Myocardial Effects of Type 2 Diabetes, Co-morbidities, and Changing Loading Conditions: a Clinical Study by Tissue Velocity Echocardiography

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A dissertation submitted to the Royal Institute of Technology (KTH)
in partial fulfilment of the requirements for the degree of Teknisk Doktor
Stockholm, 25th May 2007

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ISBN 978-91-7178-647-0
TRITA-STH Report 2007:2
ISSN 1653-3836
ISRN KTH / STH / --07:2--SE

“If you think about disaster, you will get it. Brood about death and you hasten your demise. Think positively and masterfully, with confidence and faith, and life becomes more secure, more fraught with action, richer in achievement and experience”

Swami Vivekananda, Spiritual leader

“When we do research, we never apprehend the truth; we merely reduce the level of our error”

Karl Popper, Mathematician scientist.

Acknowledgement

First and foremost I have to mention *Lars Ake Brodin,* the guru in every sense. His extremely creative mind, incisive comments, ability to make you think whether scientific or on any other topic was always an inspiration and coupled with his humour made it a memorable experience. The only topic I can teach you is about the game of cricket!
Samir Saha, full of ideas and with his passion for science was the very epitome of energy. His approach to integrate science and clinical practice was indeed a revelation. Without his guidance the completion of this thesis would have been all the more difficult. No issue was too big or too small for him to help me in any situation.
Jacek Nowak with his keen analytical mind, a great eye for detail, along with his logical and meticulous approach left a strong impact on me. His witty and forthright manner, words of encouragement and support in times of difficulty are much appreciated.
I am deeply indebted to *Britta Lind* for the constant support, and kindly gestures which made my stay extremely pleasant. Despite her busy schedule, she always had time to help me or enquire about me. With her knowledge and expertise, she was there to solve any matter, be it on echo, a technical issue or any issue related to my thesis. A big thank you!
I also acknowledge *Miguel Quintana* for his critical comments and the help he extended which enabled me to complete my early scientific works.

Fredrik Brolund made my later visits to Stockholm more delightful and to a large extent I have understood the nuances of Swedish way of life better now. I thank him for the warm hospitality he has always extended to me. The discussions on various topics we had will keep me reminiscing for a long time!
The infectious enthusiasm and ideas of *Reidar Winter* were always a pleasant break in my work, and the administrative help from *Eva Brimark, Lasse Persson, and Jolanta Selin* made my task easier. My thanks to the numerous other people since 2003 in the clinical physiology department with whom I came into contact and who made my stay pleasant.

I am thankful to the *Swedish Institute* who supported me with their scholarship, without whom, this would not have been possible, and I have to make a special mention of *Karin Dif* for her deep understanding and the prompt support she gave when I needed it the most. My thanks to *Håkan Elmquist*, *Karolinska Institutet* and the *Swedish Heart Lung foundation* for their support.

My special thanks to whom I consider my main mentor, *Ramesh S S* at my home institute in Bangalore, India. His immense faith and trust in me kept me going all these years, and without his invaluable advice and support this journey would never have been completed. His extraordinary work ethics, clinical expertise, limitless energy, principles, and combined with a wonderfully humane nature is something I would always aspire for.
I also express my gratitude to all the technologists and colleagues who took personal interest in my work and made my job easier in the echo lab.

Lastly and most importantly a mention about my family, my mother, *Geetha,* and father, *Govind Gowda*, for all the hardship they had to endure and the sacrifices they made along the way. What they strived for and what has been achieved, this thesis in many ways is a tribute to them. It was also a difficult period for my wife *Lakshmi* and daughter, *Ridhima,* and son, *Aayush* during my long absences and hopefully the lost months can be made up in the times to come. My brother *Navin* and his wife *Jennifer* were also of great support to me.

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Abstract

Ever since the validation of the tissue velocity echocardiography (TVE) technique more than a decade ago the modality has been used rather successfully in various clinical situations, at rest as well as during stress echocardiography. Hitherto, dobutamine stress echocardiography has been the hallmark of all forms of stress procedures, now with TVE, quantification of the longitudinal motions of the left ventricle shows far superiority, with improved sensitivity and specificity in the functional diagnosis of coronary artery disease. Morever there has been continued interest in this technique for even assessing subclinical myocardial systolic and diastolic function in clinical scenarios like diabetes, hypertension and chronic kidney disease.

The aim of the present study was to evaluate left ventricular myocardial functions by applying TVE in human subjects having type 2 diabetes with or without co-morbidities and during changing loading conditions. The effects of changing loading conditions were analyzed during hemodialysis and following oral administration of an AT_1 receptor blocker. The studied subjects included individuals with type 2 diabetes as well as those with associated hypertension, coronary artery disease, microalbuminuria and end-stage renal disease. All patients with type 2 diabetes and co-morbidities underwent TVE enhanced dobutamine stress echocardiography, while load dependant left ventricular functions were analyzed at rest. There were 270 subjects in the study of type 2 diabetes, and associated cardiovascular diseases, and 101 subjects in the study of changing loading conditions.

Patients with type 2 diabetes revealed subclinical left ventricular dysfunction characterized by reduced functional reserve. This influence becomes quantitatively more pronounced in the presence of coexistent coronary artery disease and hypertension. The coexistence of type 2 diabetes and hypertension appears to have additive negative effect on both systolic and diastolic left ventricular function, even in the absence of coronary artery disease. The presence of microalbuminuria in type 2 diabetes patients does not worsen diminished myocardial functional reserve. A single session of hemodialysis improves left ventricular function in patients with end-stage renal disease only in the absence of type 2 diabetes and co-morbidities, while a single dose of an AT_1 receptor blocker valsartan results in reduction of afterload, and subsequently, in improvement of left ventricular function. TVE appears to be a sensitive tool for objective assessment of left ventricular function and can be successfully applied for the clinical evaluation of the effect of type 2 diabetes and co-morbidities on myocardial performance.

List of Included manuscripts

Study 1

Govind S, Brodin LA, Nowak J, Quintana M, Raumina S, Ramesh SS, Keshava R, Saha S. Isolated type 2 diabetes mellitus causes myocardial dysfunction that becomes worse in the presence of cardiovascular diseases: results of the myocardial doppler in diabetes (MYDID) study 1. Cardiology. 2005;103(4):189-95.

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Study 2

Govind SC, Roumina S, Brodin LA, Nowak J, Ramesh SS, Saha SK. Differing myocardial response to a single session of hemodialysis in end-stage renal disease with and without type 2 diabetes mellitus and coronary artery disease. Cardiovasc Ultrasound. 2006;4(1):1-9.

(Reprinted with permission from Biomed Central)

Study 3

Govind S, Saha S, Brodin LA, Ramesh SS, Arvind SR, Quintana M. Impaired myocardial functional reserve in hypertension and diabetes mellitus without coronary artery disease: Searching for the possible link with congestive heart failure in the myocardial Doppler in diabetes (MYDID) study II. Am J Hypertens. 2006;19(8):851-857.

(Reprinted with permission from American Journal of Hypertension, Ltd)

Study 4

Govind SC, Brodin LA, Nowak J, Ramesh SS, Saha SK. Acute administration of a single dose of valsartan improves left ventricular functions: a pilot study to assess the role of tissue velocity echocardiography in patients with systemic arterial hypertension in the TVE-valsartan study I. Clin Physiol Funct Imaging. 2006;26(6):351-6.

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Study 5

Govind SC, Brodin LA, Nowak J, Arvind SR, Ramesh SS, Anita Netyö, Saha SK.Microalbuminuria and Left Ventricular Functions in Type 2 Diabetes: A Quantitative Assessment by Stress Echocardiography in the Myocardial Doppler in Diabetes (MYDID) Study III.

(Submitted to International Journal of Cardiology in 2007*)*

Abbreviations

TVE	Tissue velocity echocardiography
DSE	Dobutamine stress echocardiography
TVE-DSE	Tissue velocity enhanced dobutamine stress echocardiography
LV	Left ventricle
PSV	Peak systolic velocity
E wave	Early diastolic filling velocity by conventional echo
A wave	Late diastolic filling velocity by conventional echo
E'	Early diastolic filling velocity by TVE
A'	Late diastolic filling velocity by TVE
E/ E'	Estimated LV filling pressure
IVCT	Isovolumic contraction time
IVRT	Isovolumic relaxation time
ET	Ejection time
Tei index	IVCT+IVRT/ET (myocardial performance index)
DM	Type 2 diabetes
HTN	Hypertension
CAD	Coronary artery disease
CVD	Cardiovascular disease
CVD-DM	Type 2 diabetes without cardiovascular disease,uncomplicated DM
CVD+DM	Type 2 diabetes with cardiovascular disease, complicated DM
MA	Microalbuminuria
DM-MA	Type 2 diabetes without microalbuminuria
DM+MA	Type 2 diabetes with microalbuminuria
HD	Hemodialysis
ESRD	End-stage renal disease
ms	milliseconds
cm/s	centimeters/second
AT_1 blocker	Angiotensin receptor blocker

Introduction

1 Principles of tissue velocity echocardiography and its clinical application

The Doppler Principle, the fundamental tool for all Doppler-related computations of tissue motions, static or dynamic, states that the frequency of ultrasound reflected from a stationary object is identical to the transmitted frequency. If an object is moving towards the ultrasonic transducer, the reflected frequency will be higher than the transmitted frequency, and conversely, if an object is moving away from the transducer, the reflected frequency will be lower. The difference between the transmitted and received frequencies is known as the Doppler shift.

The Doppler data that are generated are typically displayed as velocity rather than the actual frequency shift [1]. With this principle, the conventional Doppler techniques assess the velocity of blood flow by measuring high frequency, low amplitude signals from small, fast moving blood cells. In tissue Doppler Echocardiography (henceforth termed as Tissue Velocity Echocardiography or TVE, as explained below) the same principles are used to quantify the higher amplitude, lower velocity signals of myocardial tissue motion [2, 3], displayed typically as a velocity profile with motions towards the transducer generating signals above the zero crossing line and that away from the transducer below the zero crossing line

There are two ways of deriving velocity information from the myocardium: one is by using the pulsed-wave Doppler technique [2] and the other is colour tissue velocity technique [3]. The latter modality requires off-line extraction of velocity curves from acquired colour cine-loops. In today's ultrasound equipments the former is nearly always available while in some equipment both modalities could be available as in-built tools. Whatever methods are used to derive information of tissue motion the basic measured variable is velocity, and hence the technique should be named Tissue Velocity Echocardiography [4]. By further mathematical processing of the velocity data, a number of parametric images could be obtained such as strain and displacement imaging.

One essential requirement to obtain adequate motion information from colour cine-loops is that the acquisition of the images should be digitized at a frame rate (generally over 100 Hz) adequate to obtain information of even the very subtle motions of the heart during isovolumic phases of the cardiac cycle [5]. Such motions are essential for the "reshaping" of the cardiac geometry prior to unloading and loading of the heart respectively during systole and diastole. The reason for high sampling frequency lies in the fact that when the velocity curves undergo Fourier transformation it mostly contains a frequency component above 50Hz. Therefore, according to the samplings theorem described by Shannon [6], the acquisition should be at least double the frequency. In clinical testing it has been validated that a sample frame rate of 110 appear to cover the physio-pathological situations where myocardial velocities of less than 20 ms duration are impossible to detect because of the fact that the upper limit of human eye is between 10-12 Hz. A diagnostic capability to cover those limits has not been possible with slow motion presentation of video recordings because these have never exceeded 40 Hz [4]. The multiple variables are obtained on the TVE software (Figure-1).

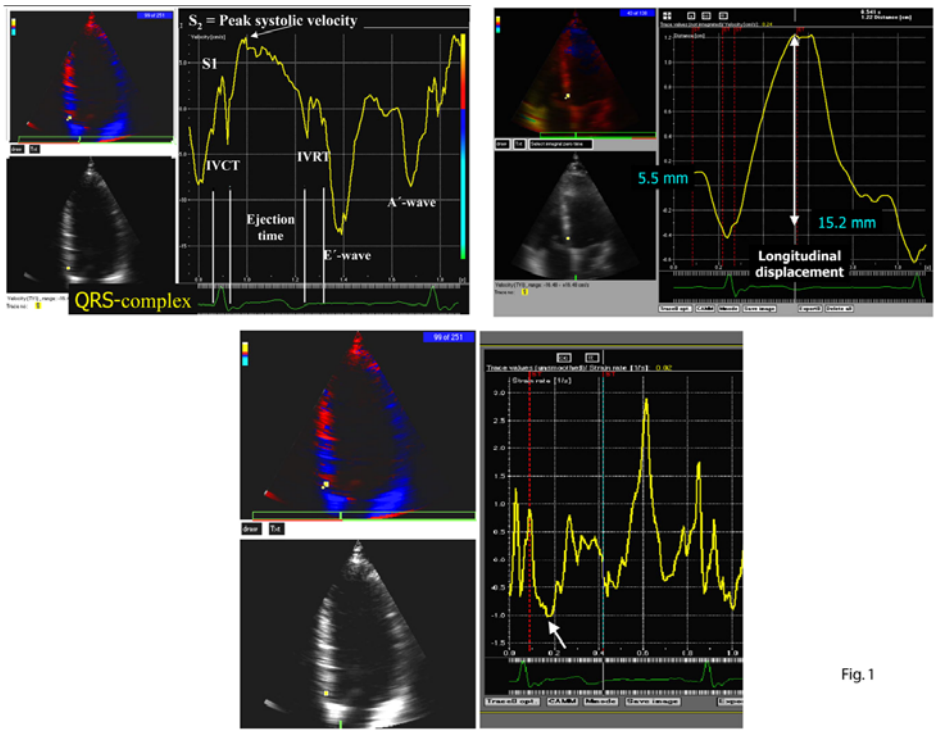


Fig. 1

Figure 1: *TVE images of velocity curve (upper left), displacement curve (upper right), and strain rate curve (lower).*

Myocardial velocity is the most widely used and validated parameter, and its derivatives like strain rate imaging and strain which is being increasingly discussed, provides information on deformation and along with other additional parameters like myocardial displacement and tissue synchronization imaging add to its capability. These parameters are characterized by their ability to quantify myocardial motion.

Ever since the validation of the technique more than a decade ago [7, 8] the modality has been used rather successfully in a variety of clinical situations, from investigations of athlete's heart to implantation of bi-ventricular pacing (cardiac resynchronization therapy) and even in preclinical diagnosis of genetic diseases such as hypertrophic cardiomyopathy [9]. More importantly it is now widely used in the diagnosis of diastolic dysfunction. There has been continued interest in its utility for assessing subclinical myocardial systolic and diastolic function of the ventricles in clinical scenarios like diabetes, hypertension (HTN), hypothyroidism [10, 11, 12]. One of the other successful clinical applications of TVE has been the use of E/E´ratio (the ratio of early diastolic transmitral to early diastolic tissue velocity first proposed by Nagueh) that can non-invasively diagnose left ventricular filling pressure with high prognostic value [13] as well as non-invasive differentiation of constrictive and restrictive physio-pathologies [9].

It may be mentioned here that just by quantification of longitudinal velocities measured from around the mitral annulus nearly all of the above mentioned diseases could be monitored, diagnosed, and prognosticated. This is clearly a step forward compared with what was observed earlier by Alam et al by estimating the annular excursion during systole using M-mode in the intersection of mitral leaflets and LV cursor [14, 15]. The current version of the TVE software however is not suitable enough to negotiate radial and/or circumferential velocities. However, a non-Doppler based software called Speckle tracking echocardiography [16, 17], which is presently under validation, can compute multidirectional motions of the heart in a more physiological way commensurate with the complex fibre arrangement of the heart.

2 Type 2 diabetes: Does left ventricular dysfunction play a central role in adverse outcome?

DM which is now being termed as an epidemic is spiralling upwards at an alarming rate, raising worldwide concern about its devastating effects on health and healthcare systems. It is estimated that presently there are 200 million diabetics and it is expected to reach 360 million by 2030 as per World Health Organisation projections [18], with the largest number of cases going to be seen in China, India and USA. More than 90% of these are likely to be type 2 diabetes (DM) individuals. People with diabetes are at increased risk for cardiovascular diseases and have worse outcomes after surviving a cardiovascular event. They are at 2-4 fold risk of developing cardiovascular problems which unfortunately is the most common cause of mortality in this population [19]. The risk of myocardial infarction and its sequelae is well known, but there is also a subset of this group which does not have coronary artery disease (CAD), but has a disconcertingly higher morbidity and mortality. Hence risk stratification of diabetics is essential to pre-empt any major events.

Among the many investigations available today to investigate DM, functional imaging with echocardiography which is easily accessible and cost effective now stands better equipped with availability of newer applications like TVE to objectively quantify myocardial function. Evaluation of DM and co-morbidities by dobutamine stress echo (DSE) and the behaviour of altered loading conditions is an excellent model to study by applying TVE in a clinical setting.

Multiple mechanisms are implicated for the high cardiovascular (CVD) morbidity and mortality in DM. Apart from the traditional risk factors; other culpable factors include congestive heart failure and end stage renal failure. Biomarkers like albuminuria further multiply the CVD risks faced. It is recognized that DM predisposes to impaired left ventricular (LV) systolic and diastolic dysfunction [20]. Multiple mechanisms have been held forth for this, apart from CAD, HTN, left ventricular hypertrophy (LVH) and obesity, they also include diseases of the coronary microvasculature, endothelial dysfunction and the much discussed entity “diabetic cardiomyopathy” which is attributed to metabolic and possibly structural abnormalities within the heart muscle. The propensity of DM to affect the heart has resulted in separate guidelines being issued [21]. There is specific long-term target end organ damage due to its microvascular disease and these patients are at a high risk of cardiovascular disease, cerebrovascular disease and

peripheral artery disease. Hypertension, a frequent co-morbidity, is known to affect patients with DM two to three times more than non-diabetic subjects. DM and HTN are additive risk factors for atherosclerosis and conversely HTN is more predisposed to DM. And apart from traditional risk factors, microalbuminuria in DM is associated with an increase in cardiovascular mortality and is considered as a risk indicator [22]. Abnormal myocardial function in DM but without overt heart disease manifestations has been uncovered using TVE [10]

The reportedly excess cardiovascular morbidity and mortality in subjects with type 2 diabetes cannot be explained by the conventional risk factors such as dyslipidemia, overweight, ageing etc. Moreover, there is only a weak link between hyperglycemia, though considered to be the principal metabolic culprit, and incidence of excess cardiovascular disease [23] in DM. Though a number of other factors involving the various endogenous systems such as inflammatory, endothelial, cytokines, and neuro-endocrine pathways have been proposed to be responsible for the excess occurrence of cardiovascular mortality, it seems increasingly likely that factors within the myocardium itself could play a decisive role in individual subjects. This myocardial dysfunction, enthusiastically termed as "diabetic cardiomyopathy" [24] or more cautiously termed as diabetic heart muscle disease [25] may play a central role in the worse outcome in patients with DM with acute coronary syndrome. The proposal is supported by the findings in a Swedish study [26] in which it has been shown that subjects with DM presenting with acute coronary syndrome have worse outcome compared with the non-diabetic subjects well controlled for treatment strategies (percutaneous intervention), serum troponin levels, and left ventricular ejection fractions. This study probably highlights the fact that there could be some factors in the myocardium itself that have resulted in reportedly poorer outcome in their study in the diabetic group, but other factors such as microvascular pathologies and ischemic preconditioning should however be not be forgotten in this context. Whether this intrinsic myocardial disease causes the reduction of velocity or whether there are other factors such as increased afterload secondary to aortic stiffness resulting from macrovascular complications of type 2 diabetes, remains to be seen [27].

3 Dobutamine stress echocardiography and functional diagnosis of coronary artery disease

Though dobutamine stress echocardiography has hitherto been the hallmark of all forms of stress procedures to detect coronary artery disease indirectly by left ventricular wall motion abnormalities during provocation, the procedure seriously lacks reproducibility even when the images are analysed by cross-continental experts [28]. It is rather intriguing to note that by TVE quantification of longitudinal motions of the left ventricle during dobutamine provocation, the European [29] and the Australian investigators [30] have been successful in objective and non-invasive diagnosis of coronary disease with a far superior sensitivity, specificity, and reproducibility for all forms of coronary artery disease. Both the landmark studies have used longitudinal velocity for quantification of myocardial motions during dobutamine stress. In the MYDISE (Myocardial Doppler in Stress Echocardiography) sub study [31] however, displacement imaging (tissue tracking) resulted in best sensitivity and specificity for the circumflex disease. It must be emphasised, however, that the left ventricular segmental velocities should be measured at peak stress and should be adjusted for heart rate, gender, and age [29]. This objective quantification of dobutamine stress echocardiography has been termed as "quantitative stress echocardiography".

Evidence continues to mount that TVE and the extrapolated measurements of strain and torsion can be of great value in assessing regional myocardial performance in the setting of acute ischemia. In a combined experimental and clinical study [32] the ability of the TVE recording modalities of velocity, strain, and displacement was compared to quantitatively assess regional myocardial systolic function. They measured these variables at baseline and during occlusion of the left anterior descending coronary artery in 10 open-chested dogs with sonomicrometry as a standard. They demonstrated that systolic strain correlated very well with segmental shortening and work and differentiated both moderately and severely ischemic myocardium from normal. They also studied 10 patients with acute anterior infarction and 15 control subjects. In this clinical study they observed that systolic strain differentiated well between infarcted and normal myocardium, whereas displacement and ejection velocity showed overlap. They have concluded that TVE provides an excellent modality to assess the function of segmental ischemic myocardium and that strain measurements are clearly superior to other TVE variables in this regard.

4 Determinants of cardiac performance and their influence on TVE

For the heart to pump an effective output under normal physiological conditions generally 4 different factors act together in synergy. These are; 1. Heart rate, 2. Preload, 3. Afterload, and 4. Contractility. All TVE variables are influenced by these determinants. The classic example of how changes in contraction and heart rate alters the myocardial velocity in humans has been illustrated in the MYDISE study [29] that described how increases in heart rate and contraction by dobutamine during stress echocardiography increased the myocardial velocity in healthy subjects while failing to do so in patients with significant coronary artery disease.

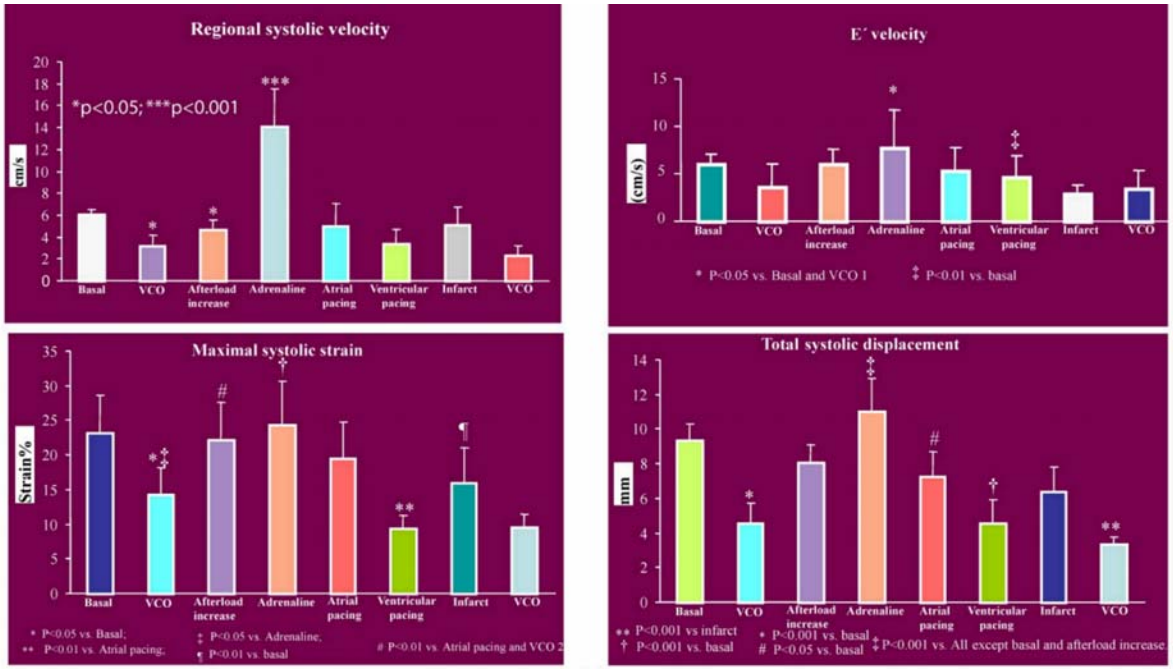


Figure 2: *Bar diagram showing evidence of influence of changes in loading conditions in the TVE variables.*

VCO = Vena caval occlusion; Infarct = Myocardial injury caused by left anterior descending artery occlusion in the coronary vasculature. Changes in regional ***systolic velocities (upper left)*** *and* ***early diastolic velocities (upper right)*** *as well as consequent changes in* ***strain% (lower left)*** *and total* ***systolic displacements (lower right)*** *are appreciated. Statistical significances are marked by symbols.*

In animal experiments, it has been shown by our group [33] that not only myocardial systolic functions but also myocardial diastolic functions measured by regional TVI are subject to changes in preload, afterload, contraction, and coronary blood flow occlusion (Figure 2). However, the question of load changes is not a simple and straightforward one and depends on the extent of changes in the loading conditions. For example, in subacute or minimal banding of the aorta in animals, do not markedly change the myocardial velocity though a striking decrease is seen in more severe banding of the aorta [34]. It has also been reported that not all TVE variables react in the same fashion in response to alteration of a loading conditions, as has been seen in paediatric population [35, 36]. In one study patients that underwent interventional atrial septal defect (ASD) closure: at baseline, patients with ASD had significantly higher right ventricular systolic velocities than controls whereas isovolumic contraction acceleration was similar in patients with ASD and controls. In the catheterization laboratory post intervention, conventional function parameters remained stable but systolic myocardial velocities decreased significantly in all segments. Diastolic velocities decreased in LV segments but not in the right ventricle. In contrast to velocities, isovolumic acceleration was stable during ASD device closure. On follow-up at 24 hours, myocardial velocities had normalized. The authors concluded that device closure of ASD resulted in an acute transient decrease of regional myocardial velocities in the LV and right ventricle, whereas the load-insensitive marker isovolumic acceleration remained stable. Therefore, the velocity changes may represent a response to altered left and right ventricular loading conditions [35].

5 Preload and its effects on TVE variables

Besides congenital heart diseases with pathological shunting, another intriguing model of studying the influence of loading conditions in humans is end-stage renal disease that requires renal replacement therapy quite often in the form of hemodialysis (HD), a procedure that results in acute decrease in preload by virtue of removal of excess fluids and solutes. Immediately after HD, a highly significant decrease in the left ventricular internal dimension is noted [37], confirming the acute reduction of left ventricular end diastolic volume, bringing the left ventricle to work according to the Frank Starling´s principle provided that it is free from background diseases. While simultaneously measuring regional myocardial velocities, it has been characteristically noted that the systolic velocities increased by about 10-15% immediately post HD in patients without background cardio- metabolic diseases, presence of which may mask or even blunt the improvement in systolic and diastolic functions of the heart.

6 Effects of afterload manipulations on left ventricular systolic and diastolic velocities

Primary arterial hypertension is presumed to be a classic clinical model of studying the effect of afterload on left ventricular systolic and diastolic function. It is therefore not surprising that relatively large number of studies have been performed to assess the cardiac functional status in human subjects with hypertension, using tissue velocity echocardiography. The studies have provided evidence of decreased myocardial systolic and diastolic velocities in patients with hypertension, evidently because of increased afterload resulting from chronically elevated systemic arterial blood pressure. Though left ventricular hypertrophy and associated diastolic dysfunction have been the usual areas of interest in hypertension research [38], application of TVE has provided significant information on left ventricular systolic dysfunction even in the absence of left ventricular hypertrophy [39, 40]. A combination of both systolic and diastolic dysfunction has also been noted [41]. Not only that, one study from Hong Kong has also shown prognostic values of TVE variables in human subjects with hypertension. In that study the early diastolic mitral annular velocity measured by TVE provided prognostic information, incremental to the clinical and standard echocardiographic variables [42].

In an experimental project a group of Japanese investigators [43] have studied the effects of afterload increase on regional wall motion velocities in human volunteers by infusing intravenous angiotensin II. By using pulsed-wave tissue Doppler imaging they have shown that a 30% increase in mean blood pressure following angiotensin II infusion, an acute increase in afterload caused a significant decrease in longitudinal fiber shortening during the isovolumic contraction phase, and circumferential fiber shortening during the ejection phase. LV relaxation during early diastole (early diastolic LV wall motion velocities along both axes) also decreased significantly.

Because of the robustness of the TVE in studying subjects with hypertension it is now possible to study the effects of blood pressure lowering agents on the outcome of treatment measured by tissue Doppler echocardiography. In one such study known as SILVHIA the investigators have shown differential effects of drug treatment (beta blocker versus angiotensin receptor blocker) on the improvement of diastolic TVE parameters [44]. In the newly launched VALIDD study [45] the investigators are on the way to test the hypothesis that TVE derived left ventricular relaxation properties could be improved following treatment with angiotensin receptor blocker valsartan. The

investigators believe that the ratio of early transmitral velocity to early myocardial velocity (E/E´ ratio) could be a more robust variable than the conventional transmitral Doppler data that are vulnerable to even small changes of heart rate and loading conditions. However, it remains to be seen whether the technique is still reliable enough to be used in the primary care centers to monitor patients with hypertension as has been claimed by some investigators [46]. It should be emphasized here that all the studies mentioned above have been performed using pulsed-wave Doppler not the colour Doppler. The two modalities though provide similar information, the latter modality not only allows off-line analyses of digitized images, it also probably has other advantages such as in quantification of isovolumic motions of the heart by acquisition of images at high frame rates.

Aims

The aims of the present thesis were as follows:

General Aims

1) To evaluate LV myocardial functions in clinical situations by applying TVE
2) The presence of DM, co-morbidities or changed loading conditions alters LV myocardial function as measured by TVE

Specific Aims

1) To determine whether isolated DM alters myocardial function differently than common cardiovascular diseases and to what extent a combination of the illnesses might lead to additional worsening of myocardial function.
2) Longitudinal myocardial functions as assessed by TVE in response to hemodialysis are quantitatively different in differing clinical settings irrespective of similar degree of changes in loading conditions.
3) To assess the effects of HTN, DM, and its combination on LV systolic and diastolic functional reserve using TVE during dobutamine stress echo in patients without CAD.
4) To characterize LV myocardial functional changes consequent upon afterload reduction by the angiotensin receptor blocker valsartan.
5) To assess whether microalbuminuria could cause additional myocardial dysfunction in patients with isolated DM.

Subjects and Methods

Studies 1, 2, 4 and 5 were prospective while *Study 3* was a sub study and all were non randomized. Patients were eligible based on the inclusion and exclusion criteria. All patients who had clinical indications for their procedures were screened and those eligible were included.

Exclusion criteria:

- Primary myocardial disease
- Significant pericardial disease
- Significant valvular disease
- Sustained arrhythmias
- LVEF less than 40 %
- Pacemakers
- Structural heart disease
- Pregnancy or serious medical illness.

Diagnoses of the illnesses were supported from the clinical data available in the hospital database, medication and treatment history. Patients diagnosed to have illnesses were on prescribed treatment prior to recruitment. Systemic arterial hypertension was defined as two measurements of brachial artery blood pressure more than 140/90 mm of Hg as per JNC criteria [47]. Type 2 diabetes was defined as fasting plasma glucose concentration as 126 mg/dl and above, and for whole blood as 110 mg/dl and above, as per World Health Organization criteria [48]. CAD was defined by clinical history of typical angina or previous myocardial infarction documented by electrocardiography (ECG) or presence of regional wall motion abnormality by echocardiography or positive dobutamine stress echocardiography or those having angiographic evidence of significant coronary artery disease. Controls were defined as those with low pretest probability of CAD, no diseases, had atypical symptoms or were asymptomatic, not on any medications, with normal clinical examination and normal relevant clinical investigations.

The Ethical Committee of the Karolinska University Hospital at Huddinge, Stockholm, and the Institutional Review Board of the BMJ Heart Centre, Bangalore, India approved the study protocols. All study subjects gave informed consent.

1 Study population

In Study 1, subjects were selected when evaluated for either atypical chest pain or for coronary artery disease.

In Study 2, patients with primary end-stage renal disease had polycystic kidney disease, IgA nephropathy, focal segmental glomerulosclerosis, or crescentic glomerulonephritis.

In Study 3, subjects from the myocardial Doppler in diabetes *(Study1)* study population participated. Patients with CAD and above mentioned criteria were excluded.

In Study 4, 14 subjects were Swedish nationals (12 of them were native white and two were from South Asian background) and the rest were native Indian nationals. Forty-three subjects had only HTN, while 12 had HTN and stable coronary artery disease or type 2 diabetes mellitus or both. All patients had essential HTN without any past history of acute illnesses of any kind. The subjects were given an early morning single dose of 80 mg valsartan, withholding regular antihypertensive medications on the day of investigation. Recording of blood pressure (BP) was performed in supine position using the right arm.

In Study 5, male and female subjects were equally distributed except in the DM without microalbuminuria (DM-MA) group. Patients with known CAD, HTN and other above mentioned criteria were excluded from the present analysis. Fundoscopy showed that DM-MA group had 8 patients with non-proliferative diabetic retinopathy and 2 patients had neuropathy by microfilament testing, while in the DM with microalbuminuria (DM+MA group) there were 11 patients with non-proliferative diabetic retinopathy and 3 with neuropathy.

	Study 1 *Patients/controls*	**Study 2** *Patients*	**Study 3** *Patients/control*	**Study 4** *Patients*	**Study 5** *Patients/ controls*
Total number of patients	177/22	46	106/22	55	58/13
Male/female ratio	128/71	31/15	72/56	29/26	42/29
Mean age (years)*	56±10	51±14	54±1.3	55±11	49±10
Mean body mass index (kg/m ^{2})*	26.6±4.0	———	26.6±5.2	27.9±5.0	25.2±3.2
Distribution of patients in different study groups	DM=59 HTN=20 CAD=35 DM+HTN=27 DM+CAD=16 DM+HTN+CAD=20	ESRD=17 ESRD+DM=15 ESRD+DM+CAD=14	DM=59 HTN=20 HTN+DM=27	_____	DM-MA=31 DM+MA=27

Table 1: *Subjects characteristics of all studies. *Data are mean ± SD.*

2 Echocardiography

2.1 Echocardiography equipment and image acquisition.

In *Studies 1-4,* Echocardiography was performed using commercially available Vivid 5 equipment (GE Vingmed, Horten, Norway) with a preinstalled Echopac software program and Vivid 7 equipment in *Studies 4-5*.

The LV images were acquired in parasternal long and short axis, as well as in apical four- and two-chamber projections. Due precautions were taken during Doppler study, optimal image and frame rates were considered and images were obtained whenever possible at end respiration with breath holding. The LV with TVE enhanced images were obtained with colour scale adjusted to 20 cm to avoid aliasing, at an average frame rate of 130 for post processing on Vivid 5 equipment and an average frame rate of 177 on Vivid 7 equipment, and were digitally stored on the echo machine. The data was then transferred, and stored on commercially available magnetic optical disks. Digitally stored cine loops

during three to five consecutive heart cycles were analyzed either on a Mac or a PC based workstation.

2.2 Conventional echo measurements

In S*tudies 1-5*, eligible subjects underwent initial routine conventional transthoracic echocardiography after prior clinical evaluation. LV dimensions, wall thicknesses and LV mass were measured by M-mode in the parasternal long-axis views. Mitral inflow velocities were measured by conventional pulsed wave Doppler, by positioning the sample volume at the level of the tips of mitral leaflets in the apical 4-chamber views. All 2-D and M-mode measurements were made according to the American Society of Echocardiography guidelines [49.] The transmitral peak early (E) and late (A) diastolic velocities, and E/A ratio were measured along with pulmonary systolic, diastolic and atrial reversal flow velocities. As an additional diastolic variable, velocity propagation of early mitral inflow by color M-mode was also measured [50]. 2-D LV ejection fraction was measured by modified Simpson's approach

LV myocardial performance index or Tei index was measured; it was defined as the sum of isovolumic contraction time and isovolumic relaxation time divided by ejection time. Global Tei index was calculated by taking the average data from 4 LV bases [51].

LV filling pressure was estimated as E/E' ratio by measuring transmitral inflow (E) velocity and this was divided by E' by pulse Doppler at the septal and lateral annulus from the apical 4-chamber projection to obtain the E/E´ratio [52].

2.3 Dobutamine stress echocardiography

In *Studies 1, 3 and 5*, standard gray scale images with superimposed color Doppler were acquired in apical four- and two-chamber projections. Cine loops containing three consecutive cardiac cycles were analyzed off-line at rest and during peak dobutamine stress. DSE was performed using a graded standard 3-min stage (5–40 g/kg per min) protocol. End points of DSE were the achievement of 85% of maximum heart rate, severe wall motion abnormalities or subjective intolerance. Patients who failed to achieve target heart rate were given atropine in increments of 0.3 mg up to a maximum of 1.8 mg. For visual analysis, the left ventricle was divided into 16 segments. The wall motion of each segment during DSE was scored as follows: hyperkinetic, normal, hypokinetic, akinetic, or dyskinetic. A test was considered normal in the absence of a new-onset wall motion

abnormality in at least two consecutive segments. A test was considered eligible for the analysis if at least 12 of 16 segments were interpretable. All the echocardiograms were analyzed by the main author, while in *Study 1 and 3* along with the main author they were also independently analyzed by another co-author. A second interpretation was done by an independent investigator wherever there was ambiguity, and consensus opinion prevailed.

2.4 TVE measurements

In *Studies 1-5*, quantification of longitudinal wall motion of the LV was made according to the protocol followed in the MYDISE study [29]. The LV long-axis regional systolic and diastolic function (septum, lateral, inferior and anterior) was assessed from the apical views from which four basal and mid segments (septum, lateral, inferior, and anterior) were analyzed. Care was taken when the cursor was placed in the basal segment so as to exclude the mitral annulus. A typical velocity profile could be obtained by positioning the sample volume in any of the segments. Peak systolic (PSV), early diastolic (E'), and late diastolic (A') myocardial velocities (cm/s) were measured at the peaks of the respective waves (Figure 1) on the myocardial velocity curves in the individual LV walls. LV diastolic function was assessed by velocity of the E' wave at maximal stress. The corresponding variables were measured by taking average of the four LV bases which was likely to be indicative of global function.

The LV short-axis systolic and diastolic functions were assessed from the parasternal short-axis images, from which only the mid posterior segments (parasternal short axis image) were analyzed in order to compute radial data. Measured variables were isovolumic contraction times (IVCT) and velocities, isovolumic relaxation times (IVRT), ejection times (ET, defined by the period between aortic valve opening and closure), and filling times (summation of early and late diastolic filling times) were calculated from the tissue velocity profile. Filling time (FT) was measured from the end of IVRT to the beginning of IVCT (Figure 1). Events of the cardiac cycle were recorded according to methods described previously [53].

The myocardial wall displacement (mm) in the long- and short-axis was obtained by automated temporal integration of the velocity profile during systole.

Strain and strain rate imaging are TVE variables that can quantify deformation [54]. Strain represents deformation of an object (myocardial wall) and is often expressed in

percentage of change from end-diastolic dimension. Positive strain represents lengthening or stretching while negative strain is shortening or compression. Strain rate (SR) is the instantaneous strain per unit time (cm/s/cm, or 1/sec) and has the same direction as the strain, i.e. negative strain rate during shortening and positive strain during lengthening. Strain rate therefore represents velocity of regional myocardial deformation, thereby also reflecting myocardial contraction when measured at peak systole. Strain (%) is the integral of strain rate. To calculate strain rate the area of interest along the sample scale was chosen to be ≈ 15 mm since this appears to provide the best signal to noise ratio. The longitudinal basal, mid and apical segments of septum, lateral, inferior and anterior walls were averaged to assess global strain rate. Radial strain rate was calculated from the LV posterior wall in the short axis projection.

3 Biochemical analyses

In all studies, biochemical analyses were done prior to recruitment. Lipid profile was done as a fasting test. Blood sugars were done as either non-fasting or fasting samples.

In *Study 5,* urinary albumin was measured by a morning spot collection on two different days. The urinary albumin creatinine ration (UACR) was measured by modified Jaffe's method using the immunoturbidimetry method. The UACR measured in a spot urine sample is highly correlated with 24-hour urine albumin. MA was defined by urine albumin: creatinine ratio as more than 30 and less than 300 μg albumin / mg creatinine as described earlier [55, 56].

4 Electrocardiography

In *Studies 1-5,* electrocardiography was performed using commercial GE MAC "series" equipment that were enabled to show automated values, and subsequently used for interpretation after manual corrections were made whenever needed. All ECG's were performed prior to inclusion and during dobutamine stress echocardiography stages and whenever it required.

5 Coronary angiogram

In *Studies 1, 2, 3 and 4,* coronary angiogram was carried out by standard Judkins technique. Significant stenosis was defined as >70 % intra-luminal obstruction either visually or quantitatively by intra vascular ultrasound.

6 Hemodialysis

In *Study 5,* all patients were on maintenance dialysis, done twice weekly. Duration of maintenance dialysis varied from 10 months to 7 years. Echocardiography was done within 60 minutes of pre- and post dialysis. Hemodialysis was performed using Fresemins-76 dialyzer with dialyzate flow rate of 500 ml/min. Sodium bicarbonate dialysis was done with dialyzate flow of 500 ml/min and an average blood flow of 250 ml/ min.

7 Statistical analysis

A PC based STATISTICA version 6.0 (Statsoft, Tulsa, OK) was used for data analysis. A p value of <0.05 was considered significant. Data are expressed as mean ± SD

In Study 1, one-way ANOVA followed by post hoc Tukey honest test was performed to compare the differences between groups. Reproducibility was tested using Pearson's correlation coefficient equation and calculation of methodological error. Regression analyses were performed to assess correlation between two variables. Unpaired t-test was used to study comparisons between complicated and uncomplicated DM

In Study 2, one-way ANOVA followed by Scheffe's test was performed to compare the differences among the three groups. Paired t-test was performed to compare pre-and post-dialysis data within groups. Intra-observer variation was similar to our previous study.

In Study 3, one-way ANOVA followed by post hoc Tukey honest test was performed to compare the differences between groups. To study the possible determinants of LV global myocardial systolic function expressed as peak systolic velocity at peak stress, a linear regression analysis was performed introducing variables from demographic, biochemical and conventional echo data. Only the variables showing a correlation with a p value of <0.1 were entered in a multiple forward stepwise regression analysis.

In Study 4, paired t-test was performed to compare the pre- and post-valsartan data.

In Study 5, one-way ANOVA followed by post hoc Scheffe's tests were performed to compare the differences between the groups.

8 Reproducibility: intra- and inter-observer variability

In *Study 1*, there were no significant differences between the two measurements made on systolic and diastolic variables on two different occasions (all $p > 0.05$). Adjusted R^2 values were 0.71 for systolic and 0.81 for E velocity at rest, and those at maximum were 0.93 and 0.70, respectively (all $p < 0.001$). Methodological error was assessed by using the formula E = SD of mean difference·100/total mean·√2. The error was calculated as the variation of a single measurement based on double measurements. Peak systolic velocity, E' and A' diastolic velocities showed an error of 7, 8 and 11%, respectively.

Results

1 Effects of type 2 diabetes and co-morbidities on left ventricular myocardial function *(Studies 1, 3, and 5)*

1.1 Demographic data

In *Study 1,* patients with DM along with both CAD and HTN were the oldest (63 ± 8 years) while those with only DM (53 ± 10 years) were the youngest, whereas in *Study 3 and 5,* there was no significant variation. Duration of diabetes (years) showed that patients with both isolated DM (7.2±5.2) and DM+HTN (7.4±4.2) had a shorter duration compared with both DM+CAD (12.5±5.1) and DM+CAD+HTN (15.8±5.5), the latter having the longest duration, while in study 5 it showed no significance among the groups (5.6±4.7, DM-MA vs. 8.2±5.1 DM+MA).The body mass index did not differ among the groups.

1.2 Biochemical data

Plasma glucose was inadequately controlled, but levels never exceeded more than 200 mg/dl in *Study 1, 3 and 5,* while glycosylated haemoglobin (%) levels, done only in *Study 5,* were mildly elevated (8.1±1.7 in DM-MA and 8.3±1.6 in DM+MA groups). Expectedly serum creatinine in *Study 1* was significantly higher in DM+CAD+HTN compared with DM and DM+HTN and also in the DM+MA group in *Study 5*, while in *Study 3* it was more associated with HTN as it was seen to be increased in subjects with both HTN and HTN + DM. Microalbuminuria levels (µg/mg) showed a significant difference among the diabetic subjects and were as follows, 1.2±4.0 (controls), 7.2±4.0 (DM-MA) and 127.5±41.6 (DM+MA) with $p<0.001$. Lipid levels did not vary among the diabetic groups, but were higher when compared with the controls.

1.3 Conventional echocardiography data

LV size was within normal limits, but was comparatively increased when DM was associated with CAD. LV mass was significantly higher in DM+CAD+HTN when compared with controls. Statistically significant differences were found in septal thickness, with lowest in controls, and highest in DM+CAD+HTN group, while posterior

wall thickness did not vary. LV ejection fraction was lowest in DM+CAD (56 ± 6%) compared with all other groups and was highest in controls.

Differences were noted in transmitral E velocity, wherein in *Study 1* it did not differ among the patient groups, while significant differences were observed in the other two studies. Highest velocities were recorded in controls. Transmitral A velocities showed statistically significant differences in all the studies, with the highest velocities recorded in DM+CAD+HTN. Consequently, E/A ratio was lowest in DM+CAD+HTN, suggesting significant diastolic dysfunction. Tei index was introduced as an additional parameter in the diabetic population in *Study 5* and was found to be higher in them compared with controls (0.40±0.1; control, 0.53±0.1;DM-MA, 0.52±0.1;DM+MA with $p < 0.001$). The effect of DM on ECG was also analyzed in *Study 5* at rest, but it showed no significant differences in the heart rate, PR interval, QRS duration, and QTc interval in any of the groups. None of these patients had ST-T abnormalities on their ECG's.

1.4 Tissue velocity enhanced-dobutamine stress echocardiography data at rest and during peak stress

In *Study 1*, average peak systolic velocity at rest (Figure 3) was significantly lower only in CAD when compared with controls (5.7 ± 1.2 cm), but lower in DM only when associated with CAD and HTN, when compared with DM (5.3 ± 1.3 cm; $p < 0.05$). During peak stress, patients with isolated DM, CAD, and HTN had significantly lower peak systolic velocity compared with controls. When they were grouped as cardiovascular diseases (HTN ± CAD) without DM and cardiovascular diseases with DM respectively, the average peak systolic velocity were significantly lower in both the groups at rest when compared with controls and the corresponding velocities were similarly lower in both the groups when compared with controls ($p < 0.001$) during peak stress, but was more pronounced in cardiovascular diseases with DM.

A somewhat similar pattern was seen in E' (Figure 3, lower panel) velocities at rest, which were significantly lower in CAD compared with controls and in both forms of cardiovascular diseases with or without DM when compared also with controls. At maximum stress, in individual groupings (Figure-3, lower panel), E' velocity was significantly decreased in all the groups in comparison to controls, and significantly decreased in the DM+CAD, DM+HTN and DM+CAD+HTN when compared to DM, while a similar picture emerged when they were grouped as cardiovascular diseases with DM .

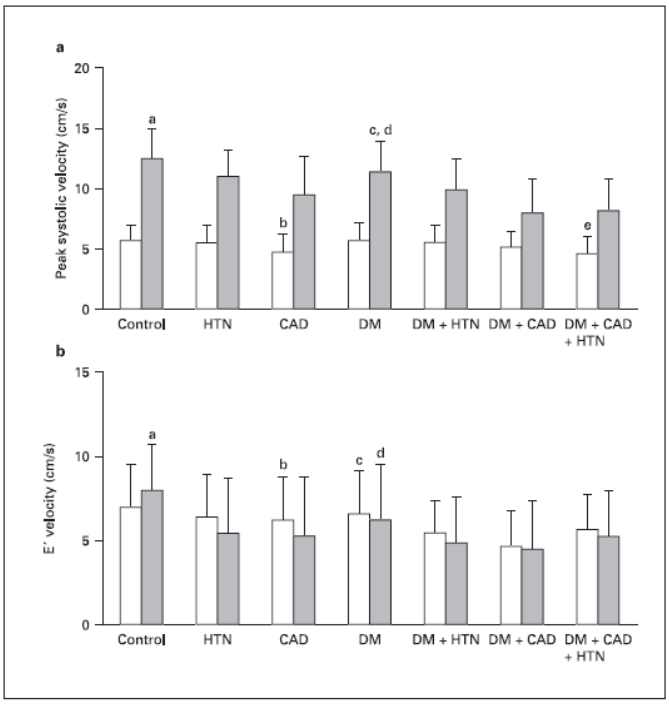


Figure 3: *Average LV systolic (a: upper panel) and diastolic (b: lower panel) velocities at rest and maximal dobutamine stress (Study 1) (white bars denote at rest and grey bars maximum stress).*

Upper panel*: [a] = p<0.001vs.CAD, HTN; [b] = p< 0.05 vs. control; [c] = p< 0.01 vs. control; [d] = p< 0.001 vs. DM+CAD, DM+HTN and DM+CAD+HTN; [e] = p<0.001 vs. DM.* ***Lower panel****: [a] = p<0.001 vs. all; [b] = p< 0.05 vs. control; [c] = p< 0.001 and [d] = p< 0.001 vs. DM+CAD, DM+HTN and DM+CAD+HTN; Data are mean ± SD. HTN:hypertension; CAD:coronary artery disease; DM:Type 2 diabetes*

In *Study 3*, peak systolic velocity at rest showed no significant changes, while in contrast at peak stress, (Figure 4) there were statistically significant differences among the groups, and a possible additive effect could also be observed. The E′ wave velocity at rest was lower in patients with HTN + DM compared with the other groups. This difference was more pronounced at peak stress. Strain rate and strain at rest and at peak stress followed a similar pattern as that of velocity and was lower in patients with HTN and HTN + DM compared with other groups.

In this study additionally, the determinants of global LV myocardial systolic reserve, expressed as peak systolic velocity at peak stress, were also calculated. Along with the presence of DM and HTN, the following variables showed a linear correlation with a non significant p value (≤ 0.1): age, HTN, DM, plasma glucose, plasma creatinine levels, LV mass index, posterior wall thickness, relative wall thickness, and A wave velocity, and

E/A velocity ratio. To avoid variables containing redundant information, posterior wall thickness, relative wall thickness, and E/A velocity ratio were not introduced in the multiple forward stepwise regression analysis. In this analysis only the following variables remained statistically significant: age ($F = 7.9$, $p \leq 0.001$), LV mass index ($F = 8.4$, $p \leq 0.005$), the presence of HTN ($F = 5.9$, $p = 0.01$), and plasma glucose levels ($F = 4.8$, $p = 0.03$). When female gender, plasma cholesterol levels, and heart rate at peak stress were introduced in the model, only the following variables remained statistically significant: age ($F = 8.6$, $p = 0.004$), LV mass index ($F = 11.8$, $p = 0.001$), and HTN ($F = 4.9$, $p=0.03$).

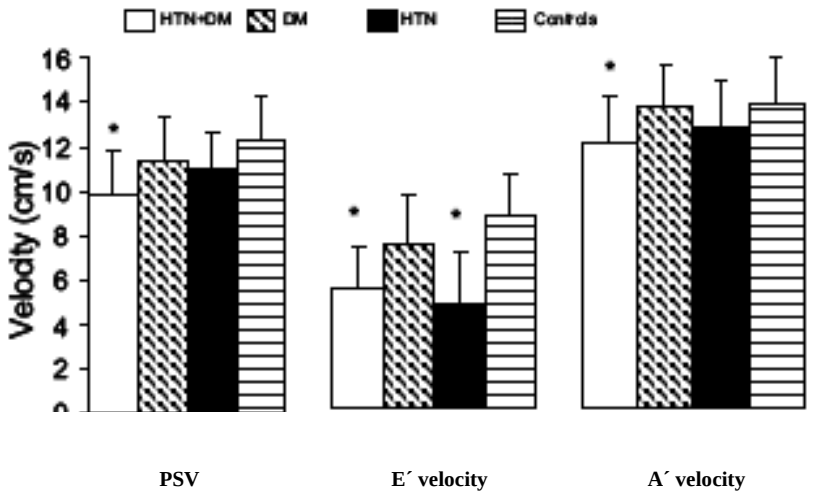


Figure 4: *Peak systolic velocity, early E' and late A' diastolic velocities obtained as the average of the four left ventricular basal segments at peak dobutamine stress echocardiography (Study 3).*

**p<0.05:HTN+DM vs controls or HTN versus controls. Data are mean ± SD. HTN: Hypertension; DM: Type 2 diabetes; PSV: peak systolic velocity*

In *Study 5*, similar patterns of earlier studies were observed wherein average peak systolic velocity at rest (Figure 5) was the same in all the groups, but at peak stress it was significantly diminished in the diabetic subjects irrespective of microalbuminuria when compared with the controls, but did not differ between the DM groups. Similarly, average E´ velocity was significantly lower in DM at rest as well as during peak stress, when compared with controls. Average A´ velocity did not differ neither at rest nor peak stress. LV filling pressure estimated as E/E´ ratio was significantly higher in the DM groups compared with the controls.

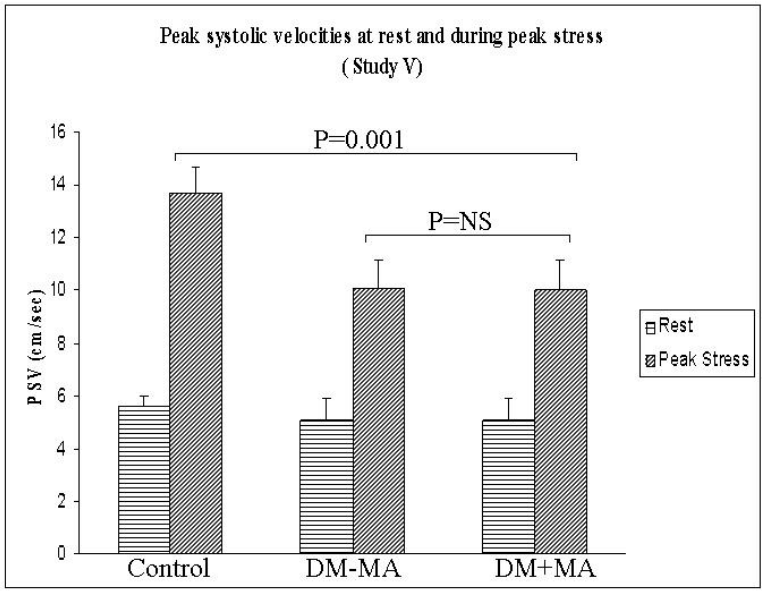


Figure 5: *Average of Peak systolic velocity of the four left ventricular basal segments at rest and peak dobutamine stress echocardiography (Study 5)*

Data are mean ± SD. DM-MA: diabetes without microalbuminuria; DM+MA: diabetes without microalbuminuria; PSV: peak systolic velocity

1 Effects of acute changes in loading conditions on left ventricular functions during hemodialysis and following administration of AT_1 receptor blocker *(Study 2 and 4)*

2.1 Demographic data

In *Study 2*, the age (years) of the subjects respectively in the 3 groups were 40 ± 16 (ESRD), 57 ± 7 ESRD+DM), and 56 ± 9 (ESRD+DM+HTN) ($p < 0.05$ ESRD vs. others) and diabetic subjects had an average duration of 14±6 years. There were no changes in the heart rate (R-R interval) and systolic and diastolic blood pressures at pre- or post-hemodialysis, but body weight decreased significantly in all the groups at post-hemodialysis.

In *Study 4*, there was a significant reduction of BP (mm Hg) after administration of valsartan, 147 ± 10 vs. 137 ± 10 systolic BP and 90 ± 7 vs. 86 ± 7 diastolic BP (all $p<0{\cdot}01$) respectively. The R–R interval (ms) also correspondingly decreased post valsartan, from 838 ± 150 to 796 ± 151 ($P<0{\cdot}01$), respectively.

2.2 Biochemical data

Study 2 showed mildly elevated plasma glucose (mg/dl) in the diabetic population, 169.9±74.0 and 161.1±67.0 in ESRD+DM, and ESRD+DM+CAD groups respectively. Serum creatinine was raised in all the groups with the lowest seen in ESRD+DM+CAD group ($p < 0.05$). Blood hemoglobin levels were low in all the groups, with no significant variation. Serum sodium, potassium, calcium, and phosphate did not show any significant changes among the groups

2.3 Conventional echocardiography findings

In *Study 2* LVH at pre-dialysis was seen in 7 ESRD, 10 ESRD+DM, and 12 ESRD+DM+CAD subjects. LV ejection fraction did not show any significant change within the groups at pre- and post-hemodialysis. LV internal dimensions showed significant decrease in all the groups post-hemodialysis (Figure 10). E/A ratio was low in all the groups at post-hemodialysis (all $p < 0.05$).

In *Study 4* pre valsartan echocardiography data showed mild LVH with septal wall being 12 ± 5 mm, and LV posterior wall 12 ± 5 mm. The LV chambers were of normal dimension and LV mass was 199 ± 57 gm. The mean LV ejection fraction was 74 ± 8·7 %. Transmitral velocities (cm/s) E, pre- and post- valsartan were 75 ± 19 and 77 ± 18 respectively, and A velocities were 84 ± 15 and 88 ± 20, while the corresponding deceleration times (ms) of the early transmitral velocity was 174 ± 64 and 172 ± 76 (all p>0·05).

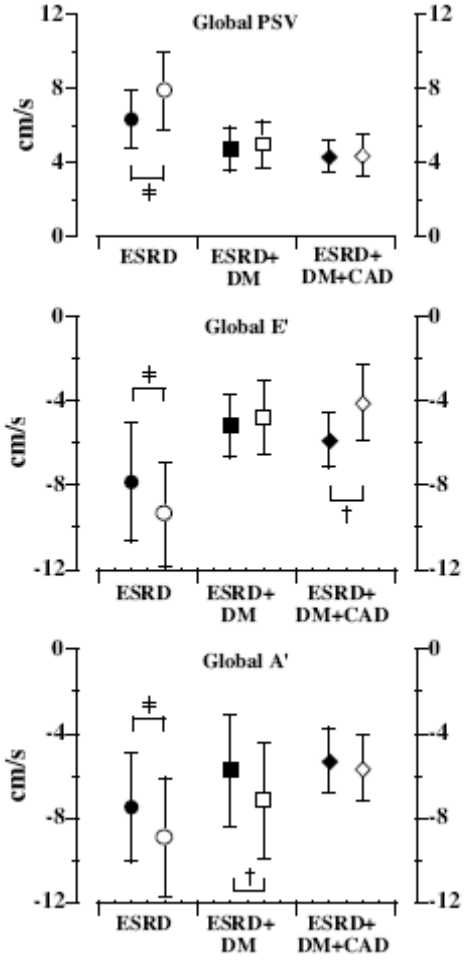
2.4 Tissue velocity echocardiography findings during hemodialysis

Comparisons of pre- and post-hemodialysis effects on LV systolic and diastolic functions (Figure 6) shows the average peak systolic velocity was significantly increased in the ESRD group after dialysis but remained unchanged in the other two groups. After dialysis, the average E' velocity was increased in ESRD group, but decreased in ESRD+DM+CAD group. LV strain rate pre- and post-dialysis (Figure 7) shows that subjects with ESRD as well as those with only DM had increased strain rate following dialysis, while the ESRD+DM+CAD group did not show any significant change.

Additionally, radial images were also analyzed; pre-dialysis did not show any significant difference among the groups. Post-dialysis measurements however revealed peak systolic velocity, E' velocity and strain rate to be increased in the ESRD group. Peak systolic velocity and strain rate were increased in the ESRD+DM+CAD group. E'/A' ratio was significantly lower in all the groups.

It was shown that LV displacement at pre-dialysis was higher in ESRD group when compared with ESRD+DM+CAD; this was somewhat similar to what was seen in peak systolic velocity. Measurement of isovolumic contraction time, ejection time, isovolumic relaxation time and diastolic filling time at pre-dialysis did not differ. However after dialysis, ESRD and ESRD+DM+CAD group showed global and regional decrease of ejection time, while the isovolumic relaxation time (ms) was prolonged globally (73.5 ± 9.8 vs. 101.4 ± 19.5 ; $p < 0.001$). Isovolumic contraction times however did not differ.

LV filling pressures (E/E') at pre-dialysis, showed no significant differences among the groups. At post-dialysis however, there was a decrease only in the ESRD group (23.2 ± 15.8 vs. 14.4 ± 8.8; $p < 0.05$), suggesting improved myocardial performances



Global longitudinal myocardial systolic (PSV), early (E') and late diastolic (A') velocities, pre- and post-hemodialysis. † $p < 0.01$; ‡ $p < 0.001$. Results were obtained by taking average of 4 left ventricular basal segments pre- (closed symbols) and post- (open symbols) hemodialysis. Data are mean ± SD

Figure 6: *TVE findings of LV systolic and diastolic velocities at pre- and post-hemodialysis (Study 2). ESRD:end-stage renal disease;DM:type 2 diabetes;CAD:coronary artery disease*

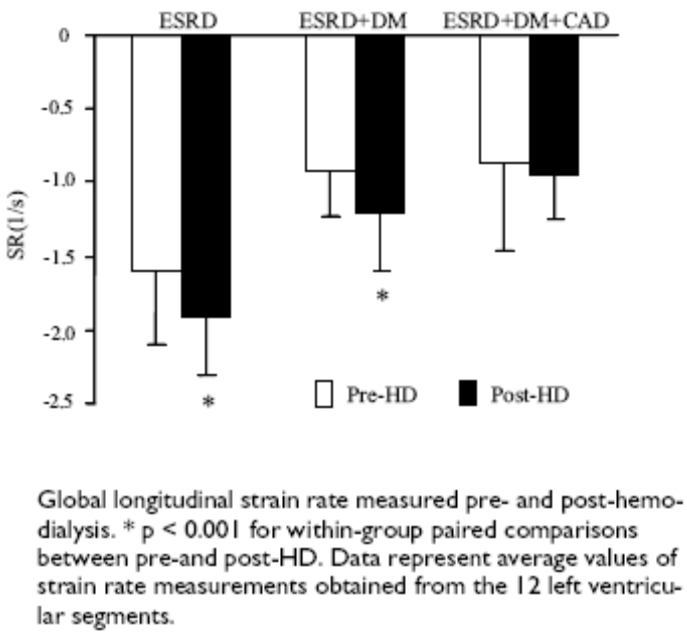


Figure 7: *TVE findings of LV longitudinal strain at pre- and post-hemodialysis (Study-2). ESRD:end-stage renal disease;DM:type 2 diabetes;CAD:coronary artery disease*

2.5 Tissue velocity echocardiography findings following administration of valsartan

Longitudinal peak systolic velocities pre- and post-valsartan in the four LV bases (Figure 8) as well as the average velocities were significantly higher post- valsartan compared with pre-valsartan. The lower panel shows the radial peak systolic velocities obtained from the parasternal long- and short-axis projections as well as average of the mean velocities taken from the two LV segments. As in the longitudinal directions, radial velocities were also significantly higher post valsartan.

Longitudinal strain rate did not differ, although there was a marginally greater strain rate only in the anterior wall. By contrast, the radial strain rates were significantly greater post valsartan

E' and A' diastolic velocities, E'/A' ratio, isovolumic contraction velocities and isovolumic times did not differ post valsartan. However, ejection and filling times were significantly shorter post compared with pre valsartan. Tei index did not differ.

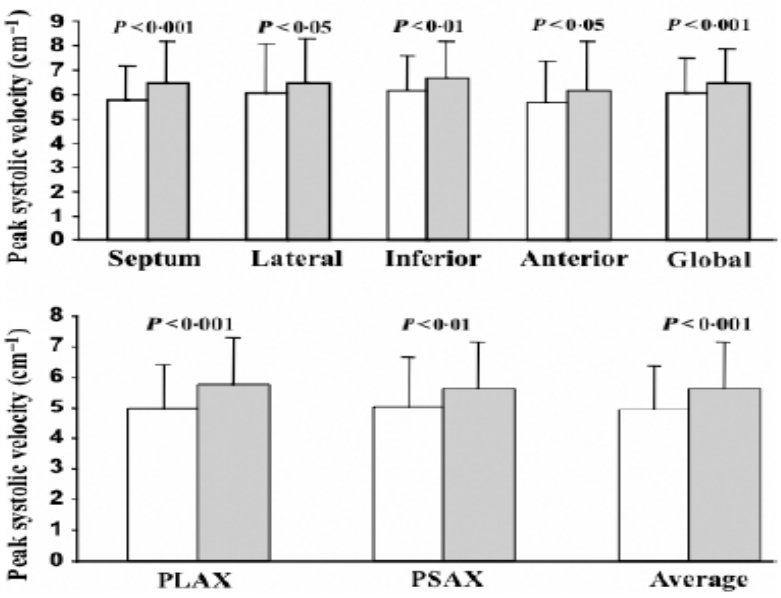


Figure 8: *Longitudinal (upper panel) and radial (lower panel) peak systolic velocities, pre- and post-valsartan*

Longitudinal peak systolic velocities were measured at the left ventricular bases. Radial velocities were obtained from the LV posterior wall from parasternal long (PLAX) and short axis (SAX) views. P values denote significant changes pre and post valsartan.

Discussion

1. Conventional echocardiography and left ventricular myocardial function

DM is associated with a high incidence of premature myocardial infarction and heart failure with disproportionately higher adverse outcome compared with the background population [57]. While LV systolic dysfunction reflected by decreased LV ejection fraction, may be a well-recognized and widely used marker of such adverse outcome, the reports on isolated diastolic dysfunction with normal LV ejection fraction as defined by conventional Doppler methods in diabetic subjects [58, 59, 60] has renewed interest in the study of pathophysiology and prognosis of cardiac complications of DM.

M-Mode echocardiography, 2-D grey scale imaging and standard Doppler which constitute conventional echocardiography has been used for over many decades now. Although these modalities form the backbone in routine clinical echocardiography, its dependency on optimal images, inability to objectively assess LV function at regional and global levels as well as its loading and heart rate dependency make conventional echocardiography an incomplete tool in clinical situations. In the study of LV diastolic function the limitations of transmitral inflow, being load dependent are well known [50]. Pulmonary venous flow profile is inconsistent and dependent on image quality, and color M-mode study as a supplemental method aiming to provide global diastolic filling status of left ventricle has also been unsatisfactory. There is no single parameter for quantification of LV diastolic dysfunction; hence it is essential to combine multiple parameters for this purpose.

TVE which is less sensitive to these limitations and regional in approach has uncovered the shortcomings of the conventional Doppler approach, particularly in patients with "pseudonormal" flow pattern whereby a combined application of both the modalities has made it extremely useful to separate it from true normal patterns of transmitral flow. Although manoeuvres like saline infusion or Valsalva can distinguish between normal from pseudonormal patterns of transmitral flow, application of TVE is definitely a more convenient option to address the issue [61, 62]. The method has been successfully tested to study diastolic status in a variety of other clinical conditions like atrial fibrillation [63],

microvascular angina [64] , and myocardial ischemia [65]. LV systolic function is more difficult to assess because of the lower sensitivity of its standard parameters like LV ejection fraction.

1.1 Assessment of left ventricular systolic function

LV ejection fraction despite its fallibilities, but still a widely used surrogate of systolic function, displayed varying results in *Studies 1, 2, 3 and 5*, showing inadequacy of this technique. Though there are reports by conventional echocardiography mentioning a combined systolic and diastolic dysfunction, a majority of studies show that the DM patients had only diastolic disturbance and a normal systolic function as estimated by a normal LV ejection fraction; even if this does not necessarily mean that the systolic function was normal.

It is interesting to note that HTN and DM patients showing signs of LV diastolic dysfunction had higher LV mass and lower myocardial systolic function as evidenced by wall stress measurements [66] and Tei index in patients with HTN and DM was higher despite a normal LV ejection fraction [67]. Epidemiologic studies have shown some degree of impairment in myocardial structure and LV function among patients with DM or HTN. Those studies have dealt mostly with LV mass, geometry, and diastolic function [60, 66, 68, 69, 70.] However, the modest changes observed in some of the studied variables preclude its use in clinical practice. In addition, the most widely used parameters in clinical practice, namely the LV ejection fraction, did not differ between patients and controls, although in *Study 3* we could demonstrate some form of LV myocardial geometric remodeling, as shown by the increased relative wall thickness in patients with HTN + DM compared with the other groups. In *Study* 5 the Tei index, a marker of global myocardial performance, was also higher in the diabetic population compared with the controls.

1.2 Assessment of left ventricular diastolic function

Using conventional Doppler criteria of diastolic dysfunction at baseline, we have shown in our studies that patients with DM, HTN and CAD have milder forms of LV diastolic dysfunction, while patients with end-stage renal disease expectedly had a more severe form of this dysfunction. In *Study 1* where there was a more heterogenous population, it did not differentiate controls from isolated DM, but when DM was associated with HTN and CAD, it required more atrial contribution compared to other groups. In *Study 3* conventional Doppler did slightly better in differentiating controls with associated co

morbidities, whereas in *Study 5* the diabetic subjects had diastolic dysfunction at rest but no difference among themselves. Taking the *Studies of 1,3 and 5* together there was no significant variation of the measured parameters of LV diastolic dysfunction among the diseased groups, which is contrary to one study [60] which showed significant diastolic dysfunction with a combined DM+HTN versus single disease, but this could be explained by the fact that it was a population based survey of large numbers of asymptomatic subjects whereas our patients were hospital referred, examined over a shorter period by a single operator and single machine, lower patient number and were symptomatic, indicating that they were more severely diseased and hence more likely to have uniformity of findings. Conventional Doppler technique was able to detect changes in diastolic function when preload was reduced as in *Study 2*, but not so when afterload was reduced in *Study 4*.

Could there be other improved ways and easier methods, which could better diagnose these patients with more information in different settings? In *Study 1, 3 and 5*, patients exhibit features of LV diastolic dysfunction at rest, but the limitation of conventional echocardiography in view of its load and heart rate dependency precludes its use in stress echocardiography, wherein TVE is better suited to fulfill this role.

1.3 Left ventricular filling pressure

Though deceleration time has the time honoured status of being the single most powerful predictor of adverse outcome, it has very poor correlation with LV filling pressure in presence of normal ejection fraction [71]. Mitral annular velocity E', is reflective of changes in LV long axis dimensions and volume which is in turn related to myocardial relaxation. E' is relatively preload independent, and is therefore more suitable for assessing diastolic function. Combining conventional Doppler methods with TVE assistance, LV filling pressure estimation is easily obtainable and is relatively less dependant on operator, patient's co-operation and loading conditions. Increased E/ E' is one of the best independent predictors of future heart failure events and provides incremental information to LV ejection fraction, along with indexed left atrial volume [72]. The findings in our study are discussed below.

2. Tissue velocity enhanced dobutamine stress echocardiographic assessment of left ventricular myocardial function

2.1 Type 2 diabetes

One of the most interesting aspects of clinical echocardiography today is its ability to uncover illnesses of the heart by challenging it with different forms of stress, both physical and pharmacological. Although most stress studies throughout the world are performed to assess wall motion and systolic functions, stress echocardiography can also be used to study diastolic parameters [73]. Studies of diastology using transcatheter and transthoracic echocardiographic approach have clearly documented the close relationship of left atrial filling status and the characteristic alterations of mitral inflow patterns [74]. The MYDISE study [29] and the Brisbane study [30] have shown the importance of tissue velocity enhanced dobutamine stress echocardiography in improving sensitivity and specificity for diagnosis of CAD. Pharmacological stress echocardiography with its wide availability and lower cost is useful to analyze global as well as regional systolic function and has been studied extensively in various clinical situations for more accurate assessment of systolic and diastolic functions of the heart at rest as well as during dobutamine stress [9, 29].

In *Study* 1, one of the principal finding is that, isolated DM significantly reduces myocardial velocity response during dobutamine stress compared with controls. The findings of subclinical forms of the myocardial dysfunction in isolated DM in our study is in agreement with those observed by Fang et al [75] though we did not find decreased velocities at baseline in systole unlike in the studies of Andersen et al. [76] and Vinereanu et al. [10].

In *Study* 3, a normal LV longitudinal function at rest in patients with DM was seen. However, global LV myocardial systolic reserve assessed during dobutamine stress echocardiography was depressed in patients with DM. In *Study 5,* the diabetic subjects had diminished left ventricular functional reserve assessed by stress echocardiography compared with the controls, while no differences were observed between the diabetic populations.

2.2 Type 2 diabetes and the co-morbid conditions of hypertension and coronary artery disease

The findings in *Study 1* showed that in DM complicated by CAD and/or HTN, the velocity response becomes lower. Also, the pooled data suggest that all forms of cardiovascular disease worsen myocardial systolic and diastolic function in the presence of DM. These findings are not at all surprising considering the fact that these patients have the longest duration of DM, worse glycemic control, and highest levels of creatinine It should not be forgotten in this context that the patients with DM+CAD+HTN had the greatest LV mass index. However, the increased LV mass index is unlikely to cause disturbed diastolic function, because the extent of hypertrophy in the study population is not as great as commonly seen in other forms of cardiomyopathy [77]. Additionally, our data do not corroborate the findings of isolated diastolic dysfunction defined by other investigators by conventional Doppler criteria [59, 60] that have been claimed to be markers of diabetic cardiomyopathy [78]. However, standard Doppler echocardiography may uncover disturbed LV performance measured by Tei index particularly when DM and HTN coexist [79]. This approach is probably more suitable, because longitudinal functional studies have shown systolic function to be closely linked to diastolic function of the heart [79], thereby questioning the existence of isolated diastolic dysfunction. The strength of this study lies in the fact that it shows, for the first time, evidence of worsening of myocardial systolic and diastolic velocity response in DM complicated by CAD and/or HTN.

In *Study 3*, global LV myocardial systolic reserve assessed by TVE during dobutamine stress was depressed suggesting a subclinical LV dysfunction in patients with HTN+DM compared with the controls. The coexistence of both diseases seems to have an additive harmful effect. This subclinical LV systolic dysfunction may play an important role in the pathogenesis of congestive heart failure with preserved LV ejection fraction which has been observed in the elderly population, in women and groups with extremely high prevalence of DM and HTN [69, 70, 80] .The strength of this study lies in the fact that it has been demonstrated that HTN and DM, even in the absence of significant CAD, induces deterioration of the LV systolic and diastolic functions that can be assessed by clinically meaningful variables. Using TVE, it has been demonstrated that patients with DM or HTN have a depressed LV longitudinal myocardial function, whereas the radial LV myocardial function is increased [10, 81]. This increment in the radial LV myocardial function has been proposed as a compensatory mechanism to maintain LV hemodynamics in the presence of depressed longitudinal functions, which are presumably

caused by ischemic and fibrotic processes of the subendocardial longitudinal fibers [82, 83] .

In *Study 3*, age, LV mass index, the presence of HTN, and plasma glucose levels were independent determinants of LV systolic myocardial function. However, when gender, plasma cholesterol levels, and heart rate at peak stress were introduced in the model, plasma glucose level was no longer an independent predictor. These statistical associations do not establish any causal relationship but needs to be tested in larger and prospective studies. In the study by Fang et al. [84] only glycosylated hemoglobin and treatment with angiotensin converting enzyme inhibitors independently predicted LV systolic myocardial function. However, the exclusion of LV hypertrophy, a common finding in diabetics even in the absence of hypertension [60], and inclusion of as many as 25 covariates in the model, makes the results of the multivariate analysis less accurate. We did not include in our model any treatment because the patients were not randomized to any therapy and because the treatment options for both DM and HTN were diverse with multiple combinations. Separating the relative importance of each risk factor in clinical practice is, however, almost impossible, because up to 40% of patients with DM are on antihypertensive medication [70] , and up to 73% of patients have a blood pressure more than 130/80 mm Hg [80]. In light of this evidence it seems clear that the addition of DM to HTN has an additive harmful effect with regard to LV myocardial systolic function and in the pattern of LV filling, as shown in the present and another study [60] .

Inappropriately high LV mass, i.e the mass exceeding the compensatory needs for workload is associated with delayed LV relaxation and systolic dysfunction [85]. Disproportionally high LV mass was associated with often preclinical manifestations of cardiac disease in the absence of traditionally defined echocardiographic LV hypertrophy and concentric geometry [86]. Reductions in longitudinal mitral annular systolic and diastolic velocities in HTN with or without LV hypertrophy have been reported and the reduction is likely to parallel increases in LV mass [40]. This also could have influenced our data, since some of our patients had mild LV hypertrophy. Though insulin resistance is associated with myocardial structural changes in DM, HTN and CAD, it is unclear whether it maybe an independent determinant of these abnormalities.

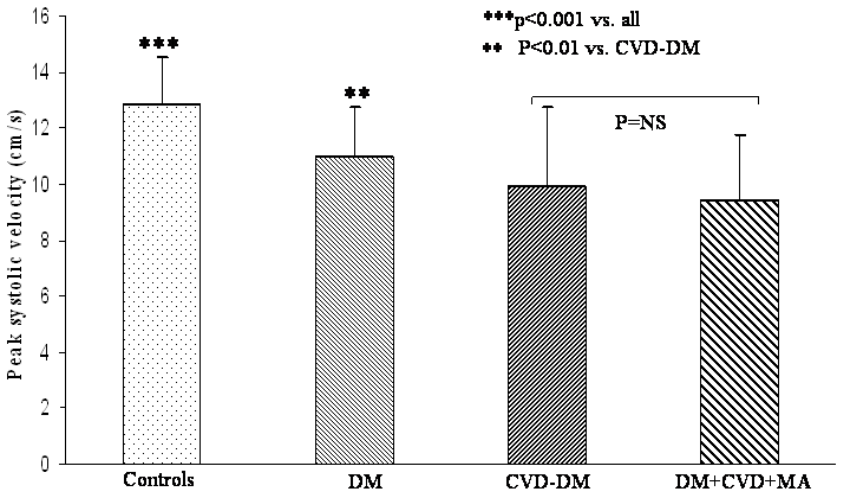


Figure 9: *Average of peak systolic velocity of the four left ventricular basal segments at peak dobutamine stress echocardiography in the combined populations of Study 1, 3, and 5, showing DM, and those with and without co-morbidities.*

DM: type 2 diabetes; CVD-DM: cardiovascular diseases without DM; CVD+DM+MA: cardiovascular diseases with DM and microalbuminuria

2.3 Type 2 diabetes and microalbuminuria

Changes in the glomeruli have been identified as analogous to those seen in the myocardium [87]. But in *Study 5* the diabetic subjects, irrespective of the presence of microalbuminuria, had diminished left ventricular functional reserve assessed by quantified stress echocardiography compared with the controls while no differences were observed between the two diabetic populations. The role of microalbuminuria and its relationship with cardiovascular disease is a question that has not been clearly answered [88]. However, the importance of microalbuminuria as a strong predictor of cardiovascular risk in diabetes has been clearly stated [89]. A recent study by Vasan et al [90] reinforced the opinion that microalbuminuria can be used as a biomarker for risk stratification of cardiovascular events. However, the effects of microalbuminuria on left ventricular systolic and diastolic functions have not been studied using advanced echocardiographic methods such as TVE.

The diabetic subjects did not show any difference in regional LV systolic function at rest in comparison with the controls emphasizing the fact that their left ventricular geometry was not altered. However, the diabetic subjects had evidence of diastolic dysfunction at

rest as measured by TVE (E´ velocity and E´/A´ ratio). The latter variables persisted also at peak stress. The findings of this study support those of others where evidence of diastolic dysfunction using conventional Doppler techniques has been identified [91]. The hallmark of diastolic dysfunction leading to heart failure is the elevated left ventricular end diastolic pressure. The ratio of early transmitral E velocity to regional early E´ diastolic velocity measured either at the septal or lateral mitral annulus (E/E´) has now been increasingly used as a surrogate and non-invasive marker of elevated LV filling pressure both for diagnostic [52] and prognostic [92] purposes. In the current study the E/E´ ratio was found to be significantly higher in the diabetic population with and without microalbuminuria compared with the controls.

The lack of significant difference in DM subjects with or without microalbuminuria could be attributed to the relatively shorter duration of diabetes and absence of co-morbidities. Our studies, *Study 1 and 3* have shown how co-morbid conditions negatively influence the myocardial velocity response in subjects with DM. Since microalbuminuria is a form of incipient diabetic nephropathy where glomerular filtration rate remains normal, it can be presumed that the global burden of the disease was not heavy enough in our DM subjects to have more negative velocity response even in the presence of microalbuminuria. Another factor that may contribute to the explanation in the current study is the fact that patients in the DM group have been treated with angiotensin-converting enzyme inhibitors and the lipid-lowering agent, atorvastatin. Since both the drugs have so called "pleotropic" effects and since angiotensin receptor blocker improves myocardial systolic velocities, the longitudinal myocardial functions were most probably preserved in the DM groups, though was significantly lower compared with the controls. But despite this, microalbuminuria should not be ignored and hence should be treated according to current practices. In fact it has been shown in a study that a reduced glomerular filtration rate in asymptomatic diabetic patients was associated with a two-fold increase in cardiac events and reduced glomerular filtration rate independent of albuminuria was a significant predictor of cardiac events [55].

To summarise (Figure 9), in *Studies I and 3,* the procedure has been used not only to identify sub-clinical disease in isolated type 2 diabetes, but could also detect the disease burden quantitatively in the patients complicated by CAD and/or HTN. The results of the two studies conform well to the epidemiological evidence collected from all over the world over the past quarter of a century that shows worse prognosis in type 2 diabetic subjects having concomitant cardiovascular complications [93]. Another study, in many

ways similar to ours also shares the same view [94]. However, in *Study 5* type 2 diabetic subjects with microalbuminuria but without cardiovascular complications did not show additional left ventricular dysfunctions over and beyond those already caused by DM alone.

2.4 Diastolic stress echocardiography

Very recently, interest has been focussed on whether left ventricular filling pressure (LV end diastolic pressure) could be estimated during exercise stress echocardiography. In one study, Burgess et al [95] investigated 37 patients undergoing left heart catheterization and found a good correlation between E/E´, and LV end diastolic pressure at rest as well as during exercise. Not only that the exercise-induced changes in E/E´ratio can also reflect exercise tolerance: higher the ratio earlier would be the stoppage of exercise. The data provide preliminary evidence supporting the use of diastolic stress echocardiography in selected patient population [96]. In *Study 1, 3 and 5*, we have measured the early regional E´ velocity in patients with DM and co-morbidities and have found that those with complications have lowered E´ velocity at rest and during dobutamine stress, emphasising the importance of quantification of diastolic function during dobutamine stress particularly in patients with DM having cardiovascular complications.

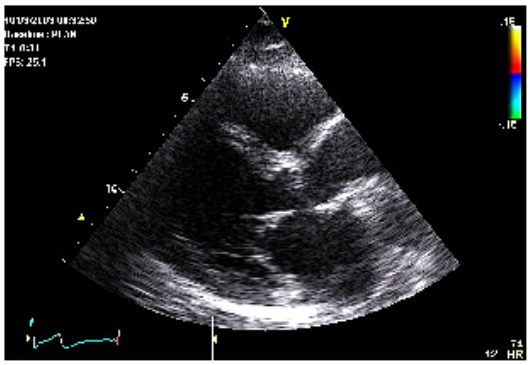
3. Effect of changing loading conditions on the left ventricle

3.1 Hemodialysis in end-stage renal disease

Patients with DM of long duration are frequently affected with end-stage renal disease and a great majority of them also have coexistent CAD [97]. Since there is no uniform consensus regarding CAD screening in patients with chronic kidney disease [98, 99], our patients were diagnosed on the basis of clinical examination and investigations. Patients with end-stage renal disease are at risk of having a coronary event as well and often die of cardiac causes [100, 101, 102]. Detailed mechanisms behind cardiac deaths have been relatively well described, but exact changes in the regional contractile behaviors of the LV are not clearly established in end-stage renal disease, particularly during on-going renal replacement therapy such as hemodialysis, a procedure destined to temporarily remove excess fluids and toxins.

Though it has been already demonstrated in patients undergoing hemodialysis that concomitant changes in the TVE variables are load dependant [103, 104, 105, 106], it is however still not known whether end-stage renal disease with coexistent DM and/or CAD, i.e., conditions in which regional myocardial systolic and diastolic velocities are already diminished [10] have differing myocardial response following dialysis-dependent acute changes in preload. The principal findings in *Study 2* show that firstly, left ventricular longitudinal systolic and diastolic myocardial velocities and systolic strain rate become higher in end-stage renal disease group post dialysis; secondly, these variables are either unchanged or even become worse in presence of co-morbidities; thirdly, radial velocities and strain rate also becomes higher in end-stage renal disease while minimal compensatory over-activities of the radial systolic functions (velocity and strain rate) are noted in the end-stage renal disease with DM+CAD group. Finally, despite significant reduction in dialysis-dependent preload, LV filling pressure, as assessed by E/E' ratio, decreased only in end-stage renal disease group, indicating that acute changes in loading conditions influence the TVE variables in a different way depending on the background diseases.

Before hemodialysis (A)



After hemodialysis (B)

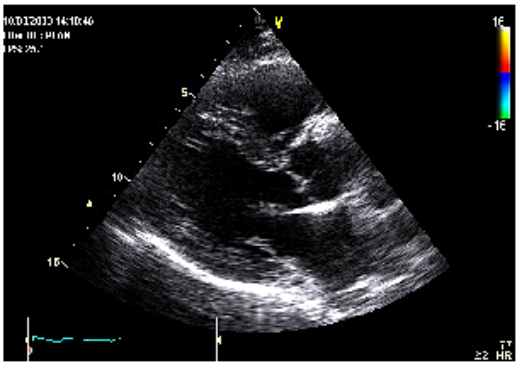


Figure 10: *2-D image of the left ventricle before (A) and after dialysis (B). LV dimension before dialysis was 56 mm, which reduced to 43 mm after one single session of hemodialysis*

The improved LV functions in end-stage renal disease groups in the present study confirm the findings reported by others [107, 108], and could be explained by the fact that after dialysis there is improved myocardial perfusion, that occurs most probably not only because of removal of fluids, solutes and toxins [37], but also because of improvement in myocardial interstitial oedema [109]. Another reason that can be attributed to this improvement is that LV hypertrophy was not seen in this group as frequently as in the other two groups. A modest increase in Tei index was however observed in this group of patients post hemodialysis, suggesting somewhat decreased myocardial performance, not uncommonly seen even in patients with isolated end-stage renal disease [110]. However, the absolute value was far below the threshold of 0.91 seen in patients with heart failure [111].A subtle form of diabetic heart muscle disease recognizable by TVE at rest as has been shown [25], possibly reflecting microvascular disease. Moreover, it has been reported that the patients with end-stage renal disease having DM and angiographically normal coronary arteries have a lower coronary flow reserve than patients with DM alone [112]. Decreased lumen area, vascular remodeling and increased LV mass may explain this attenuated coronary flow reserve. These mechanisms are often related to microvascular disease of DM, hypertensive heart disease, and CAD [113, 114], and may have influenced the current post dialysis data in the groups with concomitant DM and CAD. In addition, when DM is associated with significant CAD the LV myocardial velocities progressively decrease as shown in *Study 1*. It is therefore not surprising that patients with end-stage

renal disease complicated with DM+CAD had additional disturbances, such as prolonged isovolumic relaxation time, diminished systolic displacement, unchanged LV filing pressures and significantly higher Tei index post hemodialysis

3.2 Administration of the AT_1 receptor blocker, valsartan

The findings in *Study 4* are, regional longitudinal myocardial systolic velocities were significantly higher 5 hours after administration of 80 mg valsartan in all the four LV basal segments and, strain rate, a marker of regional contraction, was higher when computed radially and thirdly, the ejection time was increased while the LV filling times decreased significantly post valsartan. Combination of these effects could well reflect the more efficient performance of the LV pump function.The mechanism of the currently observed improved systolic function following a single, low dose of valsartan appears to be secondary to decreased afterload. One can of course argue whether the improvements in left ventricular functions were related to pure afterload reduction or whether there could be also intramyocardial effects of the drug resulting in improved functions, since the renin–angiotensin–aldosterone system is also present in the heart [115]. But the present results do not provide enough data to conclusively settle the issue.

Our data suggest that LV longitudinal and radial velocities as well as radial strain improved post valsartan, reflecting overall improvement of LV functions. Also, the decreased ejection and filling times in the study probably indirectly reflect more efficient pump function of the left ventricle even though the inotropic status of the myocardium was probably not changed at the dosage used as reflected by the lack of change in the isovolumic contraction velocities pre- and post-valsartan. As TVE is essentially a non-invasive tool, it is however difficult to determine exactly which haemodynamic and functional parameters were influenced by valsartan. Nonetheless, the data suggest that an acute oral administration of even a low, single dose of valsartan not only significantly decreased systolic and diastolic blood pressure, but also improved myocardial longitudinal systolic velocities and radial strain rate. Absence of effects on the isovolumic contraction velocity, however, limits the arguments in favour of a true inotropic effect of valsartan.

The post valsartan segmental disparity (longitudinal versus radial) of strain rate data in our patients having mild degree of LV hypertrophy can be attributed either to regional remodelling i.e. collagen accumulation which is often seen in arterial HTN [116], or by the existence of complex and often heterogeneous myocardial fibre architecture that may

make TVE, a one-dimensional modality in its current stage of development, less suitable for identification of pathologies in multiple myocardial fibres. The results therefore suggest that the increase in short-axis function may likely represent improvement in intrinsic myocardial contractile function regionally and probably these fibres are less affected by the hypertensive burden. The segmental disparity can also be due to the limitations of strain rate imaging technique, i.e. its angle dependency and respiratory drift during image acquisition. However, calculations of global strain involving multiple LV segments (not only the basal ones that we used in this study), might provide a more reliable answer to this apparent disparity between longitudinal and global strain rate.

In the SILVHIA study [44] the angiotensin receptor blocker irbesartan was studied along with atenolol for evaluating diastolic function using TVE. They reported that TVE is more sensitive than conventional echocardiography in detecting diastolic changes and both the drugs, in addition to reducing BP, improve diastolic function through different mechanisms. The LIFE trial stated that losartan has direct cardiac benefits because BP lowering was comparable with atenolol, but this was debated by some authors who said that there was no direct benefit and it was put down to just LV afterload effect, though this mechanism was not tested in LIFE or HOPE trial [117]. The DETECTIV pilot study will assess the efficacy of valsartan 160 mg daily on markers of early disease in individuals with detectable cardiovascular abnormalities and this might throw more light on this important issue [118].

4. Mechanism of myocardial dysfunction in diabetes

It is clearly established that the foremost differential diagnosis of decreased regional myocardial systolic velocity response during peak dobutamine stress echocardiography is CAD [29] . This was seen in *Study 1,* not only at peak stress echo, but also at rest. Among various other conditions, pathological hypertrophy has also been implicated [77]. The concept that diabetes affects cardiac structure and function independent of blood pressure or coronary artery disease or whether these factors are more likely to exert a synergistic action on the myocardium is a continuing source of debate. Thus the proposed mechanisms for diminished myocardial functional reserve in diabetes are multifactorial, and these are described in brief as follows.

4.1 Alterations in load

The changes seen in the LV myocardial functions in our studies appear likely to be due to alterations in load. Also, the role of increased peripheral vascular resistance, which was not done in our studies, cannot be ruled out. One report mentioned that when LV systolic performance as assessed by load and rate independent indices in young, isolated diabetics with normal blood pressure and no CAD, there was no evidence of cardiomyopathy in these patients [119].

Isovolumic contraction velocity is a readily obtainable non-invasive parameter, which correlates with the classical invasive measurement of global LV contractility [120].We did a small sub-analysis (unpublished) measuring isovolumic contraction velocity at rest and peak dobutamine stress echo, controls versus isolated DM, and did not find any significant difference between them. The diminished myocardial velocity response therefore could possibly be explained by an afterload effect. But whether isovolumic contraction velocity which is more independent of load than velocity is an ideal parameter to reflect contractility, and also whether dobutamine is the appropriate stressor is not clear at present. Another alternative would have been to use isovolumic acceleration, which is supposedly superior to isovolumic contraction velocity [121], but this was not measured in our study.

Increased afterload affects both systolic and diastolic performance, with progressive systolic dysfunction and shortening of diastolic filling time, worsened by increased heart rate. There is ventricular remodelling in patients with cardiovascular diseases. Unlike in myocardial infarction which can acutely hasten the LV remodelling, it is much more of a

gradual process in patients with DM and HTN [122]. One can surmise that initially there is a reduced systolic and diastolic function as the subendocardial fibres are the first to be affected and as LV hypertrophy appears, there is further compromise, but then this gets offset by compensatory increase in radial contraction, thus restoring the LV ejection fraction to a normal range. But as remodelling progresses there is increase in LV volumes and decrease in LV ejection fraction, to an obvious state of systolic dysfunction. Worsening global diastolic dysfunction of LV is associated with a progressive decline in longitudinal systolic function [123]. A radionuclide study also showed that preserved LV ejection fraction was often associated with LV systolic dysfunction and LV diastolic dysfunction [124].

4.2 Arterial stiffness

Arterial stiffness, though not done in our study, has been proposed to be another mechanism, wherein it is shown that apart from DM and HTN having an adverse effect on LV geometry and function, the combination of both results in greater arterial stiffness, myocardial dysfunction and LV hypertrophy [125]. Reduction of compliance leads to an increase in afterload on the heart, an increase in pulse pressure and in turn, LV hypertrophy and diastolic dysfunction. Diabetes is accompanied by an early reduction in arterial distensibility which becomes greater if there is a BP rise. The stiffening of aorta and the decreased arterial compliance increases cardiovascular morbidity, and an interesting observation [27] is that this happens early in DM thereby exposing them to high risk of cardiovascular complications, with improper glycemic control and longer duration of DM adding to the risk. One study done very recently from our centre reported that early in the course of DM increased aortic stiffness is evident. This alters the phasic coronary flow together with decreased LV systolic and diastolic functions.

4.3 Structural and metabolic changes

Structural changes have been demonstrated in the diabetic myocardium of animals and these have been explained by increased fibrosis, myocyte atrophy and apoptosis [126]. The most prominent histopathological finding is fibrosis. These changes supposedly parallel functional changes of diabetic heart disease in the absence of CAD or HTN. But biopsy studies of the diabetic myocardium have so far been restricted to animal models. Non invasive techniques have been used to identify fibrosis and in one study [127] it is reported that fibrosis can be quantified using ultrasonic backscatter. Altered substrate metabolism in the diabetic heart has also been implicated due to a primary defect in the stimulation of

glycolysis and glucose oxidation, thereby injuring the heart muscle [128]. Elevated free fatty acid levels which enhance insulin resistance and triggers cell death is believed to be one of the major contributing factors in the pathogenesis of diabetes. More work is awaited to further explain these concepts.

4.4 Microvascular disease

Apart from changes stated above, among other mechanisms microangiopathy in DM has been a subject of intense scrutiny. Structural abnormalities of the small vessels like basement membrane and arteriolar thickening and the resulting functional abnormalities of these vessels have been investigated. One study using myocardial contrast echo and strain rate imaging has reported that though they have impaired myocardial blood flow with normal coronary arteries the abnormal transmural flow and subclinical longitudinal myocardial dysfunction are not related [129]. They suggest that microvascular disease may not be the predominant causative factor in diabetic heart disease. The endothelial function in the coronary bed of the diabetic heart has also been shown to be altered [130]. Thus, the hemodynamics in the small vessels may be pathogenetically involved in decreased LV functional reserve. Despite this the clinical importance of these vascular changes in DM is unknown.

4.5 Cardiovascular autonomic neuropathy

Another important but overlooked mechanism is cardiovascular autonomic neuropathy in DM where the autonomic fibres that innervate the heart and its vasculature results in abnormal heart rate control and vascular dynamics [131]. This causes impaired exercise tolerance, inappropriate heart rate and BP response, which is more often late findings, resulting in diminished cardiac output. The blunted LV myocardial response could also be attributed to this. The same study point out that autonomic neuropathy is associated with LV diastolic dysfunction.

5. Limitations

In *Study 1, 3 and 5*, although coronary angiography was not used to rule out significant CAD, a negative dobutamine stress echocardiography was used to rule out the presence of CAD with approximately 90% accuracy according to published literature. In *Study 1 and 3*, the grade of metabolic control was assessed by plasma blood glucose samples, which is probably not as effective as glycosylated hemoglobin as a marker of long term metabolic control. LV radial contraction was not assessed in *Study 1, 3 and 5,* and therefore the results of previous studies published by other investigators could not be confirmed. Since plasma insulin levels were not measured and neither a homeostatic model assessment was done, it is not known how many of the studied patients had insulin resistance syndrome, which might have contributed to depressed myocardial systolic and diastolic functions. In *Study 3* the higher number of men in the DM group may have influenced the results introducing a higher than expected value for peak systolic velocity at peak stress. These finding needs to be confirmed in future studies.

In *Study* 2, younger age in the end-stage renal disease group could have influenced the increase in systolic and diastolic velocities and strain rate. However, published data from our group as well as those from others indicate that presence of DM and CAD has ominous effects on LV functions irrespective of age. We therefore believe that the age differences have not influenced the obtained results. We have studied only one randomly chosen dialysis session and hence it is difficult to reflect on the natural history of progression of LV dysfunction in these patients.

Information on detailed medical therapy was not available in some patients and to what extent underlying medical treatment could have altered the outcome of the studies is not known. Left atrial volume which has increasingly been recognized as a reliable marker of diastolic function was not done. In *Study 4,* it would have been more comprehensive if dose response had been done to get additional insight into the mechanism of intramyocardial effects, if any, of AT_1 blocker valsartan.

6. Conclusions

1. TVE is a sensitive tool for objective assessment of left ventricular function and can be successfully applied for the clinical evaluation of the effect of type 2 diabetes and co-morbidities.

2. TVE based quantification of stress echocardiography images improves the diagnostic yield by providing better possibilities to objectively detect both regional and global disturbances of myocardial function.

3. The application of TVE technique in patients with type 2 diabetes reveals the occurrence of subclinical left ventricular dysfunction characterized by reduced functional reserve. This influence becomes quantitatively more pronounced in the presence of coexistent cardiovascular diseases such as coronary artery disease and hypertension.

4. The coexistence of type 2 diabetes and hypertension appears to have additive negative effect on both systolic and diastolic left ventricular function, even in the absence of coronary artery disease.

5. The presence of microalbuminuria in patients with type 2 diabetes does not further aggravate diminished myocardial functional reserve.

6. A single session of hemodialysis improves left ventricular function in patients with end-stage renal disease only in the absence of type 2 diabetes and other co-morbid conditions.

7. A single dose of an AT_1 receptor blocker valsartan results in reduction of afterload and, subsequently, in improvement of left ventricular function.

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Errata

Paper 1

Table 1, PWDd (DM) 11.7±11.3, should read as 11.7±1.3

Table 2, all units expressed as mg%, should read as mg/dl

Table 2, Serum triglyceride (DM+CAD+HTN) 1501.0±28.0 should read as 150.0±28.0

Table 2, duration of diabetes, years, DM+HTN 7.2±5.2, should read as 7.4±4.2 and duration of diabetes, years, DM 7.4±4.2, should read as 7.2±5.2

Controls 23, should read as 22

Paper 2

Table 2, Hemoglobin (mg/dl) should read as Hemoglobin (g/dl)

Table 2, Serum Sodium in ESRD+DM group 131.72±35.31, should read as 131.72±3.53

Table 3, Diastolic BP in ESRD (Pre) 86±40, should read as 86±4