3$). They came to medical attention because of a history of sustained ventricular arrhythmias. Myocarditis or another cardiomyopathic process could have caused these arrhythmias and could also have influenced their pattern of NOS gene expression. At the time of study however, their hemodynamic phenotype was consistent with athlete's heart [6] as evident from an enlarged LV end-diastolic volume, a normal LV ejection fraction, a low heart rate and a slight increase in LV wall thickness. Histological examination of their biopsies also failed to reveal any evidence of myocarditis. A major inflammatory or cardiomyopathic contribution to their pattern of NOS gene expression therefore seems unlikely.

6. Conclusions

Dilated cardiomyopathy patients with preserved LVSW and low LV diastolic stiffness have LV endomyocardial NOS2 and NOS3 gene expression equal to professional cyclists and higher than patients with low LVSW and high LV diastolic stiffness. A NO-mediated decrease in LV diastolic stiffness and a concomitant rise in LV preload reserve explains the beneficial effect of higher endo-

myocardial NOS2 and NOS3 gene expression on the hemodynamic phenotype of dilated cardiomyopathy.

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References

[1] Mann DL. Mechanisms and models in heart failure. Circulation 1999;100:999–1008.
[2] the Heart Muscle Disease Study Group, Pinamonti B, Di Lenarda A, Sinagra GF, Camerini F. Restrictive left ventricular filling pattern in dilated cardiomyopathy assessed by Doppler echocardiography: Clinical, echocardiographic and hemodynamic correlations and prognostic implications. J Am Coll Cardiol 1993;22:808–815.
[3] Rihal CS, Nishimura RA, Hatle LK, Bailey KR, Tajik AJ. Systolic and diastolic dysfunction in patients with clinical diagnosis of dilated cardiomyopathy: relation to symptoms and prognosis. Circulation 1994;90:2772–2779.
[4] Heymes C, Vanderheyden M, Bronzwaer JGF, Shah AM, Paulus WJ. Endomyocardial nitric oxide synthase and left ventricular preload reserve in dilated cardiomyopathy. Circulation 1999;99:3009–3016.
[5] Wittstein IS, Kass DA, Pak PH et al. Cardiac nitric oxide production due to angiotensin-converting enzyme inhibition decreases beta-adrenergic myocardial contractility in patients with dilated cardiomyopathy. J Am Coll Cardiol 2001;38:429–435.
[6] Maron BJ, Crawford MH. The athlete's heart. Cardiol Clin 1992;10:197–339.
[7] Sadoshima JI, Xu Y, Slayter HS, Izumo S. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. Cell 1993;75:977–984.
[8] Yasue H, Yoshimura M, Sumida H et al. Localization and mechanism of secretion of B-type natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and patients with heart failure. Circulation 1994;90:195–203.
[9] Kapadia SR, Oral H, Lee J, Nakano M, Taffet GE, Mann DL. Hemodynamic regulation of tumor necrosis factor-α gene and protein expression in adult feline myocardium. Circ Res 1997;81:187–195.
[10] Knowlton AA, Eberli FR, Brecher P et al. A single myocardial stretch or decreased fiber shortening stimulates the expression of heat shock protein 70 in the isolated, erythrocyte-perfused rabbit heart. J Clin Invest 1991;88:2018–2025.
[11] Li J, Hampton T, Morgan JP, Simons M. Stretch-induced VEGF expression in the heart. J Clin Invest 1997;100:18–24.
[12] Kass DA, Midei M, Brinker J, Maughan WL. Influence of coronary occlusion during PTCA on end-systolic and end-diastolic pressure–volume relations in humans. Circulation 1990;81:447–460.
[13] Mirsky I, Rankin JS. The effects of geometry, elasticity, and external pressures on the diastolic pressure–volume and stiffness–stress relations. Circ Res 1979;44:601–611.
[14] Bourdillon PD, Lorell BH, Mirsky I et al. Increased regional myocardial stiffness of the left ventricle during pacing-induced angina in man. Circulation 1983;67:316–323.
[15] Bronzwaer JGF, De Bruyne B, Ascoop CAPL, Paulus WJ. Comparative effects of pacing-induced and balloon coronary occlusion ischemia on left ventricular diastolic function in man. Circulation 1991;84:211–222.
[16] Gaasch WH. Passive elastic properties of the left ventricle. In: Gaasch WH, LeWinter M, editors, Left Ventricular Diastolic Dysfunction and Heart Failure, Malvern, USA: Lea and Febiger, 1994, pp. 143–149.
[17] Haywood GA, Tsao PS, von der Leyen HE et al. Expression of inducible nitric oxide synthase in human heart failure. Circulation 1996;93:1087–1094.
[18] Taketani S, Sawa Y, Taniguchi K et al. C-Myc expression and its role in patients with chronic aortic regurgitation. Circulation 1997;96:II83–II89.
[19] Hirota H, Chen J, Betz UA et al. Loss of gp130 cardiac muscle cell survival pathway is a critical event in the onset of heart failure during biomechanical stress. Cell 1999;97:189–198.
[20] Mann DL. The role of inflammatory mediators in the failing heart. Heart Failure Rev 2001;6:69–151.
[21] Weber KT. Extracellular matrix remodeling in heart failure. A role for de novo angiotensin II generation. Circulation 1997;96:4065–4082.
[22] Li YY, Feldman AM, Sun Y, Mc Tiernan CF. Differential expression of tissue inhibitors of metalloproteinases in the failing human heart. Circulation 1998;98:1728–1734.
[23] Spinale FG, Coker ML, Krombach SR et al. Matrix metalloproteinase inhibition during the development of congestive heart failure. Effects on left ventricular dimensions and function. Circ Res 1999;85:364–376.
[24] Pauschinger M, Knopf D, Petschauer S et al. Dilated cardiomyopathy is associated with significant changes in collagen Type I/III ratio. Circulation 1999;9:2750–2756.
[25] Pinsky DJ, Cai B, Yang X et al. The lethal effects of cytokine-induced nitric oxide on cardiac myocytes are blocked by nitric oxide synthase antagonism or transforming growth factor β. J Clin Invest 1995;95:677–685.
[26] Calderone A, Takahashi N, Izzo Jr. NJ, Thaik CM, Colucci WS. Pressure- and volume-induced left ventricular hypertrophies are associated with distinct myocyte phenotypes and differential induction of peptide growth factor mRNAs. Circulation 1995;92:2385–2390.
[27] Cotton JM, Kearney MT, Mac Carthy PA et al. Effects of nitric oxide synthase inhibition on basal function and the force–frequency relationship in the normal and failing human heart in vivo. Circulation 2001;104:2318–2323.
[28] Hare JM, Stamler JS. NOS: modulator, not mediator of cardiac performance. Nat Med 1999;5:273–274.
[29] Paulus WJ, Frantz S, Kelly RA. Nitric oxide and cardiac contractility in human heart failure: time for reappraisal. Circulation 2001;104:2260–2262.
[30] Drexler H, Kästner S, Strobel A et al. Expression, activity and functional significance of inducible nitric oxide synthase in the failing human heart. J Am Coll Cardiol 1998;32:955–963.
[31] Calderone A, Thaik CM, Takahashi N, Chang DLF, Colucci WS. Nitric oxide, atrial natriuretic peptide, and cyclic GMP inhibit the growth-promoting effects of norepinephrine in cardiac myocytes and fibroblasts. J Clin Invest 1998;101:812–818.
[32] Hasenfuss G, Schillinger W, Lehnart SE et al. Relationship between Na^+–Ca^{2+}-exchanger protein levels and diastolic function in failing human myocardium. Circulation 1999;99:641–648.
[33] Bernstein RD, Ochoa FY, Xu X et al. Function and production of nitric oxide in the coronary circulation of the conscious dog during exercise. Circ Res 1996;79:840–848.
[34] Sessa WC, Pritchard K, Seyedi N, Wang J, Hintze TH. Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. Circ Res 1994;74:349–353.
[35] Locke M, Tanguay RM, Klabunde RE, Ianuzzo CD. Enhanced postischemic myocardial recovery following exercise induction of HSP 72. Am J Physiol 1995;269:H320–325.

[36] Mantymaa P, Arokoski J, Porsti I et al. Effect of endurance training on atrial natriuretic peptide gene expression in normal and hypertrophied hearts. J Appl Physiol 1994;76:1184–1194.

[37] Standley PR, Stanley MA, Senechal P. Activation of mitogenic and antimitogenic pathways in cyclically stretched arterial smooth muscle. Am J Physiol 2001;281:E1165–1171.

[38] Serneri GGN, Boddi M, Modesti PA et al. Increased cardiac sympathetic activity and insulin-like growth factor-I formation are associated with physiological hypertrophy in athletes. Circ Res 2001;89:977–982.

[39] Kelly RA, Balligand JL, Smith TW. Nitric oxide and cardiac function. Circ Res 1996;79:363–380.

[40] Andrew PJ, Mayer B. Enzymatic functions of nitric oxide synthases. Cardiovasc Res 1999;43:521–531.

[41] Brahmajothi MV, Campbell DL. Heterogeneous basal expression of nitric oxide synthase and superoxide dismutase isoforms in mammalian heart. Circ Res 1999;85:575–587.

[42] Bloch W, Fleischmann BK, Lorke DE et al. Nitric oxide synthase expression and role during cardiomyogenesis. Cardiovasc Res 1999;43:675–684.

[43] Brookes PS, Zhang J, Dai L et al. Increased sensitivity of mitochondrial respiration to inhibition by nitric oxide in cardiac hypertrophy. J Mol Cell Cardiol 2001;33:69–82.

[44] Yang B, Larson DF, Shi J, Gorman M, Watson RR. Modulation of iNOS activity in senescent cardiac dysfunction (Abstract). Circulation 2001;104:II435.

[45] Montgomery H, Clarkson P, Dollery CM et al. Association of angiotensin-converting enzyme gene I/D polymorphism with change in left ventricular mass in response to physical training. Circulation 1997;96:741–747.

[46] Lewis NP, Tsao PS, Rickenbacher PR et al. Induction of nitric oxide synthase in the human allograft is associated with contractile dysfunction of the left ventricle. Circulation 1996;93:720–729.

[47] Wildhirt SM, Weis M, Schulze C et al. Expression of endomyocardial nitric oxide synthase and coronary endothelial function in human cardiac allografts. Circulation 2001;104(Suppl I):336–343.

[48] Mancini GBJ, Henry GC, Macaya C et al. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease. The TREND Study. Circulation 1996;94:258–265.

[49] Fukuchi M, Hussain SNA, Giaid A. Heterogeneous expression and activity of endothelial and inducible nitric oxide synthases in end-stage human heart failure. Their relation to lesion site and β-adrenergic receptor therapy. Circulation 1998;98:132–139.

Chapter 3.3

Myocardial Contractile Effects of Nitric Oxide

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Myocardial Contractile Effects of Nitric Oxide

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Abstract. **Recent experimental and clinical research solved some of the controversies surrounding the myocardial contractile effects of NO. These controversies were: (1) does NO exert a contractile effect at baseline? (2) is NO a positive or a negative inotrope? (3) Are the contractile effects of NO similar when NO is derived from NO-donors or from the different isoforms of NO synthases (NOS)? (4) Does NO exert the same effects in hypertrophied, failing or ischemic myocardium?**

Transgenic mice with cardioselective overexpression of NOS revealed NO to produce a small reduction in basal developed LV pressure and a LV relaxation-hastening effect mainly through myofilamentary desensitization. Similar findings had previously been reported during intracoronary infusions of NO-donors in isolated rodent hearts and in humans. The LV relaxation hastening effect was accompanied by increased diastolic LV distensibility, which augmented LV preload reserve especially in heart failure patients. This beneficial effect on diastolic LV function always overrode the small NO-induced attenuation in LV developed pressure in terms of overall LV performance. In most experimental and clinical conditions, contractile effects of NO were similar when NO was derived from NO-donors or produced by the different isoforms of NOS. Because expression of inducible NOS (NOS2) is frequently accompanied by elevated oxidative stress, NO produced by NOS2 can lead to peroxynitrite-induced contractile impairment as observed in ischemic or septic myocardium. Finally, shifts in isoforms or in concentrations of myofilaments can affect NO-mediated myofilamentary desensitization and alter the myocardial contractile effects of NO in hypertrophied or failing myocardium.

Key Words. **nitric oxide, myocardium, contractility, diastole, heart failure, nitric oxide synthase**

Introduction

In vascular tissue, the contractile effects of nitric oxide (NO) are easily appreciated consisting of relaxation of isolated vascular strips or vasodilation of perfused organs. In cardiac tissue, the contractile effects of NO are far less obvious and have been the subject of considerable confusion and debate. Some of the most important controversies surrounding the myocardial contractile effects of NO were: (1) Does NO exert a myocardial contractile effect under baseline conditions or only following adrenergic or cholinergic stimulation? (2) Can the contractile effects of NO be labelled as positively or negatively inotropic? (3) Are the contractile effects of NO similar for NO derived from NO-donor substances, for NO produced by NOS3 (eNOS, "endothelial-constitutive" NOS) or for NO produced by NOS2 (iNOS, "cytokine-inducible" NOS)? (4) Does hypertrophy, aging, transplantation, heart failure, ischemia or sepsis alter the observed contractile effects of NO?

Some of the controversies arose from comparison of data derived from different experimental set-ups ranging from isolated cardiomyocytes, papillary muscle strips, perfused rodent hearts, anaesthetized open-chest dogs to humans at the time of cardiac catheterization. In all these set-ups, myocardial contractility was assessed in an unequal and often incomplete way. Isolated cardiomyocytes are contracting at low load (i.e. friction on a glass surface). Extent of muscle shortening (Δl) or velocity of shortening ($\Delta l/dt$) recorded in isolated cardiomyocytes is therefore hard to compare with peak force (F) or speed of force development (dF/dt) in isometrically contracting papillary muscles. Effects on diastolic LV function can easily be appreciated in perfused hearts, open-chest dogs or humans but are difficult to detect in isolated cardiomyocytes or muscle strips because of the controlled preloading conditions. Interference by NO in parasympathetic or sympathetic outflow to the heart is absent in isolated preparations but unavoidable in humans.

The present review summarizes the current evidence for myocardial contractile effects of NO thereby highlighting the aforementioned controversies and shortcomings of the different experimental settings.

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Contractile Effects of NO in Normal Myocardium

Systolic Function

In isolated cardiomyocytes, administration of exogenous NO was first reported to be associated at baseline with a small negative inotropic effect, evident from a reduction in extent and velocity of shortening [1]. In the same preparation, inhibition of endogenous NOS3 had no baseline effect but reduced extent and velocity of shortening following adrenergic stimulation [2]. In isolated isometrically contracting cardiac muscls strips, NO produced by NOS3 of adjacent endocardial cells or derived from high, but not low, doses of NO-donors exerted a similar small negative inotropic effect evident from a reduction in peak isometric force development [3–5] without change in rate of rise of force development (dF/dt_{max}) (Fig. 1). This reduction in peak isometric force development resulted from an earlier onset of isometric relaxation, which abbreviated duration of contraction or time left for isometric tension build-up. This unique pattern of effect of NO on isometric force development revealed the underlying mechanism: a NO-induced reduction in myofilamentary calcium sensitivity because of phosphorylation of troponin I by cGMP dependent protein kinase.

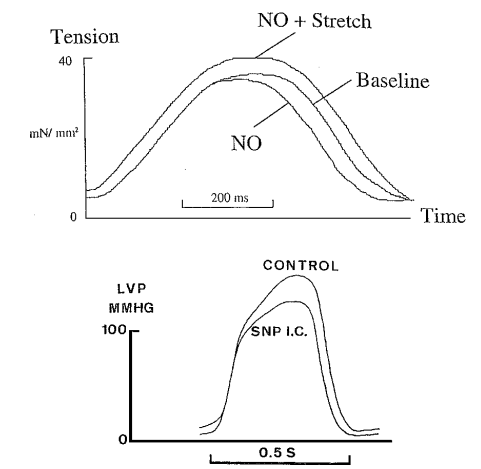

Fig. 1. *Top: Contractile effects of NO on isometric tension development in an isolated cat papillary muscle. NO slightly reduces peak tension because of earlier onset of isometric relaxation and fails to alter the rate of rise of tension. The contractile effects of NO are abolished by a simultaneous rise in muscle preload (NO + stretch). Bottom: The contractile effects of NO derived from an intracoronary NO-donor infusion (sodium nitroprusside; SNP-IC) in a normal human subject. In analogy to the contractile effects in a cat papillary muscle, there is a reduction in peak LV pressure (LVP), an earlier onset of isovolumic relaxation and no effect on the rate of rise of LV pressure. There is also a drop in diastolic LV pressures during SNP-IC.*

A similar relaxation hastening effect was also observed in experimental preparations using whole heart models. In isolated ejecting guinea-pig hearts perfused with buffer solution and contracting with constant preload, afterload and heart rate, both NO derived from an NO-donor [6] and NO released from the coronary endothelium following stimulation with bradykinin or substance P [7] induced an earlier onset and faster rate of LV relaxation and a small reduction in peak LV pressure without change in LV dP/dt_{max}. These changes could not be reproduced with the cGMP-independent vasodilator nicardipine and were therefore unrelated to coronary vasodilation or to coronary perfusion. These effects were also observed after addition to the perfusate of angiotensin converting enzyme inhibitors probably because of slower bradykinin breakdown and were abolished by addition of haemoglobin, which inactivates NO [8].

Experiments [9] in transgenic mice with cardioselective overexpression of NOS3 and a 60-fold increase in NOS activity (^{3}H-L-Citrulline production) confirmed NO to produce a 30% reduction in basal LV developed pressure mainly through myofilamentary desensitization and to a lesser extent through alterations in sarcolemmal calcium fluxes [10,11] or sarcoplasmic calcium handling [12–15]. A similar 10% reduction in basal LV developed pressure was also observed in transgenic mice with cardioselective overexpression of NOS2 and a 20-fold increase in ^{3}H-L-Citrulline production [16]. In these mice, addition of L-arginine to the perfusion corrected the substrate deficit for NOS2 and induced a further 10% drop in LV developed pressure. A similar drop in LV developed pressure during addition of L-arginine to an intracoronary substance P infusion had previously been reported in the human allograft [17] (Fig. 2).

A NO-induced and myofilamentary desensitization-mediated LV relaxation hastening effect was also observed in the human heart during intracoronary infusions of NO-donor substances or of substance P, which releases NO from the coronary endothelium [18–20]. In analogy to the experimental preparations, this relaxation-hastening effect abbreviated LV contraction, reduced the magnitude of the late-systolic reflected LV pressure wave and caused a ±10 mmHg drop in LV end-systolic pressure at unaltered LV end-systolic volume (Fig. 1). There were no changes in the rate of rise of LV pressure (LV dP/dt_{max}). The small drop in LV end-systolic pressure at unaltered LV end-systolic volume implied a rightward and downward shift

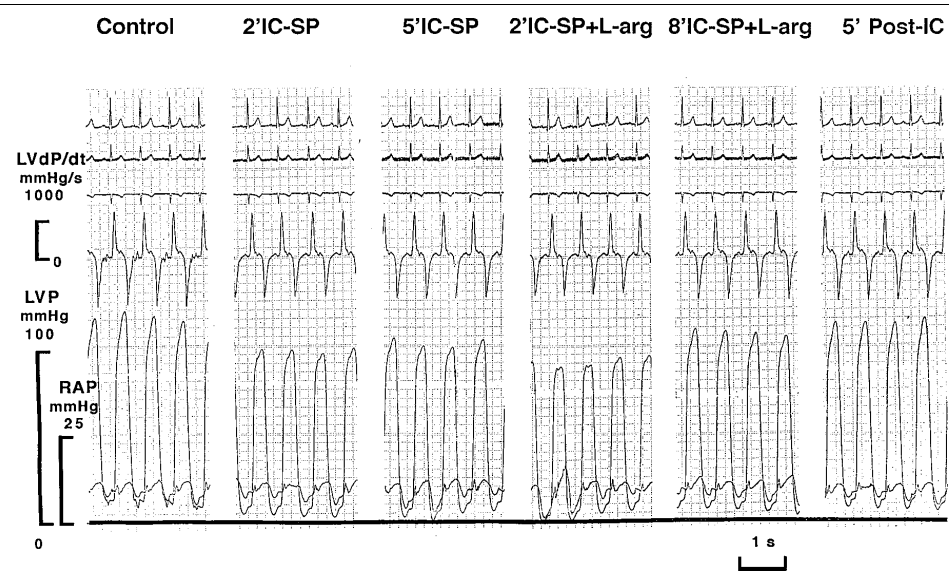

Fig. 2. *Three lead electrocardiogram, LV dP/dt, LV pressure (LVP) and right atrial pressure (RAP) recorded in a human allograft in control conditions, after 2 and 5 min of intracoronary (IC) substance P (SP) infusion, after 2 and 8 min of intracoronary (IC) substance P + L-arginine infusion (SP + L-arg) and 5 min after cessation of IC infusion. SP releases NO from the coronary endothelium, which reduces peak LV pressure without changing LV* dP/dt_{max} *or RAP. Addition of L-arg to the SP infusion potentiates these effects. Reproduced with permission from [17].*

of the LV end-systolic pressure-volume relation and was therefore theoretically consistent with a small negative inotropic effect. The unaltered LV $dP/dt_{\max}$ and the simultaneous fall in LV end-diastolic pressure however argue against significant hemodynamic deterioration as a result of these NO-induced negative inotropic effects on LV end-systolic performance. In fact, the NO-induced effects on LV end-systolic performance could well be beneficial for the ejecting left ventricle as they reduced mechanical work wasted in contracting against late-systolic reflected arterial pressure waves.

When isolated cardiomyocytes [21] or isometrically contracting papillary muscle strips [5] were exposed to low doses of NO-donors, a positive inotropic effect was observed probably resulting from cGMP-induced inhibition of phosphodiesterase 3 and a concomitant rise in cAMP or from direct nitrosylation of sarcolemmal or sarcoplasmic proteins. Similar positive inotropic effects of NO have been reported in anaesthetized dogs [22] and in the normal human heart, in which an intracoronary infusion of L-NMMA induced a modest (14%) drop in LV $dP/dt_{\max}$ [23]. The dose-dependent transition from a positive to a negative inotropic effect occurred at lower doses of the NO-donor in the presence of an intact endocardium and in the presence of cholinergic or ß-adrenergic stimulation [5] (Fig. 3). Larger negative inotropic effects in the presence of a functioning endocardium implies additive effects of NO derived from NO-donors and of NO produced by NOS3 of adjacent endothelial cells. Larger negative inotropic effects following cholinergic stimulation implies additive effects at the level of the second messenger cGMP, whose intracellular concentration rises both following NO-donor administration and cholinergic stimulation. The larger negative inotropic effect following ß-adrenergic stimulation suggests additive effects also at the level of the myofilaments because of simultaneous phosphorylation of troponin I by cGMP dependent protein kinase and by cAMP dependent protein kinase. The latter observation has far reaching consequences and explains why many experimental and clinical studies detected no or only small effects of NO under baseline conditions but reported large changes following ß-adrenergic stimulation. In the human heart, an intracoronary infusion of substance P following pretreatment with dobutamine resulted in a fall in LV end-systolic pressure, which was twice as large as the fall observed in control conditions and accompanied by

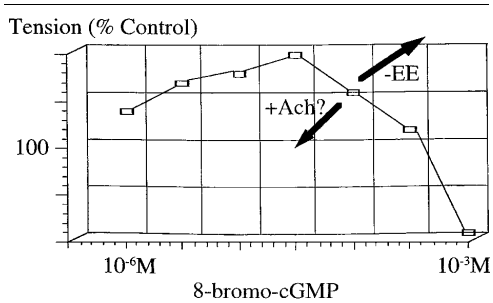

Fig. 3. *Dose response curve of peak isometric tension of an isolated cat papillary muscle versus concentration of an analogue of cGMP (8-bromo-cGMP), the second messsenger of NO. At low doses of cGMP or of NO there is a positive inotropic effect, at high doses of cGMP of of NO there is a negative inotropic effect. Removal of the endocardial endothelium (−EE) shifts the descending limb of the dose response curve to the right, addition of acetylcholine (+Ach) shifts it to the left. This implies additive effects of exogenously administered cGMP to the endogenously produced cGMP by the endothelium or following Ach administration.*

a 6% fall in LV dP/dt_{max} [24] (Fig. 4). A similar augmentation by dobutamine of NO-related myocardial contractile effects was also observed during intracoronary infusion of L-NMMA, which potentiated the dobutamine-induced rise in LV dP/dt_{max} by 51% [25] but failed to have any effect in the same patient population in control conditions [23]. Larger NO-related negative inotropic effects following ß-adrenergic stimulation also highlight the importance of the contraction mode of the experimental preparation. Differences in pre- or afterload can indeed affect calcium sensitivity of crossbridges and can counteract both cGMP- or cAMP-induced myofilamentary desensitization and concomitant changes in timing of myocardial contraction-relaxation sequence [26,27] (Fig. 1).

Diastolic Function

NO related phosphorylation of troponin I also increases myocardial diastolic distensibility through prevention of calcium-independent diastolic crossbridge cycling. Such an increase in diastolic distensibility, evident from a longer diastolic cell length at constant preload, has been observed in isolated cardiomyocytes following exposure to cGMP, the second messenger of NO or following exposure to sodium nitroprusside [28,29]. The increase in diastolic cell length and the concomitant reduction in cell shortening following exposure to sodium nitroprusside have been attributed not only to cGMP-mediated myofilamentary desensitization but also to myofilamentary desensitization related to a fall in intracellular pH. This fall in intracellular pH suggested NO, via cGMP, to disable forward sodium-proton exchange [29]. The reverse mechanism, intracellular alkalization, has been observed following administration of angiotensin II or endothelin [30] and following cardiac muscle stretch, which leads to autocrine production of angiotensin II and endothelin [31]. As discussed earlier, cardiac muscle stretch can indeed effectively counteract the NO-induced effects on timing of myocardial contraction-relaxation sequence [27] (Fig. 1). Moreover, in a beating rabbit heart, a sudden increase in LV diastolic stretch because of release of a caval occluder, leads to an instantaneous increase in diastolic intramyocardial NO concentrations measured by a porphyrinic sensor inserted in the LV wall of the beating rabbit heart [32]. Hence, a rise in LV preload triggers not only myocardial autocrine production of angiotensin II but also of NO, which can reverse the angiotensin II-induced changes in intracellular pH and in myofilamentary calcium sensitivity.

In isolated ejecting guinea-pig hearts, a perfusate containing the specific NOS inhibitor N^G-monomethyl-L-arginine, raised LV end-diastolic pressure and reduced preload recruitable LV stroke volume probably because of an acute left and upward shift of the diastolic LV pressure-volume relation [33]. In the same preparation, an increase in LV preload, caused a rise in the NO concentration of the coronary effluent [34] confirming the preload triggered enhancement of myocardial NO production recorded from porphyrinic sensors inserted in the wall of the beating rabbit heart [32]. NO-related changes in diastolic LV distensibility have been observed not only following acute administration of a NOS inhibitor but also following chronic NOS blockade. In rats receiving eight weeks of treatment with the NOS inhibitor L-NAME, the diastolic LV pressure-volume relation shifted upward with a significant reduction in LV unstressed volume and no increase in LV mass despite the elevated blood pressure [35].

In the human heart, the relaxation-hastening effect of NO was also accompanied by an increase in LV diastolic distensibility. During intracoronary infusions of NO-donors or of substance P, the LV diastolic pressure-volume relation shifted right- and downward consistent with increased LV diastolic distensibility [18–20]. Similar downward shifts of the diastolic LV pressure-volume relation had previously been observed during intravenous administration of NO-donors and had always been attributed to a sequence of events consisting of venodilation, right ventricular unloading and biventricular interaction [36,37]. The downward shifts of the diastolic LV

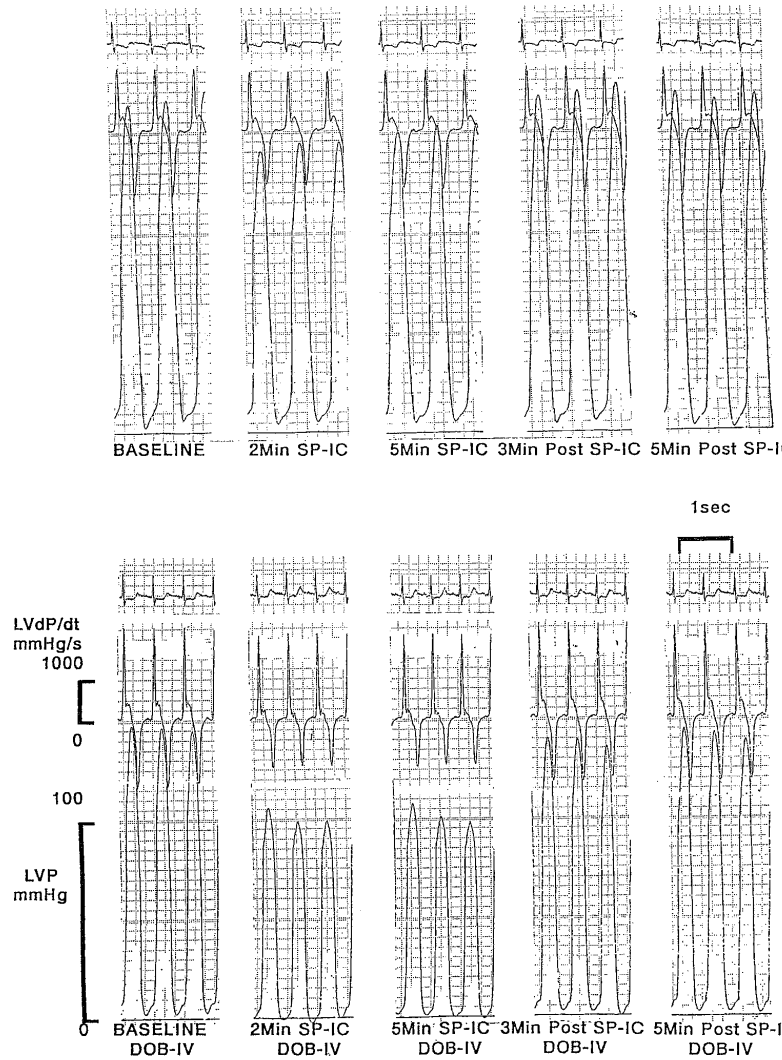

Fig. 4. *Single lead electrocardiogram, LV dP/dt and LV pressure (LVP) recorded in a human allograft at baseline, after 2 and 5 minutes of intracoronary (IC) substance P (SP) infusion and 3 and 5 minutes following infusion in the absence (top panel) and in the presence of an intravenous infusion of dobutamine (DOB-IV) (bottom panel). During intravenous infusion of dobutamine, an IC-SP infusion results in a larger drop in LV pressure than before (50 mmHg vs. 15 mmHg). Reproduced with permission from [98].*

pressure-volume relation observed during intracoronary infusion of NO-donors did however not result from such a sequence of events but from a direct myocardial effect of NO (probably phosphorylation of troponin I and reduced calcium-independent diastolic crossbridge cycling) for the following reasons: (1) the doses of NO-donors infused intracoronarily were too low to elicit significant systemic vasodilator effects; (2) a right atrial infusion of the same dose of NO-donor failed to reproduce the observed shift in the diastolic LV pressure-volume relation; (3) the shift in the

diastolic LV pressure-volume relation persisted even after transforming the measured LV diastolic pressure to transmural LV diastolic pressure and (4) the fall in LV end-diastolic pressure was accompanied by an increase in LV end-diastolic volume and a fall in heart rate, both of which were incompatible with peripheral vasodilator effects.

Contractile Effects of NO in Hypertrophied and Senescent Myocardium

In hypertrophied cardiomyocytes isolated from aortic-banded rats, exposure to the NO-donor sodium nitroprusside failed to alter diastolic cell length, to reduce the amplitude of cell shortening or to lower intracellular pH, all of which were observed in cardiomyocytes from normal rats following exposure to sodium nitroprusside [29]. In a guinea-pig model of compensated LV hypertrophy induced by aortic banding, the LV relaxant effects of endothelially released NO were significantly blunted despite a preserved response to exogenous NO [38]. This defective contractile response was unrelated to alterations in coronary flow but reflected the impact on LV performance of an hypertrophy-induced endothelial dysfunction and concomitant reduction of NO bioactivity. Reduced NO bioactivity in the hypertrophied heart could have resulted from a decrease of NOS3 expression or from inactivation of NO by reactive oxygen species (ROS) such as superoxide anion. In the guinea-pig model of aortic banding [38] and in the spontaneously hypertensive rat [39], NOS3 expression was unaltered or even upregulated. This made increased ROS production the most likely cause for the reduction of NO bioactivity and for the defective LV relaxant response to coronary endothelial stimulation. Increased ROS production could have resulted from dysfunctional NOS because of deficiencies in cofactor or substrate (i.e. tetrahydrobiopterin or L-arginine) or from upregulation of other enzymes such as NADH/NADPH oxidases [40]. In the same guinea pig model of compensated LV hypertrophy produced by aortic banding, neither tetrahydrobiopterin nor L-arginine corrected the defective LV relaxant response to coronary endothelial stimulation. The latter was however restored by vitamin C, deferroxamine or superoxide dismutase and in line with these observations, protein expression of the NADPH oxidase subunits (gp91-phox and p67-phox) and myocardial NADPH oxidase activity were shown to be significantly increased in the banded animals [41].

In patients with LV hypertrophy of aortic stenosis, intracoronary administration of a NO-donor caused a marked fall in LV end-diastolic pressure and in LV end-diastolic chamber stiffness [19]. The fall in LV end-diastolic pressure observed in the aortic stenosis patients was larger than the fall observed in normal controls (−39 vs. −21%) but the fall in peak and end-systolic LV pressures was smaller than in control subjects. The larger fall in LV end-diastolic pressure observed in the aortic stenosis patients could have resulted from their higher baseline LV end-diastolic pressure and not from a higher myocardial sensitivity to the distensibility-increasing effect of NO. When the left ventricle is operating on the steeper portion of its diastolic pressure-volume relation, a NO-induced rightward shift of the diastolic pressure-volume relation will result in a larger reduction in LV end-diastolic pressure than when the left ventricle is operating on the flat portion of its diastolic pressure-volume relation. The smaller fall in peak and end-systolic LV pressures was consistent with a lower sensitivity of hypertrophied human myocardium to the relaxation-hastening effect of NO and confirmed the aforementioned reduction in contractile response to NO observed in cardiomyocytes of aortic banded rats [29]. Lower sensitivity of hypertrophied myocardium to the relaxation-hastening effect of NO could possibly be explained by lower concentrations in hypertrophied myocardium of troponin I, whose breakdown is accelerated whenever the left ventricle is chronically subjected to elevated LV filling pressures [42]. Other mechanisms for a lower sensitivity of hypertrophied myocardium to the contractile effects of NO include a lower baseline cGMP concentration in hypertrophied myocardium, which would make it operate on the flat portion of its biphasic dose-reponse to NO [5,43] (Fig. 3), or blunted downstream signaling in hypertrophied myocardium from cGMP to sodium-proton exchange [29]. Both the lower myocardial sensitivity to NO and the lower myocardial bioactivity of NO could be important mechanisms for the diastolic dysfunction of the hypertrophied LV.

The effects of aging on myocardial NOS activity are controversial. In one study, myocardial NOS3 activity was increased in the aging rat heart [44]. Despite increased myocardial NOS3 activity, left ventricular diastolic distensibility was reduced as evident from an elevation of left ventricular filling pressures. Similar to the human allograft [17], the reduction in diastolic left ventricular distensibility of the senescent rat heart reacted favourably to administration of L-arginine. Both observations warrant further investigations on the clinical use of L-arginine for the treatment of aging-induced diastolic left ventricular dysfunction. Other studies documented an aging-induced reduction of NOS3 activity [45] and upregulation of NOS2 activity [46].

Contractile Effects of NO in Transplanted Myocardium

In transplanted myocardium, myocardial contractile effects of NO were the subject of intense research because of the early demonstration of NOS2 gene expression in transplanted myocardium [47] and because of the numerous experimental studies, which linked NOS2 gene expression to contractile dysfunction in experimental preparations [48–52]. In transplant recipients free of rejection or graft vasculopathy, a Doppler echocardiographic index of LV performance revealed a significant association between systolic, diastolic or combined LV dysfunction and intensity of NOS2 gene expression in simultaneously procured LV biopsies [47]. An invasive study in transplant recipients obtained microtip LV pressure recordings and simultaneous LV angiograms at the time of annual coronary angiography but found similar indices of baseline LV function in transplant recipients with low or high myocardial NOS2 mRNA [53]. This study however observed a larger relaxation-hastening effect and a larger reduction in peak and end-systolic LV pressure in transplant recipients with high myocardial NOS2 mRNA following intravenous infusion of the ß-agonist dobutamine. These findings resembled the contractile effects of NOS3-derived NO. The latter were also studied in transplant recipients using intracoronary infusions of substance P, which releases NO from the coronary endothelium. The relaxation hastening effect and the concomitant fall in peak and end-systolic LV pressure of NOS3-derived NO were small under baseline conditions but rose drastically following pretreatment with dobutamine (Fig. 4) [24]. The similar contractile effects during dobutamine infusion of NOS2- or NOS3-derived NO also supports substantial bioavailability of NOS2-derived NO. Bioavailability of NOS2-derived NO has been questioned because of the simultaneous presence in transplanted myocardium of rejection-related oxidants, which could combine with NO to generate peroxynitrite [54] and which could thereby prevent NO from exerting its direct effects on the cardiomyocytes. The myocardial bioavailability of NOS2-derived NO was also indirectly demonstrated in transplant recipients by higher myocardial cGMP concentrations [47] and more recently by higher transcardiac nitrite/nitrate production [55] in the presence of upregulated NOS2 gene expression.

Rejection related oxidants or lower baseline myocardial concentrations of cGMP or of cAMP could explain the smaller LV relaxation-hastening effect of intracoronary NO-donor infusions in transplant recipients compared to a normal control population subjected to a similar infusion protocol [56]. In the cardiac allograft, baseline diastolic LV dysfunction predicted a poor NO-induced LV relaxation-hastening effect. The inverse correlation between the NO-induced LV relaxation-hastening effect and baseline diastolic LV dysfunction could be related to varying degrees of oxidative stress, which could induce diastolic LV dysfunction because of sarcoplasmic membrane damage and a reduction of the LV-relaxation hastening effect because of lower NO bioavailability. Low baseline cGMP concentration could result from reduced coronary NOS3 release because of graft vascular disease and could explain the characteristically shrunken appearance of the transplanted LV [57] in analogy to the small LV cavity size of rat hearts following chronic treatment with NOS inhibitors [35]. Low baseline cAMP concentration in the cardiac allograft could result from cardiac denervation and could act in concert with the presence of oxidants and low baseline cGMP concentration to blunt the NO-donor induced LV contractile effects in the cardiac allograft.

Contractile Effects of NO in Failing Myocardium

In analogy to transplanted myocardium, the early reports of increased myocardial NOS2 [58–64] and NOS3 [65] expression in failing human hearts stimulated research on the myocardial contractile effects of NO in failing myocardium because of the widely proposed but unfortunately unproven paradigm of excess NO production contributing to contractile depression.

In dogs with pacing-induced heart failure, myocardial NOS activity was significantly increased compared to control dogs. Administration of a NOS inhibitor to cardiomyocytes isolated from these hearts had no effect on baseline myocardial performance but augmented the inotropic response to ß-adrenergic stimulation [66]. Similar results were obtained in muscle strips of explanted human cardiomyopathic hearts: L-NMMA administration had no effect on baseline isometric force development but increased the dobutamine-induced rise in contractile performance [62]. Other investigators however failed to confirm these results and observed no changes in the contractile response to ß-adrenoreceptor stimulation following administration of a NOS inhibitor to cardiomyocytes isolated from cardiomyopathic human hearts [67]. Moreover, in the pacing-induced heart failure dog model, the fall in LV stroke work and the rise in LV end-diastolic pressure observed after 4 weeks of pacing was accompanied by a fall in cardiac NO production as evident from a smaller transcardiac nitrite/nitrate gradient [68]. In this same

model, dampening of the myocardial ß-adrenergic response has recently been attributed to increased cGMP levels resulting not from excess NO-induced guanylyl cyclase activity but from reduced phosphodiesterase 5-mediated cGMP breakdown [69].

In patients with dilated cardiomyopathy and biopsy proven endomyocardial NOS2 gene expression, intracoronary infusion of L-NMMA had no effect on LV contractile performance [23]. In the same patient population, an intracoronary infusion of L-NMMA had previously been shown to potentiate the dobutamine-induced rise in LV dP/dt_{max} [25]. In the pacing-induced heart failure dog model, the latter finding was confirmed and related to abundance of caveolin protein in failing myocardium [70]. Caveolin protein abundance in failing myocardium reduces NOS3 activity and interferes with translocation of receptor-agonist complexes from the sarcolemma. The beneficial outcome of statins in heart failure could, apart from their effects on rho proteins, also relate to reduction of caveolin and concomitant enhancement of NOS3 activity [71]. In dilated cardiomyopathy patients with elevated LV filling pressures, intracoronary infusion of substance P enhanced myocardial NOS2 activity, slightly reduced LV end-systolic pressure but improved overall hemodynamic status as evident from higher LV stroke volume and LV stroke work [64]. These beneficial NO-related contractile effects resulted from a simultaneous NO-induced increase in diastolic LV distensibility. Low intensity of LV endomyocardial NOS2 and NOS3 gene expression was recently demonstrated to coincide with a hemodynamic phenotype of dilated cardiomyopathy patients characterized by elevated LV diastolic stiffness and reduced LV stroke work [72]. In contrast to this hemodynamic phenotype with poor prognosis, dilated cardiomyopathy patients with low LV diastolic stiffness and preserved LV stroke work had higher intensity of NOS2 and NOS3 gene expression, which attained the level observed in athlete's heart [72] (Fig. 5). Low LV diastolic stiffness, reversible right- and downward displacement of the diastolic LV pressure-volume relation and high LV preload reserve are typical features of athlete's heart and could be NO-mediated because of the documented upregulation of NOS activity and expression following regular physical exercise [73,74]. Simultaneous improvement in diastolic LV distensibility also overrode the NO-induced attenuation of the LV contractile response to dobutamine in terms of overall hemodynamic status of dilated cardiomyopathy patients. In two studies [24,75], agonist-induced coronary endothelial release of NO during intravenous administration of dobutamine resulted in a small reduction of LV contractility indices such as LV dP/dt_{max} and LV elastance but without

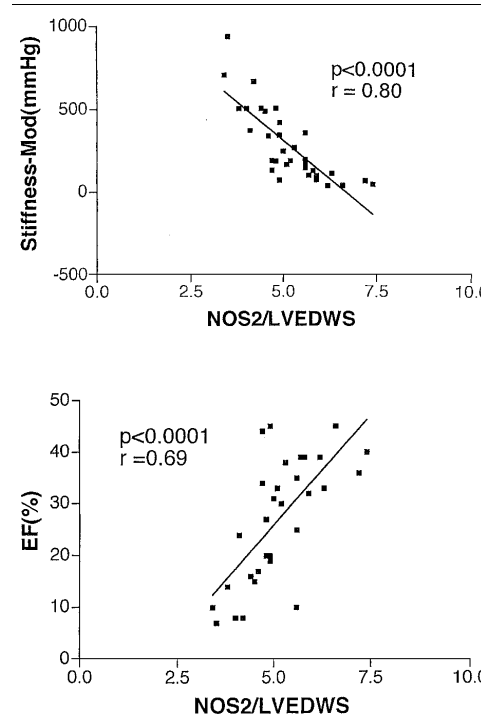

Fig. 5. *In patients with non-ischaemic dilated cardiomyopathy, an inverse relation was observed between LV diastolic stiffness (stiffness-Mod) and intensity of endomyocardial NOS2 gene expression normalised for LV end-diastolic wall stress (LVEDWS) (Top). A similar relation was also observed for LV ejection fraction (EF) [72]. Both relations imply better hemodynamic status in patients with dilated cardiomyopathy when myocardial NOS2 gene expression is elevated.*

hemodynamic deterioration as evident from unaltered LV stroke volume and reduced LV end-diastolic pressure. The fall in LV end-diastolic pressure was accompanied by an unchanged LV end-diastolic volume and was therefore consistent with an improvement in LV diastolic distensibility. NO-related modulation of diastolic LV distensibility was also observed in the pacing-induced heart failure dog model [68]. In this model, a fall in myocardial NO production occurred after 4 weeks of pacing. This fall was accompanied by a steep rise in LV end-diastolic presssure because of reduced diastolic LV distensibility and a drop in LV stroke volume. From these observations in patients with dilated cardiomyopathy and in dogs with pacing-induced heart failure, it seems justified to conclude that there is no objective

evidence for NO, whether derived from NOS2 or NOS3, to account for hemodynamic deterioration of failing myocardium, either at baseline or during ß-adrenoreceptor stimulation [43,76].

Contractile Effects of NO in Ischemic and Septic Myocardium

NO exerts marked protective effects on isolated reoxygenated cardiomyocytes mostly through prevention of reoxygenation-induced hypercontracture [77]. These effects are cGMP dependent because addition of methylene blue, an inhibitor of soluble guanylyl cyclase, reduces NO-induced protection. Although a specific negative contractile effect of NO can not be excluded, cGMP-induced phosphorylation of troponin I could certainly contribute through prevention of calcium-independent diastolic crossbridge cycling [78]. Other mechanisms include reduction of osmotic fragility of the sarcolemma damaged by increased oxidative stress because of radical scavenging properties of NO [79].

In opposition to the uniform findings in isolated cardiomyocytes, reports on the outcome of inhibition of NO synthase in ischaemic-reperfused hearts are controversial. Especially under acidotic conditions, as present during low-flow ischemia, peroxynitrite anions, which result from reaction of NO with superoxide anion, get protonated and subsequently decay rapidly to generate hydoxyl radicals [80], which are highly toxic for sarcolemmal or sarcoplasmic membranes. Consistent with this sequence of events, inhibition of NO synthesis has been reported to reduce reoxygenation injury in anaesthetized piglets [81] and to reduce infarct size in rabbits [82]. In contrast to these initial results, subsequent studies reported endogenous release of NO to protect against ischemia-reperfusion. Both in the ischemic-reperfused rat [83] and rabbit [84] heart, inhibition of endogenous NO synthesis by L-nitro arginine increased the ischemia related contractile deficit possibly because of a NO-mediated scavanging effect of free radicals with a concomitant reduction in ischemic injury.

In the search for the contractile effects of endogenous NO during ischemia-reperfusion, pharmacological inhibition of NO was replaced by the use of transgenic NOS2 or NOS3 knock-out mice. Here again, the results were nonuniform: some investigators found exacerbated ischemia-reperfusion injury in NOS knock-out animals while other investigators observed a protective effect of endogenous NO production [85,86]. Using an isolated mouse heart preparation, an interesting crosstalk between NOS2 and NOS3 during ischemia-reperfusion was recently reported [87]: in NOS3 knock-out mice subjected to 30 minutes of global ischemia followed by reperfusion, a paradoxical increase of NO production was observed because of superinduction of NOS2. This increased NO production, blunted the hyperdynamic response during early reperfusion and protected the heart against myocardial injury as evident from a reduction in infarct size. Similar findings were observed in wild-type mice treated with the NO-donor S-nitroso-N-acetylpenicillamine (SNAP). In these experiments, NO-induced myofilamentary desensitization because of cGMP-induced phosphorylation of troponin I served as a brake for the myocardial hypercontractile response to early reperfusion thereby reducing energy demand and ATP deficit.

Apart from affecting the initial contractile response to ischemia-reperfusion injury, NO could also influence long-term left ventricular remodeling after myocardial infarction [88]. In the early-postinfarction period, left ventricular contractile performance in wild-type and NOS2 knock-out mice was similar, but 4 months after infarction, NOS2 knock-out mice had superior hemodynamics and better survival because of reduced apoptotic cell death and myocyte loss in the noninfarcted zone [89]. Similar findings were reported by other investigators, who noticed an earlier increase in left ventricular end-diastolic diameter and an earlier decrease in fractional shortening at 1 month post-infarction in wild type mice compared to NOS2 knock-outs [90]. In these end-stage mice-infarct models, circulating levels of free radicals become very elevated [91] and the observed late deterioration of left ventricular performance could therefore result not from NO itself but from elevated oxidative stress, which triggers peroxynitrite production and hydroxyl-related membrane damage in the presence of NO [92,93]. The superior long-term outcome following myocardial infarction in wild-type mice compared to NOS3 knock-out mice also argues against direct NO-related deleterious effects on long-term post-infarct LV remodeling [94]. Moreover, in endothelial cells, NO confers protection against apoptotic cell death via s-nitrosylation of caspases. In these cells, aging-induced loss of NOS3 activity increases sensitivity to oxidized LDL or tumor necrosis factor α induced apoptosis [95].

A detrimental role of peroxynitrite rather than NO was also demonstrated in septic or endotoxemic hearts. Once established, the depressed cardiac function after endotoxin administration was not reversed by NOS inhibition [96] and myocardial function of the endotoxemic heart remained depressed even after return of NOS2 protein levels to control value [97]. The large quantities of NO produced by NOS2 during the initial episode of endotoxemia could however have

promoted the formation of peroxynitrite, which could have irreversibly impaired contractile performance through long-lasting modifications of proteins such as actin, sarcoplasmic Ca-ATPase or sarcoplasmic Ca-release channel (ryanodine receptor).

Conclusions

Recent experimental and clinical research clarified some of the controversies surrounding the myocardial contractile effects of NO. In transgenic mice with cardioselective overexpression of NOS3 [9] or of NOS2 [16] and with a 60- to 20-fold increase in myocardial NOS activity, NO produced a small reduction in basal LV developed pressure and a LV relaxation-hastening effect mainly through myofilamentary desensitization and to a lesser extent through alterations in calcium handling. Similar findings had previously been reported during intracoronary infusions of NO-donors and of substance P, which releases NO from the coronary endothelium, in isolated rodent hearts [6,7], in normal control subjects [18,20] and in patients with aortic stenosis [19]. The LV relaxation hastening effect was always accompanied by increased diastolic LV distensibility, which augmented LV preload reserve especially in dilated cardiomyopathy patients [64]. In this patient population, low endomyocardial NOS expression was accompanied by an unfavourable hemodynamic phenotype characterized by elevated LV diastolic stiffness and reduced LV stroke work [72]. The beneficial effect of NO on diastolic LV function always overrode the small NO-induced attenuation in LV developed pressure in terms of overall LV performance [43]. In most experimental and clinical conditions, contractile effects of NO were similar when NO was derived from NO-donors or produced by the different isoforms of NOS [24,53]. Because expression of inducible NOS (NOS2) is frequently accompanied by elevated oxidative stress, NO produced by NOS2 can lead to peroxynitrite formation and irreversible contractile impairment as observed in post-infarct LV remodeling [89,90] or sepsis [97]. Finally, presence of ROS and shifts of myofilaments to isoforms with reduced susceptibility to cGMP-mediated desensitization can alter the myocardial contractile effects of NO in hypertrophied or failing myocardium [29,38].

References

1. Brady AJ, Warren JB, Poole-Wilson PA, Williams TJ, Harding SE. Nitric oxide attenuates cardiac myocyte contraction. *Am J Physiol* 1993;265:H176–H182.
2. Balligand JL, Kelly RA, Marsden PA, Smith TW, Michel T. Control of cardiac muscle cell function by an endogenous nitric oxide signalling system. *Proc Natl Acad Sci USA* 1993;90:347–351.
3. Brutsaert DL, Meulemans AL, Sipido KR, Sys SU. Effects of damaging endocardial surface on the mechanical performance of isolated cardiac muscle. *Circ Res* 1988;62:358–366.
4. Smith JA, Shah AM, Lewis MJ. Factors released from endothelium of the ferret and pig modulate myocardial contraction. *J Physiol* 1991;439:1–14.
5. Mohan P, Brutsaert DL, Paulus WJ, Sys SU. Myocardial contractile response to nitric oxide and cGMP. *Circulation* 1996;93:1223–1229.
6. Grocott-Mason R, Fort S, Lewis MJ, Shah AM. Myocardial relaxant effect of exogenous nitric oxide in the isolated ejecting heart. *Am J Physiol* 1994;266:H1699–H1705.
7. Grocott-Mason R, Anning P, Evans H, Lewis MJ, Shah AM. Modulation of left ventricular relaxation in the isolated ejecting heart by endogenous nitric oxide. *Am J Physiol* 1994;267:H1804–H1813.
8. Anning PB, Grocott-Mason R, Lewis MJ, Shah AM. Enhancement of left ventricular relaxation in the isolated rat heart by an angiotensin-converting enzyme inhibitor. *Circulation* 1995;92:2660–2665.
9. Brunner F, Andrew P, Wölkart G, Zechner R, Mayer B. Myocardial contractile function and heart rate in mice with myocyte-specific overexpression of endothelial nitric oxide synthase. *Circulation* 2001;104:3097–3102.
10. Mery PF, Pavoine C, Belhassen L, Pecker F, Fischmeister R. Nitric oxide regulates cardiac Ca^{++} current. Involvment of cGMP-inhibited and cGMP-stimulated phosphodiesterases through guanylyl cyclase activation. *J Biol Chem* 1993;268:26286–26295.
11. Campbell DL, Stamler JS, Strauss HC. Redox modulation of L-type calcium channels in ferret ventricular myocytes. Dual mechanism regulation by nitric oxide and S-nitrosothiols. *J Gen Physiol* 1996;108:277–293.
12. Finkel MS, Oddis CV, Mayer OH, Hattler BG, Simmons RL. Nitric oxide synthase inhibitor alters papillary muscle force-frequency relationship. *J Pharmacol Exp Ther* 1995;272:945–952.
13. Kaye DM, Wiviott SD, Balligand JL, Simmons WW, Smith TW, Kelly RA. Frequency-dependent activation of a constitutive nitric oxide synthase and regulation of contractile function in adult rat ventricular myocytes. *Circ Res* 1996;78:217–224.
14. Stoyanovsky D, Murphy T, Anno PR, Kim Y-M, Salama G. Nitric oxide activates skeletal and cardiac ryanodine receptors. *Cell Calcium* 1997;21:19–29.
15. Ziolo MT, Katoh H, Bers DM. Expression of inducible nitric oxide synthase depresses ß-adrenergic-stimulated calcium release from the sarcoplasmic reticulum in intact ventricular myocytes. *Circulation* 2001;104:2961–2966.
16. Heger J, Gödecke A, Flögel U, Merx MW, Molojavyi A, Kühn-Velten WN, Schrader J. Cardiac-specific overexpression of inducible nitric oxide synthase does not result in severe cardiac dysfunction. *Circ Res* 2002;90:93–99.
17. Paulus WJ, Kästner S, Vanderheyden M, Shah AM, Drexler H. Myocardial contractile effects of L-arginine in the human allograft. *J Am Coll Card* 1997;29:1332–1338.
18. Paulus WJ, Vantrimpont PJ, Shah AM. Acute effects of nitric oxide on left ventricular relaxation and diastolic distensibility in humans. *Circulation* 1994;89:2070–2078.

19. Matter CM, Mandinov L, Kaufmann PA, Vassalli G, Jiang Z, Hess OM. Effects of NO-donors on LV diastolic function in patients with severe pressure-overload hypertrophy. *Circulation* 1999;99:2396–2401.
20. Paulus WJ, Vantrimpont PJ, Shah AM. Paracrine coronary endothelial control of left ventricular function in humans. *Circulation* 1995;92:2119–2126.
21. Kojda G, Kottenberg K, Nix P, Schulter KD, Piper HM, Noack E. Low increase in cGMP induced by organic nitrates and nitrovasodilators improves contractile response of rat ventricular myocytes. *Circ Res* 1996;78:91–101.
22. Preckel B, Kojda G, Schlack W, Ebel D, Kottenberg K, Noack E, Thämer V. Inotropic effects of glyceryl trinitrate and spontaneous NO donors in the dog heart. *Circulation* 1997;96:2675–2682.
23. Cotton JM, Kearney MT, Mac Carthy PA, Grocott-Mason RM, McClean DR, Heymes C, Richardson PJ, Shah AM. Effects of nitric oxide synthase inhibition on basal function and the force-frequency relationship in the normal and failing human heart *in vivo*. *Circulation* 2001;104:2318–2323.
24. Bartunek J, Shah AM, Vanderheyden M, Paulus WJ. Dobutamine enhances cardiodepressant effects of receptor-mediated coronary endothelial stimulation. *Circulation* 1997;95:90–96.
25. Hare JM, Givertz MM, Creager MA, Colucci WS. Increased sensitivity to nitric oxide synthase inhibition in patients with heart failure: Potentiation of ß-adrenergic inotropic responsiveness. *Circulation* 1998;97:161–166.
26. Coudray N, Beregi JP, Lecarpentier Y, Chemla D. Effects of isoproterenol on myocardial relaxation rate: Influence of the level of load. *Am J Physiol* 1993;265:H1645–H1653.
27. Mohan P, Paulus WJ, S.U.Sys. Cardiac muscle length increase abolishes early relaxation induced by cardiac agents. *Eur Heart J* 1994;15:145 (Abstr Suppl).
28. Shah AM, Spurgeon HA, Sollott SJ, Talo A, Lakatta EG. 8-Bromo-cGMP reduces the myofilament response to Ca^{2+} in intact cardiac myocytes. *Circ Res* 1994;74:970–978.
29. Ito N, Bartunek J, Spitzer KW, Lorell BH. Effects of the nitric oxide donor sodium nitroprusside on intercellular pH and contraction in hypertrophied myocytes. *Circulation* 1997;95:2303–2311.
30. Ito N, Kagaya Y, Weinberg EO, Barry WH, Lorell BH. Differing effects of endothelin on contraction, intracellular pH and intracellular Ca^{2+} in hypertrophied and normal rat myocytes. *J Clin Invest* 1997;99:125–135.
31. Cingolani HE, Alvarez BV, Ennis IL, Camilion de Hurtado MC. Stretch-induced alkalinization of feline papillary muscle: An autocrine-paracrine system. *Circ Res* 1998;33:775–780.
32. Pinsky DJ, Patton S, Mesaros S, Brovkovych V, Kubaszewski E, Grunfeld S, Malinski T. Mechanical transduction of nitric oxide synthesis in the beating heart. *Circ Res* 1997;81:372–379.
33. Prendergast BD, Sagach VF, Shah AM. Basal release of nitric oxide augments the Frank-Starling response in the isolated heart. *Circulation* 1997;96:1320–1329.
34. Sagach VF, Shimanskaya TV, Sagach VV, Bogomolets AA. Coronary endothelium dysfunction and heart failure. *J Heart Fail* 1998;5:79 (Abstr.).
35. Matsubara BB, Matsubara LS, Zornoff AM, Franco M, Janicki JS. Left ventricular adaptation to chronic pressure overload induced by inhibition of nitric oxide synthase in rats. *Basic Res Cardiol* 1998;93:173–181.
36. Carroll JD, Lang RM, Neumann AL, Borow KM, Rajfer SI. The differential effects of positive inotropic and vasodilator therapy on diastolic properties in patients with congestive cardiomyopathy. *Cicrulation* 1986;74:815–825.
37. Kingma L, Smiseth OA, Belenkie I, Knudtson ML, Mc Donald RPR, Tyberg JV. A mechanism for the nitroglycerin-induced downward shift of the left ventricular diastolic pressure-diameter relationship of patients. *Am J Cardiol* 1986;57:673–677.
38. MacCarthy PA, Shah AM. Impaired endothelium-dependent regulation of ventricular relaxation in pressure-overload cardiac hypertrophy. *Circulation* 2000;101:1854–1860.
39. Nava E, Noll G, Lüscher TF. Increased activity of constitutive nitric oxide synthase in cardiac endothelium in spontaneous hypertension. *Circulation* 1995;91:2310–2313.
40. Lang D, Mosfer SI, Shakesby A, Donaldson F, Lewis MJ. Coronary microvascular endothelial cell redox state in left ventricular hypertrophy. The role of angiotensin II. *Circ Res* 2000;86:463–469.
41. McCarthy PA, Grieve DJ, Li JM, Dunster C, Kelly FJ, Shah AM. Impaired endothelial regulation of ventricular relaxation in cardiac hypertrophy. Role of reactive oxygen species and NADPH oxidase. *Circulation* 2001;104:2967–2972.
42. Feng J, Schaus BJ, Fallavollita JA, Lee TC, Canty JM, Jr. Preload induces troponin I degradation independently of myocardial ischemia. *Circulation* 2001;103:2035–2037.
43. Paulus WJ, Frantz S, Kelly RA. Nitric oxide and cardiac contractility in human heart failure: Time for reappraisal. *Circulation* 2001;104:2260–2262.
44. Zieman SJ, Gerstenblith G, Lakatta EG, Rosas GO, Vandegaer K, Ricker KM, Hare JM. Upregulation of nitric oxide-cGMP pathway in aged myocardium: Physiological response to l-arginine. *Circ Res* 2001;88:97–102.
45. Gao F, Christopher TA, Lopez BL, Friedman E, Cai G, Ma XL. Mechanism of decreased adenosine protection in reperfusion injury of aging rats. *Am J Physiol* 2000;279:H329–H338.
46. Yang B, Larson DF, Shi J, Gorman M, Watson RR. Modulation of iNOS activity in senescent cardiac dysfunction. *Circulation* 2001;104(II):435 (Abstract).
47. Lewis NP, Tsao PS, Rickenbacher PR, Xue C, Johns RA, Haywood GA, von der Leyen H, Trindade PT, Cooke JP, Hunt SA, Billingham ME, Valantine HA, Fowler MB. Induction of nitric oxide synthase in the human allograft is associated with contractile dysfunction of the left ventricle. *Circulation* 1996;93:720–729.
48. Finkel MS, Odis CV, Jacob TD, Watkins SC, Hattler BG, Simmons RL. Negative inotropic effects of cytokines on the heart mediated by nitric oxide. *Science* 1992;257:387–389.
49. Brady AJB, Poole-Wilson PA, Harding SE, Warren JB. Nitric oxide production within cardiac myocytes reduces their contractility in endotoxemia. *Am J Physiol* 1992;26:H1963–H1996.
50. Balligand JL, Ungureanu D, Kelly RA, Kobzik L, Pimental D, Michel T, Smith TW. Abnormal contractile function due to induction of nitric oxide synthesis in rat cardiac myocytes follows exposure to activated macrophage-conditioned medium. *J Clin Invest* 1993;91:2314–2319.
51. Evans HG, Lewis MJ, Shah AM. Interleukin 1-ß modulates myocardial contraction via dexamethasone-sensitive production of nitric oxide. *Cardiovasc Res* 1993;27:1486–1490.

52. Kinugawa K, Takahashi T, Kohmoto O, Yao A, Aoyagi T, Momomura S, Hirota Y, Serizawa T. Nitric oxide-mediated effects of interleukin-6 on $[Ca^{2+}]_i$ and cell contraction in cultured chick ventricular myocytes. *Circ Res* 1994;75:285–295.
53. Paulus WJ, Kästner S, Pujadas P, Shah AM, Drexler H, Vanderheyden M. Left ventricular contractile effects of inducible nitric oxide synthase in the human allograft. *Circulation* 1997;96:3436–3442.
54. Ronson RS, Nakamura M, Vinten-Johansen J. The cardiovascular effects and implications of peroxynitrite. *Cardiovasc Res* 1999;44:47–59.
55. Wildhirt SM, Weis M, Schulze C, Conrad N, Pehlivanli S, Rieder G, Enders G, von Scheidt W, Reichart B. Expression of endomyocardial nitric oxide synthase and coronary endothelial function in human cardiac allografts. *Circulation* 2001;104(Suppl I):336–343.
56. Paulus WJ. Nitric oxide and cardiac contraction: Clinical studies. In: Lewis MJ and Shah AM, eds. *Endothelial Modulation of Cardiac Function*. Amsterdam: Harwood Academic Publishers 1997;35–51.
57. Paulus WJ, Bronzwaer JGF, Felice H, Kishan N, Wellens F. Deficient acceleration of left ventricular relaxation during exercise after heart transplantation. *Circulation* 1992;86:1175–1185.
58. de Belder AJ, Radomski M, Why H, Richardson PJ, Bucknall CA, Salas E, Martin JF. Nitric oxide synthase activities in human myocardium. *Lancet* 1993;341:84–85.
59. Haywood GA, Tsao PS, von der Leyen HE, Mann MJ, Keeling PJ, Trindade PT, Lewis NP, Byrne CD, Rickenbacher PR, Bishopric NH, Cooke JP, McKenna WJ, Fowler MB. Expression of inducible nitric oxide synthase in human heart failure. *Circulation* 1996;93:1087–1094.
60. Habib FM, Springall DR, Davies GJ, Oakley CM, Yacoub MH, Polak JM. Tumour necrosis factor and inducible nitric oxide synthase in dilated cardiomyopathy. *Lancet* 1996;347:1151–1155.
61. Satoh M, Nakamura M, Tamura G, Makita S, Segawa I, Tashiro A, Satodate R, Hiramori K. Inducible nitric oxide synthase and tumor necrosis factor-alpha in myocardium in human dilated cardiomyopathy. *J Am Coll Cardiol* 1997;29:716–724.
62. Drexler H, Kästner S, Strobel A, Studer R, Brodde OE, Hasenfuss G. Expression, activity and functional significance of inducible nitric oxide synthase in the failing human heart. *J Am Coll Cardiol* 1998;32:955–963.
63. Fukuchi M, Hussain SNA, Giaid A. Heterogeneous expression and activity of endothelial and inducible nitric oxide synthases in end-stage human heart failure. Their relation to lesion site and ß-adrenergic receptor therapy. *Circulation* 1998;98:132–139.
64. Heymes C, Vanderheyden M, Bronzwaer JGF, Shah AM, Paulus WJ. Endomyocardial nitric oxide synthase and left ventricular preload reserve in dilated cardiomyopathy. *Circulation* 1999;99:3009–3016.
65. Stein B, Eschenhagen T, Rüdiger J, Scholz H, Förstermann U, Gath I. Increased expression of constitutive nitric oxide synthase III, but not inducible nitric oxide synthase II, in human heart failure. *J Am Coll Cardiol* 1998;32:1179–1186.
66. Yamamoto S, Tsutsui H, Tagawa H, Saito K, Takahashi M, Tada H, Yamamoto M, Katoh M, Egashira K, Takeshita A. Role of myocyte nitric oxide in ß-adrenergic hyporesponsiveness in heart failure. *Circulation* 1997;95:1111–1114.
67. Harding SE, Davies CH, Money-Kyrle AM, Poole-Wilson PA. An inhibitor of nitric oxide synthase does not increase contraction or ß-adrenoreceptor sensitivity of ventricular myocytes from failing human heart. *Cardiovasc Res* 1998;40:523–529.
68. Recchia FA, McConnell PL, Bernstein RD, Vogel TR, Xu X, Hintze TH. Reduced nitric oxide production and altered myocardial metabolism during the decompensation of pacing-induced heart failure in the conscious dog. *Circ Res* 1998;83:969–979.
69. Senzaki H, Smith CJ, Juang GJ, Isoda T, Mayer SP, Ohler A, Paolocci N, Tomaselli GF, Hare JM, Kass DA. Cardiac phosphodiesterase 5 (cGMP-specific) modulates beta-adrenergic signaling *in vivo* and is downregulated in heart failure. *FASEB J* 2001;15:1718–1726.
70. Hare JM, Lofthouse RA, Juang GJ, Colman L, Ricker KM, Kim B, Senzaki H, Cao S, Tunin RS, Kass DA. Contribution of caveolin protein abundance to augmented nitric oxide signaling in conscious dogs with pacing-induced heart failure. *Circ Res* 2000;86:1085–1092.
71. Bauersachs J, Galuppo P, Fraccarollo D, Christ M, Ertl G. Improvement of left ventricular remodeling and function by hydroxymethylglutaryl coenzyme A reductase inhibition with cerivastatin in rats with heart failure after myocardial infarction. *Circulation* 2001;104:982–985.
72. Bronzwaer JGF, Zeitz C, Visser CA, Paulus WJ. Endomyocardial nitric oxide synthase and the hemodynamic phenotypes of human dilated cardiomyopathy and of athlete's heart. *Cardiovasc Res* 2002;55:270–278.
73. Sessa WC, Pritchard K, Seyedi N, Wang J, Hintze TH. Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. *Circ Res* 1994;74:349–353.
74. Bernstein RD, Ochoa FY, Xu X, Forfia P, Shen W, Thompson CI, Hintze TH. Function and production of nitric oxide in the coronary circulation of the conscious dog during exercise. *Circ Res* 1996;79:840–848.
75. Wittstein IS, Kass DA, Pak PH, Maughan WL, Fetics B, Hare JM. Cardiac nitric oxide production due to angiotensin-converting enzyme inhibition decreases beta-adrenergic myocardial contractility in patients with dilated cardiomyopathy. *J Am Coll Cardiol* 2001;38:429–435.
76. Hare JM, Stamler JS. NOS: Modulator, not mediator of cardiac performance. *Nat Med* 1999;5:273–274.
77. Schlüter KD, Weber M, Schraven E, Piper HM. NO donor SIN-1 protects against reoxygenation-induced cardiomyocyte injury by a dual mechanism. *Am J Physiol* 1994;267:H1461–H1466.
78. Shah AM, Silverman HS, Griffiths EJ, Spurgeon HA, Lakatta EG. cGMP prevents delayed relaxation at reoxygenation after brief hypoxia in isolated cardiac myocytes. *Am J Physiol* 1995;268:H2396–H2404.
79. Schlüter KD, Jacob G, Ruiz-Meana M, Garcia-Dorado D, Piper HM. Protection of reoxygenated cardiomyocytes against osmotic fragility by NO donors. *Am J Physiol* 1996;271:H428–H434.
80. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide. *PNAS* 1990;87:1620–1624.
81. Matheis G, Sherman MP, Buckberg GD, Haybron DM, Young HH, Ignarro LJ. Role of L-arginine-nitric oxide pathway in myocardial reoxygenation injury. *Am J Physiol* 1992;262:H616–H620.

82. Patel VC, Yellon DM, Singh KJ, Neild GH, Woolfson RG. Inhibition of nitric oxide limits infarct size in the *in situ* rabbit heart. *Biochem Biophys Res Comm* 1993;194: 234–238.
83. Beresewicz A, Karwatowska-Prokopczuk E, Lewartowski B, Cedro-Ceremuzynska K. A protective rol of nitric oxide in isolated ischaemic/reperfused rat heart. *Cardiovasc Res* 1995;30:1001–1008.
84. Williams MW, Taft CS, Ramnauth S, Zhao ZQ, Vinten-Johansen J. Endogenous nitric oxide (NO) protects against ischaemia-reperfusion injury in the rabbit. *Cardiovasc Res* 1995;30:79–85.
85. Jones SP, Girod WG, Palazzo AJ, Granger DN, Grisham MB, Jourd'Heuil D, Huang PL, Lefer DJ. Myocardial ischemia-reperfusion injury is exacerbated in absence of endothelial nitric oxide synthase. *Am J Physiol* 1999;276:H1567–H1573.
86. Igarashi J, Nishida M, Hoshida S, Yamashita N, Kosaka H, Hori M, Kuzuya T, Tada M. Inducible nitric oxide synthase augments injury elicited by oxidative stress in rat cardiac myocytes. *Am J Physiol* 1998;274:C245–C252.
87. Kanno S, Lee PC, Zhang Y, Ho C, Griffith BP, Shears LL II, Billiar TR. Attenuation of myocardial ischemia/reperfusion injury by superinduction of inducible nitric oxide synthase. *Circulation* 2000;101:2742–2748.
88. Jugdutt BI. Effect of nitrates on myocardial remodeling after acute myocardial infarction. *Am J Cardiol* 1996;77:17C–23C.
89. Sam F, Sawyer DB, Xie Z, Chang DL, Ngoy S, Brenner DA, Siwik DA, Singh K, Apstein CS, Colucci WS. Mice lacking inducible nitric oxide synthase have improved left ventricular contractile function and reduced apoptotic cell death late after myocardial infarction. *Circ Res* 2001;89:351–356.
90. Feng Q, Lu X, Jones DL, Shen J, Arnold JM. Increased inducible nitric oxide synthase expression contributes to myocardial dysfunction and higher mortality after myocardial infarction in mice. *Circulation* 2001;104:700–704.
91. Tsutsui H, Ide T, Hayashidani S, Suematsu N, Shiomi T, Wen J, Nakamura K, Ichikawa K, Utsumi H, Takeshita A. Enhanced generation of reactive oxygen species in the limb skeletal muscles from a murine infarct model of heart failure. *Circulation* 2001;104:134.
92. Yasmin W, Strynadka KD, Schulz R. Generation of peroxynitrite contributes to ischemia-reperfusion injury in isolated rat hearts. *Cardiovasc Res* 1997;33:422–432.
93. Wang W, Sawicki G, Schulz R. Peroxynitrite-induced myocardial injury is mediated through matrix metalloproteinase-2. *Cardiovasc Res* 2002;53:165–174.
94. Scherrer-Crosbie M, Ullrich R, Bloch KD, Nakajima H, Nasseri B, Aretz T, Lindsey ML, Vançon AC, Huang PL, Lee RT, Zapol WM, Picard MH. Endothelial nitric oxide synthase limits left ventricular remodeling after myocardial infarction in mice. *Circulation* 2001;104:1286–1291.
95. Hoffmann J, Haendeler J, Aicher A, Rossig L, Vasa M, Zeiher A, Dimmeler S. Aging enhances the sensitivity of endothelial cells toward apoptotic stimuli. *Circ Res* 2001;89:709.
96. Decking U, Flesche C, Godecke A, Schrader J. Endotoxin-induced contractile dysfunction in guinea pig hearts is not mediated by nitric oxide. *Am J Physiol* 1995;268:H2460–H2465.
97. Mebazaa A, De Keulenaer GW, Paqueron X, Andries LJ, Ratajczak P, Lanone S, Frelin C, Longrois D, Payen D, Brutsaert DL, Sys SU. Activation of cardiac endothelium as a compensatory component in endotoxin-induced cardiomyopathy: Role of endothelin, prostaglandins and nitric oxide. *Circulation* 2001;104:3137–3144.
98. Paulus WJ, Shah AM. NO and cardiac diastolic function. *Cardiovasc Res* 1999;43:595–606.

Chapter 4

Diastolic left ventricular dysfunction and a steeper slope of the left ventricular diastolic pressure-volume relationship

Chapter 4.1

Myocardial Fibrosis Blunts Nitric Oxide Synthase-Related Preload Reserve in Human Dilated Cardiomyopathy

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Myocardial fibrosis blunts nitric oxide synthase-related preload reserve in human dilated cardiomyopathy

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Bronzwaer, Jean G. F., Christophe Heymes, Cees A. Visser, and Walter J. Paulus. Myocardial fibrosis blunts nitric oxide synthase-related preload reserve in human dilated cardiomyopathy. *Am J Physiol Heart Circ Physiol* 284: H10–H16, 2003; 10.1152/ajpheart.00401.2002.—The purpose of the study was to investigate interactions between myocardial nitric oxide synthase (NOS) and myocardial fibrosis, both of which determine left ventricular (LV) preload reserve in patients with nonischemic dilated cardiomyopathy (DCM). In previous animal experiments, chronic inhibition of NOS induced myocardial fibrosis and limited LV preload reserve. Twenty-eight DCM patients underwent LV catheterization, balloon caval occlusions (BCO; $n = 8$), intracoronary substance P infusion ($n = 8$), and procurement of LV endomyocardial biopsies for determinations of collagen volume fraction (CVF), of gene expression of NOS2, NOS3, heme oxygenase (HO)-1, and TNF-α, and of NOS2 protein. CVF was unrelated to the intensity of NOS2, NOS3, HO-1, or TNF-α gene expression or of NOS2 protein expression. Preload recruitable LV stroke work (PR-LVSW) correlated directly with NOS2 gene expression ($P = 0.001$) and inversely with CVF ($P = 0.04$). High CVF (>10%) reduced baseline LVSW and PR-LVSW at each level of NOS2 gene expression. In DCM, myocardial fibrosis is unrelated to the intensity of myocardial gene expression of NOS, antioxidative enzymes (HO-1), or cytokines (TNF-α) and blunts NOS2-related recruitment of LV preload reserve.

collagen; diastole; myocardial contraction

HIGHER LEFT VENTRICULAR (LV) endomyocardial nitric oxide (NO) synthase (NOS) gene expression enables patients with dilated cardiomyopathy (DCM) to recruit LV preload reserve as evident from their higher LV stroke work (LVSW) at elevated LV filling pressures (13). This ability to recruit LV preload reserve could result from an acute direct myocardial action of NO, because intracoronary infusion of substance P, which releases NO from the coronary endothelium, induces an instantaneous increase in LVSW in DCM patients with elevated LV filling pressures (13). This increase in LVSW is accompanied by a rightward shift of the diastolic LV pressure-volume relation. A similar NO-related rightward shift of the diastolic LV pressure-volume relation was previously observed in control and aortic stenosis patients after intracoronary infusion of a NO donor (20, 24) and in DCM patients after intracoronary infusion of enalaprilat (37). Acute effects of NO on myocardial diastolic distensibility have also been reported in experimental preparations. In isolated rat cardiomyocytes, exposure to cGMP, the second messenger of NO, increases diastolic cell length (32), and, in isolated guinea pig hearts, a coronary perfusate containing a specific NOS inhibitor results in an acute reduction of LV stroke volume because of a leftward shift of the diastolic LV pressure-volume relation (27).

The ability of NO to preserve LV diastolic distensibility could result not only from its acute direct myocardial actions but also from chronic effects on LV hypertrophy and on collagen turnover within the cardiac interstitium. In rats receiving chronic treatment with a NOS inhibitor, the diastolic LV pressure-volume relation shifts upward with a decrease in LV volume relative to wall thickness and no increase in LV mass despite the elevated blood pressure (1, 19). Chronic inhibition of NO synthesis also induces progressive myocardial interstitial and perivascular fibrosis through a signaling cascade involving endothelin, angiotensin II, aldosterone, and transforming growth factor-β (3, 34).

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The present study investigates interactions between the intensity of myocardial NOS gene expression and myocardial fibrosis in patients with nonischemic DCM in relation to their ability to recruit LV preload reserve. Baseline hemodynamic measures and the change in LVSW during balloon caval occlusion were correlated with the intensity of NOS2 and NOS3 gene expression, intensity of NOS2 protein expression, and collagen volume fraction (CVF) in simultaneously procured LV endomyocardial biopsies. To reproduce interactions between myocardial NO content and CVF, the hemodynamic response to an intracoronary infusion of substance P (13), which releases NO from the coronary endothelium, was also analyzed in relation to CVF of the LV endomyocardial biopsy.

METHODS

Patients and study protocol. Twenty-eight patients with nonischemic DCM were included. The group consisted of 8 women and 20 men (Table 1). At the time of study, heart failure therapy was maintained and consisted of ACE inhibitors (ACEI; $n = 28$), diuretics ($n = 25$), and β-blockers ($n = 2$). Cutoff values for DCM were a LV end-diastolic volume (LVEDV) index > 102 ml/m^2 (normal average value + 2SD) and a LV ejection fraction (LVEF) ≤45%. All patients had higher than normal baseline LV end-diastolic wall stress (LVEDWS) [≥85 kdyn/cm^2 = LVEDWS in a LV with a LVEDV at the upper limit of normal and a LV end-diastolic pressure (LVEDP) at the upper limit of normal (=16 mmHg) (38)]. A control group of three patients was also studied. Two male patients (age 44 and 24 yr, LVEF: 54% and 63%) were referred for myocarditis, and one female patient (age 72 yr, LVEF 57%) was referred for amyloidosis. Histological examination of the LV endomyocardial biopsies revealed no evidence of myocarditis or amyloidosis nor were there other abnormalities.

LV pressure and the first derivative of LV pressure (LV dP/dt) were derived from a high-fidelity micromanometer-tipped catheter. In eight patients (Table 1, *patients 21–28*), a 5-min intracoronary infusion of substance P (20 pmol/min) was performed (13). Substance P causes receptor-mediated coronary endothelial release of NO. At the end of the infusion period, LV hemodynamic and angiographic measures were repeated and compared with baseline values. In eight patients (Table 1, *patients 1–8*), a conductance catheter (Leycom Sigma) was used for continuous LV volume and pressure measurement. In these patients, a 8-Fr balloon catheter was introduced into the right atrium. Transient pullback of the inflated balloon into the inferior vena cava created multiple, variably preloaded beats. LV endomyocardial biopsies were obtained using a disposable transfemoral bioptome (Cordis). The study protocol was approved by the local review board (VU-University Medical Center, Amsterdam, The Netherlands), and informed consent for the study protocol was obtained from all patients.

RT-PCR for NOS2, NOS3, heme oxygenase-1, and TNF-α mRNA. Snap-frozen biopsies were obtained in 20 patients (Table 1, *patients 1–20*) for determination of NOS2 ($n = 20$), NOS3 ($n = 20$), heme oxygenase (HO)-1 ($n = 14$), and TNF-α mRNA ($n = 10$). RNA extraction, internal standard preparation, and oligonucleotides used for RT-PCR and quantitative RT-PCR protocol, using a defined amount of specific RNA mutant as an internal standard, have been extensively described previously (13). For HO-1, the primers chosen were

Table 1. *Patient characteristics*

Patient	Age, yr	Dig	HR, beats/min	LVPSP, mmHg	LVEDP, mmHg	LV dP/dt_{max}, mmHg/s	LVEDVI, ml/m^2	LVEF, %
1	67	−	75	108	14	785	392	10
2	37	−	105	102	28	1,021	167	10
3	47	−	71	128	21	560	124	35
4	65	−	69	97	28	550	289	7
5	45	−	73	90	29	525	123	15
6	56	+	105	129	25	1,455	189	16
7	39	+	74	115	27	859	177	20
8	56	+	96	137	25	1,230	183	19
9	72	+	55	126	12	1,280	143	32
10	70	−	77	137	21	1,240	106	39
11	55	−	95	97	33	672	105	24
12	70	+	78	95	20	850	160	17
13	45	−	88	108	42	725	106	38
14	66	−	90	139	19	1,225	107	25
15	26	−	92	79	33	425	180	8
16	72	+	82	127	26	1,225	146	35
17	61	+	82	80	22	450	239	30
18	65	+	88	91	24	456	162	33
19	32	+	78	100	35	645	178	44
20	34	+	78	108	14	1,350	115	32
21	54	−	88	108	22	745	162	28
22	47	+	95	100	29	740	168	22
23	72	−	82	144	36	920	170	32
24	40	−	94	84	27	640	133	21
25	38	+	90	140	34	795	114	29
26	52	+	107	137	30	1,350	120	45
27	63	−	104	121	25	1,150	178	25
28	69	−	115	95	31	840	134	23

Dig, use of digitalis; HR, heart rate; LVPSP, left ventricular (LV) peak systolic pressure; LVEDP, LV end-diastolic pressure; LV dP/dt_{max}, maximal first derivative of LV pressure; LVEDVI, LV end-diastolic volume index; LVEF, LV ejection fraction.

5′-CAGGCAGAGAATGCTGAGTTC-3′ (sense) and 5′-GCTTCACATAGCGCTGCA-3′ (antisense), amplifying the 79- to 429-bp region of HO-1. The expression of TNF-α was quantified as the ratio of the target band intensity to the β-actin band intensity.

Quantitative morphometry and immunohistochemistry. The extent of interstitial fibrosis was determined on elastica von Gieson-stained sections of at least three LV endomyocardial biopsies placed in 5% formalin with the use of an automated image analyzer (Prodit). Patients were divided into low and high extents of myocardial fibrosis in accordance to the CVF. A CVF = 10% corresponded to the median value of the CVF data and was used as the cutoff value to separate the low from the high extent of myocardial fibrosis. NOS2 protein expression was analyzed by immunohistochemistry using a commercially available antibody (NOS2, BioMol). Antibodies were detected by an indirect peroxidase antibody conjugate technique, and NOS2 staining was graded semiquantitatively on a scale of 0 to 4.

Data analysis. LVSW was derived from the area within the LV pressure-volume diagram (13). Preload recruitable LVSW (PR-LVSW) equaled the slope of a linear curve fit to the LVSW-versus-LVEDP data of the variably preloaded beats induced by transient pullback of the inflated balloon into the inferior vena cava. Baseline LVSW correlated closely ($P < 0.001$, $r = 0.90$) with PR-LVSW. This correlation justifies the use of baseline LVSW as a measure of LV preload reserve in the entire patient population.

Circumferential LV end-diastolic wall stress (LVEDWS) was computed using a thick wall ellipsoid model of the LV (23) as follows

$$\text{LVEDWS} = \text{P}D/2h \times [1 - (h/D) - (D^2/2L^2) \times 1.332 \text{ dyn cm}^2]$$

where P is LVEDP, h is LV echocardiographic end-diastolic wall thickness, and D and L are LV end-diastolic diameter and length at the midwall, respectively.

To assess diastolic LV myocardial material properties, a radial myocardial LV stiffness modulus (Stiffness-Mod) (6) was calculated from the microtip LV pressure and the echocardiographic LV wall thickness using the formula

$$\text{Stiffness-Mod} = \Delta\sigma_R/\Delta\epsilon_R = -\Delta P/(\Delta h/h) = -\Delta P/\Delta \ln h$$

and assuming the increment in radial stress ($\Delta\sigma_R$) to be equal but opposite in sign to the increment in LV pressure (P) at the endocardium, and the increment in radial strain ($\Delta\epsilon_R$) to be equal to the increment in wall thickness (Δh) relative to the instantaneous wall thickness. Because $\Delta h/h = \Delta \ln h$, Stiffness-Mod was equal to the slope of a P-versus-ln h plot.

All results are given as means ± SE. Univariate and multivariate linear regression analysis were performed with the use of the SPSS software package.

RESULTS

Determinants of LV preload reserve. Univariate linear regression analysis showed the intensity of NOS2 gene expression to correlate with baseline LVSW ($P = 0.0002$, $r = 0.75$; Fig. 1*A*) and with PR-LVSW ($P = 0.001$, $r = 0.90$), NOS2 immunostaining to correlate with baseline LVSW ($P = 0.003$, $r = 0.63$), and CVF to inversely correlate with baseline LVSW ($P = 0.02$, $r = 0.48$) and with PR-LVSW ($P = 0.04$, $r = 0.70$). Patients with CVF < 10% had higher baseline LVSW ($P < 0.01$) and higher PR-LVSW ($P < 0.03$) than patients with CVF > 10%.

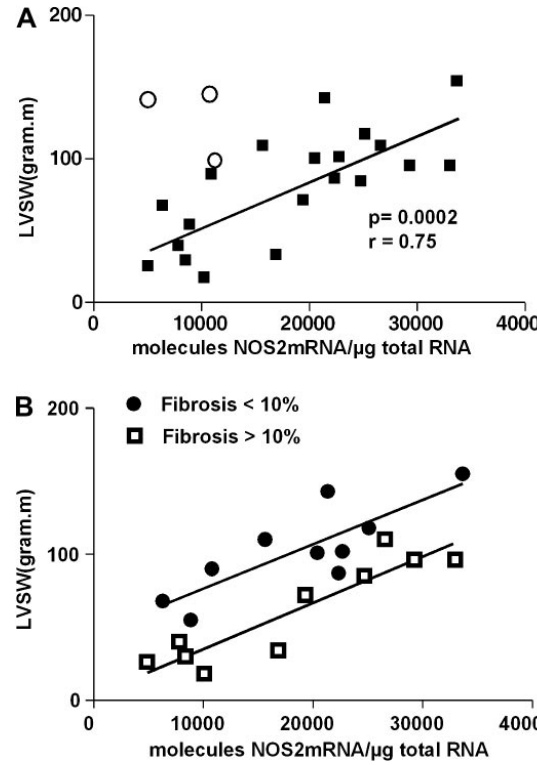

Fig. 1. *A*: linear relation between baseline left ventricular (LV) stroke work (LVSW) and the intensity of nitric oxide synthase (NOS)2 gene expression in the dilated cardiomyopathy (DCM) study population and in control patients (○). *B*: relation between baseline LVSW and NOS2 gene expression is shifted upward in patients with low-level myocardial fibrosis [collagen volume fraction (CVF) < 10%].

Determinants of LV fibrosis. No significant correlations were observed between CVF and the intensity of NOS2, NOS3, HO-1, or TNF-α gene expression or NOS2 immunostaining.

Interaction between NOS, fibrosis, and LV preload reserve. In a multivariate linear regression analysis, in the evaluation of the relation of baseline LVSW to the intensity of NOS3, NOS2, HO-1, and TNF-α gene expression and to the extent of myocardial fibrosis, only the intensity of NOS2 gene expression ($P < 0.0005$) and the extent of myocardial fibrosis ($P = 0.001$) significantly correlated with baseline LVSW.

When patients with CVF < 10% were separated from patients with CVF > 10%, the relation between baseline LVSW and NOS2 gene expression was significantly ($P < 0.05$) shifted upward in patients with CVF < 10% compared with patients with CVF > 10% (Fig. 1*B*). This upward shift implies a higher LVSW for a given value of NOS2 gene expression in patients with CVF < 10%. In patients with CVF < 10%, a relation was observed between PR-LVSW and NOS2 gene ex-

pression ($P < 0.01$, $r = 0.85$). Patients with CVF > 10% fell below the 95% confidence interval of this relation.

When patients with CVF < 10% were separated from patients with CVF > 10%, a significant correlation between baseline LVSW and the intensity of NOS3 gene expression was observed only in patients with CVF < 10% ($P = 0.04$, $r = 0.64$) (Fig. 2*A*). In patients with CVF > 10%, NOS3-related augmentation of LVSW was no longer demonstrable. Reduced NOS3-related augmentation of LVSW in patients with a high extent of interstitial fibrosis was also evident during intracoronary infusion of substance P, which induces coronary endothelial release of NO. In patients with CVF > 10%, the substance P-induced increase in LVSW was significantly smaller than in patients with CVF < 10% [ΔLVSW: 10 ± 3 g·m for CVF > 10% vs. 35 ± 5 g·m for CVF < 10% ($P = 0.02$)].

When patients with CVF < 10% were separated from patients with CVF > 10%, a significant correlation between the intensity of HO-1 gene expression and baseline LVSW was observed only in patients with CVF < 10% ($P = 0.04$, $r = 0.69$; Fig. 2*B*). In patients with CVF > 10%, these relations were absent.

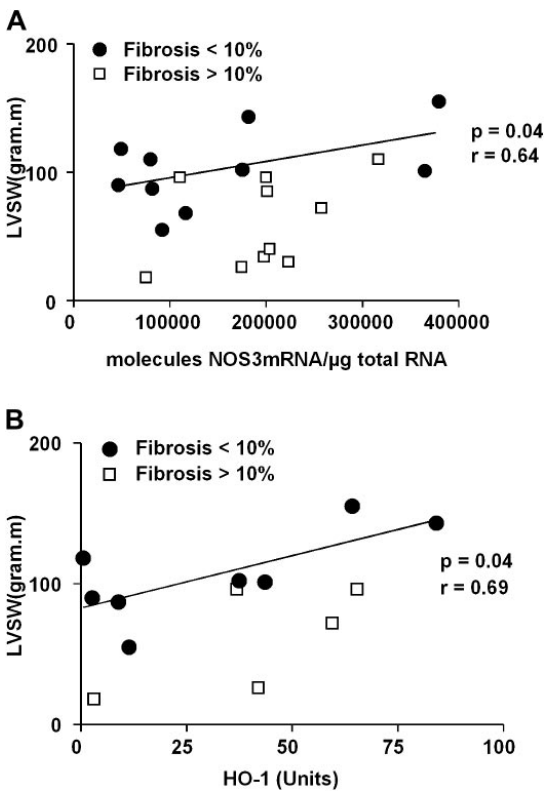

Fig. 2. *A*: linear relation between baseline LVSW and the intensity of NOS3 gene expression is observed in patients with low-level myocardial fibrosis (CVF < 10%) but not in patients with high-level myocardial fibrosis (CVF > 10%). *B*: linear relation between baseline LVSW and the intensity of heme oxygenase (HO)-1 gene expression is observed in patients with low-level myocardial fibrosis (CVF < 10%) but not in patients with high-level myocardial fibrosis (CVF > 10%).

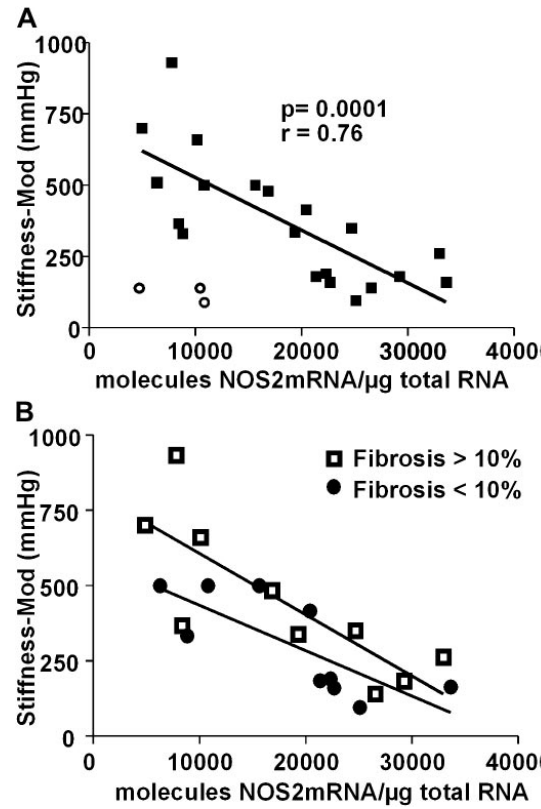

Fig. 3. *A*: linear relation between the LV stiffness modulus (Stiffness-Mod) and intensity of NOS2 gene expression in the DCM study population and in control patients (○). *B*: the relation between the LV Stiffness-Mod and NOS2 gene expression is shifted downward in patients with low-level myocardial fibrosis (CVF < 10%).

Interaction between NOS, fibrosis, and LV myocardial stiffness. Multivariate linear regression analysis was used to evaluate the relation of LV myocardial stiffness to the intensity of NOS3, NOS2, HO-1, and TNF-α gene expression and to the extent of myocardial fibrosis. Only the intensity of NOS2 gene expression ($P = 0.001$; Fig. 3*A*) and the extent of myocardial fibrosis ($P = 0.04$) significantly correlated with the LV Stiffness-Mod. When patients with CVF < 10% were separated from patients with CVF > 10%, the relation between Stiffness-Mod and NOS2 gene expression was significantly ($P < 0.05$) shifted downward in patients with CVF < 10% compared with patients with CVF > 10% (Fig. 3*B*). This downward shift implies a lower Stiffness-Mod for a given value of NOS2 gene expression in patients with CVF < 10%.

DISCUSSION

NO, fibrosis, and LV preload reserve. Patients with DCM are highly dependent on LV preload reserve to augment cardiac output during exercise. The present study identified high LV endomyocardial NOS2 and low CVF to be important for recruitment of LV preload reserve in these patients.

During exercise, patients with DCM are highly dependent on LV preload reserve because of the blunted myocardial inotropic response to catecholamines (14). The enhancement of PR-LVSW in DCM results from a rightward displacement of the diastolic LV pressure-volume relation (15). Limitation of this rightward displacement is accompanied by a restrictive LV filling pattern on the Doppler mitral inflow signal, by exacerbation of symptoms, and by poor prognosis (25). Because of the ability of NO to shift the diastolic LV pressure-volume relation rightward (24, 37), a low intensity of endomyocardial NOS gene expression could limit rightward displacement of the diastolic LV pressure-volume relation and PR-LVSW (7, 13). A high extent of myocardial fibrosis could similarly limit LV distensibility and PR-LVSW (36).

The findings of the present study are consistent with NO being physiologically involved in the LV hemodynamic response to a rise in preload and with myocardial fibrosis effectively counteracting this physiological response. A physiological role for NO in the LV response to preload augmentation was recently established in experiments that measured the intramyocardial NO concentration using a microporphyrinic sensor in the LV wall of the beating rabbit heart (26). In these experiments, a LV volume load due to release of a caval occluder resulted in a prompt rise in intramyocardial NO concentration. Pretreatment with a NOS inhibitor (27) reduced both PR- LVSW and LV diastolic distensibility, and L-arginine resulted in opposite effects.

The present study investigated relations between myocardial fibrosis and the intensity of NOS gene expression not only because NO could reduce the collagen turnover of fibroblasts but also because myocardial fibrosis could affect myocardial NOS gene expression. Fibrosis of the failing myocardium is indeed accompanied by disruption of the collagen weave around individual myocytes leading to malalignment or slippage. This disruption of the collagen weave alters myocardial gene expression because of an altered cascade of stress signals descending from the collagen fibers to sarcolemmal integrins, cytoskeletal proteins, and nuclear membranes. Both a previous study (30) and the present study failed to observe a relation between the extent of myocardial fibrosis and NOS2 gene expression.

The present study also failed to observe a relation between the extent of myocardial fibrosis and NOS3 gene expression or HO-1 gene expression, which is upregulated in the failing myocardium and could augment the bioavailability of NO by protection against oxidative stress (28). The lack of relations between myocardial fibrosis and NOS3 or antioxidative enzymes such as HO-1 does not exclude the involvement of NOS3 or antioxidative enzymes in the development of myocardial fibrosis but suggests excessive myocardial fibrosis in DCM to result more from upregulation of stimulatory pathways such as angiotensin II, endothelin, and aldosterone than from downregulation of inhibitory pathways (36).

Upregulation of NOS2. A potential mechanism for the observed upregulation of NOS2 could be a compensatory rise for reduced expression of NOS3 (9). Recent experiments in NOS3 knockout mice indeed observed a superinduction of NOS2 triggered by the oxidative stress of an ischemia-reperfusion episode (16). This superinduction of NOS2 provided an important protective effect against ischemia-reperfusion injury with preservation of systolic and diastolic LV performance. Superior hemodynamic status in the presence of myocardial NOS2 upregulation was also observed in the present study, and this result confirmed the findings of a recent clinical study that failed to observe a change in LV dP/dt_{max} in DCM patients during intracoronary N^G-monomethyl-L-arginine infusion despite NOS2 gene expression (8, 22). In addition, recent experimental findings have demonstrated that the heart can tolerate high levels of NOS2 activity without detrimental functional consequences (12).

The myocardial upregulation of NOS2 observed in the present study could also be part of the reexpression of the fetal gene program frequently observed in hypertrophied or failing myocardium. NOS2 is indeed abundantly expressed in the fetus and gets downregulated before birth (2).

Myocardial cytokines could be important determinants of LV preload reserve because of their ability to induce NOS2 (30) and to increase activity of matrix metalloproteinases with a concomitant acceleration of collagen breakdown (33). In the present study, myocardial TNF-α gene expression appeared unrelated to LVSW, myocardial NOS2 gene expression, or the extent of myocardial fibrosis.

Therapeutic implications. The present study shows recruitment of LV preload reserve in patients with DCM to relate to the high intensity of myocardial NOS gene expression and to the low extent of myocardial fibrosis. ACEI, β-blockers, and spironolactone have all been shown to both upregulate myocardial NOS gene expression and to induce regression of endomyocardial fibrosis. Some of the benefits of ACEI, β-blockers, and spironolactone in the treatment of chronic heart failure could therefore be ascribed to their ability to maintain LV preload reserve of the cardiomyopathic heart because of upregulation of NOS3 activity and prevention of myocardial fibrosis. ACEI augment coronary endothelial NO activity acutely as evident from intracoronary enalaprilat infusion during pacing tachycardia (21) and chronically as evident from the Trial on Reversing Endothelial Dysfunction, which showed improved endothelial-dependent vasodilator responses during long-term quinapril therapy (18). Apart from beneficial effects on LV preload reserve, ACEI-induced upregulation of myocardial NO content could also

favourably modify substrate utilization and mitochondrial respiration of failing myocardium (17, 29, 35). In patients with hypertensive heart disease, 6 mo of lisinopril therapy induced significant regression of myocardial fibrosis and improvement of diastolic LV function (5). DCM patients on β-blocker therapy had a higher intensity of NOS3 gene expression in their endomyocardial biopsies (11, 13). β-Blocker therapy could also favourably affect myocardial fibrosis through reduction of angiotensin II-related activation of matrix metalloproteinases (31). In patients with heart failure, spironolactone improved endothelial dysfunction by increasing NO bioactivity (10), and, in the Randomized Aldactone Evaluation Study, serum levels of markers of cardiac fibrosis were significantly reduced by spironolactone therapy (39).

Study limitations. LV endomyocardial NOS gene expression was derived from a single biopsy sample, which therefore did not take into account spatial heterogeneity of gene expression (4). In a previous study (13), however, multiple biopsies from different LV sites were obtained in the same patient and the variability of endomyocardial NOS2 and NOS3 gene expression was low (7% and 15%).

In the present study, LVSW and Stiffness-Mod were correlated with endomyocardial NOS mRNA and protein and not with measures of endomyocardial NO activity, such as myocardial cGMP concentration or transcardiac nitrite/nitrate production. Only the latter could have accounted for posttranscriptional and posttranslational modification of NOS, for substrate deficiency, or for the reduced bioavailability of NO because of elevated oxidative stress.

The LV endomyocardial biopsies used for determination of interstitial myocardial fibrosis were considered representative of the whole LV myocardium and therefore assumed homogeneity in LV myocardial structure.

In conclusion, in the cardiomyopathic heart, a high intensity of myocardial NOS gene expression and low-level myocardial fibrosis appeared to be essential for maintaining LV preload reserve. Part of the benefit of ACEI, β-blockers, and spironolactone in the treatment of chronic heart failure could be related to their ability to upregulate NOS2 gene expression and to regress myocardial fibrosis. Upregulation of myocardial NOS2 and regression of myocardial fibrosis are important targets for prevention of progression of cardiomyopathic LV dysfunction.

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REFERENCES

1. **Bartunek J, Weinberg EO, Tajima M, Rohrbach S, Katz SE, Douglas PS, and Lorell BH.** Chronic N^G-nitro-L-arginine methyl ester-induced hypertension: novel molecular adaptation to systolic load in absence of hypertrophy. *Circulation* 101: 423–429, 2000.
2. **Bloch W, Fleischmann BK, Lorke DE, Andressen C, Hops B, Hescheler J, and Addicks K.** Nitric oxide synthase expression and role during cardiomyogenesis. *Cardiovasc Res* 43: 675–684, 1999.
3. **Boffa JJ, Tharaux PL, Placier S, Ardaillou R, Dussaule JC, and Chatziantoniou C.** Angiotensin II activates collagen type I gene in the renal vasculature of transgenic mice during inhibition of nitric oxide synthesis: evidence for an endothelin-mediated mechanism. *Circulation* 100: 1901–1908, 1999.
4. **Brahmajothi MV and Campbell DL.** Heterogeneous basal expression of nitric oxide synthase and superoxide dismutase isoforms in mammalian heart: implications for mechanisms governing indirect and direct nitric oxide-related effects. *Circ Res* 85: 575–587, 1999.
5. **Brilla CG, Funck RC, and Rupp H.** Lisinopril-mediated regression of myocardial fibrosis in patients with hypertensive heart disease. *Circulation* 102: 1388–1393, 2000.
6. **Bronzwaer JG, De Bruyne B, Ascoop CA, and Paulus WJ.** Comparative effects of pacing-induced and balloon coronary occlusion ischemia on left ventricular diastolic function in man. *Circulation* 84: 211–222, 1991.
7. **Bronzwaer JG, Zeitz C, Visser CA, and Paulus WJ.** Endomyocardial nitric oxide synthase and the hemodynamic phenotypes of human dilated cardiomyopathy and of athlete's heart. *Cardiovasc Res* 55: 270–278, 2002.
8. **Cotton JM, Kearney MT, MacCarthy PA, Grocott-Mason RM, McClean DR, Heymes C, Richardson PJ, and Shah AM.** Effects of nitric oxide synthase inhibition on basal function and the force-frequency relationship in the normal and failing human heart in vivo. *Circulation* 104: 2318–2323, 2001.
9. **Drexler H, Kastner S, Strobel A, Studer R, Brodde OE, and Hasenfuss G.** Expression, activity and functional significance of inducible nitric oxide synthase in the failing human heart. *J Am Coll Cardiol* 32: 955–963, 1998.
10. **Farquharson CA and Struthers AD.** Spironolactone increases nitric oxide bioactivity, improves endothelial vasodilator dysfunction, and suppresses vascular angiotensin I/angiotensin II conversion in patients with chronic heart failure. *Circulation* 101: 594–597, 2000.
11. **Fukuchi M, Hussain SN, and Giaid A.** Heterogeneous expression and activity of endothelial and inducible nitric oxide synthases in end-stage human heart failure: their relation to lesion site and beta-adrenergic receptor therapy. *Circulation* 98: 132–139, 1998.
12. **Heger J, Godecke A, Flogel U, Merx MW, Molojavyi A, Kuhn-Velten WN, and Schrader J.** Cardiac-specific overexpression of inducible nitric oxide synthase does not result in severe cardiac dysfunction. *Circ Res* 90: 93–99, 2002.
13. **Heymes C, Vanderheyden M, Bronzwaer JG, Shah AM, and Paulus WJ.** Endomyocardial nitric oxide synthase and left ventricular preload reserve in dilated cardiomyopathy. *Circulation* 99: 3009–3016, 1999.
14. **Higginbotham MB, Sullivan MJ, Coleman RE, and Cobb FR.** Regulation of stroke volume during exercise in patients with severe left ventricular dysfunction: importance of the Starling mechanism (Abstract). *J Am Coll Cardiol* 9: 58A, 1987.
15. **Holubarsch C, Ruf T, Goldstein DJ, Ashton RC, Nickl W, Pieske B, Pioch K, Ludemann J, Wiesner S, Hasenfuss G, Posival H, Just H, and Burkhoff D.** Existence of the Frank-Starling mechanism in the failing human heart. Investigations on the organ, tissue, and sarcomere levels. *Circulation* 94: 683–689, 1996.
16. **Kanno S, Lee PC, Zhang Y, Ho C, Griffith BP, Shears LL, and Billiar TR.** Attenuation of myocardial ischemia/reperfusion injury by superinduction of inducible nitric oxide synthase. *Circulation* 101: 2742–2748, 2000.
17. **Loke KE, Laycock SK, Mital S, Wolin MS, Bernstein R, Oz M, Addonizio L, Kaley G, and Hintze TH.** Nitric oxide modulates mitochondrial respiration in failing human heart. *Circulation* 100: 1291–1297, 1999.
18. **Mancini GB, Henry GC, Macaya C, O'Neill BJ, Pucillo AL, Carere RG, Wargovich TJ, Mudra H, Luscher TF, Klibaner MI, Haber HE, Uprichard AC, Pepine CJ, and Pitt B.** Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coro-

nary artery disease. The TREND Study. *Circulation* 94: 258–265, 1996.
19. **Matsubara BB, Matsubara LS, Zornoff LA, Franco M, and Janicki JS.** Left ventricular adaptation to chronic pressure overload induced by inhibition of nitric oxide synthase in rats. *Basic Res Cardiol* 93: 173–181, 1998.
20. **Matter CM, Mandinov L, Kaufmann PA, Vassalli G, Jiang Z, and Hess OM.** Effect of NO donors on LV diastolic function in patients with severe pressure-overload hypertrophy. *Circulation* 99: 2396–2401, 1999.
21. **Mohri M, Tagawa H, Egashira K, and Takeshita A.** Intracoronary enalaprilat improves metabolic coronary vasodilation in patients with idiopathic dilated cardiomyopathy. *J Cardiovasc Pharmacol* 35: 249–255, 2000.
22. **Paulus WJ, Frantz S, and Kelly RA.** Nitric oxide and cardiac contractility in human heart failure: time for reappraisal. *Circulation* 104: 2260–2262, 2001.
23. **Paulus WJ, Heyndrickx GR, Buyl P, Goethals MA, and Andries E.** Wide-range load shift of combined aortic valvuloplasty-arterial vasodilation slows isovolumic relaxation of the hypertrophied left ventricle. *Circulation* 81: 886–898, 1990.
24. **Paulus WJ, Vantrimpont PJ, and Shah AM.** Acute effects of nitric oxide on left ventricular relaxation and diastolic distensibility in humans. Assessment by bicoronary sodium nitroprusside infusion. *Circulation* 89: 2070–2078, 1994.
25. **Pinamonti B, Di Lenarda A, Sinagra G, and Camerini F.** Restrictive left ventricular filling pattern in dilated cardiomyopathy assessed by Doppler echocardiography: clinical, echocardiographic and hemodynamic correlations and prognostic implications. Heart Muscle Disease Study Group. *J Am Coll Cardiol* 22: 808–815, 1993.
26. **Pinsky DJ, Patton S, Mesaros S, Brovkovych V, Kubaszewski E, Grunfeld S, and Malinski T.** Mechanical transduction of nitric oxide synthesis in the beating heart. *Circ Res* 81: 372–379, 1997.
27. **Prendergast BD, Sagach VF, and Shah AM.** Basal release of nitric oxide augments the Frank-Starling response in the isolated heart. *Circulation* 96: 1320–1329, 1997.
28. **Raju VS, Imai N, and Liang CS.** Chamber-specific regulation of heme oxygenase-1 (heat shock protein 32) in right-sided congestive heart failure. *J Mol Cell Cardiol* 31: 1581–1589, 1999.
29. **Recchia FA, McConnell PI, Loke KE, Xu X, Ochoa M, and Hintze TH.** Nitric oxide controls cardiac substrate utilization in the conscious dog. *Cardiovasc Res* 44: 325–332, 1999.
30. **Satoh M, Nakamura M, Tamura G, Makita S, Segawa I, Tashiro A, Satodate R, and Hiramori K.** Inducible nitric oxide synthase and tumor necrosis factor-alpha in myocardium in human dilated cardiomyopathy. *J Am Coll Cardiol* 29: 716–724, 1997.
31. **Senzaki H, Paolocci N, Gluzband YA, Lindsey ML, Janicki JS, Crow MT, and Kass DA.** β-Blockade prevents sustained metalloproteinase activation and diastolic stiffening induced by angiotensin II combined with evolving cardiac dysfunction. *Circ Res* 86: 807–815, 2000.
32. **Shah AM, Spurgeon HA, Sollott SJ, Talo A, and Lakatta EG.** 8-Bromo-cGMP reduces the myofilament response to Ca^{2+} in intact cardiac myocytes. *Circ Res* 74: 970–978, 1994.
33. **Siwik DA, Chang DL, and Colucci WS.** Interleukin-1beta and tumor necrosis factor-alpha decrease collagen synthesis and increase matrix metalloproteinase activity in cardiac fibroblasts in vitro. *Circ Res* 86: 1259–1265, 2000.
34. **Tomita H, Egashira K, Ohara Y, Takemoto M, Koyanagi M, Katoh M, Yamamoto H, Tamaki K, Shimokawa H, and Takeshita A.** Early induction of transforming growth factor-beta via angiotensin II type 1 receptors contributes to cardiac fibrosis induced by long-term blockade of nitric oxide synthesis in rats. *Hypertension* 32: 273–279, 1998.
35. **Trochu JN, Bouhour JB, Kaley G, and Hintze TH.** Role of endothelium-derived nitric oxide in the regulation of cardiac oxygen metabolism: implications in health and disease. *Circ Res* 87: 1108–1117, 2000.
36. **Weber KT.** Targeting pathological remodeling: concepts of cardioprotection and reparation. *Circulation* 102: 1342–1345, 2000.
37. **Wittstein IS, Kass DA, Pak PH, Maughan WL, Fetics B, and Hare JM.** Cardiac nitric oxide production due to angiotensin-converting enzyme inhibition decreases beta-adrenergic myocardial contractility in patients with dilated cardiomyopathy. *J Am Coll Cardiol* 38: 429–435, 2001.
38. **Yamakado T, Takagi E, Okubo S, Imanaka-Yoshida K, Tarumi T, Nakamura M, and Nakano T.** Effects of aging on left ventricular relaxation in humans. Analysis of left ventricular isovolumic pressure decay. *Circulation* 95: 917–923, 1997.
39. **Zannad F, Alla F, Dousset B, Perez A, and Pitt B.** Limitation of excessive extracellular matrix turnover may contribute to survival benefit of spironolactone therapy in patients with congestive heart failure: insights from the randomized aldactone evaluation study (RALES). Rales Investigators. *Circulation* 102: 2700–2706, 2000.

Chapter 4.2

Nitric Oxide's Role in the Heart: Control of Beating or Breathing?

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Introduction

Nitric oxide(NO) is universally accepted as an important regulator of vascular tone, capillary permeability and platelet adhesion. NO's myocardial actions are unfortunately less well understood despite the growing clinical awareness that progressive dysfunction of the failing heart could well result from an imbalance between myocardial NO and oxidative stress induced by excess neurohormonal and inflammatory mediators(32). The appreciation of a beneficial role of NO in failing myocardium was a recent turnaround(8,11,15,33) and resulted mainly from an appraisal of NO's favorable effects on cardiac energetics(51) and on LV diastolic distensibility(35) clearly outweighing the negative inotropy of NO reported in the very initial studies looking at its contractile effects. These studies had observed a reduction of extent and velocity of shortening of isolated cardiomyocytes following administration of exogenous NO(3) and an increase in extent and velocity of shortening of adrenergically-stimulated cardiomyocytes following inhibition of endogenous NO production(1). At that time, these experiments were thought to provide an explanation for simultaneously published clinical observations, which reported inducible NO synthase(NOS2) activity in patients with nonischemic dilated cardiomyopathy(10). These initial experimental studies and the potential link to myocardial dysfunction of nonischemic dilated cardiomyopathy were however rapidly rebutted by several investigators reporting positive inotropic effects of low doses of exogenous NO or of cGMP(23,29) and by clinical studies reporting myocardial NOS2 expression in ischemic cardiomyopathy, in valvular heart disease and even in athlete's heart(5,16). These clinical studies also found higher NOS2 expression in heart failure patients with lower functional class, larger stroke work and preserved LV diastolic distensibility. Nevertheless, the idea of NO being deleterious because of a negative inotropic effect gained widespread acceptance and subsequently hindered a correct appreciation of NO's favorable effects on failing myocardium.

NO-induced myocardial contractile depression: time for acquittal!

Detailed analysis of the time course of isometric contraction of isolated cat papillary muscle strips revealed endogenous NO, released from the endothelium, to affect cardiac muscle contraction in a unique way (29,47): NO induced an earlier onset of isometric tension decay, which reduced peak isometric tension(T) without effect on the rate of rise of tension (Figure 1a). This effect was attributed to a NO-induced reduction in myofilamentary calcium sensitivity because of phosphorylation of troponin I by cGMP dependent protein kinase as evident from simultaneous recordings in isolated cardiomyocytes of cell lengthening and of calcium transient(44). In these cardiomyocytes, diastolic cell length was not clamped and following administration of NO or

of cGMP diastolic cell length consistently increased (20,44). This finding implied a rightward shift of the passive length-tension relation of the cardiomyocyte. This shift was also explained by NO-induced phosphorylation of troponin I, which prevented calcium-independent diastolic crossbridge cycling and concomitant diastolic stiffening of the myocardium. Both the *relaxation-hastening* and *distensibility-increasing* effects of NO were confirmed in the human heart. During intracoronary infusions of low doses of NO-donors(35) or of substance P(36), which releases NO from the coronary endothelium, there was an earlier onset of isovolumic LV pressure decay(Figure 1b), lower LV peak-systolic, end-systolic and end-diastolic pressures and rightward displacement of the diastolic LV pressure-volume relation(Figure 1c). In patients with a hypertrophied LV of aortic stenosis, the NO-induced rightward displacement of the diastolic LV pressure-volume relation was larger than in controls(27). In patients with dilated cardiomyopathy, the rightward displacement of the diastolic LV pressure-volume relation was accompanied by a significant increase in LV stroke volume because of improved recruitment of LV preload reserve (18). The lower LV end-systolic pressure at unaltered LV end-systolic volume observed during intracoronary infusions of NO-donors or of substance P implied a downward shift of the LV end-systolic pressure-volume relation and was therefore consistent with a negative inotropic effect of NO. The unaltered LV dP/dtmax at larger LV end-diastolic volume was also theoretically consistent with lower myocardial inotropic state. The simultaneous fall in LV end-diastolic pressure and the rise in LV stroke volume or stroke work however argue against significant cardiac contractile depression as a result of these NO-induced effects. Finally, direct positive inotropic effects of NO were recently demonstrated in normal control patients(7), in whom an intracoronary infusion of L-NMMA induced a modest(14%) drop in LV dP/dt max. In the same study, intracoronary L-NMMA failed to alter LV dP/dt max in dilated cardiomyopathy patients despite myocardial expression of NOS2 in simultaneously procured endomyocardial biopsies.

Following β-adrenoreceptor stimulation of isolated cardiac muscle strips, the NO-induced *relaxation-hastening* effect was larger probably because of simultaneous phosphorylation of troponin I by cAMP dependent and cGMP dependent protein kinases(29). In dilated cardiomyopathy patients, similar cooperative effects of NO and of β-adrenoreceptor stimulation were reported: during concomitant intravenous infusion of dobutamine, intracoronary infusion of substance P caused a larger drop in LV end-systolic pressure(±30 mmHg) and a small(6%) reduction in LV dP/dtmax (34). Both effects were again accompanied by a fall in LV end-diastolic pressure implying absence of hemodynamic deterioration. Similar findings had been observed during concomitant intravenous infusion of dobutamine and intracoronary infusion of the NO synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA)(14,46) or of the angiotensin-converting enzyme inhibitor enalaprilat(50). Intracoronary L-NMMA infusion raised LV dP/dtmax and increased the slope of the LV end-systolic pressure-volume relation without change in LV end-diastolic pressure again implying no substantial change in overall hemodynamic status. Intracoronary enalaprilat in the presence of angiotensin II receptor blockade caused bradykinin-

induced coronary endothelial release of NO and this also resulted in a small reduction of LV dP/dtmax accompanied by a fall in LV end-diastolic pressure and no change in LV stroke volume.

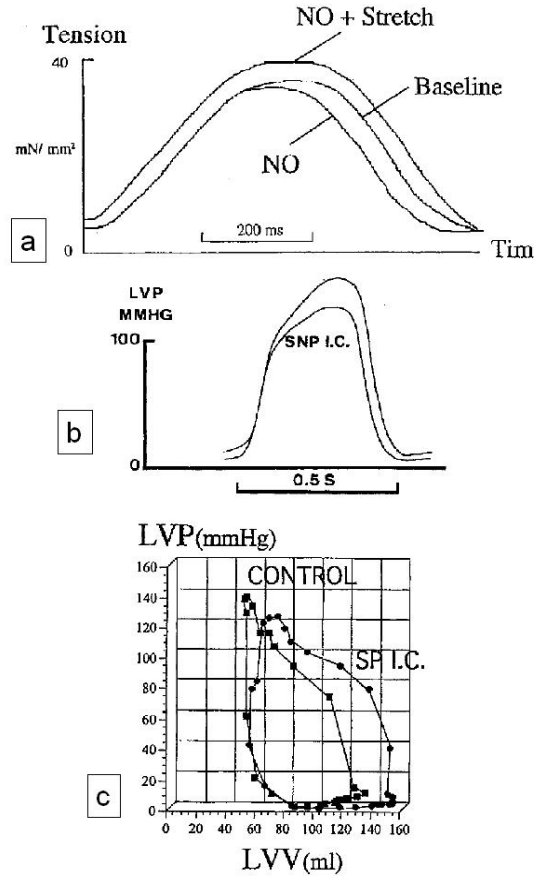

Figure 1: Figure 1a-Top panel: Effects of NO on isolated papillary muscle isometric contraction. NO has no effect on the rate of rise of isometric tension but causes earlier isometric tension decay with a concomitant small reduction in peak isometric tension. These effects are counteracted by an increase in muscle preload(NO+Stretch). Figure 1b-Middle panel: Effects of an intracoronary(I.C.) infusion of sodium nitroprusside(SNP) on LV pressure in the normal human heart. There is no effect on the rate of rise of LV pressure but an earlier onset of isovolumic LV pressure decay with concomitant reduction in peak and end-systolic LV pressures. Figure 1c-Bottom panel: Effects of an intracoronary infusion of substance P (SP I.C.) on the LV pressure(LVP)-volume(LVV) relation in the normal human heart. SP I.C. causes a small right and downward displacement of the end-systolic pressure-volume point and a right and downward displacement of the diastolic pressure-volume relation consistent with an increase in diastolic LV distensibility. Reproduced with permission from ref 32.

Hence, from these observations in dilated cardiomyopathy patients, it can be concluded that in terms of overall LV performance, improvement in diastolic LV function also overrode the NO-induced attenuation of the LV contractile response to β-adrenoreceptor stimulation.

In transgenic mice with cardioselective overexpression of endothelial NOS(NOS3)(6) and a 60-fold increase in myocardial NOS3 activity (^{3}H-L-Citrulline production), there was only a small reduction in peak LV developed pressure without hemodynamic consequence mainly because of myofilamentary desensitization. A similar small reduction in peak LV developed pressure without signs of cardiac dysfunction was also observed in transgenic mice with cardioselective overexpression of NOS2 and a 20-fold increase in myocardial ^{3}H-L-Citrulline production(17). In these mice, addition of L-arginine to the perfusion, augmented the drop in LV developed pressure to 20% of basal value again without hemodynamic consequence. In experimental volume-overload, basal isometric twitch characteristics were more depressed in papillary muscles retrieved from decompensated than from compensated rats despite similar myocardial NOS2 activity(12).

Maintenance of preload reserve: an important task for NO in the stressed heart!

In isolated ejecting guinea-pig hearts, a perfusate containing L-NMMA raised LV end-diastolic pressure and reduced preload recrutable LV stroke work because of an acute left and upward shift of the diastolic LV pressure-volume relation (39). In this preparation, use of LV preload reserve also induced a rise in the NO concentration of the coronary effluent. This preload triggered enhancement of myocardial NO production confirmed earlier observations using porphyrinic sensors inserted in the wall of the beating rabbit heart(38). In rats receiving eight weeks of treatment with a NOS inhibitor, the diastolic LV pressure-volume relation shifted upward with reduced LV unstressed volume and no increase in LV mass despite the elevated blood pressure(26). NO-related modulation of diastolic LV distensibility was also observed in the pacing-induced heart failure dog model (40), in which a fall in myocardial NO production occurred after 4 weeks of pacing. This fall was accompanied by a drop in LV stroke volume and a steep rise in LV end-diastolic pressure probably because of reduced diastolic LV distensibility.

A NO-induced diastolic LV distensibility increasing effect was observed not only in experimental models but also in the normal human heart(35), in the cardiac allograft(36), in the hypertrophied LV of aortic stenosis(27) and in the failing LV of dilated cardiomyopathy(18). In dilated cardiomyopathy patients with elevated LV filling pressures(18), enhanced myocardial NOS3 activity during intracoronary substance P infusion, increased LV stroke volume and LV stroke work. This acute increase in LV stroke work resulted from a simultaneous NO-induced increase in diastolic LV distensibility and LV preload reserve(5).

In patients with dilated cardiomyopathy, limited LV preload reserve (19) corresponds with a

restrictive LV filling pattern on the Dopplerechocardiogram(37). This phenotype of dilated cardiomyopathy is characterized by a worse symptomatic course and a worse prognosis. Low intensity of LV endomyocardial NOS2 and NOS3 gene expression was recently demonstrated to coincide with this hemodynamic phenotype(5). In contrast, dilated cardiomyopathy patients with maintained LV preload reserve, normal Dopplerechocardiographic LV filling dynamics and low LV diastolic stiffness had a high intensity of LV endomyocardial NOS2 and NOS3 gene expression, comparable to the intensity observed in athlete's heart(5). Low LV diastolic stiffness and high LV preload reserve are also typical features of athlete's heart and could also be NO-mediated because of the well documented upregulation of NOS3 activity and expression by intense physical exercise(2,42). Further evidence for a beneficial effect of high endomyocardial NO activity on prognosis of dilated cardiomyopathy was recently provided by studies looking at NOS3 gene polymorphism in man. In these studies, dilated cardiomyopathy patients of a genotype with high NOS3 activity had a more benign course of their disease than patients of a genotype with low NOS3 activity(28). These findings also resemble the superior long-term outcome of LV remodeling following myocardial infarction in wild-type mice compared to NOS3 knock-out mice(41). Finally, the improved prognosis of dilated cardiomyopathy patients treated with ACE inhibitors or β blockers is paralleled by an upregulation of their endomyocardial NOS3 activity(18).

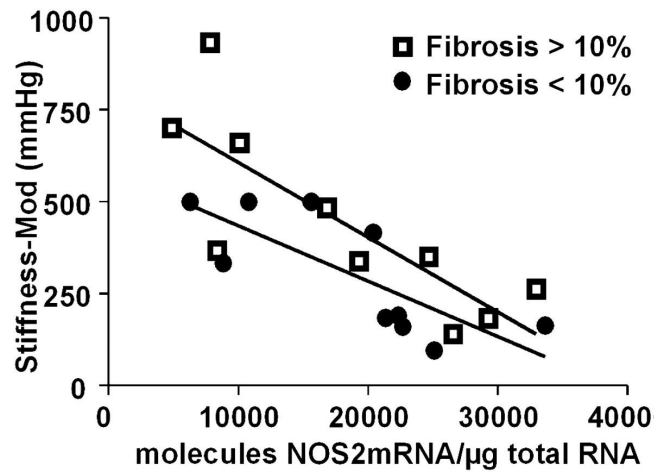

Figure 2: Additive and opposite effects of intensity of NOS2 gene expression and of myocardial fibrosis on diastolic LV stiffness in the failing human heart. The relation between the LV stiffness modulus (Stiffness-Mod) and NOS2 gene expression is shifted downward in patients with low level myocardial fibrosis [Collagen Volume Fraction(CVF) < 10%].

A beneficial effect of high endomyocardial NO activity on diastolic LV distensibility of the

cardiomyopathic heart could result not only from NO-induced phosphorylation of troponin I and a concomitant reduction of diastolic crossbridge cycling but also from prevention of endomyocardial fibrosis. Chronic inhibition of NO synthesis has indeed been demonstrated to induce progressive myocardial fibrosis through a signaling cascade involving endothelin, angiotensin II, aldosterone and transforming growth factor β. A recent study looked at the interaction between endomyocardial NOS gene expression and fibrosis in patients with dilated cardiomyopathy (4).

This study found no correlation between endomyocardial NOS mRNA and collagen volume fraction and observed additive but opposite effects of intensity of NOS gene expression and of fibrosis on diastolic LV stiffness(Figure 2). The lack of correlation between NOS expression and collagen does not exclude involvement of NOS in the development of myocardial fibrosis in dilated cardiomyopathy but suggests excessive deposition of collagen in cardiomyopathic hearts to result more from upregulation of stimulatory pathways such as endothelin, angiotensin II or aldosterone than from down regulation of inhibitory pathways such as NO or natriuretic peptides.

NO's control of diastole and energetics: Two of a kind?

The altered energetics of failing myocardium are characterized by 1) reduced creatine kinase activity and phosphocreatine levels, 2) loss of control of myocardial mitochondrial respiration leading to excessive oxygen consumption and 3) a switch in preferential myocardial substrate utilization from free fatty acids to glucose. Apart from creatine kinase activity (13), NO appears to correct all these derangements of myocardial energetics(49). NO can bind to heme moieties of proteins involved in mitochondrial respiration. Reduced inhibition of these enzymes explains the higher myocardial oxygen consumption in conscious dogs during NOS inhibition(45) and in terminal stages of experimental(51) and clinical heart failure(25), which are characterized by low cardiac NO production(18,40). In failing human myocardium, this excessive myocardial oxygen consumption reacted favorably to ACE inhibitor, amlodipine or neutral endopeptidase inhibitor(25) all of which are known to raise myocardial NO content through inhibition of kinin degradation. In pacing-induced heart failure, at the time of transition to decompensation, a drop in myocardial NO production is observed, which coincides with a switch in myocardial substrate utilization from free fatty acids to glucose(40). A similar switch was also observed in transgenic mice with absent NOS gene expression(48). The altered energetics of failing myocardium are paralleled by a shift of myofilamentary gene expression towards isoforms with higher calcium sensitivity and lower ATPase activity. This shift in gene expression enhances contractile efficiency of failing myocardium even in the presence of a deranged oxygen consumption(21). In the human cardiomyopathic heart, administration of L-NMMA does not affect its efficiency(46) despite the augmented LV contractile response to β-adrenergic stimulation.

NO's effects on LV contractile performance appear to be synergistic with its effects on

energetics through prevention of inappropriate contractile augmentation in the setting of reperfusion, through prevention of myocardial energy wastage induced by LV contraction against late-systolic reflected arterial pressure waves and through prevention of diastolic LV stiffening, which preserves adequate subendocardial coronary perfusion.

In NOS3 knock-out mice subjected to 30 minutes of global ischemia followed by reperfusion, a paradoxical increase in NO production was observed because of superinduction of NOS2(22). The accompanying increase in NO's myofilamentary desensitizing action reduced the hyper dynamic myocardial contractile response characteristic of early reperfusion. This protected the reperfused heart because of reduced myocardial energy demand at a time of low energy availability. Similar NO-mediated flow-metabolism-function matching was also observed in the high oxygen demand model of pacing-induced heart failure(30). In the early pacing period, when myocardial NO production and NOS2 gene expression were high, there were parallel reductions in coronary blood flow, myocardial oxygen consumption and LVdP/dt max. In a later phase, when myocardial NO production and NOS2 gene expression declined, flow-metabolism-function matching was disrupted because of a continuing fall in LVdP/dt max despite higher coronary blood flow and myocardial oxygen consumption. Using the same model, other investigators had previously observed a reduction in diastolic LV distensibility as evident from a steep rise in LVEDP, when myocardial NO production started to decline(40). Taken together, these three studies suggest NO to be responsible for myocardial flow-metabolism-function matching through modulation of LV preload reserve. NO-induced prevention of reperfusion-related LV dysfunction was also evident in-vitro from the NO-conferred protection of isolated cardiomyocytes against reoxygenation contracture (43).

In the human heart, intracoronary infusions of NO-donors or of substance P revealed a NO-induced reduction in late-systolic LV pressure generation because of earlier onset of LV isometric relaxation(Figure 1b)(35,36). This reduction in late-systolic LV pressure generation corresponded with a reduced LV contractile effort against reflected arterial pressure waves. This reduced LV contractile effort prevented myocardial energy wastage induced by late-systolic afterload augmentation . These beneficial effects of NO on myocardial contractile performance and energetics are in concert with NO's well established effects on the vasculature. A high vascular NO activity induces arteriolar vasodilation and improves arterial distensibility, both of which reduce magnitude and traveling speed of reflected arterial pressure waves. A high myocardial NO activity provides appropriate timing of isometric LV relaxation, thereby preventing an amplifying effect elicited by late-systolic LV contraction against the reflected arterial pressure wave(31).

Myocardial mechanical deformation during systole is an important stimulus for NO release by cardiac endothelial cells(24). Using an intramyocardial porphyrinic NO sensor, beat-to-beat release of NO was recorded in the rabbit heart with a brisk rise at end-systole to optimally hasten myocardial relaxation and to lower LV filling pressures(38). The latter effects are beneficial for diastolic coronary perfusion especially at higher heart rates when there is a disproportionally

larger reduction in diastolic coronary perfusion period. In the same preparation, an increase in LV preload was accompanied by a higher beat-to-beat release of NO allowing for a larger desensitizing effect of NO counteracting the preload-induced augmentation of myofilamentary calcium sensitivity. When preload rises in the setting of contractile dysfunction, as occurs in failing myocardium, systolic mechanical deformation and the NO transient will fail to increase leaving preload-induced augmentation of myofilamentary calcium sensitivity unopposed. Because of induction of the fetal gene program, failing myocardium already expresses myofilamentary isoforms with increased calcium sensitivity. This isoform shift, a high LV preload and a low NO transient will all cause additive augmentation of myofilamentary calcium sensitivity and can therefore predispose failing myocardium to slow relaxation kinetics and diastolic crossbridge cycling, both of which compromise diastolic coronary perfusion and myocardial oxygen supply. In such a setting, the expression of NOS2, which produces NO independently of mechanical deformation, could be beneficial because it would interrupt the vicious circle of reduced mechanical deformation, reduced NOS3-dependent NO production and deranged diastolic LV function(18) and energetics(9).

Conclusions

In heart failure patients, beneficial effects of NO on diastolic LV function always overrode a small NO-induced attenuation of LV developed pressure in terms of overall hemodynamic status, either at baseline or following β-adrenergic stimulation. The absence of hemodynamic deterioration in transgenic mice overexpressing either myocardial NOS2 or NOS3 confirms these clinical observations. In failing myocardium, NO's correction of diastolic LV dysfunction reinforces NO's energy sparing effects and the concerted action of NO on both diastolic LV dysfunction and deranged energetics could well be instrumental for preventing relentless deterioration of failing myocardium.

References

1. **Balligand JL, Kelly RA, Marsden PA, Smith TW, and Michel T**. Control of cardiac muscle cell function by an endogenous nitric oxide signaling system. *Proc Natl Acad Sci U S A* 90: 347-351, 1993.
2. **Bernstein RD, Ochoa FY, Xu X, Forfia P, Shen W, Thompson CI, and Hintze TH**. Function and production of nitric oxide in the coronary circulation of the conscious dog during exercise. *Circ Res* 79: 840-848, 1996.
3. **Brady AJ, Warren JB, Poole-Wilson PA, Williams TJ, and Harding SE**. Nitric oxide attenuates cardiac myocyte contraction. *Am J Physiol* 265: H176-H182, 1993.
4. **Bronzwaer JG, Heymes C, Visser CA, and Paulus WJ**. Myocardial fibrosis blunts nitric oxide

synthase-related preload reserve in human dilated cardiomyopathy. *Am J Physiol Heart Circ Physiol* 284: H10-H16, 2003.

5. **Bronzwaer JG, Zeitz C, Visser CA, and Paulus WJ**. Endomyocardial nitric oxide synthase and the hemodynamic phenotypes of human dilated cardiomyopathy and of athlete's heart. *Cardiovasc Res* 55: 270-278, 2002.
6. **Brunner F, Andrew P, Wolkart G, Zechner R, and Mayer B**. Myocardial contractile function and heart rate in mice with myocyte-specific overexpression of endothelial nitric oxide synthase. *Circulation* 104: 3097-3102, 2001.
7. **Cotton JM, Kearney MT, MacCarthy PA, Grocott-Mason RM, McClean DR, Heymes C, Richardson PJ, and Shah AM**. Effects of nitric oxide synthase inhibition on Basal function and the force-frequency relationship in the normal and failing human heart in vivo. *Circulation* 104: 2318-2323, 2001.
8. **Cotton JM, Kearney MT, and Shah AM**. Nitric oxide and myocardial function in heart failure: friend or foe? *Heart* 88: 564-566, 2002.
9. **Dai L, Brookes PS, Darley-Usmar VM, and Anderson PG**. Bioenergetics in cardiac hypertrophy: mitochondrial respiration as a pathological target of NO*. *Am J Physiol Heart Circ Physiol* 281: H2261-H2269, 2001.
10. **de Belder AJ, Radomski MW, Why HJ, Richardson PJ, Bucknall CA, Salas E, Martin JF, and Moncada S**. Nitric oxide synthase activities in human myocardium. *Lancet* 341: 84-85, 1993.
11. **Drexler H**. Nitric oxide synthases in the failing human heart: a doubled-edged sword? *Circulation* 99: 2972-2975, 1999.
12. **Gealekman O, Abassi Z, Rubinstein I, Winaver J, and Binah O**. Role of myocardial inducible nitric oxide synthase in contractile dysfunction and beta-adrenergic hyporesponsiveness in rats with experimental volume-overload heart failure. *Circulation* 105: 236-243, 2002.
13. **Gross WL, Bak MI, Ingwall JS, Arstall MA, Smith TW, Balligand JL, and Kelly RA**. Nitric oxide inhibits creatine kinase and regulates rat heart contractile reserve. *Proc Natl Acad Sci U S A* 93: 5604-5609, 1996.
14. **Hare JM, Givertz MM, Creager MA, and Colucci WS**. Increased sensitivity to nitric oxide synthase inhibition in patients with heart failure: potentiation of beta-adrenergic inotropic responsiveness. *Circulation* 97: 161-166, 1998.
15. **Hare JM, and Stamler JS**. NOS: modulator, not mediator of cardiac performance. *Nat Med* 5: 273-274, 1999.
16. **Haywood GA, Tsao PS, der Leyen HE, Mann MJ, Keeling PJ, Trindade PT, Lewis NP, Byrne CD, Rickenbacher PR, Bishopric NH, Cooke JP, McKenna WJ, and Fowler MB**. Expression of inducible nitric oxide synthase in human heart failure. *Circulation* 93: 1087-1094, 1996.
17. **Heger J, Godecke A, Flogel U, Merx MW, Molojavyi A, Kuhn-Velten WN, and Schrader J**. Cardiac-specific overexpression of inducible nitric oxide synthase does not result in severe cardiac dysfunction. *Circ Res* 90: 93-99, 2002.
18. **Heymes C, Vanderheyden M, Bronzwaer JG, Shah AM, and Paulus WJ**. Endomyocardial nitric oxide synthase and left ventricular preload reserve in dilated cardiomyopathy. *Circulation* 99: 3009-3016, 1999.
19. **Higginbotham MB, Sullivan MJ, Coleman RE, and Cobb FR**. Regulation of stroke volume during exercise in patients with severe left ventricular dysfunction: importance of the Starling mechanism. *J Am Coll Cardiol* 9, 58**A**. 1987.
20. **Ito N, Bartunek J, Spitzer KW, and Lorell BH**. Effects of the nitric oxide donor sodium nitroprusside on intracellular pH and contraction in hypertrophied myocytes. *Circulation* 95: 2303-2311, 1997.

21. **Kameyama T, Chen Z, Bell SP, Vanburen P, Maughan D, and LeWinter MM**. Mechanoenergetic alterations during the transition from cardiac hypertrophy to failure in Dahl salt-sensitive rats. *Circulation* 98: 2919-2929, 1998.
22. **Kanno S, Lee PC, Zhang Y, Ho C, Griffith BP, Shears LL, and Billiar TR**. Attenuation of myocardial ischemia/reperfusion injury by superinduction of inducible nitric oxide synthase. *Circulation* 101: 2742-2748, 2000.
23. **Kojda G, Kottenberg K, Nix P, Schluter KD, Piper HM, and Noack E**. Low increase in cGMP induced by organic nitrates and nitrovasodilators improves contractile response of rat ventricular myocytes. *Circ Res* 78: 91-101, 1996.
24. **Lamontagne D, Pohl U, and Busse R**. Mechanical deformation of vessel wall and shear stress determine the basal release of endothelium-derived relaxing factor in the intact rabbit coronary vascular bed. *Circ Res* 70: 123-130, 1992.
25. **Loke KE, Laycock SK, Mital S, Wolin MS, Bernstein R, Oz M, Addonizio L, Kaley G, and Hintze TH**. Nitric oxide modulates mitochondrial respiration in failing human heart. *Circulation* 100: 1291-1297, 1999.
26. **Matsubara BB, Matsubara LS, Zornoff LA, Franco M, and Janicki JS**. Left ventricular adaptation to chronic pressure overload induced by inhibition of nitric oxide synthase in rats. *Basic Res Cardiol* 93: 173-181, 1998.
27. **Matter CM, Mandinov L, Kaufmann PA, Vassalli G, Jiang Z, and Hess OM**. Effect of NO donors on LV diastolic function in patients with severe pressure-overload hypertrophy. *Circulation* 99: 2396-2401, 1999.
28. **McNamara DM, Postova L, Holubkov R, Janosko K, MacGowan GA, Murali S, Mathier M, and London B**. Asp298 variant of endothelial nitric oxide synthase and heart failure survival: comparison of ischemic and nonischemic cardiomyopathy. *Circulation* 106, II-328**A**. 2002.
29. **Mohan P, Brutsaert DL, Paulus WJ, and Sys SU**. Myocardial contractile response to nitric oxide and cGMP. *Circulation* 93: 1223-1229, 1996.
30. **Nikolaidis LA, Hentosz T, Doverspike A, Huerbin R, Stolarski C, Shen YT, and Shannon RP**. Mechanisms whereby rapid RV pacing causes LV dysfunction: perfusion-contraction matching and NO. *Am J Physiol Heart Circ Physiol* 281: H2270-H2281, 2001.
31. **O'Rourke MF, and Mancia G**. Arterial stiffness. *J Hypertens* 17: 1-4, 1999.
32. **Paulus WJ**. The role of nitric oxide in the failing heart. *Heart Fail Rev* 6: 105-118, 2001.
33. **Paulus WJ, Frantz S, and Kelly RA**. Nitric oxide and cardiac contractility in human heart failure: time for reappraisal. *Circulation* 104: 2260-2262, 2001.
34. **Paulus WJ, and Shah AM**. NO and cardiac diastolic function. *Cardiovasc Res* 43: 595-606, 1999.
35. **Paulus WJ, Vantrimpont PJ, and Shah AM**. Acute effects of nitric oxide on left ventricular relaxation and diastolic distensibility in humans. Assessment by bicoronary sodium nitroprusside infusion. *Circulation* 89: 2070-2078, 1994.
36. **Paulus WJ, Vantrimpont PJ, and Shah AM**. Paracrine coronary endothelial control of left ventricular function in humans. *Circulation* 92: 2119-2126, 1995.
37. **Pinamonti B, Di Lenarda A, Sinagra G, and Camerini F**. Restrictive left ventricular filling pattern in dilated cardiomyopathy assessed by Doppler echocardiography: clinical, echocardiographic and hemodynamic correlations and prognostic implications. Heart Muscle Disease Study Group. *J Am Coll Cardiol* 22: 808-815, 1993.
38. **Pinsky DJ, Patton S, Mesaros S, Brovkovych V, Kubaszewski E, Grunfeld S, and Malinski T**. Mechanical transduction of nitric oxide synthesis in the beating heart. *Circ Res* 81: 372-379, 1997.
39. **Prendergast BD, Sagach VF, and Shah AM**. Basal release of nitric oxide augments the Frank-Starling

response in the isolated heart. *Circulation* 96: 1320-1329, 1997.

40. **Recchia FA, McConnell PI, Bernstein RD, Vogel TR, Xu X, and Hintze TH**. Reduced nitric oxide production and altered myocardial metabolism during the decompensation of pacing-induced heart failure in the conscious dog. *Circ Res* 83: 969-979, 1998.
41. **Scherrer-Crosbie M, Ullrich R, Bloch KD, Nakajima H, Nasseri B, Aretz HT, Lindsey ML, Vancon AC, Huang PL, Lee RT, Zapol WM, and Picard MH**. Endothelial nitric oxide synthase limits left ventricular remodeling after myocardial infarction in mice. *Circulation* 104: 1286-1291, 2001.
42. **Sessa WC, Pritchard K, Seyedi N, Wang J, and Hintze TH**. Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. *Circ Res* 74: 349-353, 1994.
43. **Shah AM, Silverman HS, Griffiths EJ, Spurgeon HA, and Lakatta EG**. cGMP prevents delayed relaxation at reoxygenation after brief hypoxia in isolated cardiac myocytes. *Am J Physiol* 268: H2396-H2404, 1995.
44. **Shah AM, Spurgeon HA, Sollott SJ, Talo A, and Lakatta EG**. 8-bromo-cGMP reduces the myofilament response to Ca2+ in intact cardiac myocytes. *Circ Res* 74: 970-978, 1994.
45. **Shen W, Xu X, Ochoa M, Zhao G, Wolin MS, and Hintze TH**. Role of nitric oxide in the regulation of oxygen consumption in conscious dogs. *Circ Res* 75: 1086-1095, 1994.
46. **Shinke T, Takaoka H, Takeuchi M, Hata K, Kawai H, Okubo H, Kijima Y, Murata T, and Yokoyama M**. Nitric oxide spares myocardial oxygen consumption through attenuation of contractile response to beta-adrenergic stimulation in patients with idiopathic dilated cardiomyopathy. *Circulation* 101: 1925-1930, 2000.
47. **Smith JA, Shah AM, and Lewis MJ**. Factors released from endocardium of the ferret and pig modulate myocardial contraction. *J Physiol* 439: 1-14, 1991.
48. **Tada H, Thompson CI, Recchia FA, Loke KE, Ochoa M, Smith CJ, Shesely EG, Kaley G, and Hintze TH**. Myocardial glucose uptake is regulated by nitric oxide via endothelial nitric oxide synthase in Langendorff mouse heart. *Circ Res* 86: 270-274, 2000.
49. **Trochu JN, Bouhour JB, Kaley G, and Hintze TH**. Role of endothelium-derived nitric oxide in the regulation of cardiac oxygen metabolism: implications in health and disease. *Circ Res* 87: 1108-1117, 2000.
50. **Wittstein IS, Kass DA, Pak PH, Maughan WL, Fetics B, and Hare JM**. Cardiac nitric oxide production due to angiotensin-converting enzyme inhibition decreases beta-adrenergic myocardial contractility in patients with dilated cardiomyopathy. *J Am Coll Cardiol* 38: 429-435, 2001.
51. **Xie YW, Shen W, Zhao G, Xu X, Wolin MS, and Hintze TH**. Role of endothelium-derived nitric oxide in the modulation of canine myocardial mitochondrial respiration in vitro. Implications for the development of heart failure. *Circ Res* 79: 381-387, 1996.

Chapter 5

Discussion

Diastolic Left Ventricular Dysfunction: Old Questions Resurface

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Submitted to Circulation Research

Introduction

The increasing incidence in aging Western societies of patients with documented congestive heart failure and a normal left ventricular(LV) ejection fraction(EF) justifies the recent surge of interest in diastolic LV dysfunction as a cause of heart failure (1,2,11,31,62,72,81-83). Mainly noninvasive imaging techniques, especially tissue Doppler imaging(49,65,79), and the use of plasma B-type natriuretic peptide(BNP)(40) are currently advancing our knowledge on diastolic LV dysfunction in heart failure. From these recent observations some questions resurface, which are similar to questions asked in the early eighties, when investigators used mainly invasive techniques to construct diastolic LV pressure-volume relations in order to understand the pathophysiology of diastolic LV dysfunction. It therefore seems timely to readdress some of these questions and to confront the new, noninvasive observations with the old framework of invasively acquired diastolic LV pressure-volume relations. Some of the questions on diastolic LV dysfunction, that have recently reemerged, are:

1. Is diastolic LV dysfunction secondary to systolic LV dysfunction?
2. Can transient elevations in LV loading induce diastolic LV dysfunction?
3. Can diastolic LV dysfunction result from heightened active diastolic cardiac muscle tone?

Is diastolic LV dysfunction secondary to systolic LV dysfunction?

In patients with a history of heart failure and a normal LVEF (EF>50%), refined Dopplerechocardiographic studies recently revealed significant depression of long axis systolic shortening(9,78) and of regional myocardial systolic velocity(79). Regional myocardial systolic velocity was significantly lower in patients with diastolic heart failure (4.6±1.3 cm/s) than in patients with diastolic LV dysfunction (5.4±1.0 cm/s) and this finding suggested worse systolic LV dysfunction, albeit subtle and still without effect on global LVEF, to significantly contribute to the appearance of heart failure symptoms even in patients labeled as having isolated diastolic heart failure because of a LVEF>50%. In accordance to the paradigm proposed by these studies, heart failure always results from a sequence of events, which starts with an afterload or tachycardia-induced(29) reduction in LV systolic performance. This forces the left ventricle to operate at a larger LV end-diastolic volume(LVEDV) to maintain LV stroke volume. In the presence of a steep or near vertical diastolic LV pressure-volume relation, a larger LVEDV leads to a substantial rise in LV end-diastolic pressure(LVEDP) and appearance of heart failure symptoms. Essential for the validity of this paradigm is the presence during the heart failure episode of a larger LVEDV and of concordant changes in LVEDV and LVEDP, with a higher LVEDP always occurring at a larger LVEDV. Several clinical and experimental studies looking at LV performance during acute heart failure episodes however failed to satisfy these criteria:

1) During an episode of acute pulmonary edema associated with arterial hypertension, the LVEDV(109±43 ml) was not different from the LVEDV(117±50 ml) observed one to three days later at the time of recompensation(19).
2) A clinical study(5), which looked at LV function during acute ischemia-induced heart failure episodes observed discordant changes in LVEDV and LVEDP when comparing in the same patient the hemodynamic effects of pacing-induced and balloon-occlusion ischemia. In this study, 7 minutes of pacing tachycardia in the presence of single vessel proximal left anterior descending coronary artery stenosis resulted in a significantly smaller LVEDV (LVEDVI=88±17 ml/m^2) despite higher LVEDP(24±7 mmHg) than 60 seconds of balloon coronary occlusion(LVEDVI=96±16 ml/m^2; LVEDP=21±8 mmHg)(Figure 1). In a similar clinical protocol(12), 60seconds of balloon coronary occlusion with hypoxemic perfusion distal to the occlusion also resulted in a slightly smaller LVEDVI (78±14 ml/m^2) at a higher LVEDP (34±7 mmHg) when compared to 60 seconds of regular balloon coronary occlusion (LVEDVI=79±15 ml/m^2;LVEDP=23±6 mmHg).
3) In anaesthetized dogs with coronary stenosis on both proximal left anterior descending and left circumflex coronary arteries(56), 3 minutes of pacing tachycardia resulted in significant increases in LV end-diastolic segment length (from 16.8±0.9 to 17.5±0.9 mm) and in LVEDP(from 9±1 to 15±1 mmHg). The increase in LV end-diastolic segment length was no longer present despite a tripling of LVEDP(from 9±1 to 27±2 mmHg) after 3 minutes of pacing tachycardia with an intravenous infusion of caffeine. Caffeine induces myocardial cytosolic calcium overload and potentiates hypoxic cardiac muscle contracture.

The constancy during acute hypertensive pulmonary edema of LVEDV and the discordant changes during acute ischemia-induced heart failure episodes of LVEDV and LVEDP, with the smaller LVEDV being observed at the higher LVEDP, argue against systolic LV dysfunction and a concomitant outward displacement of the LVEDP-LVEDV point along a single steep diastolic LV pressure-volume relation to be the unique mechanism underlying all heart failure episodes. The hemodynamic changes observed during acute pulmonary edema associated with both arterial hypertension or demand-ischemia support the existence during some acute heart failure episodes of primary diastolic LV dysfunction, which corresponds to an acute and reversible upward displacement of the diastolic LV pressure-volume relationship(Figure 1).

The opposite type of discordant changes of LVEDV and LVEDP (i.e. a larger LVEDV occurring at a lower LVEDP) was recently also reported in dilated cardiomyopathy patients during intracoronary infusion of substance P, which acutely released NO from the coronary endothelium(23). In this study, intracoronary substance P induced a fall in LVEDP from 25±3 to18±2 mmHg($p<0.0001$) and a simultaneous discordant rise in LVEDV from 220±26 to 240±29ml($p<0.05$) because of a reversible downward and rightward displacement of the LV diastolic pressure-volume relation(7). A similar fall in LVEDP without change in LVEDV was

also observed in dilated cardiomyopathy patients during intracoronary infusion of enalaprilat. These patients had been pretreated with an angiotensin-1 receptor antagonist to expose effects of enalaprilat on bradykinin degradation and on myocardial NO availability(75). Discordant changes of LVEDP and LVEDV (i.e. a fall in LVEDP accompanying a rise in LVEDV) have occasionally even been observed during intravenous administration of NO-donors despite the venous pooling induced by the systemic administration route(32).

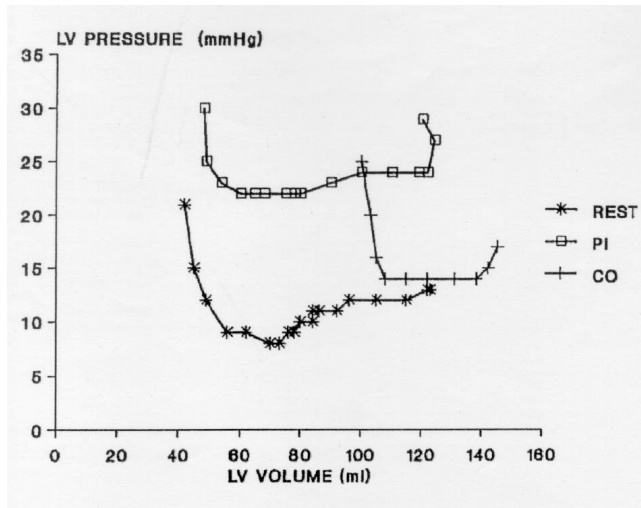

Figure 1. Discordant changes of LVEDV and LVEDP during acute ischemic heart failure episodes. In a patient with single vessel LAD coronary stenosis, pacing-induced angina(PI) results in a smaller LVEDV than 1-minute of balloon coronary occlusion(CO) despite higher LVEDP during PI than CO(5). The discordant changes of LVEDV and LVEDP during PI are inconsistent with a unique and fixed diastolic LV pressure-volume relation and support reversible upward displacement of the diastolic LV pressure-volume relation during an acute ischemic heart failure episode of PI.

Can transient elevations in LV loading induce diastolic LV dysfunction?

A recent clinical report(19) of an hypertensive episode inducing acute pulmonary edema without Doppler echocardiographic evidence of reduced LV systolic performance or of increased mitral regurgitation, revived scientific interest for the effects of high LV afterload on diastolic LV function(8,30). In animal experiments, a rise in LV afterload by vasopressor infusion or by mechanical occlusion of the aorta slowed isovolumic LV pressure decline(16,28). This slowing was especially evident with late-systolic loading increments(24,37,80). A late systolic LV loading increment elevates myocardial wall stress at a time when calcium reuptake into the SR is

completed and when no recruitment of additional cross-bridges can occur. A late systolic LV loading increment therefore increases individual crossbridge load, which slows cross-bridge cycling and decelerates LV pressure decay. In the aging human heart and especially in patients with arterial hypertension, LV systolic pressure and LV systolic wall stress always peak in late systole because of the presence of prominent arterial wave reflections(48). This loading pattern renders the aging and hypertensive human heart especially susceptible to afterload-induced deceleration of isovolumic LV relaxation. This was nicely demonstrated by Kawaguchi et al.(30) in patients with arterial hypertension, normal systolic function and a history of heart failure, who were exercised in the cardiac catheterization laboratory. Sustained isometric handgrip exercise induced a rise of LV end-systolic pressure to an average level of 201±12 mmHg and a rise in the time constant of isovolumic LV pressure decay (τ) to an average value of 86±23 ms, which corresponds to profound slowing of LV isovolumic relaxation kinetics [normal value of τ=36±6 ms(54)].

The slowing of LV isovolumic relaxation kinetics during exercise, also implies a blunting of the lusitropic actions of ß-adrenoreceptor stimulation in these patients. Load-induced blunting of the lusitropic actions of ß-adrenoreceptor stimulation has previously been reported in the human allograft(52). In transplant recipients, LVEDP rises during the initial stages of exercise because of a mismatch between venous return and chronotropic response. At later stages of exercise, both heart rate and LV dP/dt max catch up due to high levels of circulating catecholamines but τ fails to abbreviate. Even pretreatment with dobutamine does not correct this lusitropic deficiency of the transplanted heart during exercise(69). Moreover, the close correlation during exercise(51) between LVEDP and τ suggest the initial rise in LVEDP to actually counteract subsequent ß-adrenoreceptor induced acceleration of myocardial relaxation kinetics. A similar interaction had previously been reported in isolated papillary muscle strips, in whom a stretch from 95% lmax to lmax effectively reduced the acceleration of isometric tension decay during ß-adrenergic stimulation(10,43). Hence, not only excessive elevation of late-systolic load but also preload-induced blunting of the lusitropic response to ß-adrenoreceptor stimulation, could have contributed to the exercise-induced prolongation of τ in hypertensive patients as these patients also developed a large rise in LVEDP (to a value of 32±9 mmHg) during exercise(30).

Although an isolated rise in LV preload does not affect isovolumic LV pressure decay in an anesthetized dog model(17) or in man(71), a higher LV preload potentiates afterload-induced slowing of isovolumic LV pressure decay. This was recently demonstrated in an in-vivo rabbit model using single beat aortic cross-clamps(36). In this model, a high LV afterload prolonged τ and shifted the diastolic LV pressure-volume relation slightly upward(Figure 2). These effects were markedly potentiated by a concomitant elevation of LV preload. Furthermore, the value of LVEDP of the diastole following the cross-clamped beat largely exceeded the value predicted by extrapolating the exponential LV pressure decay using the prolonged τ. The latter finding argued in favor of concomitant overload-induced diastolic crossbridge cycling, which occurred simultaneously with the slow crossbridge detachment responsible for the prolonged isovolumic

relaxation. From these clinical and experimental observations, the importance of preload elevation for afterload-induced diastolic LV dysfunction becomes evident. Preload elevation causes an additional increase in myofilamentary calcium sensitivity and this contributes to diastolic LV dysfunction by: 1) abolishing PKA or PKG-induced acceleration of LV relaxation kinetics; 2) by further slowing LV isovolumic relaxation under conditions of elevated end-systolic afterload and 3) and by promoting diastolic crossbridge cycling thereby inducing rises in LV filling pressures, which exceed residual LV relaxation pressure.

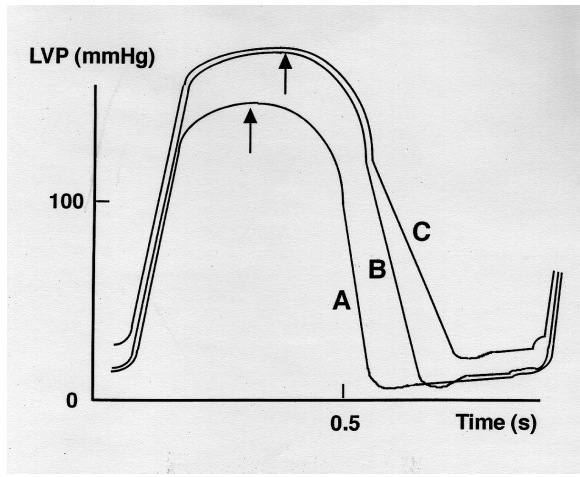

Figure 2. Schematic diagram of effects of LV afterload and LV preload on diastolic LV function(36). A rise in LV afterload(B), if it augments late-systolic LV load(see shift of arrow), slows isovolumic LV pressure decay. A concomitant elevation in LV preload(C) further slows isovolumic LV pressure decay and raises LVEDP beyond the originally imposed preload elevation.

The following sequence of events could underlie hypertension-induced pulmonary edema in patients with normal LV systolic function:

1) The LV mass-volume ratio(2.12±0.14 g/ml) of these patients is almost double the value observed in systolic heart failure(1.22±0.14 g/ml)(31).Because of this myocardial hypertrophy, the diastolic LV pressure-volume relation is steeper than normal and forces the LV to operate under basal conditions at a higher LVEDP(84). Furthermore, their basal LVEDP has also been shown to be higher than in patients with arterial hypertension, normal systolic function and no heart failure history(30).
2) These patients also have a very steep arterial elastance (slope of the ESP/SV relation)(30). A rise in arterial impedance during a bout of arterial hypertension will therefore induce a huge rise in LV end-systolic pressure and end-systolic wall stress.
3) A huge rise in LV end-systolic pressure in the presence of an elevated LVEDP

profoundly slows isovolumic relaxation kinetics and causes a further rise of LVEDP(36), which even exceeds the value predicted from an extrapolation of the slow isovolumic LV pressure decay. This further rise in LVEDP sets off a vicious circle of additional elevation of LV preload eventually leading to flash pulmonary edema.

4) Because of the steep arterial elastance relation, there will only be minimal changes in LVSV and therefore also in LVEDV and LVESV. Constancy of LV volumes during an episode of pulmonary edema has indeed been observed in patients with arterial hypertension and normal LV systolic function(19)

A prolongation of isovolumic LV pressure decay was always considered not to affect the mid- and late-diastolic LV filling pressures because in a normal human heart [with a time constant of isovolumic LV pressure decay(τ) = 36±6 ms] LV pressure generated by decaying LV contractile interaction would amount to less than 2 mmHg once 3.5 times τ or 126 ms had elapsed after closure of the aortic valve. This reasoning supposed LV pressure decay to proceed along the same mono exponential curve during the LV filling phase and led to calculation of passive early-diastolic LV pressure by subtracting extrapolated LV relaxation pressure from the measured early-diastolic LV pressure(50). Experimental proof validating this concept is however lacking. In isolated papillary muscles, residual isometric force decay after isotonic reextension was determined by the load during reextension(67). For the filling ventricle, this finding implied the persistence during the LV filling phase of a level of active myocardial force corresponding to the wall stress observed at mitral valve opening and predicted decay of active myocardial force extending well into mid- and late-diastole. The same findings had also been observed in anesthetized open-chest dogs with a mitral annulus occluder. In this preparation, LV filling substantially slowed LV pressure decay, which reached a value less than 2 mmHg not after 3.5 times τ but after 5.4 times τ(47). No data exist on mid- and late diastolic LV relaxation pressure decay in the filling human LV but in non-filling beats during inflation of the self-positioning Inoue balloon at the time of percutaneous mitral valvuloplasty the observed LV end-diastolic pressure (2±4 mmHg) significantly exceeded the value extrapolated from a mono exponential curve using the time constant of LV pressure decay (-9±11 mmHg; $p<0.001$)(59). This observation likewise suggested significant slowing of active myocardial force decay in the human heart with residual persistence of active myocardial force and of active crossbridge cycling during mid- and late-diastole.

In the human heart, modest changes in LV afterload had no effect on isovolumic LV relaxation(13,66) but a drastic reduction in LV afterload, as imposed in patients with aortic stenosis by a sequence of balloon aortic valvuloplasty and sodium nitroprusside infusion markedly slowed isovolumic LV pressure decay(55). These successive interventions resulted in a threefold reduction of LV end-systolic wall stress (from 90 ± 30.10^3 to 26 ± 6.10^3 dyne/cm^2) and a marked abbreviation of LV contraction (Figure 3). This abbreviation of LV contraction made the onset of LV isovolumic relaxation probably coincide with the terminal phase of myocardial cytosolic calcium reuptake, thereby allowing for some crossbridge reattachment, which could

explain the considerable slowing of isovolumic LV pressure decay. Apart from slowing, LV pressure decay also became nonexponential as evident from the biphasic appearance of the negative dP/dt signal (Figure 3). A similarly slow, nonexponential LV pressure decay and a biphasic negative dP/dt signal were later reported in a mouse model of hypertrophic cardiomyopathy(αMHC403/+)(20). Despite profound slowing of τ in both aortic stenosis patients following drastic unloading(73±23 ms) and in hypertensives following excessive loading during exercise(86±23 ms)(30), the aortic stenosis patients lowered their LVEDP from 23±8 to 14±8mmHg but the hypertensives raised their LVEDP from 24±5 to 32±9 mmHg. These opposite effects on LVEDP are consistent with the different effects of both loading interventions on myofilamentary calcium sensitivity, which gets downregulated by unloading but upregulated by

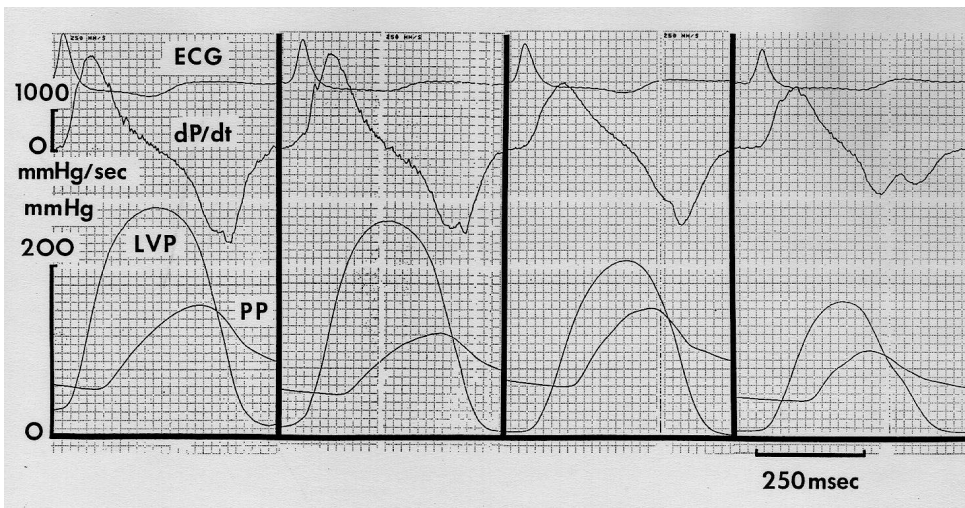

Figure 3. LV pressure(LVP), peripheral artery pressure(PP) and LV dP/dt in a patient with aortic stenosis in control conditions, in control conditions during nitroprusside infusion, after balloon aortic valvuloplasty and after balloon aortic valvuloplasty during nitroprusside infusion(55). After drastic unloading of balloon aortic valvuloplasty and nitroprusside infusion, there is marked abbreviation of LV contraction and profound slowing of isovolumic LV pressure decay. In contrast to figure 2, the slowing of isovolumic LV relaxation is accompanied by a fall in LVEDP.

loading. Hence, load-induced systolic upregulation of myofilamentary calcium sensitivity and not slowing of LV isovolumic relaxation per se appears to be essential for the induction of diastolic LV dysfunction, which therefore probably results from persistent diastolic crossbridge cycling because of upregulated myofilamentary calcium sensitivity and not from continuing slow LV relaxation pressure decay.

Can diastolic LV dysfunction result from a heightened active diastolic cardiac muscle tone?

The existence of active diastolic cardiac muscle tone had been the subject of intense clinical and experimental research in the eighties and recently resurfaced as a potential mechanism of flash pulmonary edema in the overloaded hypertensive heart(19). Active diastolic cardiac muscle tone was especially looked for in myocardial ischemia and in myocardial hypertrophy(18,53).

In Krebs-perfused isovolumic guinea-pig hearts, a switch from aerobic to hypoxic perfusion at constant perfusion pressure induced within a 5-minute period a fall in developed tension and a rise in resting tension, which under isovolumic conditions corresponded to a drop in LV distensibility(46). In blood perfused isovolumic rabbit hearts, pacing tachycardia superimposed on global low-flow ischemia simulated human demand angina and also resulted in a rise in resting tension and a drop in LV distensibility(26). A drop in LV distensibility, evident from an upward shift of the diastolic LV pressure-volume relation had previously been reported following an atrial pacing stress test in patients with multivessel coronary disease(3) and in anaesthetized dogs with two vessel coronary stenosis(63). In this last model, intravenous administration of caffeine during the pacing stress test, greatly enhanced the upward shift of the diastolic LV pressure-volume relation and suggested diastolic crossbridge cycling to be the underlying mechanism because of caffeine's known effects on myoplasmic calcium reuptake and on myofilamentary calcium sensitivity(56). Recent experiments in the aforementioned isovolumic blood-perfused rabbit heart however rebutted the idea of calcium overload as the mechanism underlying diastolic crossbridge cycling. These experiments suggested ischemia to induce reversible crossbridge-rigor bounds because quick stretches, which disrupt rigor bounds, and not a calcium desensitizer (butanedionemonoxime) corrected the ischemia-induced drops in diastolic LV distensibility(70). A large drop in diastolic LV distensibility was also observed in the human heart during simulated hypoxemia created by maintaining distal hypoxic coronary perfusion at the time of balloon coronary angioplasty(12).

Clinical observations consistent with deranged LV diastolic function caused by elevated active diastolic cardiac muscle tone were also obtained in LV hypertrophy caused by either hypertrophic cardiomyopathy or aortic stenosis(39,58). In some patients with hypertrophic cardiomyopathy, both high-fidelity tip-micromanometer LV and pulmonary capillary wedge pressure recordings revealed a unique diastolic LV pressure waveform with continuous LV pressure decline throughout diastole and minimum diastolic LV pressure occurring just prior to the onset of the a-wave of the subsequent beat(Figure 4). Continuous LV pressure decline at a moment when the LV cavity actually expands is incompatible with passive LV distension and implies the presence of active diastolic cardiac muscle tone in these patients. Further arguments for the presence of active diastolic cardiac muscle tone were the restoration of a normal diastolic LV pressure morphology (i.e. fast and slow rising LV pressure waves) following administration of calcium channel blockers(39) and the induction in other patients of a continuous diastolic LV pressure

decline following postextrasystolic potentiation(58). Both interventions again highlight the intimate link between active diastolic cardiac muscle tone and abnormal intracellular calcium kinetics of hypertrophied myocardium(Figure 5).

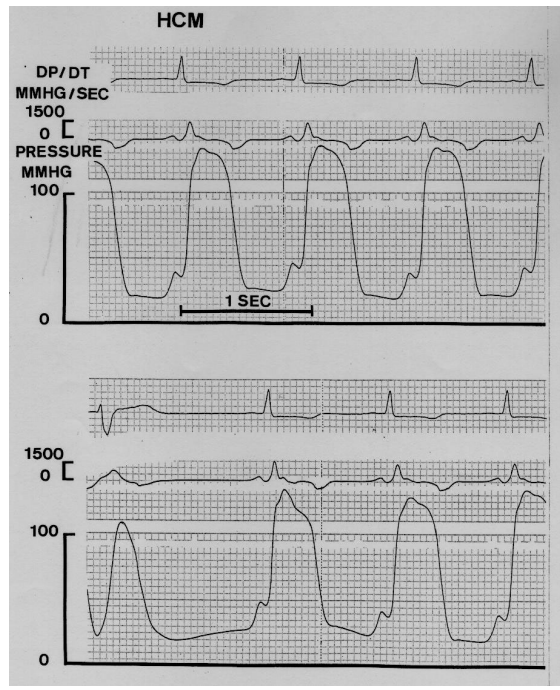

Figure 4. LV pressure and LV dP/dt in a patient with nonobstructive hypertrophic cardiomyopathy (HCM) during regular sinus rhythm(upper panel) and after a premature ventricular beat(lower panel)(57). During regular sinus rhythm and especially in the diastole following the potentiated beat(second beat of the lower panel), there is continuous LV pressure decline throughout diastole, incompatible with passive LV distension and suggestive of active diastolic cardiac muscle tone.

Such linkage seems to be present already at the level of isolated cardiomyocytes where low-grade diastolic crossbridge cycling coincided with small cytosolic calcium sparks(34). An abnormal high rate of calcium leak from the sarcoplasmic reticulum was especially evident in cardiac muscle isolated from failing hearts(42) because of a high phosphorylation state of the calcium release channel (ryanodine receptor). In muscle strips of explanted human hearts, a rise in diastolic force at higher stimulation rates was correlated with modified activity of other calcium handling proteins such as sarcoplasmic reticular calcium-ATPase and sodium-calcium exchanger(22). In pressure-hypertrophied cardiomyocytes of cats, diastolic constitutive properties (=Δstrain for a given Δstress) were also dependent on intracellular calcium concentration(85). In a perfused rat papillary muscle, an increase in coronary perfusion pressure augmented transversal muscle stress(35). This opened stretch activated ion channels with a prompt rise in

myocardial calcium influx. This effect was different from the effect of longitudinal stress, which caused an immediate increase in myofilamentary calcium sensitivity. The effect of transversal cardiac stress on myocardial calcium influx is especially relevant to arterial hypertension, which greatly elevates coronary perfusion pressure and transversal muscle stress. In the failing hypertensive heart, abnormal myocardial calcium influx and handling gets superimposed on an enhanced myofilamentary calcium sensitivity, resulting from reduced phosphorylation of myosin light chain 2 and of troponin I(68). Especially the reduced phosphorylation of troponin I could be a main contributor to diastolic LV dysfunction(4) because of the marked diastolic LV dysfunction in troponin I knock-out mice(25) and because of the accelerated myocardial troponin I degradation in isolated rat hearts following elevation of LV preload from control value to 25 mmHg(14).

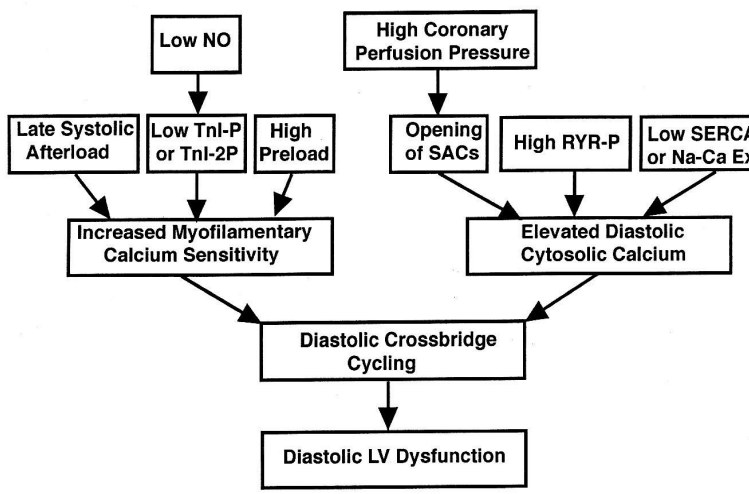

Figure 5. Increased myofilamentary calcium sensitivity and elevated diastolic cytosolic calcium predispose to diastolic crossbridge cycling and diastolic LV dysfunction in hypertrophied and failing myocardium. Myofilamentary calcium sensitivity is increased because of deficient phosphorylation of troponin I (low TnI-P or TnI-2P)(68), low NO bioavailability(41), high preload and late systolic afterload. Cytosolic diastolic calcium is elevated because of opening of stretch activated ion channels (SAC) at the high coronary perfusion pressure of arterial hypertension(35), because of diastolic calcium leakage into the cytosol from the hyperphosphorylated ryanodine receptor(RyR-P)(42) and because of reduced diastolic calcium reuptake from the cytosol by sarcoplasmic reticulum calcium-ATPase(SERCA2) and by sodium-calcium exchanger (Na^+-Ca^{2+} Ex)(22).

Because of its ability to induce cGMP-mediated phosphorylation of troponin I, myocardial NO can prevent diastolic crossbridge cycling and the induction of active diastolic LV tone. In beating rabbit hearts, release of a caval occluder raises the intramyocardial NO concentration measured by a porphyrinic sensor in the LV wall(60). In isolated ejecting guinea pig hearts, a perfusate containing a NOS inhibitor, results in an elevation of LVEDP and a reduction in preload recrutable cardiac output because of a left- and upward shift of the diastolic LV pressure-volume relation(61). Finally, in isolated cardiomyocytes, both NO and cGMP increase resting diastolic cell length because of PKG-mediated phosphorylation of myofilaments (27,64). All these experimental observations indicate a close link between muscle preload and myocardial NO content and suggest a preload-induced rise of myocardial NO to maintain LV preload reserve through PKG-mediated myofilamentary desensitization and eventual prevention of diastolic crossbridge cycling. A similar role for myocardial NO has also been deduced from clinical observations. In patients with dilated cardiomyopathy, an intracoronary infusion of substance P, which acutely releases NO from the coronary endothelium, augments LV stroke work because of improved diastolic LV distensibility evident from a rightward shift of the diastolic LV pressure-volume relation(7,23). In patients with dilated cardiomyopathy and elevated myocardial collagen volume fraction, the NO-induced augmentation of LV stroke work and improvement of LV diastolic distensibility are still present(6). The latter finding is important because it implies independent modulation of LV diastolic distensibility by intracellular elements, some of them active, and extracellular elements. A similar conclusion of overall cardiac muscle stiffness resulting from a summation of intracellular and extracellular elements has also been reached from isolated cardiac tissue preparations with intracellular elements, such as titin, responsible for myocardial stiffness within the physiological range of stress and extracellular elements preventing overstretch at extreme stresses(76).

Active diastolic cardiac muscle tone could also arise from mechanisms other than diastolic crossbridge cycling. These mechanisms include interactions between cytoskeletal proteins and myofilaments, phosphorylation of cytoskeletal proteins and induction of myofibroblasts. A specific domain of the giant cytoskeletal protein titin, which is responsible for some of its spring-like elastic properties, was recently shown to interact with the actin filament in a calcium-dependent way. This interaction accounted for viscous forces produced by the myocardium at higher stretch velocities as occur during rapid LV filling(33). Another domain of titin with similar spring-like elastic properties gets phosphorylated after ß-receptor stimulation or exposure to cyclic AMP-dependent protein kinase (PKA) and this phosphorylation is accompanied by a significant reduction in passive tension(77). Myofibroblasts arise from interstitial fibroblasts and/or pericytes at sites of myocardial fibrogenesis, express a-smooth muscle actin and can impose a contractile "smooth muscle cell" tone on surrounding tissue via cell-cell connections(e.g. gap junctions) and via cell-matrix connections (e.g. fibronexus)(74). In tissue engineered cardiac muscle constructs, ß-receptor stimulation reduced the tone of smooth muscle cells lining the strip and this resulted in a simultaneous fall in resting tension of the entire

cardiac muscle construct(86).

Because of NO's ability to improve diastolic LV distensibility through prevention of diastolic crossbridge cycling, raising myocardial NO bioavailability becomes a logical target for treatment of diastolic LV dysfunction. So far only calcium channel blockers(39), ACE inhibitors(15) and angiotensin II receptor blockers(73) have been demonstrated to exert beneficial effects on diastolic LV performance in patients with LV hypertrophy related to hypertrophic cardiomyopathy, aortic stenosis and arterial hypertension. All three classes of drugs could well exert part of their beneficial effect on diastolic LV function through elevation of myocardial NO bioavailability. In failing human myocardium, both amlodipine and ramiprilat decreased myocardial oxygen consumption through inhibition of kinin degradation and concomitant elevation of myocardial NO content(38). In hypertrophied myocardium, NO's bioavailability is reduced because of excess free radical production by endothelial NADH/NADPH oxidase(41). Angiotensin II is a potent stimulus for endothelial NADH/NADPH oxidase and administration of an angiotensin II receptor blocker could therefore augment NO's bioavailability. NO's ability to improve diastolic LV distensibility could eventually support the chronic use of NO-donors for treatment of diastolic heart failure. Chronic use of NO-donors however induces a limitation of vascular NO bioavailability and results in impairment of endothelium-dependent vasodilation and an increased incidence of adverse cardiovascular events(45). Two important mechanisms contribute to this limited vascular NO bioavailability during chronic use of NO-donors: vascular production of superoxide because of angiotensin II triggered activation of NAD(P)H oxidase(44) and dysfunction of vascular NO synthase(NOS), which produces superoxide instead of NO, probably because of lack of tetrahydrobiopterin(21). Similar mechanisms could be operative in the myocardium and could therefore render chronic use of NO-donors detrimental for diastolic LV function.

Conclusion

The smaller LV end-diastolic volume during acute hypertensive pulmonary edema than following recompensation and the discordant changes during acute ischemic heart failure in LV end-diastolic volume and pressure, with the smaller volume occurring at the higher pressure, are inconsistent with subtle reductions in myocardial systolic performance and a fixed, steep diastolic LV pressure-volume relation underlying all heart failure episodes, also those occurring with a normal LVEF. Accordingly, these findings imply the existence during some acute heart failure episodes of primary diastolic LV dysfunction, defined as transient upward displacement of the diastolic LV pressure-volume relation. The late systolic LV loading increment of a hypertensive episode and the elevated LV preload of a hypertrophied heart both predispose to diastolic LV dysfunction through slowing of isovolumic LV pressure decay and possibly also through induction of active diastolic cardiac muscle tone. The latter could result from diastolic

crossbridge cycling, from lack of phosphorylation of cytoskeletal proteins or from induction of myofibroblasts. In the hypertrophied and failing heart, raised myofilamentary calcium sensitivity, because of low troponin I phosphorylation or low NO bioavailability, and elevated diastolic cytosolic calcium, because of leakage from or slow reuptake into the sarcoplasmic reticulum, could favor such diastolic crossbridge cycling.

References

1. **Angeja BG, and Grossman W**. Evaluation and management of diastolic heart failure. *Circulation* 107: 659-663, 2003.
2. **Aurigemma GP, Gottdiener JS, Shemanski L, Gardin J, and Kitzman D**. Predictive value of systolic and diastolic function for incident congestive heart failure in the elderly: the cardiovascular health study. *J Am Coll Cardiol* 37: 1042-1048, 2001.
3. **Barry WH, Brooker JZ, Alderman EL, and Harrison DC**. Changes in diastolic stiffness and tone of the left ventricle during angina pectoris. *Circulation* 49: 255-263, 1974.
4. **Bodor GS, Oakeley AE, Allen PD, Crimmins DL, Ladenson JH, and Anderson PA**. Troponin I phosphorylation in the normal and failing adult human heart. *Circulation* 96: 1495-1500, 1997.
5. **Bronzwaer JG, de Bruyne B, Ascoop CA, and Paulus WJ**. Comparative effects of pacing-induced and balloon coronary occlusion ischemia on left ventricular diastolic function in man. *Circulation* 84: 211-222, 1991.
6. **Bronzwaer JG, Heymes C, Visser CA, and Paulus WJ**. Myocardial fibrosis blunts nitric oxide synthase-related preload reserve in human dilated cardiomyopathy. *Am J Physiol Heart Circ Physiol* 284: H10-H16, 2003.
7. **Bronzwaer JG, Zeitz C, Visser CA, and Paulus WJ**. Endomyocardial nitric oxide synthase and the hemodynamic phenotypes of human dilated cardiomyopathy and of athlete's heart. *Cardiovasc Res* 55: 270-278, 2002.
8. **Burkhoff D, Maurer MS, and Packer M**. Heart failure with a normal ejection fraction: is it really a disorder of diastolic function? *Circulation* 107: 656-658, 2003.
9. **Caruana L, Petrie MC, Davie AP, and McMurray JJ**. Do patients with suspected heart failure and preserved left ventricular systolic function suffer from "diastolic heart failure" or from misdiagnosis? A prospective descriptive study. *BMJ* 321: 215-218, 2000.
10. **Coudray N, Beregi JP, Lecarpentier Y, and Chemla D**. Effects of isoproterenol on myocardial relaxation rate: influence of the level of load. *Am J Physiol* 265: H1645-H1653, 1993.
11. **Dauterman KW, Massie BM, and Gheorghiade M**. Heart failure associated with preserved systolic function: a common and costly clinical entity. *Am Heart J* 135: S310-S319, 1998.
12. **de Bruyne B, Bronzwaer JG, Heyndrickx GR, and Paulus WJ**. Comparative effects of ischemia and hypoxemia on left ventricular systolic and diastolic function in humans. *Circulation* 88: 461-471, 1993.
13. **Diver DJ, Royal HD, Aroesty JM, McKay RG, Ferguson JJ, Warren SE, and Lorell BH**. Diastolic function in patients with aortic stenosis: influence of left ventricular load reduction. *J Am Coll Cardiol* 12: 642-648, 1988.
14. **Feng J, Schaus BJ, Fallavollita JA, Lee TC, and Canty JM, Jr.** Preload Induces Troponin I Degradation Independently of Myocardial Ischemia. *Circulation* 103: 2035-2037, 2001.
15. **Friedrich SP, Lorell BH, Rousseau MF, Hayashida W, Hess OM, Douglas PS, Gordon S, Keighley**

CS, Benedict C, Krayenbuehl HP, and et a. Intracardiac angiotensin-converting enzyme inhibition improves diastolic function in patients with left ventricular hypertrophy due to aortic stenosis. *Circulation* 90: 2761-2771, 1994.

16. **Gaasch WH, Blaustein AS, Andrias CW, Donahue RP, and Avitall B**. Myocardial relaxation. II. Hemodynamic determinants of rate of left ventricular isovolumic pressure decline. *Am J Physiol* 239: H1-H6, 1980.
17. **Gaasch WH, Carroll JD, Blaustein AS, and Bing OH**. Myocardial relaxation: effects of preload on the time course of isovolumetric relaxation. *Circulation* 73: 1037-1041, 1986.
18. **Gaasch, W. H. and M. M. LeWinter.** Textbook on diastole. Malvern, Lea&Febiger. 1993.
19. **Gandhi SK, Powers JC, Nomeir AM, Fowle K, Kitzman DW, Rankin KM, and Little WC**. The pathogenesis of acute pulmonary edema associated with hypertension. *N Engl J Med* 344: 17-22, 2001.
20. **Geisterfer-Lowrance AA, Christe M, Conner DA, Ingwall JS, Schoen FJ, Seidman CE, and Seidman JG**. A mouse model of familial hypertrophic cardiomyopathy. *Science* 272: 731-734, 1996.
21. **Gori T, Burstein JM, Ahmed S, Miner SE, Al Hesayen A, Kelly S, and Parker JD**. Folic acid prevents nitroglycerin-induced nitric oxide synthase dysfunction and nitrate tolerance: a human in vivo study. *Circulation* 104: 1119-1123, 2001.
22. **Hasenfuss G, Schillinger W, Lehnart SE, Preuss M, Pieske B, Maier LS, Prestle J, Minami K, and Just H**. Relationship between Na+-Ca2+-exchanger protein levels and diastolic function of failing human myocardium. *Circulation* 99: 641-648, 1999.
23. **Heymes C, Vanderheyden M, Bronzwaer JG, Shah AM, and Paulus WJ**. Endomyocardial nitric oxide synthase and left ventricular preload reserve in dilated cardiomyopathy. *Circulation* 99: 3009-3016, 1999.
24. **Hori M, Inoue M, Kitakaze M, Tsujioka K, Ishida Y, Fukunami M, Nakajima S, Kitabatake A, and Abe H**. Loading sequence is a major determinant of afterload-dependent relaxation in intact canine heart. *Am J Physiol* 249: H747-H754, 1985.
25. **Huang X, Pi Y, Lee KJ, Henkel AS, Gregg RG, Powers PA, and Walker JW**. Cardiac troponin I gene knockout: a mouse model of myocardial troponin I deficiency. *Circ Res* 84: 1-8, 1999.
26. **Isoyama S, Apstein CS, Wexler LF, Grice WN, and Lorell BH**. Acute decrease in left ventricular diastolic chamber distensibility during simulated angina in isolated hearts. *Circ Res* 61: 925-933, 1987.
27. **Ito N, Bartunek J, Spitzer KW, and Lorell BH**. Effects of the nitric oxide donor sodium nitroprusside on intracellular pH and contraction in hypertrophied myocytes. *Circulation* 95: 2303-2311, 1997.
28. **Karliner JS, LeWinter MM, Mahler F, Engler R, and O'Rourke RA**. Pharmacologic and hemodynamic influences on the rate of isovolumic left ventricular relaxation in the normal conscious dog. *J Clin Invest* 60: 511-521, 1977.
29. **Kass DA**. Force-frequency relation in patients with left ventricular hypertrophy and failure. *Basic Res Cardiol* 93 Suppl 1: 108-116, 1998.
30. **Kawaguchi M, Hay I, Fetics B, and Kass DA**. Combined ventricular systolic and arterial stiffening in patients with heart failure and preserved ejection fraction: implications for systolic and diastolic reserve limitations. *Circulation* 107: 714-720, 2003.
31. **Kitzman DW, Little WC, Brubaker PH, Anderson RT, Hundley WG, Marburger CT, Brosnihan B, Morgan TM, and Stewart KP**. Pathophysiological characterization of isolated diastolic heart failure in comparison to systolic heart failure. *JAMA* 288: 2144-2150, 2002.
32. **Krogmann ON, Rammos S, Jakob M, Corin WJ, Hess OM, and Bourgeois M**. Left ventricular diastolic dysfunction late after coarctation repair in childhood: influence of left ventricular hypertrophy. *J Am Coll Cardiol* 21: 1454-1460, 1993.
33. **Kulke M, Fujita-Becker S, Rostkova E, Neagoe C, Labeit D, Manstein DJ, Gautel M, and Linke WA**. Interaction between PEVK-titin and actin filaments: origin of a viscous force component in cardiac

myofibrils. *Circ Res* 89: 874-881, 2001.

34. **Lakatta EG, and Lappe DL**. Diastolic scattered light fluctuation, resting force and twitch force in mammalian cardiac muscle. *J Physiol* 315: 369-394, 1981.
35. **Lamberts RR, Van Rijen MH, Sipkema P, Fransen P, Sys SU, and Westerhof N**. Coronary perfusion and muscle lengthening increase cardiac contraction: different stretch-triggered mechanisms. *Am J Physiol Heart Circ Physiol* 283: H1515-H1522, 2002.
36. **Leite-Moreira AF, and Correia-Pinto J**. Load as an acute determinant of end-diastolic pressure-volume relation. *Am J Physiol Heart Circ Physiol* 280: H51-H59, 2001.
37. **Leite-Moreira AF, Correia-Pinto J, and Gillebert TC**. Afterload induced changes in myocardial relaxation: a mechanism for diastolic dysfunction. *Cardiovasc Res* 43: 344-353, 1999.
38. **Loke KE, Laycock SK, Mital S, Wolin MS, Bernstein R, Oz M, Addonizio L, Kaley G, and Hintze TH**. Nitric oxide modulates mitochondrial respiration in failing human heart. *Circulation* 100: 1291-1297, 1999.
39. **Lorell BH, Paulus WJ, Grossman W, Wynne J, Cohn PF, and Braunwald E**. Improved diastolic function and systolic performance in hypertrophic cardiomyopathy after nifedipine. *N Engl J Med* 303: 801-803, 1980.
40. **Lubien E, DeMaria A, Krishnaswamy P, Clopton P, Koon J, Kazanegra R, Gardetto N, Wanner E, and Maisel AS**. Utility of B-natriuretic peptide in detecting diastolic dysfunction: comparison with Doppler velocity recordings. *Circulation* 105: 595-601, 2002.
41. **MacCarthy PA, Grieve DJ, Li JM, Dunster C, Kelly FJ, and Shah AM**. Impaired endothelial regulation of ventricular relaxation in cardiac hypertrophy: role of reactive oxygen species and NADPH oxidase. *Circulation* 104: 2967-2974, 2001.
42. **Marks AR, Reiken S, and Marx SO**. Progression of heart failure: is protein kinase a hyperphosphorylation of the ryanodine receptor a contributing factor? *Circulation* 105: 272-275, 2002.
43. **Mohan P, Paulus WJ, and Sys SU** . Catecholamine-induced early relaxation is counteracted by cardiac muscle lengthening. *J Am Coll Cardiol* 21, 262**A**. 1993.
44. **Munzel T, Kurz S, Rajagopalan S, Thoenes M, Berrington WR, Thompson JA, Freeman BA, and Harrison DG**. Hydralazine prevents nitroglycerin tolerance by inhibiting activation of a membrane-bound NADH oxidase. A new action for an old drug. *J Clin Invest* 98: 1465-1470, 1996.
45. **Murakami T, and Mizuno S**. Intrinsic and extrinsic nitric oxide provides better and worse prognosis respectively for coronary artery disease. *Circulation* 106, II-296**A**. 2002.
46. **Nayler WG, Yepez CE, and Poole-Wilson PA**. The effect of beta-adrenoceptor and Ca2+ antagonist drugs on the hypoxia-induced increased in resting tension. *Cardiovasc Res* 12: 666-674, 1978.
47. **Nikolic S, Yellin EL, Tamura K, Vetter H, Tamura T, Meisner JS, and Frater RW**. Passive properties of canine left ventricle: diastolic stiffness and restoring forces. *Circ Res* 62: 1210-1222, 1988.
48. **O'Rourke MF, and Mancia G**. Arterial stiffness. *J Hypertens* 17: 1-4, 1999.
49. **Ommen SR, Nishimura RA, Appleton CP, Miller FA, Oh JK, Redfield MM, and Tajik AJ**. Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures: A comparative simultaneous Doppler-catheterization study. *Circulation* 102: 1788-1794, 2000.
50. **Pasipoularides A, Mirsky I, Hess OM, Grimm J, and Krayenbuehl HP**. Myocardial relaxation and passive diastolic properties in man. *Circulation* 74: 991-1001, 1986.
51. **Paulus WJ**. Exercise-induced diastolic left ventricular dysfunction after heart transplantation. *Med Sport Sci* 42: 17-25, 1997.
52. **Paulus WJ, Bronzwaer JG, Felice H, Kishan N, and Wellens F**. Deficient acceleration of left ventricular relaxation during exercise after heart transplantation. *Circulation* 86: 1175-1185, 1992.
53. **Paulus, W. J., B. de Bruyne, and J. G. Bronzwaer.** Comparative effects of ischemia and hypoxemia on

left ventricular diastolic function in humans. In Lorell, B. and W. Grossman, eds. Diastolic relaxation of the heart. Boston, U.S.A., Kluwer Academic Publishers. 1994, 303-321.

54. **Paulus WJ, and for the European Study Group on Diastolic Heart Failure**. How to diagnose diastolic heart failure. *Eur Heart J* 19: 990-1003, 1998.
55. **Paulus WJ, Heyndrickx GR, Buyl P, Goethals MA, and Andries E**. Wide-range load shift of combined aortic valvuloplasty-arterial vasodilation slows isovolumic relaxation of the hypertrophied left ventricle. *Circulation* 81: 886-898, 1990.
56. **Paulus WJ, Serizawa T, and Grossman W**. Altered left ventricular diastolic properties during pacing-induced ischemia in dogs with coronary stenoses. Potentiation by caffeine. *Circ Res* 50: 218-227, 1982.
57. **Paulus, W. J., S. U. Sys, and P. Nellens.** Failure of inactivation of hypertrophied myocardium: a cause of impaired left ventricular filling in hypertrophic cardiomyopathy and aortic stenosis. In Grossman, W. and B. H. Lorell, eds. Diastolic relaxation of the heart. Boston, U.S.A., Kluwer Academic Publishers. 1987, 291-304.
58. **Paulus WJ, Sys SU, Nellens P, Heyndrickx GR, and Andries E**. Postextrasystolic potentiation worsens fast filling of the hypertrophied left ventricle in aortic stenosis and hypertrophic cardiomyopathy. *Circulation* 78: 928-940, 1988.
59. **Paulus WJ, Vantrimpont PJ, and Rousseau MF**. Diastolic function of the nonfilling human left ventricle. *J Am Coll Cardiol* 20: 1524-1532, 1992.
60. **Pinsky DJ, Patton S, Mesaros S, Brovkovych V, Kubaszewski E, Grunfeld S, and Malinski T**. Mechanical transduction of nitric oxide synthesis in the beating heart. *Circ Res* 81: 372-379, 1997.
61. **Prendergast BD, Sagach VF, and Shah AM**. Basal release of nitric oxide augments the Frank-Starling response in the isolated heart. *Circulation* 96: 1320-1329, 1997.
62. **Senni M, Tribouilloy CM, Rodeheffer RJ, Jacobsen SJ, Evans JM, Bailey KR, and Redfield MM**. Congestive heart failure in the community: a study of all incident cases in Olmsted County, Minnesota, in 1991. *Circulation* 98: 2282-2289, 1998.
63. **Serizawa T, Carabello BA, and Grossman W**. Effect of pacing-induced ischemia on left ventricular diastolic pressure-volume relations in dogs with coronary stenoses. *Circ Res* 46: 430-439, 1980.
64. **Shah AM, Spurgeon HA, Sollott SJ, Talo A, and Lakatta EG**. 8-bromo-cGMP reduces the myofilament response to Ca2+ in intact cardiac myocytes. *Circ Res* 74: 970-978, 1994.
65. **Smiseth OA**. Assessment of ventricular diastolic function. *Can J Cardiol* 17: 1167-1176, 2001.
66. **Starling MR, Montgomery DG, Mancini GB, and Walsh RA**. Load independence of the rate of isovolumic relaxation in man. *Circulation* 76: 1274-1281, 1987.
67. **Sys SU, Paulus WJ, Claes VA, and Brutsaert DL**. Post-reextension force decay of relaxing cardiac muscle. *Am J Physiol* 253: H256-H261, 1987.
68. **van der Velden J, Papp Z, Zaremba R, Boontje NM, de Jong JW, Owen VJ, Burton PB, Goldmann P, Jaquet K, and Stienen GJ**. Increased Ca(2+)-sensitivity of the contractile apparatus in end-stage human heart failure results from altered phosphorylation of contractile proteins. *Cardiovasc Res* 57: 37-47, 2003.
69. **Vantrimpont PJ, Felice H, and Paulus WJ**. Does dobutamine prevent the rise in left ventricular filling pressures observed during exercise after heart transplantation? *Eur Heart J* 16: 1300-1306, 1995.
70. **Varma N, Morgan JP, and Apstein CS**. Mechanisms underlying ischemic diastolic dysfunction: relation between rigor, calcium homeostasis, and relaxation rate. *Am J Physiol Heart Circ Physiol* 284: H758-H771, 2003.
71. **Varma SK, Owen RM, Smucker ML, and Feldman MD**. Is tau a preload-independent measure of isovolumetric relaxation? *Circulation* 80: 1757-1765, 1989.
72. **Vasan RS, Larson MG, Benjamin EJ, Evans JC, Reiss CK, and Levy D**. Congestive heart failure in

subjects with normal versus reduced left ventricular ejection fraction: prevalence and mortality in a population-based cohort. *J Am Coll Cardiol* 33: 1948-1955, 1999.

73. **Warner JG, Jr., Metzger DC, Kitzman DW, Wesley DJ, and Little WC**. Losartan improves exercise tolerance in patients with diastolic dysfunction and a hypertensive response to exercise. *J Am Coll Cardiol* 33: 1567-1572, 1999.
74. **Weber KT**. Extracellular matrix remodeling in heart failure: a role for de novo angiotensin II generation. *Circulation* 96: 4065-4082, 1997.
75. **Wittstein IS, Kass DA, Pak PH, Maughan WL, Fetics B, and Hare JM**. Cardiac nitric oxide production due to angiotensin-converting enzyme inhibition decreases beta-adrenergic myocardial contractility in patients with dilated cardiomyopathy. *J Am Coll Cardiol* 38: 429-435, 2001.
76. **Wu Y, Cazorla O, Labeit D, Labeit S, and Granzier H**. Changes in titin and collagen underlie diastolic stiffness diversity of cardiac muscle. *J Mol Cell Cardiol* 32: 2151-2162, 2000.
77. **Yamasaki R, Wu Y, McNabb M, Greaser M, Labeit S, and Granzier H**. Protein kinase A phosphorylates titin's cardiac-specific N2B domain and reduces passive tension in rat cardiac myocytes. *Circ Res* 90: 1181-1188, 2002.
78. **Yip G, Wang M, Zhang Y, Fung JW, Ho PY, and Sanderson JE**. Left ventricular long axis function in diastolic heart failure is reduced in both diastole and systole: time for a redefinition? *Heart* 87: 121-125, 2002.
79. **Yu CM, Lin H, Yang H, Kong SL, Zhang Q, and Lee SW**. Progression of systolic abnormalities in patients with "isolated" diastolic heart failure and diastolic dysfunction. *Circulation* 105: 1195-1201, 2002.
80. **Zatko FJ, Martin P, and Bahler RC**. Time course of systolic loading is an important determinant of ventricular relaxation. *Am J Physiol* 252: H461-H466, 1987.
81. **Zile MR**. Heart failure with preserved ejection fraction: is this diastolic heart failure?*. *J Am Coll Cardiol* 41: 1519-1522, 2003.
82. **Zile MR, and Brutsaert DL**. New concepts in diastolic dysfunction and diastolic heart failure: Part I: diagnosis, prognosis, and measurements of diastolic function. *Circulation* 105: 1387-1393, 2002.
83. **Zile MR, and Brutsaert DL**. New concepts in diastolic dysfunction and diastolic heart failure: Part II: causal mechanisms and treatment. *Circulation* 105: 1503-1508, 2002.
84. **Zile MR, Gaasch WH, Carroll JD, Feldman MD, Aurigemma GP, Schaer GL, Ghali JK, and Liebson PR**. Heart failure with a normal ejection fraction: is measurement of diastolic function necessary to make the diagnosis of diastolic heart failure? *Circulation* 104: 779-782, 2001.
85. **Zile MR, Richardson K, Cowles MK, Buckley JM, Koide M, Cowles BA, Gharpuray V, and Cooper G**. Constitutive properties of adult mammalian cardiac muscle cells. *Circulation* 98: 567-579, 1998.
86. **Zimmermann WH, Schneiderbanger K, Schubert P, Didie M, Munzel F, Heubach JF, Kostin S, Neuhuber WL, and Eschenhagen T**. Tissue engineering of a differentiated cardiac muscle construct. *Circ Res* 90: 223-230, 2002.

Chapter 6

Summary
Samenvatting

Summary

Diastolic left ventricular (LV) distensibility is determined by the material properties of the LV wall and by LV geometry (i.e., LV shape, LV volume and LV wall thickness). These material properties are influenced both by the physical structure of the LV myocardium and by the dynamic process of myocardial relaxation. The material properties of the myocardium dictate the strain that follows a given stress, and determine position and shape of the myocardial stress-strain relationship. The material properties, together with the LV geometry, also determine position and shape of the diastolic LV pressure-volume relationship. Diastolic LV distensibility is best characterized by this diastolic LV pressure-volume relationship. The crucial role of diastolic LV distensibility in relation to the heart failure syndrome are discussed in chapter 2, 3 and 4 of this thesis.

Chapter 2 of this thesis discusses diastolic left ventricular dysfunction, characterized by an upward shift of the left ventricular diastolic pressure-volume relationship.

Chapter 2.1 describes the effects of myocardial ischemia, either pacing-induced or by coronary occlusion, on the diastolic properties of the same LV anterior wall segment in 12 patients with single-vessel proximal left anterior descending coronary artery stenosis at rest, immediately after 7 ± 1.2 minutes of pacing, and at the end of a 1- minute balloon occlusion of coronary angioplasty (CO).

Shifts of the diastolic LV pressure-length relation, derived from simultaneous tip-micromanometer LV pressure recordings and digital subtraction LV angiograms, were used as an index of regional diastolic LV distensibility of the anterior wall segment. The diastolic LV Pressure(P)-Radial Length(RL) plot of the ischemic segment was shifted upward for portions of the plot that overlapped with the diastolic LV P-RL plot at rest. This upward shift at the end of CO was significantly smaller than that immediately after pacing. At the end of CO, a correlation was observed for the ischemic segment between percentage systolic shortening and upward shift of the diastolic LV pressure-radial length plot.

The upward shift of the diastolic LV pressure-radial length plot, which was used as an index of decreased regional diastolic LV distensibility, was larger immediately after pacing than at the end of CO. Persistent systolic shortening of ischemic myocardium seems to be a prerequisite for a decrease in diastolic distensibility of the ischemic segment because of the higher percentage systolic shortening of the ischemic segment immediately after pacing, and because of the correlation at the end of CO between the upward shift of the diastolic LV pressure-radial length plot and percentage systolic shortening of the ischemic segment.

Chapter 2.2 describes the different effects of low- flow ischemia, pacing-induced ischemia, and hypoxemic perfusion on LV performance in humans. During the initial phase of an ischemic insult, left ventricular (LV) performance depends on the complex interaction between oxygen deprivation, vascular turgor, and accumulation of metabolites.

In experimental preparations, low-flow ischemia decreases systolic shortening and increases diastolic LV distensibility, whereas pacing- induced ischemia or hypoxic perfusion produces smaller decreases in systolic shortening but decreases LV diastolic distensibility. Therefore, the different effects of low-flow ischemia, pacing-induced ischemia, and hypoxemic perfusion on LV performance was studied in 20 patients with a significant stenosis in the left anterior descending coronary artery.

Micromanometer-tip LV pressure recordings, LV angiography, and coronary sinus blood sampling were obtained at rest and during pacing-induced ischemia, low-flow ischemia due to balloon coronary occlusion, and hypoxemia induced by balloon coronary occlusion with hypoxemic perfusion distal to the occlusion. LV stroke work index was lower at the end of balloon coronary occlusion than during pacing-induced ischemia and was lower at the end of balloon coronary occlusion than at the end of hypoxemia induced by balloon coronary occlusion with hypoxemic perfusion distal to the occlusion.

LV end-diastolic pressure rose from 16±5 mm Hg at rest to 23±6 mm Hg at the end of balloon coronary occlusion. However, LV end-diastolic pressure was lower at the end of balloon coronary occlusion than during pacing-induced ischemia and was lower at the end of balloon coronary occlusion than at the end of hypoxemia induced by balloon coronary occlusion with hypoxemic perfusion distal to the occlusion. LV end-diastolic volume index increased at the end of balloon coronary occlusion. Left ventricular end-diastolic volume index increased to values similar to those for balloon coronary occlusion during pacing-induced ischemia and at the end of hypoxemia induced by balloon coronary occlusion with hypoxemic perfusion distal to the occlusion. Higher values of LV end-diastolic pressure and unchanged values of LV end-diastolic volume index for pacing-induced ischemia and hypoxemia induced by balloon coronary occlusion with hypoxemic perfusion distal to the occlusion, compared with balloon coronary occlusion, suggested a lower end- diastolic LV distensibility during pacing-induced ischemia and during hypoxemia, as compared with low-flow ischemia. Upward shifts of individual diastolic LV pressure-volume curves during pacing-induced ischemia (9 of 11 patients) and at the end of hypoxemia induced by balloon coronary occlusion with hypoxemic perfusion distal to the occlusion (7 of 9 patients), compared with balloon coronary occlusion, were also consistent with lower LV diastolic distensibility during pacing-induced ischemia and during hypoxemia, compared with low-flow ischemia.

Coronary sinus lactate, H^+, and K^+ levels increased after balloon deflation (balloon coronary occlusion and hypoxemia induced by balloon coronary occlusion with hypoxemic perfusion distal to the occlusion) and during pacing-induced ischemia. Thus, during low-flow ischemia, LV systolic performance was lower and LV diastolic distensibility larger than during pacing-induced ischemia or hypoxemia. The variable response of the human myocardium to different types of ischemia was probably related to the degree of vascular turgor and accumulation of tissue metabolites.

Chapter 2.3 covers a pathophysiologic perspective of the comparative effects of ischemia and

hypoxemia on left ventricular diastolic function in humans.

In *Chapter 2.4* the presence of a deficient acceleration of left ventricular relaxation is reported during exercise after heart transplantation. The exercise-induced rise in left ventricular filling pressures after cardiac transplantation is considered to be the result of a blunted heart rate response, of elevated venous return, and of unfavorable passive late-diastolic properties of the cardiac allograft. In contrast to passive late-diastolic left ventricular properties, the effect of left ventricular relaxation on the exercise-induced rise in left ventricular filling pressures of the cardiac allograft has not yet been studied.

In the present study, the response of left ventricular relaxation to exercise was investigated in transplant recipients and compared with left ventricular relaxation observed in normal control subjects exercised to the same heart rate. Moreover, the response of left ventricular relaxation of the cardiac allograft to beta-adrenoreceptor stimulation, to reduced left ventricular afterload, and to increased myocardial activator calcium was investigated by infusion of dobutamine and of nitroprusside and by postextrasystolic potentiation. Twenty-seven transplant recipients were studied 1 year (n = 17), 2 years (n = 7), 3 years (n = 2), and 4 years (n = 1) after transplantation. All patients were free of rejection and of significant graft atherosclerosis at the time of study. Tip-micromanometer left ventricular pressure recordings and cardiac hemodynamics were obtained at rest, during supine bicycle exercise stress testing, during dobutamine infusion at a heart rate matching the heart rate at peak exercise, during nitroprusside infusion, and after postextrasystolic potentiation.

Tip-micromanometer left ventricular pressure recordings were also obtained in a normal control group at rest and during supine bicycle exercise stress testing to a heart rate, which matched the heart rate of the transplant recipient group at peak exercise. Left ventricular relaxation rate was measured by calculation of a time constant of left ventricular pressure decay (T) derived from an exponential curve fit to the digitized tip-micromanometer left ventricular pressure signal. In the transplant recipients, exercise abbreviated T and caused a rise of left ventricular minimum diastolic pressure.

In normal control subjects, exercise induced a larger abbreviation of T and a smaller drop in left ventricular minimum diastolic pressure than was found in the transplant recipients. In the transplant recipients, the change in T from rest to exercise was variable, ranging from an abbreviation, as observed in normal controls, to a prolongation and was significantly correlated with the change in RR interval on the ECG and the change in left ventricular end-diastolic pressure.

In a first subset of transplant recipients, dobutamine infusion resulted in a heart rate equal to the heart rate at peak exercise, a left ventricular end-diastolic pressure (lower than at peak exercise) and a T value, which was shorter than both the resting value and the value observed at peak exercise. In a second subset of transplant recipients, nitroprusside infusion and postextrasystolic potentiation resulted in a significant prolongation of T and a characteristic negative dP/dt upstroke pattern with downward convexity as previously observed in left ventricular hypertrophy. Exercise after cardiac transplantation resulted in a smaller acceleration

of left ventricular relaxation than in a normal control group exercised to the same heart rate. These transplant recipients, who made the largest use of left ventricular preload reserve during exercise, showed least acceleration of left ventricular relaxation. This association between a rise of left ventricular end-diastolic pressure and slower left ventricular isovolumic relaxation was also evident in the individual transplant recipient from the slower isovolumic relaxation during exercise than during dobutamine infusion despite equal heart rates.

After postextrasystolic potentation during nitroprusside infusion, a slow left ventricular relaxation with downward convexity of the dP/dt signal was observed in the cardiac allograft. This finding suggests depressed function of the sarcoplasmic reticulum in left ventricular myocardium after transplantation, which could be related either to decreased adrenergic tone or to preceding ischemic injury during organ retrieval or to hypertrophy caused by cyclosporine induced arterial hypertension.

Chapter 3 of this thesis discusses diastolic left ventricular dysfunction, characterized by a lack of rightward shift of the left ventricular diastolic pressure-volume relationship.

In *chapter 3.1* the functional significance of a modified NOS gene expression for left ventricular (LV) contractile performance was investigated in patients with dilated nonischemic cardiomyopathy. Patients with heart failure have modified myocardial expression of nitric oxide synthase (NOS), as is evident from induction of calcium-insensitive NOS isoforms. In patients with dilated, nonischemic cardiomyopathy, invasive measures of LV contractile performance were derived from LV microtip pressure recordings and angiograms and correlated with intensity of gene expression of inducible (NOS2) and constitutive (NOS3) NOS isoforms in simultaneously procured LV endomyocardial biopsies. LV endomyocardial expression of NOS2 was linearly correlated with LV stroke volume, LV ejection fraction, and LV stroke work. In patients with elevated LV end-diastolic pressure, a closer correlation was observed between endomyocardial expression of NOS2 and LV stroke volume, LV ejection fraction, and LV stroke work. LV endomyocardial expression of NOS3 was linearly correlated with LV stroke volume and LV stroke work. To establish the role of nitric oxide (NO) as a mediator of the observed correlations, substance P (which causes endothelial release of NO) was infused intracoronarily. In patients with elevated LV end-diastolic pressure, an intracoronary infusion of substance P increased LV stroke volume and LV stroke work and shifted the LV end-diastolic pressure-volume relation to the right. It is concluded, that in patients with dilated cardiomyopathy, an increase in endomyocardial NOS2 or NOS3 gene expression augments LV stroke volume and LV stroke work because of a NO-mediated rightward shift of the diastolic LV pressure-volume relation and a concomitant increase in LV preload reserve.

In *Chapter 3.2*, because nitric oxide (NO) reduces diastolic LV stiffness, diastolic LV stiffness and LV systolic performance are related to intensity of endomyocardial NO synthase (NOS) gene expression in dilated cardiomyopathy and in athlete's heart. In dilated cardiomyopathy and in athlete's heart, progressive LV dilatation is accompanied by rightward displacement of the diastolic LV pressure-volume relation. In dilated cardiomyopathy, an increase in diastolic LV

stiffness can limit this rightward displacement, thereby decreasing LV systolic performance. Microtip LV pressures, conductance-catheter or angiographic LV volumes, echocardiographic LV wall thicknesses and snap-frozen LV endomyocardial biopsies were obtained in 33 patients with dilated cardiomyopathy and in three professional cyclists referred for sustained ventricular tachycardia. Intensity of LV endomyocardial inducible NOS (NOS2) and constitutive NOS (NOS3) gene expression was determined using quantitative reverse transcription-polymerase chain reaction (RT-PCR). Dilated cardiomyopathy patients with higher diastolic LV stiffness-modulus and lower LV stroke work had lower NOS2 and NOS3 gene expression at any given level of LV end-diastolic wall stress. The intensity of NOS2 and NOS3 gene expression observed in athlete's heart was similar to dilated cardiomyopathy with low LV diastolic stiffness-modulus and preserved LV stroke work. High LV endomyocardial NOS gene expression is observed in athlete's heart and in dilated cardiomyopathy with low diastolic LV stiffness and preserved LV stroke work. Favorable effects on the hemodynamic phenotype of high LV endomyocardial NOS gene expression could result from a NO-mediated decrease in diastolic LV stiffness and a concomitant rise in LV preload reserve.

In *Chapter 3.3* findings from recent experimental and clinical research are covered, which solved some of the controversies with respect to the myocardial contractile effects of NO. These controversies were: (1) does NO exert a contractile effect at baseline? (2) Is NO a positive or a negative inotrope? (3) Are the contractile effects of NO similar when NO is derived from NO-donors or from the different isoforms of NO synthases (NOS)? (4) Does NO exert the same effects in hypertrophied, failing or ischemic myocardium? Transgenic mice with cardioselective overexpression of NOS revealed NO to produce a small reduction in basal developed LV pressure and a LV relaxation-hastening effect mainly through myofilamentary desensitization. Similar findings had previously been reported during intracoronary infusions of NO-donors in isolated rodent hearts and in humans. The LV relaxation hastening effect was accompanied by increased diastolic LV distensibility, which augmented LV preload reserve, especially in heart failure patients. This beneficial effect on diastolic LV function always overrode the small NO-induced attenuation in LV developed pressure in terms of overall LV performance. In most experimental and clinical conditions, contractile effects of NO were similar when NO was derived from NO-donors or produced by the different isoforms of NOS. Because expression of inducible NOS (NOS2) is frequently accompanied by elevated oxidative stress, NO produced by NOS2 can lead to peroxynitrite-induced contractile impairment as observed in ischemic or septic myocardium. Finally, shifts in isoforms or in concentrations of myofilaments can affect NO-mediated myofilamentary desensitization and alter the myocardial contractile effects of NO in hypertrophied or failing myocardium.

Chapter 4 of this thesis discusses diastolic left ventricular dysfunction, characterized by a steeper slope of the left ventricular diastolic pressure-volume relationship.

The purpose of the study reported in *chapter 4.1* was to investigate interactions between myocardial nitric oxide synthase (NOS) and myocardial fibrosis, both of which determine left ventricular (LV) preload reserve in patients with nonischemic dilated cardiomyopathy. In previous animal experiments, chronic inhibition of NOS induced myocardial fibrosis and limited

LV preload reserve. Twenty-eight dilated cardiomyopathy patients underwent LV catheterization, balloon caval occlusions, intracoronary substance P infusion, and procurement of LV endomyocardial biopsies for determinations of collagen volume fraction, of gene expression of NOS2, NOS3, heme oxygenase, and TNF-alpha, and of NOS2 protein. Collagen volume fraction was unrelated to the intensity of NOS2, NOS3, heme oxygenase, or TNF-alpha gene expression or of NOS2 protein expression. Preload recruitable LV stroke work correlated directly with NOS2 gene expression and inversely with collagen volume fraction. High collagen volume fraction (>10%) reduced baseline LV stroke work and preload recruitable LV stroke work at each level of NOS2 gene expression. In dilated cardiomyopathy , myocardial fibrosis is unrelated to the intensity of myocardial gene expression of NOS, antioxidative enzymes (heme oxygenase), or cytokines (TNF-alpha) and blunts NOS2-related recruitment of LV preload reserve.

Chapter 4.2 reports on heart failure patients, in which beneficial effects of NO on diastolic LV function always overrides a small NO-induced attenuation of LV developed pressure in terms of overall hemodynamic status, either at baseline or following ß-adrenergic stimulation. The absence of hemodynamic deterioration in transgenic mice over expressing either myocardial NOS2 or NOS3 confirms these clinical observations. In failing myocardium, NO's correction of diastolic LV dysfunction reinforces NO's energy sparing effects and the concerted action of NO on both diastolic LV dysfunction and deranged energetics could well be instrumental for preventing relentless deterioration of failing myocardium. Another beneficial effect of high endomyocardial NO activity on diastolic LV distensibility of the cardiomyopathic heart could result not only from NO-induced phosphorylation of troponin I and a concomitant reduction of diastolic crossbridge cycling but also from prevention of endomyocardial fibrosis. Chronic inhibition of NO synthesis has indeed been demonstrated to induce progressive myocardial fibrosis through a signaling cascade involving endothelin, angiotensin II, aldosterone and transforming growth factor β.

Chapter 5 discusses left ventricular dysfunction in relation to different changes in the left ventricular pressure-volume relationships. New, mainly noninvasive observations on diastolic LV dysfunction are confronted in this chapter with the old framework of diastolic LV pressure-volume relations to confirm their validity or to clarify their cause. Some questions recently emerged:

1. Is diastolic LV dysfunction always secondary to systolic LV dysfunction ?
2. Can transient elevations in LV loading induce diastolic LV dysfunction ?
3. Can diastolic LV dysfunction result from heightened active diastolic cardiac muscle tone?

Samenvatting

Het lijkt voor de hand te liggen, dat de kwaliteit van de pompfunctie van het hart is af te meten aan de kracht waarmee de hartspier samentrekt. Iemand met een ‘zwakke’ hartspier kan zich minder goed inspannen en is eerder kortademig dan iemand met een krachtig samentrekkende hartspier. Een ‘zwakke’ hartspier verplaatst immers minder bloed per hartslag. Studies hebben aangetoond, dat medicamenten die de hartspier versterken een averechts effect hebben en zelfs de mortaliteit doen toenemen. Is het wellicht beter een ‘zwakke’ hartspier te ontlasten in plaats van ‘op te zwepen’? Het is inderdaad gebleken, dat medicamenten die bloedvaten verwijden, wel een gunstige invloed hebben op zowel het klachtenpatroon van mensen met een ‘zwak’ hart, als op hun overlevingskans. Door het verwijden van bloedvaten ondervindt een ‘zwak’ hart minder weerstand bij het uitpompen van bloed en wordt dus ontlast.

De laatste jaren is er echter ook belangstelling ontstaan voor de soepelheid van de hartspier. Hoe soepeler de spier, des te gemakkelijker de linker ventrikel (hartkamer) zich vult met bloed, anders gezegd: de druk tijdens de vulling van de linker ventrikel zal lager zijn bij een soepele in vergelijking met een stugge hartspier en hoe lager de druk tijdens de vulling van de linker ventrikel, des te minder iemand kortademig is als hij of zij zich inspant. Het onderzoek dat ten grondslag ligt aan dit proefschrift heeft inzichten opgeleverd in de factoren die een stugge of soepele hartspier tot gevolg hebben en waarom bepaalde hartspierafwijkingen bij de ene patiënt wel en bij de andere geen aanleiding geven tot het ontstaan van lichamelijke klachten. Het onderzoek heeft aangetoond dat de kracht waarmee de hartspier samentrekt, ook wel 'systolische functie' genoemd, mogelijk zelfs van ondergeschikt belang is en dat de soepelheid of stugheid van de hartspier, ook wel 'diastolische functie' genoemd, bij patiënten met hartfalen daarentegen een cruciale rol lijkt te spelen bij het ontstaan van lichamelijke klachten.

De mate van diastolische distensibiliteit (soepelheid) van de hartspier wordt bepaald door de eigenschappen van het materiaal van die spier, door de spierwanddikte, de grootte en de vorm van de linker ventrikel. Deze eigenschappen van het spiermateriaal worden beïnvloed door de fysieke eigenschappen (bijvoorbeeld door de verhouding van spierweefsel/bindweefsel) en door de dynamische processen in de hartspier als deze relaxeert (zich ontspant) na een contractie (samentrekking). Deze relaxatie kan vertraagd zijn, waardoor de spierwand van de linker ventrikel minder soepel is als de linker ventrikel zich vult met bloed na een contractie. Een soepele hartspier zorgt er dus voor dat de linker ventrikel een relatief groot bloedvolume kan ontvangen bij een relatief lage vullingsdruk. Om deze diastolische distensibiliteit in maat en getal uit te drukken wordt de linker ventrikeldruk afgezet in een grafiek tegen het linker ventrikelvolume, de zogenaamde druk-volume relatie. De linker ventrikeldruk moet dan wel op hetzelfde moment worden gemeten als het linker ventrikelvolume. Dit kan momenteel alleen met een 'combinatie catheter' die geplaatst wordt in de linker ventrikel en tegelijkertijd linker ventrikeldruk en ventrikelvolume kan registreren.

De diastolische linker ventrikeldistensibiliteit wordt grafisch weergegeven door de diastolische linker ventrikeldruk-volume relatie en veranderingen in de positie van deze druk-volume relatie zijn specifiek voor onderliggende ziekteprocessen die respectievelijk in *hoofdstuk 2, 3* en *4* van

dit proefschrift aan bod komen. Een verschuiving van de diastolische linker ventrikeldruk-volume relatie naar links en naar boven betekent een afname van de diastolische linker ventrikeldistensibiliteit, de hartspier is stugger, de linker ventrikelvulling wordt bemoeilijkt. Een verschuiving van de diastolische linker ventrikeldruk-volume relatie daarentegen naar rechtsonder betekent een afname van de stugheid van de hartspier, hetgeen de diastolische vulling van de linker ventrikel vergemakkelijkt. Een bijkomend voordeel van een soepele hartspier, is de eigenschap van de hartspier, dat hoe meer hij uitrekt, zoals bij een groter vullingsvolume van de linker ventrikel, des te groter de contractiekracht zal zijn. Deze eigenschap wordt ook wel de 'preload reserve' genoemd en is door Frank en Starling rond 1900 voor het eerst beschreven. Deze preload reserve is dus groter naarmate de spierwand van de linker ventrikel soepeler is. Grafisch weergegeven is dat een verschuiving van de diastolische linker ventrikeldruk-volume relatie naar rechtsonder.

In *hoofdstuk 2* wordt het mechanisme beschreven dat ten grondslag ligt aan het naar boven verschuiven van de diastolische linker ventrikeldruk-volume relatie.
Hoofdstuk 2.1 beschrijft het effect van ischemie ('zuurstoftekort') van de hartspier als gevolg van

1 een te hoog zuurstofgebruik bij een opgelegde hoge hartfrequentie met behulp van een pacemaker bij patiënten met een coronair arteriestenose (vernauwing van de kransslagader).
2 een te laag zuurstofaanbod door occlusie (afsluiting) van deze kransslagader tijdens een PTCA (dotterbehandeling) van diezelfde coronair arteriestenose. Gevonden werd dat de diastolische linker ventrikeldruk-volume relatie meer naar boven verschuift na pacing ischemie dan na occlusie ischemie. Het grotere verlies aan diastolische linker ventrikeldistensibiliteit na pacing gaat gepaard met behoud van linker ventrikel systolische functie. Na occlusie ischemie was de diastolische linker ventrikeldistensibiliteit minder gestoord in combinatie met een fors gestoorde linker ventrikel systolische functie.

Hoofdstuk 2.2 beschrijft de verschillende effecten van occlusie ischemie, pacing ischemie en hypoxemische perfusie in combinatie met occlusie ischemie. Hypoxemische perfusie wordt verkregen door tijdens een PTCA met een balloncatheter het bloedvat af te sluiten (ballonocclusie) en via een kanaal in die balloncatheter een fysiologische zoutoplossing toe te dienen. Deze zoutoplossing bevat ongeveer 38 maal minder zuurstof (sterk hypoxemisch) dan slagaderlijk bloed. Toediening van deze zoutoplossing heeft tot gevolg dat de verschillende stofwisselingsproducten als gevolg van het 'zuurstoftekort' van de hartspier worden uitgewassen, in tegenstelling tot de situatie met ballonocclusie alléén, waarbij alle stofwisselingsproducten zich achter de ballon ophopen. Hierdoor wordt het mogelijk de effecten van deze stofwisselingsproducten op de diastolische en systolische linker ventrikelfunctie te bestuderen. Tijdens pacing ischemie, occlusie ischemie en occlusie ischemie met hypoxemische perfusie worden diastolische linker ventrikeldruk-volume relaties geconstrueerd met behulp van simultane linker ventrikelcontrastinjecties voor volumemeting en linker ventrikeldrukmetingen met een cathetertipmanometer en wordt er bloed afgenomen uit de sinus coronarius, het bloedvat waardoor het bloed het hart weer 'verlaat'.

Tijdens pacing ischemie en hypoxemische perfusie met occlusie ischemie is de diastolische linker ventrikeldistensibiliteit duidelijk minder dan tijdens occlusie ischemie alleen. Net als in

de studie die beschreven is in hoofdstuk 2.1 vinden we ook hier een combinatie van verminderde linker ventrikel systolische functie en een betere diastolische linker ventrikeldistensibiliteit tijdens occlusie ischemie in vergelijking met pacing ischemie en hypoxemische perfusie. De verschillende reacties van de menselijke hartspier op verschillende soorten ischemie zijn mogelijk het gevolg van de mate waarin de bloedvaten van het hart zijn gevuld (vasculaire turgor) of van de ophoping van stofwisselingsproducten in de hartspier als gevolg van een gebrekkige doorbloeding tijdens PTCA ballonocclusie.

Hoofdstuk 2.3 zet de bevindingen van hoofdstuk 2.1 en 2.2 in perspectief.

In *hoofdstuk 2.4* worden de effecten bestudeerd van lichamelijke inspanning op de kwaliteit van relaxatie van de linker ventrikelhartspier bij patiënten na harttransplantatie. De hartfrequentie bij patiënten met een donorhart neemt tijdens lichamelijke inspanning niet veel toe. Als gevolg hiervan zullen de linker ventrikel vullingsdrukken meer stijgen dan gewoonlijk. Ook is de hartspier bij patiënten met een donorhart vaak wat stugger dan normaal. Hierdoor zullen de vullingsdrukken ook wat hoger zijn. Onbekend en onderwerp van deze studie is: Waarom is de hartspier van deze patiënten stugger? Komt dit door de gewijzigde structurele eigenschappen van de hartspier, of door een afwijkende relaxatie van de spier? De relaxatie van de linker ventrikelhartspier wordt gemeten tijdens inspanning en bij dobutamine (=een hartspier 'versterkend' medicijn) toediening bij patiënten met een donorhart en bij patiënten met een normale linker ventrikelfunctie, die om een andere reden een hartcatheterisatie ondergaan. De snelheid van relaxatie van de linker ventrikelhartspier wordt bepaald door de tijdsconstante Tau waarmee de linker ventrikeldruk daalt en wordt uitgedrukt in milliseconden. Inspanning na harttransplantatie resulteert in een kleine toename van de relaxatiesnelheid in vergelijking met de normale controlepatiënten. De patiënten met een donorhart maken het meest gebruik van hun preload reserve (= een toename van de contractiekracht van de linker ventrikelhartspier bij een diastolische vulling tot een groter linker ventrikelvolume) tijdens inspanning en hadden de kleinste toename van de relaxatiesnelheid. Deze bevindingen wijzen mogelijk in de richting van een gestoorde calciumhuishouding in de hartspiercel van het donorhart als gevolg van schade door de transplantatieprocedure, waarbij het hart noodgedwongen lange tijd zonder doorbloeding is geweest.

In *hoofdstuk 3* wordt het mechanisme beschreven dat ten grondslag ligt aan het ontbreken van een adequate verschuiving van de diastolische linker ventrikeldruk-volume relatie naar rechts.

In *hoofdstuk 3.1* wordt in een biopt (stukje hartspier) de hoeveelheid stikstofoxide synthetase (een enzym dat de aanmaak van het stikstofoxide stimuleert) bepaald en in verband gebracht met de contractiele functie van de linker ventrikel waaruit het biopt is genomen. Deze biopten worden genomen uit de linker ventrikel van patiënten met gedilateerde cardiomyopathie (een groot en 'zwak' hart). De diastolische linker ventrikeldruk-volume relatie wordt ook hier weer bepaald met de 'combinatie catheter' die geplaatst wordt in de linker ventrikel en tegelijkertijd linker ventrikeldruk en ventrikelvolume kan registreren. In deze groep patiënten met gedilateerde cardiomyopathie vonden we een groter, door de linker ventrikel verplaatst, volume (slagvolume) als er een hoger gehalte werd gevonden aan stikstofoxide synthetase of als er extra stikstofoxide werd toegediend, c.q. de aanmaak van stikstofoxide werd bevorderd. Het grotere slagvolume van

de linker ventrikel is het gevolg van het naar rechtsonder verschuiven van de diastolische linker ventrikeldruk-volume relatie en een daarmee gepaard gaande toename van de preload reserve.

In *hoofdstuk 3.2* is onderzocht of patiënten met een gedilateerde cardiomyopathie en een stugge hartspier en relatief laag linker ventrikelslagvolume ook een laag stikstofoxide synthetasegehalte hadden. Dat bleek inderdaad het geval te zijn en deze bevindingen waren ongeacht de hoogte van de wandspanning van de linker ventrikel. Bij atleten werd evenals bij patiënten met een gedilateerde cardiomyopathie en een soepele hartspier en een groot linker ventrikel slagvolume, een grotere hoeveelheid stikstofoxide synthetase aangetroffen dan bij patiënten met een stugge hartspier. Gunstige effecten op het linker ventrikelslagvolume en op de preload reserve van grote hoeveelheden stikstofoxide synthetase in de hartspier zijn mogelijk het gevolg van een soepele hartspier onder invloed van het stikstofoxide zelf.

Hoofdstuk 3.3 vat de effecten samen, die stikstofoxide heeft op het functioneren van de hartspier.

In *hoofdstuk 4* wordt het mechanisme beschreven dat ten grondslag ligt aan een steilere helling van de diastolische linker ventrikeldruk-volume relatie.

Hoofdstuk 4.1 beschrijft de relatie tussen de hoeveelheid collageen (bindweefsel) van de hartspier en de hoeveelheid aangetoond stikstofoxide synthetase in diezelfde hartspier van patiënten met een gedilateerde cardiomyopathie. De gedachte heerst dat hoe hoger het collageengehalte is, des te stugger de hartspier zal zijn. Er bleek echter geen relatie aantoonbaar tussen de hoeveelheid stikstofoxide synthetase als indicator van de stugheid van de hartspier en de hoeveelheid aangetroffen collageen. Wel bleek dat de toename van preload reserve in aanwezigheid van een grotere hoeveelheid stikstofoxide synthetase verminderd was bij een grote hoeveelheid collageen.

Hoofdstuk 4.2 behandelt de invloed van stikstofoxide op bindweefselvorming en daarmee op de structurele eigenschappen van de hartspier op lange termijn. Ook wordt hierin beschreven wat de instantane effecten zijn van het stikstofoxide op de diastolische eigenschappen van de hartspier.

In *hoofdstuk 5* volgt een discussie over functiestoornissen van de linker ventrikel in relatie tot de verschillende veranderingen in de positie van de diastolische linker ventrikeldruk-volume relatie. Er wordt daarbij ingegaan op een drietal vragen.

1 Is een diastolische functiestoornis van de linkerventrikel altijd het gevolg van een systolische functiestoornis?
2 Kan een voorbijgaande overbelasting van de linker ventrikel aanleiding geven tot een diastolische functiestoornis?
3 Kan een diastolische disfunctie van de linker ventrikel het gevolg zijn van een gestoorde relaxatie van de hartspierwand van de linker ventrikel?

Nawoord

In de eerste plaats ben ik de patiënten erkentelijk, die allen onbaatzuchtig hun medewerking hebben verleend aan de uitbreiding van invasieve procedures ten dienste van het onderzoek, dat de basis vormt van dit proefschrift.

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