

**DETERMINANTS OF MYOCARDIAL AND VASCULAR  
FUNCTION IN YOUNG SUBJECTS WITH TYPE 1 DIABETES  
MELLITUS**

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A thesis submitted in fulfillment of the requirements for the degree Doctor of  
Medicine (M. D.) of Cardiff University

**2012**

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## Abstract

Cardiovascular disease including myocardial dysfunction and vascular disease is a major cause of morbidity and mortality among patients with type 1 diabetes mellitus. Early detection of myocardial dysfunction and identification of its determinants may be helpful in preventing diabetic heart muscle disease.

In this thesis, I studied myocardial and vascular function in 53 subjects – 19 with type 1 diabetes mellitus (aged  $21 \pm 4$  years, HbA1c  $8.8 \pm 1.6$  %) and 34 controls (aged  $25 \pm 3$ ). I measured myocardial functional reserve using myocardial velocity imaging during dobutamine in 18 patients and 21 controls.

The main findings of this thesis were:

1. Longitudinal shortening of the left ventricle was reduced in type 1 diabetes mellitus. (medial mitral annular excursion  $1.2 \pm 0.2$  vs.  $1.4 \pm 0.2$  cm,  $p = 0.01$ ).
2. During dobutamine, long-axis peak systolic velocity was lower in type 1 diabetes by 20% at 10 and 13% at 20  $\mu\text{g}/\text{kg}/\text{minute}$  (both  $p = 0.05$ ; ANOVA,  $p = 0.003$ ) but systolic velocities at peak dobutamine were similar and thus myocardial functional response was.
3. Longitudinal displacement was reduced in subjects with type 1 diabetes mellitus both at rest and during dobutamine stress. (by 15%,  $p = 0.001$ ).
4. Early diastolic relaxation was lower in type 1 diabetes, measured globally as the mitral E/A ratio ( $1.5 \pm 0.4$  cm/s vs.  $1.8 \pm 0.4$  cm/s,  $p = 0.02$ ) or regionally as the early diastolic velocity of the medial mitral annulus ( $e'$   $-13.0 \pm 2.7$  cm/s vs.  $-14.8 \pm 2.0$  cm/s,  $p = 0.02$ ).

5. Features of adiposity and adverse lipid profiles, more than glycaemia, are major determinants also for myocardial and vascular dysfunction in young subjects with type 1 diabetes mellitus.

In conclusion, there is evidence of myocardial dysfunction in young subjects with type 1 diabetes mellitus, and early changes may be related to metabolic rather than structural changes. Control of other risk factors such as dyslipidaemia, and maintenance of normal body weight, may be important measures in preventing progression of subclinical myocardial dysfunction into overt clinical disease.

## Table of Contents

Declaration	i
Acknowledgements	ii
Abstract	iii
Index of tables	x
Legend to figures	xiii
Glossary to abbreviated terms	xvii

### **CHAPTER 1- BACKGROUND ..... 1**

1.1 Vascular disease and type 1 diabetes mellitus .....	1
1.1.1 Epidemiology .....	1
1.1.2 Pathogenesis of vascular disease in Diabetes Mellitus .....	1
1.1.2.1 Endothelial dysfunction .....	2
1.1.2.2 Hyperglycaemia .....	2
1.1.2.3 Sorbitol pathway .....	3
1.1.2.4 Advanced glycation endproducts (AGEs).....	3
1.1.2.5 Protein Kinase C (PKC) activation .....	4
1.1.2.6 Oxidative Stress .....	4
1.2 Diabetic Heart Muscle Disease and Diabetes Mellitus .....	5
1.2.1 Epidemiology .....	5
1.2.2 Pathogenesis.....	5
1.2.2.1 Substrate metabolism .....	6
1.2.2.2 Free fatty acids utilization.....	6
1.2.2.3 Calcium homeostasis.....	6
1.2.2.4 Reactive oxygen species and oxidative stress .....	7
1.2.2.5 Poly (ADP-Ribose) Polymerase (PARP) .....	8
1.2.2.6 Advanced glycation end-products (AGEs) .....	8
1.2.2.7 Structural changes in myocardium.....	9
1.2.3 Diastolic myocardial dysfunction in diabetes mellitus .....	9
1.2.4 Systolic myocardial dysfunction in diabetes mellitus .....	10
1.2.5 Radial myocardial dysfunction in diabetes mellitus .....	11

1.2.6 Myocardial velocity imaging in detecting subclinical myocardial dysfunction.....	12
1.2.7 Dobutamine stress echocardiography in subclinical myocardial dysfunction .....	12
1.2.8 Determinants of myocardial function in diabetes mellitus- current evidence .....	19
1.2.8.1 Hyperglycaemia .....	19
1.2.8.2 Brain Natriuretic peptide.....	20
1.2.8.3 Vascular Stiffness .....	21
1.2.8.4 Endothelial dysfunction .....	22
1.2.8.5 Conclusion .....	22
1.2.9 Treatment of subclinical myocardial dysfunction in diabetes mellitus .....	28
1.2.10 Conclusion .....	33
1.3 Non-invasive assessment of vascular structure and function in diabetes mellitus .....	33
1.3.1 Carotid intima media thickness (cIMT) .....	33
1.3.2 Characteristics of the vascular tree and their impact on stiffness .....	39
1.3.3 Arterial stiffness.....	39
1.3.4 Pulse wave velocity (PWV) .....	39
1.3.5 Other indices of local arterial stiffness.....	40
1.3.6 Central pulse wave analysis .....	42
1.3.7 Endothelial function.....	44
1.3.8 Wave intensity.....	45
1.3.8 Ventriculo-arterial coupling.....	47
1.4 Chapter Conclusion .....	48
<b>CHAPTER 2- HYPOTHESES AND OBJECTIVES.....</b>	<b>49</b>
2.1 Hypotheses.....	49
2.2 Study Objectives .....	49
<b>CHAPTER 3- METHODS.....</b>	<b>50</b>
3.1 Introduction.....	50
3.2 Subjects and recruitment.....	50
3.3 Study Endpoints .....	52
3.4 Study Sample size calculation .....	52
3.5 Carotid intima media thickness measurement.....	54

3.6 Wave Intensity Methods .....	55
3.7 Pulse wave analysis methods .....	57
3.8 Blood Tests .....	59
3.9 Conventional Echocardiography Methods .....	59
3.10 Dobutamine Stress Echocardiography Methods .....	72
3.11 Analysis.....	73
3.12 Reproducibility of myocardial function .....	76
3.13 Reproducibility of vascular method.....	76
3.14 Statistical Methods.....	76
<b>CHAPTER 4- RESULTS - Baseline Characteristics .....</b>	<b>78</b>
4.1 Baseline Characteristics (whole group) .....	78
4.1.1 Female: Male Ratio (whole group) .....	80
4.2 Exercise history.....	80
4.3 Discussion.....	81
<b>CHAPTER 5- RESULTS – biochemical results .....</b>	<b>84</b>
5.1 Biochemical results .....	84
5.2 Discussion on biochemical results .....	85
<b>CHAPTER 6- RESULTS - Baseline echocardiographic results.....</b>	<b>87</b>
6.1 Baseline Echocardiographic results .....	87
6.2 Discussion on baseline echocardiographic results .....	89
6.3: M- mode data .....	92
6.4 Discussion on M-mode results.....	93
<b>CHAPTER 7 – RESULTS – Reproducibility of myocardial function assessment.....</b>	<b>95</b>
7.1 Reproducibility of myocardial displacement and velocities .....	95
<b>CHAPTER 8- RESULTS -Diastolic function .....</b>	<b>103</b>
8.1 Diastolic function results .....	103
8.2 Discussion on diastolic function .....	104
<b>CHAPTER 9- RESULTS – Stress echocardiography results.....</b>	<b>109</b>
9.1 Baseline Characteristics (Stress Group).....	109
9.2 Biochemical Results.....	111
9.3. Myocardial Displacement .....	112
9.3.1 Myocardial Displacement at rest.....	112
9.3.2. Myocardial Displacement at dobutamine dose of 5 µg/kg/min .....	113

9.3.3 Displacement at dobutamine dose of 10µg/kg/min.....	114
9.3.4 Displacement at dobutamine dose of 20µg/kg/min.....	114
9.3.5 Displacement at dobutamine dose of 30µg/kg/min.....	115
9.3.6 Displacement at dobutamine dose of 40µg/kg/min.....	116
9.4 Longitudinal myocardial velocity .....	119
9.4.1 Longitudinal myocardial velocity (Systolic velocity, S) at rest .....	120
9.4.2 Longitudinal myocardial velocity (Systolic velocity, S) at dobutamine 5µg/kg/min .....	121
9.4.3 Longitudinal myocardial velocity (Systolic velocity, S) at dobutamine 10µg/kg/min .....	122
9.4.4 Longitudinal myocardial velocity (Systolic velocity, S) at dobutamine 20µg/kg/min .....	123
9.4.5 Longitudinal myocardial velocity (systolic velocity, S) at dobutamine 30µg/kg/min .....	124
9.4.6 Longitudinal myocardial velocity (systolic velocity, S) at dobutamine 40µg/kg/min .....	125
9.5 Radial myocardial function.....	127
9.6 Discussions on left ventricular systolic function and radial myocardial function .....	128
9.7 Myocardial functional reserve .....	130
9.8 Discussions on myocardial functional reserve .....	131
9.9 Early diastolic velocity during dobutamine stress .....	132
9.10 Strain and strain rate .....	133
9.11 Discussion on strain and strain rate.....	134
9.12 Heart rate, systolic and diastolic blood pressure responses during dobutamine stress.....	135
9.13 Discussion on heart rate and blood pressure response to dobutamine .....	137
9.14 Effect of exercise on myocardial function .....	138
9.15 Discussion on the effect of exercise.....	138
<b>CHAPTER 10- RESULTS - Correlates of myocardial function .....</b>	<b>139</b>
10.1 Correlates of myocardial function (stress group).....	139
10.2 Correlates of Myocardial function in type 1 diabetes mellitus .....	142
10.3 Correlates of myocardial function in control group .....	152
10.4 Discussion on correlates of myocardial function in type 1 diabetes mellitus .....	155

<b>CHAPTER 11- RESULTS - Vascular structure and function</b> .....	<b>161</b>
11.1 Reproducibility of vascular measurements .....	161
11.2 Discussion on vascular reproducibility .....	162
11.3 Vascular stiffness and cIMT .....	163
11.4 Univariate Analysis of Variance of vascular function .....	164
11.4.1 Relationship between age and diabetes/controls for cIMT .....	164
11.4.2 Relationship between age and stiffness parameter $\beta$ .....	165
11.4.3 Relationship between age and stiffness parameter $\epsilon$ .....	166
11.4.4 Relationship between age and augmentation index .....	167
11.4.5 Relationship between age and pulse wave velocity .....	168
11.5 Wave Intensity Results.....	169
11.6 Discussion on vascular structure and function.....	171
11.7 Correlates of vascular function .....	174
11.7.1 Correlates of vascular function (whole group).....	174
11.7.2 Correlates of vascular structure and function in type 1 diabetes mellitus (n=19).....	174
11.8 Discussion on correlates of vascular function.....	176
<b>CHAPTER 12 – SUMMARY AND CONCLUSIONS</b> .....	<b>177</b>
12.1 General discussion .....	177
12.2 Clinical Implications .....	183
12.3 Study limitations .....	184
12.4 Conclusions.....	184
12.5 Future directions .....	185
<b>CHAPTER 13 – BIBLIOGRAPHY</b> .....	<b>186</b>

## Index of Tables

### **Chapter 1**

Table 1.1:	Summary of selected studies of assessment of myocardial function in diabetes	14
Table 1.2:	Summary of studies of myocardial function in diabetes mellitus and its associations	23
Table 1.3:	Summary table showing treatment of subclinical myocardial dysfunction in diabetes mellitus	31
Table 1.4:	Summary table of selected studies on carotid intima media thickness cIMT in diabetes mellitus	37
Table 1.5:	Indices of local arterial stiffness	41

### **Chapter 4**

Table 4.1:	Baseline characteristics of the whole group (n=53)	78
Table 4.2:	Male to Female Ratio (whole group) (n=53)	80

### **Chapter 5**

Table 5.1:	Biochemical results (whole group)	84
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### **Chapter 6**

Table 6.1:	Baseline grey scale and pulsed Doppler echocardiographic data (whole group)	87
Table 6.2:	Baseline grey scale and pulsed Doppler echocardiographic data (stress group)	88
Table 6.3:	M- mode echocardiographic data (N=53)	92

### **Chapter 7**

Table 7.1:	Intra-observer reproducibility of PSV at basal segments (Bland-Altman Analysis)	96
Table 7.2:	Intra-observer reproducibility of displacement at basal segments (Bland-Altman Analysis)	96

### **Chapter 8**

Table 8.1:	Diastolic function (type 1 diabetes mellitus vs. control)	103
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### **Chapter 9**

Table 9.1:	Baseline characteristics of the stress group (N=39)	109
------------	---	-----

Table 9.2:	Biochemical results (stress group) (N=39)	111
Table 9.3:	Myocardial displacement (mm) of basal segments at rest	112
Table 9.4:	Myocardial displacement (mm) of basal segments at dobutamine dose of 5µg/kg/min	113
Table 9.5:	Myocardial displacement (mm) of basal segments at 10µg/kg/min	114
Table 9.6:	Myocardial displacement (mm) of basal segments at 20µg/kg/min	115
Table 9.7:	Myocardial displacement (mm) of basal segments at dobutamine dose of 30µg/kg/min	116
Table 9.8:	Myocardial displacement (mm) of basal segments at dobutamine dose of 40µg/kg/min	117
Table 9.9:	Peak Systolic Velocity (PSV) (cm/s) of basal segments at Rest	120
Table 9.10:	Peak Systolic Velocity (PSV) (cm/s) of basal segments at dobutamine 5µg/kg/min	121
Table 9.11:	Peak Systolic Velocity (PSV) (cm/s) of basal segments at dobutamine 10µg/kg/min	122
Table 9.12:	Peak Systolic Velocity (PSV) (cm/s) of basal segments at dobutamine 20µg/kg/min	123
Table 9.13:	Peak Systolic Velocity (PSV) (cm/s) of basal segments at dobutamine 30µg/kg/min	124
Table 9.14:	Peak Systolic Velocity (PSV) (cm/s) of basal segments at dobutamine 40µg/kg/min	125
Table 9.15:	Radial myocardial function (systolic velocity) (cm/s) during dobutamine stress	127
Table 9.16:	Myocardial function reserve (cm/s) in basal segments	130
Table 9.17:	Strain and strain rate in basal septal and basal lateral segments	133
Table 9.18:	Heart rate (beats/minute) response to dobutamine infusion	135
Table 9.19:	Systolic blood pressure (mmHg) response to dobutamine infusion	135

Table 9.20:	Diastolic blood pressure (mmHg) response to dobutamine infusion	135
<b>Chapter 10</b>		
Table 10.1:	Pearson's correlations between anthropometric measures, biochemical results and myocardial function (n=40)	139
Table 10.2:	Pearson's correlations between anthropometric measures, biochemical results and myocardial function in subjects with type 1 diabetes mellitus (n=19)	142
Table 10.3:	Pearson's correlations between age, HbA1, vascular function and myocardial function in type 1 diabetes mellitus (n=19)	145
Table 10.4:	Pearson's correlations between anthropometric measures, biochemical results and myocardial function in control subjects (n=21)	152
<b>Chapter 11</b>		
Table 11.1:	Intra-observer reproducibility of vascular measurements (Bland-Altman Analysis)	162
Table 11.2:	Markers of vascular stiffness and cIMT (T1DM vs. controls)	163
Table 11.3:	Wave intensity	169
Table 11.4:	Log transformed wave intensity results (all data were log transformed)	170
Table 11.5:	Correlates of vascular function (n=53)	174

## Legends to Figures

### Chapter 1

Figure 1.1: PWI output (Aloka 5500) 46

### Chapter 3

Figure 3.1: Flow diagram of study participants and assessment 53

Figure 3.2: Measurement of carotid intima-media thickness (cIMT) by high resolution ultrasound 55

Figure 3.3: Measurement of wave intensity by carotid ultrasound echo-tracking method 55

Figure 3.4: Pulse wave analysis on right radial artery with a tonometer 57

Figure 3.5: Schematic representation of the arterial pulse wave 58

Figure 3.6: Parasternal long-axis view (PLAX) for LVOT diameter and cross-sectional area calculation 60

Figure 3.7: Pulsed Doppler for mitral inflow velocities 61

Figure 3.8: M-mode of left ventricle 62

Figure 3.9: M-mode examination showing lateral mitral annular excursion 63

Figure 3.10: M-mode examination showing medial mitral annular excursion 64

Figure 3.11: M-mode examination showing tricuspid annular excursion 65

Figure 3.12: Mitral flow propagation on colour M-mode 66

Figure 3.13: IVRT measurement by placing cursor for pulsed Doppler near LVOT 67

Figure 3.14: Measurement of velocity time integral (VTI) at LVOT 68

Figure 3.15: Pulsed Doppler of lateral mitral annulus 69

Figure 3.16: Pulsed Doppler of medial mitral annulus 70

Figure 3.17: Pulsed Doppler of tricuspid annulus 71

Figure: 3.18: Displacement in basal septal and basal lateral segments 74

Figure: 3.19: Regional myocardial velocities (S, E, and A) in basal septal segments 74

Figure 3.20:	Measurement of strain in basal septal segment	75
Figure 3.21:	Measurement of strain rate in basal septal segment	75
<b>Chapter 7</b>		
Figure 7.1:	Intra-observer variability for peak systolic velocity	97
Figure 7.2:	Intra-observer variability for basal displacement analysis	97
Figure 7.3:	Intra-observer variability for diastolic velocities	98
Figure 7.4:	Bland-Altman analysis of PSV at Basal Lateral (BL) segments	99
Figure 7.5:	Bland-Altman analysis of PSV in Basal Septal (BS) segments	100
Figure 7.6:	Bland-Altman Analysis of PSV in Basal Anterior (BA) segments	101
Figure: 7.7:	Bland-Altman Analysis of PSV in Basal Posterior (BP) segments	102
<b>Chapter 8</b>		
Figure 8.1:	Diagnostic flowchart for the diagnosis of HFNEF	105
<b>Chapter 9</b>		
Figure 9.1:	Myocardial displacement (mm) of basal segments at rest	112
Figure 9.2:	Myocardial displacement (mm) of basal segments at 5 $\mu$ g/kg/min	113
Figure 9.3:	Myocardial displacement (mm) of basal segments at 10 $\mu$ g/kg/min	114
Figure 9.4:	Myocardial displacement (mm) of basal segments at 20 $\mu$ g/kg/min	115
Figure 9.5:	Myocardial displacement (mm) of basal segments at 30 $\mu$ g/kg/min	116
Figure 9.6:	Myocardial displacement (mm) of basal segments at 40 $\mu$ g/kg/min	117
Figure 9.7:	Mean basal displacement during dobutamine stress	118
Figure 9.8:	Peak Systolic Velocity (PSV) (cm/s) of basal segments at Rest	120
Figure 9.9:	Peak Systolic Velocity (PSV) (cm/s) of basal segments	121

	at dobutamine 5 $\mu$ g/kg/min	
Figure 9.10:	Peak Systolic Velocity (PSV) (cm/s) of basal segments at dobutamine 10 $\mu$ g/kg/min	122
Figure 9.11:	Peak Systolic Velocity (PSV) (cm/s) of basal segments at dobutamine 20 $\mu$ g/kg/min	123
Figure 9.12:	Peak Systolic Velocity (PSV) (cm/s) of basal segments at dobutamine 30 $\mu$ g/kg/min	124
Figure 9.13:	Peak Systolic Velocity (PSV) (cm/s) of basal segments at dobutamine 40 $\mu$ g/kg/min	125
Figure 9.14:	Mean peak systolic velocity during dobutamine stress	126
Figure 9.15:	Radial myocardial function during dobutamine stress	127
Figure 9.16:	Myocardial functional reserve (cm/s) in six basal segments	130
Figure 9.17:	Early diastolic velocity during dobutamine stress	132
Figure 9.18:	Changes in heart rate, SBP and DBP during dobutamine stress	136
<b>Chapter 10</b>		
Figure 10.1:	Correlation between BMI and E/e' (n=40)	140
Figure 10.2:	Correlation between waist circumference and E/e' (n=40)	140
Figure 10.3:	Correlation between waist-hip ratio and lateral e' (n=40)	141
Figure 10.4:	Correlation between BMI and E/e' ratio in subjects with type 1 diabetes mellitus (n = 19)	143
Figure 10.5:	Correlation between waist hip ratio and lateral e' in subjects with type 1 diabetes mellitus (n=19)	143
Figure 10.6:	Correlation between AIx and displacement	146
Figure 10.7:	Correlation between AIx and early diastolic velocity E (tissue velocity)	146
Figure 10.8:	Correlation between augmentation index and medial mitral annular tissue velocity (medial e') in type 1 diabetes mellitus (n=19)	147

Figure 10.9:	Correlation between age and late diastolic tissue velocity A	148
Figure 10.10:	Correlation between waist hip ratio and late diastolic tissue velocity (A)	148
Figure 10.11:	Correlation between mean cIMT and late diastolic tissue velocity	149
Figure 10.12:	Correlation between mean peak systolic velocity and HbA1c	150
Figure 10.13:	Correlation between BMI and late diastolic (mitral inflow) velocity (A) in control subjects (n=21)	153
Figure 10.14:	Correlation between BMI and E/A ratio in control subjects (n=21)	153
Figure 10.15:	Correlation between total cholesterol and early diastolic velocity (mitral inflow) (E) in control subjects (n=21)	154
<b>Chapter 11</b>		
Figure 11.1:	Coefficient of variation (CV) of vascular measurements	161
Figure 11.2:	Relationship between age (years) and mean cIMT (mm)	164
Figure 11.3:	Relationship between age (years) and mean $\beta$	165
Figure 11.4:	Relationship between age and stiffness parameter $\epsilon$	166
Figure 11.5:	Relationship between age and augmentation index	167
Figure 11.6:	Relationship between mean pulse wave velocity (pwv) (m/s) and age (years)	168
Figure 11.7:	Correlation between cIMT and age	175
Figure 11.8:	Correlation between cIMT and waist hip ratio	175

## Glossary of abbreviated terms

A	- late diastolic mitral inflow velocity
A'	- late diastolic velocity tissue
ACR	- albumin creatinine ratio
AGEs	- advanced glycated endproducts
AIx	- augmentation index
BA	- basal anterior myocardial segment
BAS	- basal anteroseptal myocardial segment
BCW	- backward compression wave
BEW	- backward expansion wave
BI	- basal inferior myocardial segment
BL	- basal lateral myocardial segment
BP	- basal posterior myocardial segment
BS	- basal septal myocardial segment
CO	-cardiac output
cIMT	-carotid intima-media thickness
DBP	- diastolic blood pressure
DSE	- dobutamine stress echocardiography
DT	- deceleration time of mitral flow E velocity
E	- early diastolic mitral inflow velocity
E'	- tissue early diastolic velocity
EDV	- end diastolic volume
EF	-ejection fraction
ESV	- end systolic volume
FCW	- forward compression wave
FEW	- forward expansion wave
HbA1c	- glycated haemoglobin
HDL	- high density lipoprotein
hsCRP	- high sensitivity C-reactive protein
IVRT	- isovolumic relaxation time
LDL	- low density lipoprotein
LV	- left ventricle

LVH	- left ventricular hypertrophy
LVM	- left ventricular mass
LVMi	- left ventricular mass index
LVOT	- left ventricular outflow tract
NADPH	- nicotinamide adenosine dinucleotide phosphate
NO	- nitric oxide
NOS	- nitric oxide synthase
PARP	- poly (ADP-Ribose) Polymerase
PKC	- protein kinase C
PSV	- longitudinal peak systolic velocity
PWI	- pulse wave intensity
pwv	- pulse wave velocity
ROS	- reactive oxygen species
SBP	- systolic blood pressure
SERCA	- sarcoendoplasmic reticulum calcium ATPase
SV	-stroke volume
TC	- total cholesterol
TDI	- tissue Doppler imaging
TG	- triglycerides
T1DM	- type 1 diabetes mellitus
T2DM	- type 2 diabetes mellitus
VTI	- velocity time integral
$\beta$	- stiffness index, beta
$\epsilon$	- elastic modulus, epsilon

# **CHAPTER 1- BACKGROUND**

## ***1.1 Vascular disease and type 1 diabetes mellitus***

### **1.1.1 Epidemiology**

Subjects with type 1 diabetes mellitus (T1DM) are at increased risk of cardiovascular diseases. [1] T1DM is associated with a four (in men) to eight (in women) -fold increase in coronary artery disease. [2, 3] Even though the incidence of cardiovascular disease in T1DM is lower than that in type 2 diabetes mellitus, younger people are affected.[1] The relative risk of mortality for both coronary artery disease and stroke in T1DM is significantly elevated in the United Kingdom. [4, 5] The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) demonstrated the cumulative incidence for myocardial infarction to be 14.8% and 18.1% for angina in T1DM respectively. [6] Actually, age-adjusted relative risk for CVD in T1DM may even be higher than that in type 2 diabetes mellitus. [7]

### **1.1.2 Pathogenesis of vascular disease in Diabetes Mellitus**

The exact mechanism for accelerated atherosclerosis in diabetes mellitus remains unclear. It is thought to be multifactorial. Endothelial dysfunction may be caused by hyperglycaemia and other metabolic derangements in T1DM. Atherosclerosis is essentially an inflammatory disease and subjects with diabetes mellitus are in a state of proinflammatory and prothrombotic conditions. [7, 8]

### 1.1.2.1 Endothelial dysfunction

Endothelial dysfunction is thought to be an important initial change in the pathogenesis of atherosclerosis. Endothelial dysfunction in diabetes mellitus may result from oxidative stress which could be caused by hyperglycaemia via a variety of mechanisms such as the sorbitol pathway, activation of protein kinase C (PKC), the hexosamine pathway and non-enzymatic glycation of proteins. [9]

Normal endothelium is a single layer of cells lining the inner layer of blood vessels. It is an active and complex organ which regulates vascular tone, permeability and vascular tissue homeostasis. [10] Studies have shown endothelial dysfunction in both type 1 and type 2 diabetes mellitus. [11] [12] Hurks et al reported endothelial dysfunction detected by impaired flow mediated dilatation (FMD) in young patients with type 1 diabetes mellitus before any evidence of early atherosclerosis. Lower FMD in type 1 diabetes mellitus was also reported by other researchers. [13] [14]

### 1.1.2.2 Hyperglycaemia

Hyperglycaemia may cause vascular injury via a range of mechanisms such as: increase in intracellular glucose leading to more production of sorbitol via polyol pathway, an increase in production of glucosamine-6-phosphate via hexosamine pathway and the activation of PKC (protein kinase C) via de novo synthesis of DAG (diacylglycerol). [9]

### 1.1.2.3 Sorbitol pathway

Under normal circumstances, most glucose is metabolized by glycolysis. However, in a hyperglycaemic state, a higher percentage of glucose goes through other metabolic pathways such as the sorbitol pathway due to increased substrate availability for the aldose reductase (AR) enzyme which has low affinity for glucose under physiological conditions. [15]

Glucose can be metabolized to sorbitol and fructose in most cells, resulting in increased oxidation of NADPH to NADP<sup>+</sup> and increased reduction of NAD<sup>+</sup> to NADH. This pathway results in imbalance of redox potentials and favours the formation of advanced glycation endproducts (AGEs) and enhances oxidative stress. [16] This altered redox state resembles hypoxia and it is called “pseudohypoxia”. Moreover, NADPH is essential for glutathione reductase activity and reduced availability of NADPH results in reduced glutathione reductase activity, increasing oxidant injury. [17]

### 1.1.2.4 Advanced glycation endproducts (AGEs)

Advanced glycation endproducts (AGEs) are proteins formed by non-enzymatic reaction between the carbonyl group of glucose and the N-terminus of free amino acids of proteins.[18] Non-enzymatic glycosylation of basic fibroblast growth factor in vitro reduces the mitotic activity of endothelial cells. [19] AGE has been shown to increase permeability of human endothelium in vitro. [20] AGEs are chemotactic for mononuclear cells and AGEs on the subendothelium induces monocytes migration across the endothelium.[21] Receptors for AGE (RAGE) seem to exert their effects on target organs by expression of cytokines and growth factors and expression of proinflammatory, and pro-coagulatory molecules by

endothelial cells such as thrombomodulin, tissue factor and the cell adhesion molecule VCAM-1(vascular cell adhesion molecule-1). [15]

#### 1.1.2.5 Protein Kinase C (PKC) activation

The PKCs are a family of serine/threonine kinases that act on the cell membrane in the regulation of signal transduction in a variety of cells and are usually activated by diacylglycerol (DAG). [22] PKC iso-enzyme  $\beta$  in the endothelium is activated by elevated DAG level in the presence of hyperglycaemia. [23] PKC $\beta$  reduces endothelial nitric oxide synthetase (eNOS) expression by inhibiting protein kinase B in vivo and in vitro. [24] PKC may mediate atherosclerosis by increased production of reactive oxygen species (ROS), reduced NO production and increased endothelin ET1 production. [25] In healthy humans, PKC  $\beta$  inhibition prevents impaired endothelium dependent vasodilatation caused by hyperglycaemia. [26] However, PKC  $\beta$  inhibition failed to do so in diabetes mellitus. [27]

#### 1.1.2.6 Oxidative Stress

Hyperglycaemia increases the production of reactive oxygen species (ROS) which in turn increases cellular oxidative stress. Neutrophils from poorly controlled diabetes mellitus produce excess superoxide via phospholipase A2. [28] Auto oxidation of glucose and formation of advanced glycation end products (AGEs) produce ROS in diabetes mellitus. [29] Hyperglycaemia increases the synthesis of reactive oxygen species which inactivate nitric oxide (NO). Hyperglycaemia may initiate superoxide synthesis via electron transport in mitochondria. [30]

There are many metabolic abnormalities observed in diabetes mellitus which were thought to be the basis for the pathophysiology of vascular disease in diabetes mellitus.

Understanding pathophysiology may be helpful in preventing vascular disease in diabetes mellitus.

## ***1.2 Diabetic Heart Muscle Disease and Diabetes Mellitus***

### **1.2.1 Epidemiology**

Atherosclerotic cardiovascular disease is common in diabetes mellitus. However, it is postulated that diabetic cardiomyopathy or diabetic heart muscle disease, which is independent of coronary artery disease, also exists. The Framingham Heart Study proved that diabetes mellitus is a risk factor for heart failure [31] and this was subsequently confirmed in many other studies such as the Strong Heart Study. [32] It is recognized that there is an over-representation of patients with diabetes mellitus in heart failure trials, accounting for about 25% of subjects. [33] Many other epidemiological studies such as the Cardiovascular Health Study [34] and the Euro Heart Failure Survey [35] suggested that the presence of diabetes mellitus is an independent risk factor for development of heart failure. Patients with diabetes mellitus are not only at higher risk of heart failure but also have poorer outcomes. [36]

Rubler et al first described the term “diabetic cardiomyopathy” after performing post-mortem histology in 4 patients who had heart failure in the absence of ischaemic, hypertensive and valvular heart disease. [37] There is cumulative histological and clinical evidence of heart muscle disease in diabetes mellitus, particularly in type 2 diabetes mellitus.

### **1.2.2 Pathogenesis**

The exact mechanism for the cause of myocardial dysfunction in diabetes mellitus is not known. It may be multifactorial in origin. A variety of possible mechanisms has been described;

### 1.2.2.1 Substrate metabolism

Diabetes mellitus is characterized by reduced glucose and lactate metabolism. Decreased glucose supply and utilization was found in diabetic cardiomyocytes and diabetic patients. [38] [39] Myocardial glucose transport may be impaired due to reduced myocardial concentrations of glucose transporters, GLUT1 and GLUT4. [40] In an animal model of type 2 diabetes, db/db mice, cardiac function was observed to be reduced whereas in transgenic human GLUT4 db/db mice, abnormal cardiac function was corrected. [41] Pyruvate dehydrogenase, a key enzyme for aerobic glycolysis, may also be inhibited by increased free fatty acid oxidation and its end-products, acetyl co-A. [42]

### 1.2.2.2 Free fatty acids utilization

In the presence of defective glucose metabolism, cardiac myocytes use  $\beta$  oxidation of free fatty acids (FFA) as the main source of energy. [43] Availability of FFA in diabetes may be increased by enhanced adipose tissue lipolysis and by hydrolysis of augmented myocardial triglyceride stores. Utilization of FFA by cardiac myocytes may have detrimental effects on cardiac myocytes because of the higher oxygen consumption for  $\beta$  oxidation and the production of toxic intermediates of free fatty acids, which may promote apoptosis of cardiac myocytes. [43] In monogenic db/db mice, a model of type 2 diabetes mellitus with extreme obesity and hyperglycaemia, plasma membrane FFA transports were found to be increased on cardiomyocytes, facilitating increase FFA utilization. [44]

### 1.2.2.3 Calcium homeostasis

Impaired calcium homeostasis may play an important role in the pathogenesis of diabetic cardiac muscle disease. Calcium is essential for myocardial excitation and contraction coupling. When the cardiac cell membrane is depolarized by the action potential,

calcium enters the cell through voltage dependent L-type Ca channels in the sarcolemma. This triggers the release of further calcium into the cytoplasm from sarcoplasmic reticulum through ryanodine calcium release receptors (RyR). Increased intracellular calcium facilitates the binding of calcium to myofilament, initiating cardiac contraction. For relaxation to occur, calcium needs to be removed from the cytosol. The majority of the calcium is pumped back into the sarcoplasmic reticulum by sarcoplasmic/endoplasmic reticulum  $\text{Ca}^{2+}$  ATPase (SERCA) and the rest is rejected out of the cell by the sarcolemmal ( $\text{Na}^{+}/\text{Ca}^{2+}$ ) exchange (NCX), plasma membrane  $\text{Ca}^{2+}$ -ATPase (PMCA) or mitochondrial calcium uniport. [45] In rodent models of both type 1 and type 2 diabetes mellitus, there are evidences to suggest altered expression, activity and function of all transporters involved in excitation contraction coupling namely SERCA [46], NCX [47], RyR [48] and PMCA [49]. Overexpression of SERCA in the diabetic heart improved both calcium handling [50] and cardiac function. [46]

#### 1.2.2.4 Reactive oxygen species and oxidative stress

Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and antioxidant defences. Oxidative stress may induce changes in signalling pathways or even cellular damage at high level. ROS have been implicated all stages of heart failure, from hypertrophy to fibrosis, contractile dysfunction and failure. [51] High rates of fatty acid oxidation increases ROS production by augmenting mitochondrial action potential. Under physiological conditions, ROS are removed by antioxidants. [52] In the event of excessive ROS production as observed in streptomycin induced diabetic rats and db/db mice, it causes damage to cardiac myocytes and augmented apoptosis. [53] [54] Moreover, overexpression or administration of the antioxidant, metallothionein, in rodent models of diabetes have been shown to improve morphological and function changes of diabetes heart muscle disease. [55] [56]

#### 1.2.2.5 Poly (ADP-Ribose) Polymerase (PARP)

PARP is a nuclear enzyme which is involved in DNA repair. [57] Oxidative stress secondary to hyperglycaemia damages DNA strands. [58] Extracellular glucose excess not only induces oxidative stress but also activates PARP. [59] PARP catalyses the cleavage of NAD<sup>+</sup> to nicotinamide and ADP-ribose. It uses ADP-ribose to synthesize a branched polymer, poly ADP-ribose. This process is an energy dependent process and depletes the cellular ATP. [60]

PARP may also damage myocardium by activating nuclear factor  $\kappa$ B (NF $\kappa$ B) and inducing expression of endothelin 1 (ET1), a potent vasoconstrictor, and its receptors. [61, 62] Hyperhexosaemia induced cardiac muscle hypertrophy is prevented by PARP inhibition in an animal study. [63] Inhibition of PARP has been shown to reverse endothelial and cardiac dysfunction in diabetic animals. [64, 65] However, no polymerase inhibitor is currently available to be used safely in human studies or clinical trials.

#### 1.2.2.6 Advanced glycation end-products (AGEs)

Advanced glycation end-products are formed by non-enzymatic irreversible reaction between aldose sugar and lipids or protein. Receptors for AGEs (RAGE) are found on the endothelial surface and AGE-RAGE interaction seems to induce oxidative stress and an inflammatory reaction. [66] AGEs may affect myocardial function by cross-linking with collagen in the myocardium, causing increased ventricular stiffness. [67] AGEs may also cause impaired relaxation of the myocardium through myocyte calcium handling by AGE-RAGE interaction. [68] Cross linking breaker of AGEs, ALT-117, has been shown to improve diabetes related myocardial and vascular stiffness in animal studies. [69] [70]

### 1.2.2.7 Structural changes in myocardium

Rubler et al were the first to describe structural changes observed in diabetic cardiomyopathy. [37] They found ventricular hypertrophy, fibrotic changes in myocardium and thickening of intramural arterioles, and they postulated that diabetic cardiomyopathy may be secondary to diabetic macroangiopathy as a result of abnormal myocardium metabolism in diabetes.

In animal studies, increase in myocardial mitochondria, apoptosis, intramyocardial lipid accumulation and fibrosis are common findings. Myocardial fibrosis has been described in many histological studies [71] [72] and echocardiography studies of backscatter [73] [74] in humans.

### 1.2.3 Diastolic myocardial dysfunction in diabetes mellitus

Diabetic heart muscle disease is widely regarded to be a diastolic disease.[75] [76] [77] One of the earliest studies which described diabetic cardiomyopathy as a diastolic dysfunction was carried out by Raev who studied 157 asymptomatic young subjects (mean 26.6 years) with type 1 diabetes mellitus and 54 controls, with M-mode echocardiography. He suggested that left ventricular diastolic function was impaired before systolic function. [78] Earlier studies used mitral inflow early (E) and late (A) velocities and their ratio (E/A) as a marker of diastolic dysfunction. Zarich et al showed that the E/A ratio was significantly lower in 21 type 1 diabetes mellitus than in 21 controls. [79] This finding was confirmed in other studies. [80] [81]

Moreover, some measures such as the Valsalva manoeuvre and measurement of pulmonary venous flow on echocardiography seem to have increased the sensitivity of conventional Doppler in diagnosing subclinical disease in diabetes mellitus. In a study of 46

type 2 diabetes mellitus patients without diabetic complications and cardiovascular diseases, left ventricular diastolic function was assessed by measuring the E/A ratio during the Valsalva manoeuvre and by recording pulmonary venous flow. Left ventricular diastolic dysfunction was found in 60% of the subjects of whom 28% had a pseudonormal pattern of mitral flow on Doppler assessment. [82]

It is generally accepted that diastolic dysfunction is the earliest change in diabetic heart muscle disease as parameters like IVRT, E/A, E/E' were frequently found to be abnormal in diabetes mellitus in the absence of systolic dysfunction. [83] [77] Perhaps conventional echocardiography may not be sensitive enough to detect early subtle systolic dysfunction. Moreover, these parameters are load dependent and can be affected by ventricular loading conditions.

Despite the conventional assumption that diabetic cardiomyopathy is diastolic dysfunction, with advances in technology in the area of myocardial imaging, it may be possible to assess systolic function more sensitively than before and therefore to explore subclinical myocardial dysfunction in diabetes mellitus more in its early stages.

#### 1.2.4 Systolic myocardial dysfunction in diabetes mellitus

Indeed, left ventricular longitudinal systolic dysfunction was detected in later studies using tissue Doppler imaging. Andersen et al demonstrated abnormal longitudinal systolic velocity in a study of 32 type 2 diabetes mellitus with normal ejection fraction. [84] Mean peak systolic velocity and mean peak systolic strain rate were significantly lower in diabetic group. Abnormal systolic velocities in diabetes mellitus were confirmed in further studies by Vinereanu et al [85] and Fang et al [86]. Ernande et al questioned the conventional acceptance of isolated diastolic dysfunction as an early change in diabetes cardiomyopathy as

they demonstrated both systolic and diastolic dysfunction on echocardiography and speckle tracking in 114 patients with type 2 diabetes mellitus.[87]

However, not all the studies were positive for subclinical dysfunction in diabetes mellitus. Konduracka et al studied 185 type 1 diabetes mellitus and 105 non-diabetic controls by using conventional Doppler and tissue Doppler imaging; 89% of the studied subjects had diabetic retinopathy and 45% had cardiovascular autonomic neuropathy. They did not find any significance difference in either systolic or diastolic dysfunction between controls and diabetics. [88] In fact, they even looked at 17 deceased patients who had had diabetes mellitus for more than 20 years. Even though autopsy showed microscopic evidence of small vessel disease in the myocardium, echocardiographic changes were reported to be normal for those patients as well.

#### 1.2.5 Radial myocardial dysfunction in diabetes mellitus

Radial systolic function has not been widely investigated. The results are conflicting. Two studies [85, 89] by Vinereanu et and Fang et al respectively showed reduced longitudinal systolic function but increased radial function in diabetes mellitus. “Radial compensation” to reduction in longitudinal function was postulated. It implies that longitudinal fibres in subendocardium are more affected and ischaemic pathology, possibly microvascular disease, as a causative factor is suspected. It was regarded that midwall fibres which are responsible for radial systolic function are more or less spared in the early disease process. However, in a more recent study by Ernande et al, reduction in both radial and longitudinal strain on speckle tracking was reported in type 2 diabetes mellitus.[90] Similar findings were observed by Nakai et al in a group of type 2 diabetes mellitus. [91] In contrast,

Ng et al reported normal radial strain with reduced longitudinal function in type 2 diabetes mellitus on speckle tracking. [92]

There are limited numbers of studies which assessed radial function and the reports are conflicting. The role of radial myocardial function in subclinical myocardial dysfunction in diabetes mellitus, particularly in type 1 diabetes mellitus, remains an area to explore further.

#### 1.2.6 Myocardial velocity imaging in detecting subclinical myocardial dysfunction

Echocardiography is the most commonly used non-invasive tool for the investigation of cardiac structure and function at present. Myocardial velocity imaging, also known as tissue Doppler imaging, is a new technique which gives quantitative assessment of regional myocardial function. It is well validated, reproducible and readily assessable. [93] Traditional Doppler Echocardiography is based on the Doppler effect, i.e. a change in the frequency of ultrasound signal reflected from moving objects. Doppler echocardiography is used to assess blood flow whose reflected signal has low amplitude and high frequency. On the other hand, cardiac structure has high amplitude and low frequency signal. Tissue motion creates Doppler shift and the machine can be equipped with filters which can exclude the low amplitude high frequency signal. In myocardial velocity imaging, to record low velocity tissue motion, gain amplification is reduced and high pass filters are bypassed so that the tissue signal directly enter the autocorrelator. Both systolic and diastolic function can be assessed and quantified. [94] It has been used to detect subclinical myocardial dysfunction in many conditions including diabetes mellitus (see Table 1.1).

#### 1.2.7 Dobutamine stress echocardiography in subclinical myocardial dysfunction

Attempts have been made to find a way to diagnose earlier myocardial dysfunction. Stress echocardiography seems to be promising in detecting myocardial functional reserve. Vinereanu et al demonstrated that longitudinal peak systolic velocity was low at rest and at peak stress with dobutamine in a study of 35 asymptomatic patients with type 2 diabetes mellitus. [85] However, this finding of impaired myocardial functional reserve in diabetes mellitus was not in agreement with the findings of Fang et al. [86] They reported reduced peak systolic longitudinal velocity at rest in type 2 diabetics but normal response to dobutamine. A study by Von Bibra et al supported findings by Vinereanu et al. They studied 43 diabetic patients with dobutamine stress echocardiography, and longitudinal systolic velocity was lower at peak stress in diabetics when compared with controls (Vs.  $10.7 \pm 2.7$  vs.  $13.6 \pm 3.4$ ). [95] Further studies are needed to evaluate myocardial functional reserve, which may reveal further insight into the pathophysiological processes of diabetes cardiomyopathy, particularly in type 1 diabetes mellitus. A summary of echocardiographic studies for myocardial function in diabetes mellitus are listed in table 1.1.

**Table 1.1: Summary table of selected studies of assessment of myocardial function in diabetes mellitus**

Author	Type	No (DM+CTRL)	Age	Duration (yr)	Methods	Findings	Comments
Suys (2004)[80]	1	80+52	6.2-21.6 4.1-20.2	F (6.8±3.7) M (5.7±3.2)	Conventional echo and tissue Doppler (e/e')	Larger left ventricular posterior wall, higher E', E/E', IVRT and A in female	No changes in male. Wide age range.
Raev (1994)[78]	1	157 +53	Mean 26.6		M-mode echocardiography Systolic function by fractional shortening, mean velocity of circumference fibre shortening and stroke index. Diastolic function by slope of anterior mitral leaflet in early diastole, IVRT and left atrium emptying index.	Diastolic dysfunction more common than systolic dysfunction. It was concluded that diabetes affects diastolic function before systolic function.	Early study to prove diastolic function is more affected in diabetes.
Romanens (1999)[96]	1	20+20	DM -35±8 Ctrl- 32±10	17±8	Conventional echo and pulsed Doppler at Mitral valve	No difference found.	
Von Bibra (2005) [95]	2	43+33	DM 60.6±7 Ctrl- 59.7±7.5	NA	Tissue Doppler at rest and stress (dipyridamole and/or dobutamine)	Dobutamine stress resulted in lower Vs. and Ve in diabetics	Patients with IHD were included.

Fang (2004)[97]	1+2	53+53	DM 59±9 Ctrl- 58±10	13±11	Tissue Doppler imaging. Significant IHD excluded by stress echo.	Longitudinal Sm, Em, strain and strain rate were significantly lower in diabetic patients whereas radial Sm, strain and strain rate were higher in diabetic patients.	Radial compensation was postulated.
Di Cori (2007)[98]	1	40+40	DM 28±4.2 Ctrl- 30±4.1	8.9±3.7	Conventional echo and tissue Doppler. Integrated backscatter.	Peak systolic strain lower in diabetic subjects.	HbA1c 7.4±1.2
Poirier (2001)[82]	2	46	38-67	0.25 to 32	Conventional echo and transmitral Doppler flow velocity. Pseudonormal patterned identified by measuring pulmonary venous flow during Valsalva manoeuvre.	Left ventricular diastolic dysfunction was found in 60%, of whom 28% had pseudonormalization.	Highlighted the importance of excluding pseudonormalization. Significant age difference in LVDD group.
Fang (2003)[86]	1+2	41+41 (5 patients with type1)	DM- 56±8 Ctrl- 59±9	11±10	Tissue Doppler with dobutamine stress was used. (up to 40µg/kg/min).	PSV lower in diabetic subjects at rest. However, no significant difference in systolic function in response to dobutamine. Em was lower at rest and during stress in DM.	

Muranaka (2009) [99]	NA	Ctrl-16 DM-17 DM+HT-22	DM-63±10 DM+HT-61±7 Ctrl-62±10	DM-10±9.8 DM+HT-10.3±8.4	Myocardial velocity imaging is used to measure strain rate in left ventricle and left atrium.	Lower LVs, LVe, Las and LAe in DM group. DM+HT group has lower LVe and LAe than DM group.	
Andersen (2003)[84]	2	32+32	53±7	0.5-20	Myocardial velocity imaging was used to measure longitudinal systolic function and SR. Tissue tracking score index was used.	Mean PSV and peak SR were lower in DM group.	Smokers in DM group > controls. IHD excluded by history. Early study to demonstrate systolic function could also be impaired in DCMP.
Shivalkar (2006) [100]	1	100+75	DM-46.6±9.7 Ctrl-42±11	2-36	FMD, IMT and tissue Doppler of 12 left ventricular segments were measured.	FMD was reduced and IMT was increased in diabetic group. FMD correlated with number of myocardial segments with reduced function.	Subgroup analysis in under 50 did not show any difference in vascular function between the two groups.
Vinereanu (2003) [85]	2	35+35	DM-57±10 Ctrl-56±12	NA	Conventional echo was performed. Dobutamine stress echo with tissue Doppler was	PSV was reduced in DM both at rest and at peak stress. Myocardial functional reserve was also reduced. Radial	Subclinical dysfunction was also related to serum lipids and

					used to measure longitudinal and radial myocardial velocity.	function was higher in diabetics.	HbA1c. Radial compensation was suggested.
Celentano (1995) [101]	2	N=25 IGT=15 DM=24	DM-57±4 IGT-61±6 Ctrl-58±5	NA	Conventional echo was done. Subjects were divided into three groups depending on glucose tolerance.	Left ventricular end systolic diameter indexed for BSA was higher in IGT and DM groups whereas peak E/A was lower.	Changes were observed even in impaired glucose tolerance group.
Karamitsos (2006) [102]	1	66+66	35±10	20±9	Conventional echo and tissue Doppler were performed to measure mitral and tricuspid flow and mitral and tricuspid annular velocities.	Tricuspid flow E/A ratio and tricuspid annular diastolic velocities were reduced in DM group.	Evidence of RV involvement as well as LV.
Baldi (2006) [83]	2	13+15	DM-45.3±6.2 Ctrl-47.6±5.2	NA	Conventional and Tissue Doppler Maximal oxygen consumption was assessed within 2 wks of echo.	No difference in S', E, A and E/A. However, E', E/E' and E'/A' were lower in type 2 DM subjects.	High BMI in both groups.
Konduracka (2007) [88]	1	185+105	DM-34.8±7.9 Ctrl-34.5±7.8	22.8±8.7	Conventional echo and tissue Doppler were performed. Histological studies were done on 17 hearts of	Apart from systolic S pulmonary flow/diastolic D pulmonary flow ratio and E/E', all other echo parameters were not different	Essentially a negative study.

					T1DM patients. NT-proBNP level was also measured.	between two groups. Microscopy of 17 hearts revealed early atherosclerotic changes in large epicardial coronary arteries, and mild fibrosis.	
Karamitsos (2008) [103]	1	44+21	DM+CAN - 18 DM-CAN-24 Ctrl-21	24±7 21±6	Conventional echo and tissue Doppler. Cardiac autonomic neuropathy identified by ECG using R-R interval variation and Valsalva manoeuvre.	Diastolic dysfunction more pronounced in CAN patients as evident by lower Em and Em/Am ratio and higher mitral inflow A.	
Palmieri (2006) [104]	1	20+24	DM 34±10 Ctrl 33±9	2-30 Median 14	Colour tissue Doppler study Low dose dobutamine given up to 7.5µg/kg/min	Only systolic lateral mitral annulus was lower in DM at rest.	Only type1 diabetic subjects received dobutamine, not controls. *
Palmieri (2007) [105]	1	25+32+24 DM1+NT+ HT	33±10 40±8 46±9	14±8	Echo and tissue Doppler. Exercise test and SV and CO were measured during exercise using bio-impedance cardiographer.	Peak exercise SVi and COi were lower in DM group than HT&NT group.	Subnormal LV function reserve in DM which may not be apparent at rest was suggested.
Carugo (2001)	1	56+20	DM 35±2 Ctrl 32±1.8	14±11	Conventional Echo	Increase LV wall thickness, increase LVMI, increase wall thickness/EDV ratio and reduced E/A.	

## 1.2.8 Determinants of myocardial function in diabetes mellitus- current evidence

### 1.2.8.1 Hyperglycaemia

Elevated glycosylated haemoglobin was linked with impaired myocardial function. Peak systolic strain was associated with HbA1c in a study involving 219 type 2 diabetes mellitus patients without known cardiovascular disease. [106] In the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) HbA1c was significantly associated with cardiovascular mortality. [6] In the EURODIAB study, after 8 years of follow-up, HbA1c was linked to coronary artery disease. [107]

In the DCCT trial, 1441 T1DM subjects aged 13-39 years were allocated randomly to two groups, intensive glycaemic control and conventional glycaemic control. Both groups were followed up for mean duration of 6.3 years. The intensive controlled group achieved a mean HbA1c of 7.2% whereas the conventional group achieved 9.0%. At the end of DCCT trial period, intensive treatment reduced major cardiovascular events and peripheral vascular events by 41%, but not to the level of statistical significance. [108] Nevertheless, after a mean 17 years of follow-up in the DCCT/EDIC study, 144 cardiovascular events occurred in 83 patients, 46 among 31 patients originally allocated to intensive therapy and 98 among 52 patients assigned to conventional treatment. The respective event rates were 0.38 and 0.80 per 100 patient-years. (P=0.007). A life-table analysis of the cumulative incidence of a first cardiovascular event showed that intensive treatment was associated with a 42% reduction in risk in comparison with

conventional treatment. (95% confidence interval, 9 to 63%; P=0.002) The risk of first occurrence of non-fatal myocardial infarction or stroke or death from cardiovascular disease was also reduced in the intensive treatment group despite the fact that HbA1c of the two groups converged over the course of EDIC study. [109] .

In type 2 diabetes mellitus, the UKPDS [110] failed to prove that intensive glycaemic control reduced cardiovascular mortality apart from treatment with metformin in overweight type 2 diabetes mellitus patients. [111] Moreover, the results of the ACCORD trial showed that the strategy of lowering HbA1c level closer to normal was not effective in type 2 diabetes mellitus. [112]

There is evidence to suggest that intensive glycaemic control reduce cardiovascular events and mortality in type 1 diabetes mellitus. However, there is no strong evidence to suggest so in type 2 diabetes mellitus. The reason for this apparently less effective effect of glycaemic control on cardiovascular mortality in type 2 diabetes mellitus remains unclear. However, type 2 diabetes mellitus patients tend to have other associated risk factors such as dyslipidaemia and hypertension.

#### 1.2.8.2 Brain Natriuretic peptide

No significant relation between BNP and subclinical myocardial dysfunction in diabetes mellitus was found. [113]

### 1.2.8.3 Vascular Stiffness

Diabetes mellitus increases vascular stiffness. Aortic stiffness measured by M-mode echocardiography was associated with left ventricular diastolic function measured by tissue Doppler echocardiography in a study of 57 asymptomatic type 2 diabetes mellitus and 25 healthy controls. [114] Systolic and diastolic diameters of the aorta were measured with M-mode echocardiography in a parasternal long-axis view and aortic root distensibility was calculated. Aortic distensibility was significantly reduced in type 2 diabetics and was negatively associated with mitral A wave velocity, IVRT and Am velocities. It was also positively associated with mitral E wave velocity, E/A ratio, E' velocities and E'/A' ratio. An association was found between elevated left ventricle filling pressure as measured by (E/E') and central pulse pressure measured by radial tonometry in type 2 diabetes mellitus. [115]

Moreover, pulse wave velocity (PWV), a marker of vascular stiffness, measured by whole body impedance cardiography and calf impedance plethysmography, was impaired even in a group of type 2 diabetes mellitus patients with short duration of diabetes mellitus (mean duration 1.8 years) and also in patients with type 2 diabetes mellitus, PWV was associated with low average myocardial systolic velocity and mitral annular early diastolic velocity. [116]

Even though it is not possible to find causal relationships in a cross-sectional study, it is possible that similar disease processes affect both the heart and the vessels, in parallel. Long term prospective studies are needed to

determine if there are causal relationships between vascular stiffness and myocardial dysfunction. More studies are also needed to evaluate the relationship between vascular stiffness and subclinical myocardial dysfunction, particularly in type 1 diabetes mellitus.

#### 1.2.8.4 Endothelial dysfunction

100 type 1 diabetic patients underwent assessment of flow mediated dilatation (FMD), carotid intima-media thickness (cIMT), and myocardial function by tissue Doppler imaging. [100] Subjects with type 1 diabetes mellitus had significantly lower FMD and higher cIMT. FMD was also correlated with the number of segments of myocardium showing abnormal function, for both systolic and diastolic dysfunction.

#### 1.2.8.5 Conclusion

Identifying associations or determinants of subclinical myocardial function may be helpful in risk stratification. It is not yet clear which factors determine myocardial function in type 1 diabetes mellitus. Studies showing associations of myocardial function are summarised in table 1.2.

<b>Table 1.2: Summary table of studies showing myocardial function in diabetes mellitus and its associations</b>							
Author	Type	No.	Age (years)	Duration of DM (years)	Methods	Findings	Comment
Shishehbor (2003) [81]	1	25+26	45±11 46±10	26±10	Pulsed Doppler of mitral inflow and tissue Doppler of basal segment were measured together with HbA1c.	IVRT prolonged in DM. Lower E/A ratio. HbA1c was correlated with E/Em.	HR significantly higher in DM. Patients with microalbuminuria was included in the study.
Berg (1999) [117]	1	52	40±13	17±13	Conventional echo and pulsed Doppler. Measurement of Advanced glycation end products. (AGEs) and carboxymethyl lysine (CML).	AGEs were positively correlated with IVRTs and LVD whereas CML were positively correlated with IVRT, LVFS and LVEF.	No control group in the study. A wide range of patients including patients with microvascular complications. Smokers also included.
Didangelos (2003) [118]	1	24 (+DAN) 33(-DAN)	29.8±10.7 40.4±17.3	16.0±6.5 21.2±8.9	Radionuclide ventriculography was done to assess left ventricular function. Diabetic autonomic neuropathy was diagnosed by criteria from ADA and	DAN patients had impaired LV filling pattern such as a reduced peak filling rate.	Significant age difference between two groups. (DAN group older). Microalbuminuria, a recognized complication of microvascular

					American Academy of neurology.		disease and a cardiovascular risk, is present in all patients with DAN and was statistically different to the other group.
Fang (2005) [106]	2	120	54±12 (LVD) 58±10 (no LVD)	9±8 11±8	LV dysfunction identified by using colour tissue Doppler. IHD excluded by either exercise or DSE. Patients were grouped into two depending on the presence of LVD.	LV systolic dysfunction by strain was independently associated with HbA1c level and lack of ACEI treatment where diastolic dysfunction by Em was predicted by age, hypertension, insulin and metformin treatment.	
Taskiran (2004) [119]	1	10+10+10 +/- AN	49±2 45±2 47±1.0	22±3.0 21±4.0	LV diastolic function was assessed by pulse Doppler whereas systolic function by MRI.	Significant decrease in E/A ratio in AN+ compared to controls. DT shorted in AN+ compared to AN- and controls. Greater LVMI in AN+ compared with controls.	
Liomaala (2005) [116]	2	49+15	52.3±5.6 48.3±7.4	1.8	Cardiac function assessed by pulsed	Lower age-adjusted Sm and Em in DM, pwv	Significant differences in age,

					Doppler and tissue Doppler. Vascular wall elasticity by whole-body impedance cardiography.	higher in DM group. Pwv was correlated with age and HbA1c, Age, BMI and pwv correlated with Em.	BMI and blood pressure between the groups.
Seyfeli (2008) [114]	2	57+25	49±6 (DM) 46±7 (ctrl)	82±62 (months)	Tissue Doppler was used to assess myocardial function and aortic distensibility by measuring aortic diameter during systole and diastole.	Aortic distensibility and strain were significantly lower in DM and Aortic distensibility was correlated to septal basal Em/Am ratio.	Age and sex not matched. Patients on antihypertensive were included.
Karamitsos (2008) [103]	1	18+24+21 CAN+, CAN-, ctrl	40±8 38±7 37±10	24±7 21±6	Tissue Doppler imaging was used. CAN was diagnosed by using ADA and American Academy of Neurology statement.	Em, and Em/Am were significantly lower in CAN in comparison with CAN – and controls whereas Am was higher in CAN group.	Patients with microalbuminuria were included in the study.
Karamitsos (2008) [120]	1	66			Patients were grouped according to Em/Am ratio. Em/Am>1 regarded as LV diastolic dysfunction. Aortic stiffness calculated by	Aortic stiffness was increased in LVD group. Was correlated with Em/Am, IVRT and DT.	

					measuring aortic diameter from echo.		
Guo (2009) [121]	2	50+50	48.5±8.6 47.6±8.8		Conventional echo, tissue Doppler and Acoustic densitometry were used.	Em and Em/Am lower in DM. Am and E/Em were higher in DM. IVS-IBS% and LVPW-IBS% were higher in DM. HbA1c was negatively correlated with E/A, Em, Em/Am and positively correlated with E/Em,	
Astrup (2008) [122]	1	136(DM1)	52±9 (normoalbuminuria) 48±9 (diabetic nephropathy)	31±7 34±9	Cine MR	LVMI significantly higher in diabetic nephropathy group, so was NT-proBNP.	Age not matched. Nephropathic group had significantly higher usage of antihypertensive, statins and aspirin.
Yazici (2008) [123]	2	72+50	49.1±9.8 46.1±9.8	5.1±4.2	Conventional echo, colour flow Doppler M-mode and tissue Doppler.	Systolic function was normal. However, diastolic function was impaired in DM. Lower Em, and Em/Am, Higher mitral inflow A. VE was lower and E/VE higher in DM during colour M mode flow propagation.	

						HbA1 related to diastolic dysfunction such as E/A, Em/Am. LV function was correlated with age and diabetes duration.	
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### 1.2.9 Treatment of subclinical myocardial dysfunction in diabetes mellitus

There are no major interventional studies designed to treat subclinical myocardial dysfunction in diabetes mellitus. In one prospective interventional study, 36 subjects with suboptimal glycaemic control (HbA1c > 7%) were followed up for 1 year and HbA1c and echocardiographic parameters were monitored at 6 months and 12 months. [124] There was significant improvement in HbA1c (from  $10.1 \pm 1.6$  % to  $8.1 \pm 1.4$  %,  $P < 0.001$ ). Negative correlations were found between HbA1c level and percentage changes of the peak lengthening rate of LV diameter (+dD/dt), HbA1c level and percentage changes of the peak thinning rate of the LV posterior wall,(dW/dt).

Similarly, in a study by Andersen et al, 20 type 1 diabetes mellitus patients were followed up after starting on insulin pump therapy for a mean period of 45 days. [125] HbA1c improved significantly as well as mean left ventricular strain rate. ( $-1.58 \pm 0.3$  at baseline vs.  $-1.80 \pm 0.4$ ,  $p < 0.05$ ). However, peak systolic velocity was not significantly improved. Von Bibra et al also reported improvement in diastolic velocity with insulin treatment in 25 patients with type 2 diabetes mellitus over 3 week duration. [126] In a cross-over study by Von Bibra et al, 12 patients with type 2 diabetes mellitus were treated with rosiglitazone and glimepiride. Early diastolic tissue velocity E' improved significantly after 16 weeks with rosiglitazone.[127]

The effect of exercise on myocardial function in diabetes mellitus was tested by Loimaala et al. [128] 48 men with type 2 diabetes mellitus were randomized into two groups, exercise group and the conventional treatment alone group and followed up for 1 year. Patients in exercise group received supervised training 4 times a week. After one year, even though HbA1c declined significantly in the exercise group, no significant changes in myocardial function such as early mitral annular Ea, systolic velocity, systolic strain and strain rate were observed. Similarly, Hare et al studied the effect of intensive exercise on myocardial function by randomizing patients with type 2 diabetes mellitus into an intensive exercise programme or usual treatment. Patients were followed up for 3 years and at the end of the study; no difference in myocardial function between the two groups was noted. [129] In a similar study involving 223 type 2 diabetes mellitus patients, no difference in myocardial function was noted in the intensive exercise group after 1 year. [130] It was hypothesized that exercise activity might not be rigorous enough. Incomplete follow-up was another issue with the exercise intervention study.

The effect of controlling postprandial glucose on diastolic function was studied in 61 patients with type 2 diabetes mellitus by Von Bibra et al; patients were randomized into three groups of mixed insulin two times a day, basal bolus regime or bolus insulin treatment alone. Postprandial glucose was higher in the conventional treatment group and it was associated with lower E'. [131]

Treatment with ramipril was also reported to improve E' in 16 patients with type 2 diabetes mellitus when compared with those who did not have treatment with ramipril after 9 month follow up. [132]

In a retrospective echocardiographic study of 430 patients, who had coronary artery disease but who were not known to have heart failure, the use of statins was associated with favourable myocardial function such as higher  $E'$ , lower mitral inflow  $E$  velocity and lower  $E/e'$  ratio. The study involved 121 patients with diabetes mellitus.

There have been many small studies with a short duration of treatment showing improvement in subclinical myocardial function with various treatment but not exercise. Those studies are summarised in table 1.3. Whether these results can be translated into long term prevention of heart failure is debatable. Treatment with rosiglitazone by Von Bibra [127] is a good example, as rosiglitazone now has now been clearly shown to increase the risk of heart failure in patients with type 2 diabetes mellitus.[133] [134] Therefore, larger trials with longer follow-up are needed to determine the effects of interventional treatment such as improved glycaemic control on the progression of subclinical disease into clinical heart failure in diabetes mellitus.

<b>Table 1.3: Summary table showing treatment of subclinical myocardial dysfunction in diabetes mellitus</b>							
Author	Type	No.	Age (years)	Duration of DM (years)	Methods	Findings	Comments
Grandi (2006) [124]	1	36	36±10	10.8±5.6	36 patients with T1DM followed up for 12 months. Assessed at 6 months and 12 months after insulin therapy was modified for better glycaemic control. Cardiac function evaluated at each visit by echocardiography. Peak lengthening and shortening rates of LV diameter and peak thinning rate of LV posterior wall were also measured.	Peak lengthening rate, peak wall thinning rate and E/A ratio improved after 6 months. Improvement correlates with HbA1c level. No further improvement at 12 months.	9 patients dropped out between 6 month and 12 month.
Andersen (2007) [125]	1	19	22-65	8-43.	19 patients with poorly controlled DM1 were followed up after initiation of insulin pump. Followed up by measuring SR after 35-50 days.	Mean LV SR improved so did HbA1c. Changes in SR correlates with changes in HbA1c.	Wide age range for relatively small number of patients. Variable follow up period.
Loimaala (2007) [128]	2	24+24	52.8 ±6.0 52.8 ±5.2	<3	48 newly onset DM2 were randomized into two groups, conventional treatment without exercise and with supervised exercise 4 times/week. Followed up for	No significant improvement in mitral flow velocities, septal strain and strain rate.	

					12 months with echo and tissue Doppler.		
Von Bibra (2004) [126]	2	25	$60 \pm 9$	$8 \pm 7$	Either insulin was treated or oral hypoglycaemic therapy intensified. Followed up for 0.7 month.	Fasting capillary glucose level fell and diastolic velocity improved in insulin treatment group.	Very short duration. Only capillary glucose monitored and self-reported.
Hare (2011) [129]	2	186 (92+94)	$55 \pm 10$	$6.3 \pm 6.5$	223 patients were randomized into 2 groups, intensive exercise and usual treatment. 186 patients remained at the end of 3 years and analysed.	No difference between the intensive exercise group and usual treatment group.	3 year follow up
Hordern 2009 [130]	2	176	$56.1 \pm 11.7$	Not available	176 patients randomized into intensive exercise and usual care.	No difference in myocardial function after 1 year.	
Von Bibra (2008) [127]	2	12	$59 \pm 12$	3	Cross over study with 12 patients treated with glimepiride or rosiglitazone.	E' improves with rosiglitazone.	
Siegmund (2007) [132]	2	12	$52 \pm 8$	$9 \pm 6$	Type 2 diabetes mellitus patients on insulin were treated with ramipril and compared with 8 matched controls.	E' improve after 9 month follow up.	

### 1.2.10 Conclusion

There are some epidemiological, clinical and histological data to support a view that heart muscle disease exists in diabetes mellitus. However, it remains elusive regarding its natural history, aetiology and determinants of myocardial function. Radial function and myocardial functional reserve are fairly untested in type 1 diabetes mellitus. Moreover, the precise link between myocardial dysfunction and vascular disease, vascular stiffness in particular, remains elusive.

## ***1.3 Non-invasive assessment of vascular structure and function in diabetes mellitus***

### 1.3.1 Carotid intima media thickness (cIMT)

The arterial wall is made up of three layers: namely intima, media and adventitia. The intima is composed of a single continuous layer of endothelial cells. It is supported by a layer of smooth muscle cells. The medial layer consists of elastic and collagen fibres mixed with smooth muscle cells and is responsible for the elastic properties of a medium size artery. The adventitia is mainly made of fibroblasts and collagen.

Details of delineation the structure of the arterial wall can be visualized by using high resolution B mode ultrasound. Carotid intima media thickness (cIMT) can be non-invasively measured by high resolution B mode ultrasound, as demonstrated firstly by Pignoli et al in 1986. [135] Carotid IMT as measured by B mode ultrasound is regarded as a surrogate marker of

atherosclerosis. [136] Measurement of cIMT is highly reproducible [137] and it has been used as a surrogate endpoint in many interventional studies.

Carotid intima media thickness (cIMT) is the measurement between the leading edge of the lumen-intima interface to media adventitia interface. [138] cIMT can be measured on common carotid artery and is usually measured starting from 1 cm proximal to the carotid bulb. [139] It is commonly measured on the far wall of the common carotid artery. Even though reproducibility is more or less the same for the near wall and the far wall, far wall intima media thickness is more reflective of true intima media thickness from autopsy studies. [140, 141]

cIMT reflects the burden of atherosclerosis. Increased cIMT correlates with an increased absolute risk of cardiovascular disease. [142] [143] [142] Several prospective studies have shown that increased cIMT correlates with the risk of ischemic heart disease and cerebrovascular disease. [144, 145]

Carotid intima media thickness was found to be higher in young patients with type 1 diabetes mellitus and seems to be associated with higher cumulative doses of insulin. [146]

cIMT is also found to be associated with higher left ventricular mass. There is an argument that cIMT is a mere reflection of the shear forces of the vessel wall.

cIMT adjusted for age in children with type 1 diabetes mellitus was demonstrated to be thicker than controls by Krantz et al. [147] In the same study, it was also shown that subjects with diabetic complications such as microalbuminuria, hypertension or retinopathy had thicker cIMT. However,

the control group only had cIMT measurement and therefore it is not possible to know that whether the control group's BMI and other parameters were matched with the study group. Similar findings were reported by Jarvislo et al who studied 40 type 1 diabetes mellitus children and 30 age matched controls and demonstrated that cIMT was higher in type 1 diabetes mellitus. Flow mediated dilatation (FMD), a marker of endothelial function, was lower in type 1 diabetes mellitus. Those with lower FMD among type 1 diabetes mellitus have higher cIMT and LDL cholesterol levels. Atabek et al [148] reported similar findings of increased cIMT in children with type 1 diabetes mellitus. Age, duration of diabetes, BMI, waist hip ratio, systolic blood pressure and triglycerides were significantly associated with cIMT on Pearson correlation. Systolic blood pressures, duration of diabetes and waist hip ratio are independently associated with cIMT on multiple regression analysis.

Patients with type 1 diabetes mellitus of longer duration were studied by Distiller et al. [149] The mean age of the patients was 48 years and the median duration of diabetes mellitus was 26 years. On multiple regression analysis, age, duration of diabetes, hypertension and BMI were positively associated with cIMT whereas HDL cholesterol was negatively associated. On logistic analysis, age, hypertension, smoking status and retinopathy appeared to be associated with presence of plaque. HbA1c did not show any associations with cIMT in this study.

Not all the studies for cIMT in type 1 diabetes mellitus were positive. Studies by Yavuz et al [150] and Gunczler et al [151] did not show

differences in cIMT between subjects with type 1 diabetes mellitus and controls.

In a systematic review of 23 studies, which included 4019 type 2 diabetes patients and 1110 with impaired glucose tolerance, Brohall et al [152] found that cIMT was greater in diabetes in 20 out of 21 studies. They concluded that type 2 diabetes mellitus was associated with a 0.13 mm increase in cIMT compared with control subjects. cIMT progresses faster in type 2 diabetes mellitus. It progresses with time [153] and may halt progressing [154] or even regress with treatment. [155] In the DCCT/EDIC study, intensive treatment slowed down the rate of cIMT progression; the effect was sustained even 12 years after stopping intensive treatment, although the effect was attenuated after 6 years. [156]

Meta-analysis of many studies which used cIMT as an end-point failed to prove that those changes in cIMT, either progression or regression, relate to cardiovascular events. [157] [158] Whether the use of cIMT in diabetes mellitus could increase predictive value in risk stratification to an established scoring system such as Framingham Risk Score remains controversial, as results were conflicting. Currently no data are available if increased cIMT relates to subclinical myocardial dysfunction in type 1 diabetes mellitus. A summary of studies assessing cIMT in patients with diabetes mellitus is shown in table 1.4.

<b>Author</b>	<b>Type of diabetes</b>	<b>No.</b>	<b>Age (years)</b>	<b>Duration (years)</b>	<b>Method</b>	<b>Findings</b>	<b>Comment</b>
Yamasaki (1994) [159]	1+2	105-DM1 529-DM2 104-controls	4-25 31-86 7-76	0.5-17 0.5-49	Mean cIMT	cIMT significantly increase in DM1 but not in DM2 in comparison with age matched control. Duration of DM and age are associated with cIMT.	
Krantz (2004) [147]	1	142+87	16±2.6 18.8±3.1	6.6 ± 0.5 in male 7.9 ± 0.5 in female	Mean cIMT	Age adjusted IMT higher in DM1. Among DM1, cIMT was higher in those with complications.	Not age-matched. Controls older than DM1. No other parameters apart from cIMT were collected form control group.
Jarvislo ( 2004 ) [14]	1	45+30	11±2	4.4 ± 2.9	FMD and mean and maximum cIMT	Both mean and maximum cIMT higher in DM1. FMD response was lower in	
Atabek (2006) [148]	1	45+33	14.8±2.5 14.1±2.1	4.4±2.5	Mean cIMT	Increase IMT in DM1 and correlates with duration of DM	
Distiller (2006) [149]	1	148	19-76 Median-48	18-59 Median- 26	Mean cIMT was measured. Analysis done on	Median IMT 0.66 mm. Age, duration of DM, BMI and hypertension	No control group included. Wide range of patient with

					several models, depending on presence of plaque, actual IMT thickness and risk based on IMT thickness.	appear to be major risk for increase IMT in DM1.	different complications.
Gunczler (2002) [151]	1	20+20	11.9±3.6	3.4±3.3	IMT on both carotid arteries measured. Cardiac function assessed by echocardiography.	No difference in IMT or cardiac function noted. However, LDL was higher in DM1.	

### 1.3.2 Characteristics of the vascular tree and their impact on stiffness

The elastic properties of arteries vary along the vascular tree. The more proximal along the arterial tree the more elastic the vessel is and the more distal the stiffer the artery is. This heterogeneity has important physiological consequences. As a pressure wave propagates along the vascular tree it is augmented by reflected waves coming back from reflection sites such as bifurcation of arteries. This phenomenon is called “ amplification phenomenon” [160] Therefore, brachial arterial pressure may sometimes be higher than central arterial pressure. The use of brachial pulse pressure instead of central pulse pressure to calculate regional arterial stiffness may not be accurate.

### 1.3.3 Arterial stiffness

Arterial stiffness is a dynamic component of arterial performance and it is not uniform throughout the arterial tree. [161] Arterial stiffness can be assessed as local, regional and systemic stiffness. Local and regional stiffness can be assessed directly and non-invasively.

### 1.3.4 Pulse wave velocity (PWV)

Pulse wave velocity is the most simple and reproducible method to assess regional arterial stiffness. Carotid femoral PWV is considered as the gold standard measurement for arterial stiffness. It is usually measured using foot to foot velocity method using various waveforms at different sites. It can be obtained transcutaneously at the right carotid artery and right femoral artery

and the time delay measured at the feet of two wave forms. [160] The major limitation factor for the measurement of carotid femoral PWV is the inaccuracy of the distance measured. The prognostic value of aortic PWV in the general population was assessed in a prospective study by Willum-Hansen et al. [162] One thousand six hundred and seventy eight (1678) subjects between 40 and 70 years old were followed up for a median of 9.4 years. Even after adjustment for age, sex, body weight, blood pressure and smoking status, aortic PWV was proved to have prognostic significance. With one standard deviation increase in PWV i.e. 3.4m/s, the risk of cardiovascular events increased by 16 to 20%.

Increased pwv was also found to be associated with increased mortality in patients with diabetes mellitus. [163] Arterial stiffness measured by systemic arterial compliance, carotid femoral transit time and aortic augmentation index was increased in type 2 diabetes mellitus and subjects with impaired glucose tolerance. [164]

### 1.3.5 Other indices of local arterial stiffness

Direct measurement of arterial stiffness requires simultaneous measurements of changes in arterial diameter and pressure at the same site. Diameter changes can be accurately measured by using a wall tracking technique with high resolution ultrasound. However, the estimation of pressure changes could be difficult because of the amplification of pulse pressure and the inaccuracy of sphygmomanometer systems. [165] Indices of local stiffness are listed in table 1.5.

**Table 1.5: Indices of local arterial stiffness (adapted from O' Rourke et al) [166]**

<b>Index</b>	<b>Definition</b>	<b>Formula</b>
Arterial distensibility	Relative diameter ( or area ) change for a pressure increment	$( \Delta D / \Delta P \times D ) ( \text{mmHg}^{-1} )$
Arterial compliance	Absolute diameter ( or area ) change for a given pressure step at fixed vessel length	$\Delta D / \Delta P ( \text{cm} / \text{mmHg} )$ or $( \text{cm}^2 / \text{mmHg} )$
Elastic modulus	The pressure step required for ( theoretical ) 100% stretch from resting diameter at fixed vessel length	$( \Delta P \times D / \Delta D ) \text{mmHg}$
Young Modulus	The pressure step per square centimetre required for ( theoretical ) 100% stretch from resting length	$\Delta P \times D / ( \Delta D \times h ) ( \text{mmHg} / \text{cm} )$
Stiffness index $\beta$	Ratio of logarithm ( systolic/diastolic pressures) to relative change in diameter	$\text{Ln} ( P_s / P_d ) / \{ ( D_s - D_d ) / D_d \}$  ( non- dimensional)

### 1.3.6 Central pulse wave analysis

When the heart ejects, a forward running pressure wave is generated, which is reflected in the periphery and returns as a backward running wave towards the heart. The pressure measured at a point consists of the superposition of these forward and backward pressure waves. [167] When the artery stiffens with increasing age or disease, the waves travels faster. As a consequence, the reflected wave add onto the forward running wave in early systole , rather than in late systole or diastole as it happens in young subjects. This changes the morphology of central pressure wave form from so called C type in young subjects to A type in older subjects. This augmented pressure wave increases systolic pressure and thus increases the load on the heart (afterload and heart work).

Quantification of central pressure wave forms might provide more useful measures than peripheral pressure. This wave reflection is commonly expressed as the “ augmentation index”. Augmentation index is defined as the ratio of augmented pressure, attributed to the reflected wave to the amplitude of the pulse (pulse pressure).

$$AIx = 100 \times AP/PP$$

Assessment of augmentation index requires measurement of pressure wave morphology. Augmentation as a relative measure, can be derived from non-calibrated pressure tracings. [167] However, the morphology of the pressure wave needs to be valid. The identification of the inflection point where the reflected wave adds on to the forward wave is critical in calculating

augmentation index. In C type waveform, inflected waves arrive late in systole, therefore inflection point occurs after the pressure has reached systolic pressure. In these cases, augmented pressure and augmentation index are negative. In A type wave form, the inflected point occurs before systolic pressure, Therefore, augmented pressure is positive and so is augmentation index. However, in waveforms between A and C, inflection point may occur every close to or buried by the systolic peak. Visual identification of inflection point is prone to error. The most well-known method was the one described by Takazwa et al based on 4<sup>th</sup> order derivatives. The exact procedure for determining the inflection point in commercially available systems like Sphygmocor is not disclosed.

The major disadvantage of augmentation index is that it is a composite measure depending not only on the magnitude of wave reflection but also on other parameters such as subject's height, heart rate and aortic stiffness.

Lacy et al [168] studied carotid femoral PWV and augmentation index in 66 subjects with diabetes mellitus ( 25 type 1 diabetes mellitus) and in 66 controls. Despite finding a higher PWV and pulse pressure, an increase in augmentation index was not observed in the diabetic population. It was suggested that pulse wave analysis may not be as reliable as PWV in diabetic population to assess arterial stiffness. Moreover, Wilhelm et al compared augmentation index among patients with cardiovascular disease, patients with diabetes mellitus and healthy controls. [169] Even though augmentation index was higher in diabetic subjects, it did not prove to be statistically significant.

On the other hand, Haller et al [170] demonstrated that young children ( 10 -18 years) with type 1 diabetes mellitus had increased augmentation index in comparison with age matched controls. Similarly, Wilkinson et al [171] proved that augmentation index was increased in type 1 diabetes mellitus. 35 patients with type 1 diabetes mellitus of long duration (a minimum of 10 years ) were studied together with age matched controls. Augmentation index was significantly higher in the diabetic group.

### 1.3.7 Endothelial function

Endothelial dysfunction is considered to be a key early event in the atherosclerotic process. Assessment of endothelial function may help clinicians identify and stratify subjects at risk of vascular disease. Endothelial function may be assessed by non-invasive methods such as flow mediated dilatation (FMD). Flow mediated dilatation (FMD) measures the change in diameter of the brachial artery in response to shear stress induced by increased flow after the release a pressure cuff applied around the forearm for several minutes. It measures the response of vascular smooth muscle to nitric oxide by released endothelial cells. (i.e. endothelial dependent endothelial relaxation). FMD has been demonstrated to predict cardiovascular events in subjects with peripheral vascular disease in a prospective study. [172]

### 1.3.8 Wave intensity

The confluence of waves in the arterial circulation results in simultaneous changes in pressure and flow. Wave intensity (WI) is defined as the product of the time derivatives of the measured pressure ( $dP/dt$ ) and velocity ( $dU/dt$ ) spatially averaged across the vessel diameter. [173]

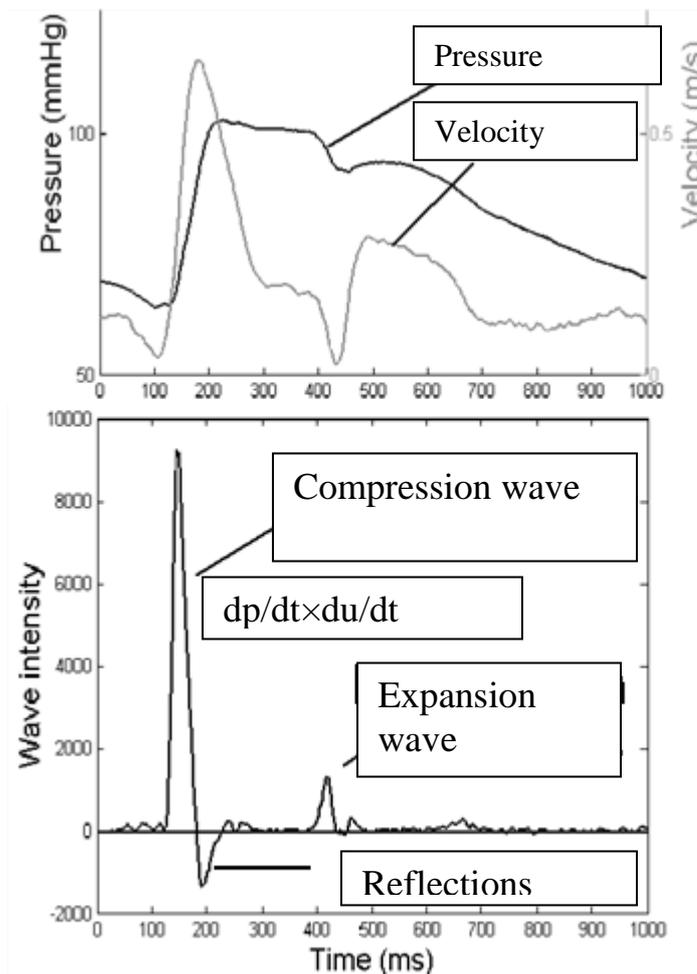
$$WI = (dP/dt) \times (dU/dt)$$

WI provides information regarding the dynamic action of the heart and blood vessels as well as their interaction. To measure WI, pressure can be obtained either invasively or non-invasively. Harada et al described a method where WI could be estimated non-invasively from a carotid artery by echo-tracking method. [174] With non-invasive measurement, local pressure wave forms can be estimated from changes in the arterial diameter and velocity can be acquired by colour flow Doppler from the same site. It is feasible to derive pressure wave from diameter change because diameter change was shown to be linearly related to changes in pressure. [175] Therefore, diameter can be calibrated for pressure using this non-invasive method.

The methods used in most clinical studies provide net WI. Studies in the aorta have shown three major components (Figure 1.1) [176] ; the first peak is due to the net forward compression wave in early systole resulting from left ventricular contraction. It is followed by net negative wave intensity, the backward compression wave, in midsystole secondary to wave reflections from reflection sites. The third wave intensity peak is the forward expansion

wave which starts in the late systole and coincides with ventricular relaxation, slows down the blood flow and reduces pressure [177].

**Figure 1.1: PWI output (Aloka 5500) – The relationship of pressure and velocity shown in the upper graph and the PWI output shown in the lower graph**



Separated forwards and backwards travelling waves can be estimated. Local wave speed is calculated from the slope of the pressure-velocity loop in early systole and within this measurement the separated waves can be calculated using an application of the water hammer equation. The

individual wave separation could provide more insight in the interaction between the left ventricle and the arterial system i.e. ventriculo-arterial coupling. This method of wave separation has been described by Rakebrandt et al, using customised software. [178]

The wave intensity method using carotid arterial wall tracking is non-invasive and does not require any assumption from models of circulation. [160] However, in this method blood pressure is normally measured in brachial artery and blood pressure and changes in arterial diameter are not measured simultaneously. The clinical utility of the WI method has been used in some clinical and epidemiological studies for their potential clinical use. Laio et al studied the link between carotid artery stiffness and the development of hypertension. [179] In the Bogalusa Heart study, increased pressure-strain modulus  $\epsilon$  was associated with concentric left ventricular hypertrophy. [180] In the SMART study (Second Manifestation of ARterial disease) carotid stiffness was associated with cardiovascular events in those patients who had had vascular disease before [181]. However, after adjustment for age, the relationship did not exist anymore.

Increased  $\beta$  and  $\epsilon$  were related to impaired longitudinal myocardial function. [182] Because of the information provided on wave separation, WI may be a useful method in assessing ventriculo-arterial coupling and may be a useful tool in predicting subclinical myocardial dysfunction.

### 1.3.8 Ventriculo-arterial coupling

Arterial stiffness should be considered together with cardiac function.

Ventricular-arterial coupling, the interaction between the heart with the

systemic vasculature is a key determinant for cardiovascular performance. [183] Both the left ventricle and arterial system could be considered as elastic chambers with known volume elastances. [165] Under normal circumstances, the left ventricle and arterial system couple optimally resulting in maximal cardiac stroke work and cardiac metabolic efficiency. However, in heart failure, due to left ventricular systolic dysfunction, ventricular-arterial coupling is impaired and pump dysfunction leads to increased impedance and decreased compliance in arterial system. Moreover, there is some evidence that ventricular-arterial coupling may impair before significant pump failure. [184] In an animal model of type 2 diabetic mice, it was reported that although mechanical efficiency and myocardial contractility were impaired, cardiac output was maintained by increased preload and decreased afterload.

#### ***1.4 Chapter Conclusion***

From this review of the literature, relating to myocardial and vascular function particularly in type 1 diabetes mellitus, several questions remain. In particular it remains unclear whether left ventricular dysfunction in early stages of myocardial dysfunction in type 1 diabetes mellitus is mainly diastolic or not; if there is any evidence of reduced myocardial functional reserve in type 1 diabetes mellitus which may suggest small vessel disease; any role of “radial compensation” in the presence of longitudinal dysfunction. Moreover, there is some evidence to suggest early atherosclerosis and conduit artery stiffness in type 1 diabetes mellitus. Nevertheless, their association with myocardial function has not been fully explored.

## **CHAPTER 2- HYPOTHESES AND OBJECTIVES**

### ***2.1 Hypotheses***

**This study addresses the following hypotheses:**

- Young subjects with type 1 diabetes mellitus have reduced myocardial function, both systolic and diastolic.
- Young subjects with type 1 diabetes mellitus have reduced myocardial functional reserve compared to controls.
- Young subjects with type 1 diabetes mellitus have increased conduit arterial stiffness and early changes of atherosclerosis such as increased cIMT.
- Changes in myocardial function and vascular function are correlated in type 1 diabetes mellitus.

### ***2.2 Study Objectives***

**The specific research objectives are:**

- To quantify regional myocardial function in young subjects with type 1 diabetes mellitus, both at rest and during dobutamine stress.
- To assess the myocardial functional reserve in young subjects with type 1 diabetes mellitus in response to dobutamine stress.
- To perform a comprehensive survey of large artery function.
- To compare subclinical myocardial and vascular function in young subjects with type 1 diabetes mellitus.

## **CHAPTER 3- METHODS**

### ***3.1 Introduction***

A study was conducted at the Wales Heart Research Institute to determine myocardial and vascular function in young subjects with type 1 diabetes mellitus.

### ***3.2 Subjects and recruitment***

Patients aged less than 30 years with type 1 diabetes mellitus were recruited from diabetes clinics at Cardiff and Vale University Health Board.

Inclusion criteria and exclusion criteria for the study subjects are as follow:

#### Inclusion Criteria

- (1) Subjects with type 1 diabetes mellitus aged 16-30 years
- (or)
- (2) Healthy subjects aged 16-30years

#### Exclusion Criteria

- (1) Known cardiovascular diseases: cerebrovascular event, peripheral vascular disease
- (2) Heart failure or documented cardiomyopathy of any cause
- (3) Pregnancy, risk of pregnancy, or current breastfeeding
- (4) Type 2 diabetes mellitus
- (5) Uncontrolled medical conditions such as thyrotoxicosis which may potentially affect cardiovascular and haemodynamic status.

(6) Renal failure ( Creatinine >150 $\mu$ mol/l or on dialysis)

From 74 eligible patients with type 1 diabetes mellitus who were approached, 19 agreed to participate in the study. 40 healthy volunteers were recruited by advertisement in Cardiff and Vale University Health Board hospitals. The recruitment is summarised in figure 1.

All subjects gave written informed consent. The study was approved by the South East Wales Research Ethics Committee and was compliant with the Declaration of Helsinki.

A detailed history was taken regarding diabetes duration and treatment. Case notes were reviewed and previous annual review results were reviewed for history of retinopathy and microalbuminuria. Detailed exercise history including type, hours per day and frequency was obtained from all the participants. From the 40 healthy volunteers who participated, 21 had dobutamine stress echocardiography and vascular studies. Among the rest, 20 had baseline echocardiographic studies, and 13 had vascular studies; 7 volunteers failed to attend for follow up vascular studies.

19 subjects with type 1 diabetes mellitus participated in the study. All but one of them had dobutamine stress echocardiography and vascular studies. One subject opted only to have baseline echocardiography and vascular studies. None of the subjects were current or ex-smokers and they were not taking any other medications apart from insulin.

### ***3.3 Study Endpoints***

*Primary endpoints of the study are:*

Myocardial functional reserve and longitudinal myocardial function

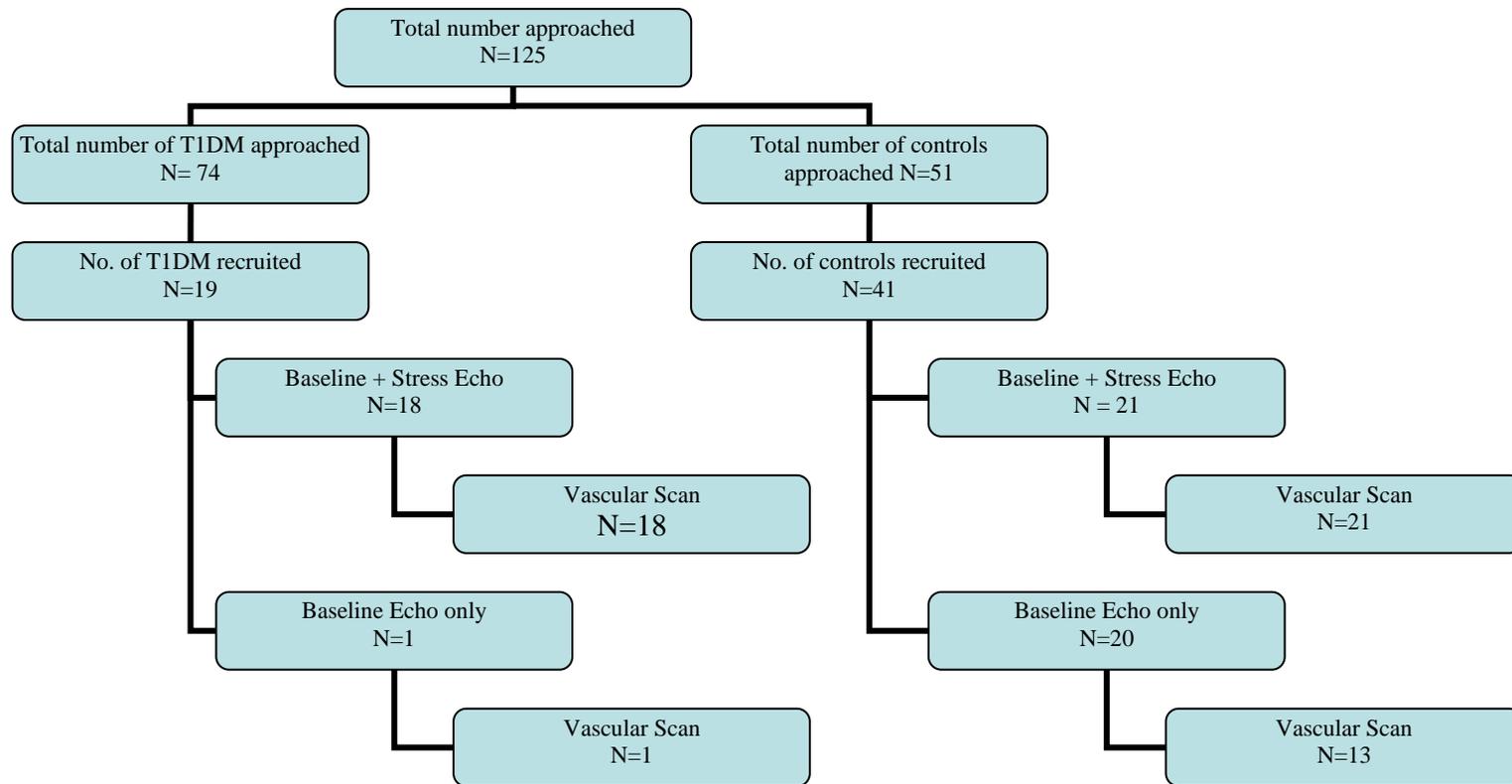
*Secondary endpoints of the study are:*

Augmentation index, pulse wave velocity, stiffness index  $\beta$ , Peterson's elastic modulus  $\epsilon$ , carotid intima media thickness (cIMT).

### ***3.4 Study Sample size calculation***

Sample size estimation was based on the previous study of type 2 diabetes mellitus for myocardial functional reserve by Vinereanu et. al. [85] The paper reports a mean myocardial functional reserve of 5.4 cm/s, SD 2.0 cm/s in type 2 diabetes mellitus compared to mean 7.7 cm/s, SD 1.7 cm/s in controls. Assuming an SD of 2.0 cm/s for these within-subjects changes, a study comparing 25 cases v. 25 controls would be sensitive to detect a difference of 1.6 cm/s or greater between cases and controls with power 80% using a test at the conventional two-sided 5% level.

**Figure 3.1: Flow diagram of study participants and assessment**



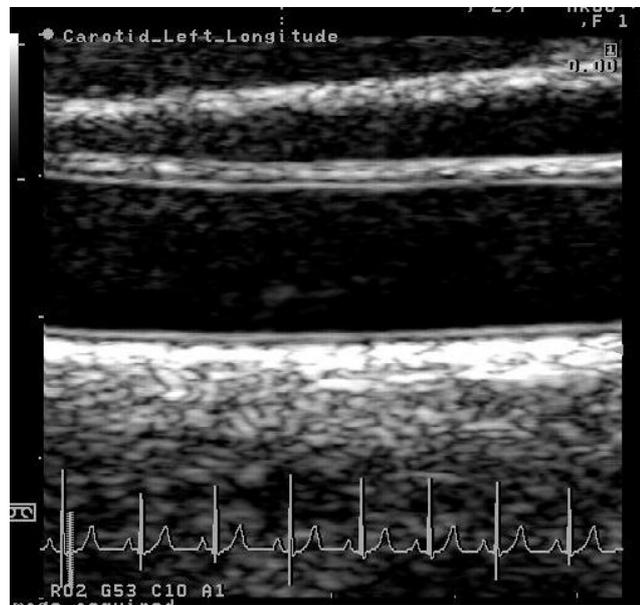
### ***3.5 Carotid intima media thickness measurement***

On visit 1 of the study, subjects came in the early morning after fasting for 10 hours. Subjects were asked to abstain from smoking and caffeine for 24 hours before the visit. Diabetic subjects were also asked not to have morning insulin and breakfast until after the scan. After arrival, subjects rested for 20 minutes in a quiet and temperature controlled environment.

*Carotid intima media thickness (cIMT) and stiffness parameters ( $\beta$  and  $\epsilon$ ) measurement:* Subjects underwent a B mode ultrasound scan of the carotid arteries on both sides of the neck together with wave intensity analysis on both sides, using an Aloka 5500 ultrasound machine and a 7.5 MHz linear array transducer, with the subject lying supine and with the neck slightly extended and turned towards the side away from the site of scanning. The common carotid artery was scanned anteriorly and longitudinally. The gain control was adjusted to obtain a clear delineation of intima, media and adventitia layers of the far wall of the common carotid artery. The images were recorded and stored on the hard drive of the Aloka machine, synchronizing with ECG.

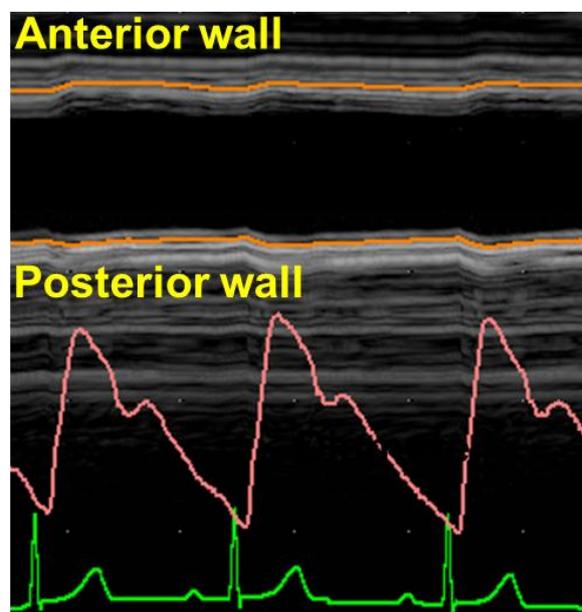
cIMT was measured on the far wall of the common carotid arteries on both sides of the neck at 1 cm below the carotid bulb. Images were also analysed off-line for cIMT using Vascular Image Analysis software. A region of interest (ROI) was placed below 1 cm of the carotid bulb manually. The ROI was placed on the best delineated region of the common carotid artery covering a minimum of 1 cm length. Intima and media were marked automatically by the software respectively with I and M line. Carotid intima media thickness was measured by the software and an average value was taken from both sides.

**Figure 3.2: B Mode ultrasound image showing carotid intima-media interface.**



### ***3.6 Wave Intensity Methods***

**Figure 3.3: Ultrasound image showing automated wall tracking of the media-adventitia boundaries of the near and far walls of the carotid artery.**



After carotid intima media thickness measurement, wave intensity measurement was carried out with the patient lying. The wave intensity measurement was also performed in the fasting state. Pulse and blood pressure were measured on the right arm. None of the subjects

were on any antihypertensive or beta blockers or calcium channel blockers or known to have diabetes.

Subjects were supine on an examination bed and the carotid arteries were scanned on both sides of the neck in the anterior triangle. The neck was slightly flexed and turned to the opposite side of scanning. The Aloka 5500 machine was used to scan carotid arteries in pulse wave intensity (PWI) mode. Blood pressure was entered manually together with the patient's height, weight and age.

The carotid arteries within 2 cm from carotid bulbs were scanned on both sides of the neck, using 7.5 MHz probe. The junction of the carotid media and adventitia was identified and adjustable gates were placed at the junction on both anterior and posterior walls of the common carotid artery. Changes in diameter were calculated automatically from beat to beat. The difference between the displacement waveforms of the anterior and posterior walls of the artery was displayed in real time. At the same site, and independently steerable ultrasound beam was positioned to obtain the mean velocity of flow at the same site. An average pressure, diameter and wave intensity were obtained over 20 beats and analysed off-line for stiffness parameter  $\beta$  and  $\epsilon$ . The values were measured on both sides of the neck and averaged.

Stiffness parameter beta ( $\beta$ ) was calculated by the formula:

$$\beta = \ln \{ (Ps/Pd) / [(Ds-Dd)/Dd] \}$$

Peterson's elastic modulus strain  $\epsilon$  was calculated by using the formula:

$$\epsilon = [ (Ps-Pd) / (Ds-Dd) ] \times Dd,$$

where Ps and Pd are systolic and diastolic pressures respectively, and Ds and Dd are maximal and minimal diameters of the arteries as measured by wall tracking method at carotid intima media junction on carotid arteries.

Wave intensity (WI) is the product of instantaneous changes in velocity and diameter.

WI can be calculated by the formula:

$$WI = (dP/dt) \times (dU/dt)$$

where dP = derivatives of pressure change and dU= derivatives of velocity in change.

### ***3.7 Pulse wave analysis methods***

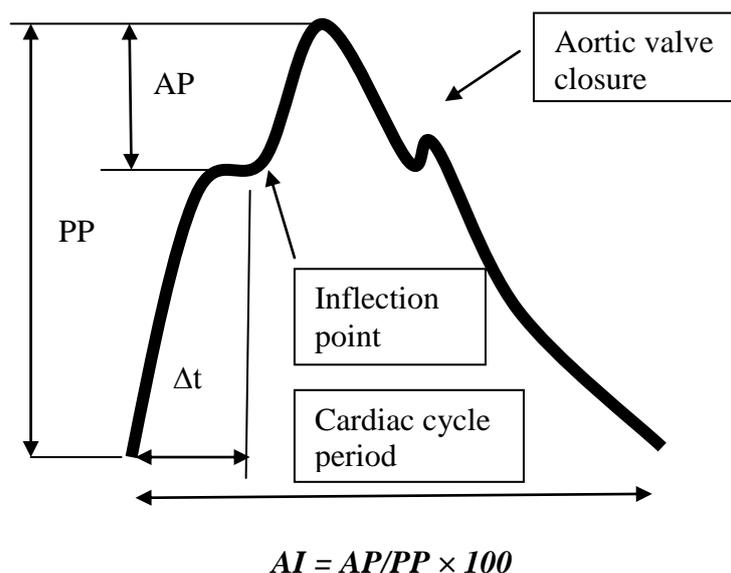
**Figure 3.4: Pulse wave analysis on right radial artery with a tonometer**



Pulse wave analysis was performed by using tonometry on the right radial artery with a Sphygmocor machine. Subjects' details and blood pressure were manually entered into the software. A high-fidelity micromanometer was used by placing the tip of the manometer on the right radial artery, perpendicular to the artery ensuring the probe flattening the artery but not occluding it. The data were transferred to a portable computer. The computer screen displayed waveforms so that the operator could recognize if the correct wave forms were obtained or not. 20 consecutive waves were obtained. The software generated peripheral and the central wave forms. Augmentation index was calculated by the software from central

wave form. Augmentation index is the difference between the first and second peaks of the central arterial wave expressed as a percentage of the pulse pressure.

**Figure 3.5: Schematic representation arterial pulse wave**



*AP = augmentation pressure, PP = pulse pressure, AI = augmentation index and  $\Delta t$  = reflection wave transit time*

Carotid-femoral pulse wave velocity was also obtained with tonometry. The distance from the sternal notch to the measurement site on the right carotid artery was measured in millimetres, using a measuring tape. The distance was entered into the computer system. Similarly, the distance from the sternal notch to the femoral artery was also measured in millimetres and entered into the system. 3 lead ECG monitor was attached to the patient and the computer system.

Arterial waveforms were obtained sequentially from the right common carotid artery and the right femoral artery. Wave transit time was calculated by the software, using the R

wave from the simultaneously recorded ECG as a reference frame. The software calculated the carotid femoral pulse wave velocity using the surface distances entered and the transit time.

### ***3.8 Blood Tests***

After the vascular studies, fasting blood samples were taken for full blood count, urea, creatinine and electrolytes, fasting glucose, lipid profiles, liver function test, high sensitivity CRP, and glycated haemoglobin (HbA1c). Serum samples were analysed at the Biochemistry Laboratory at the University Hospital of Wales, Cardiff.

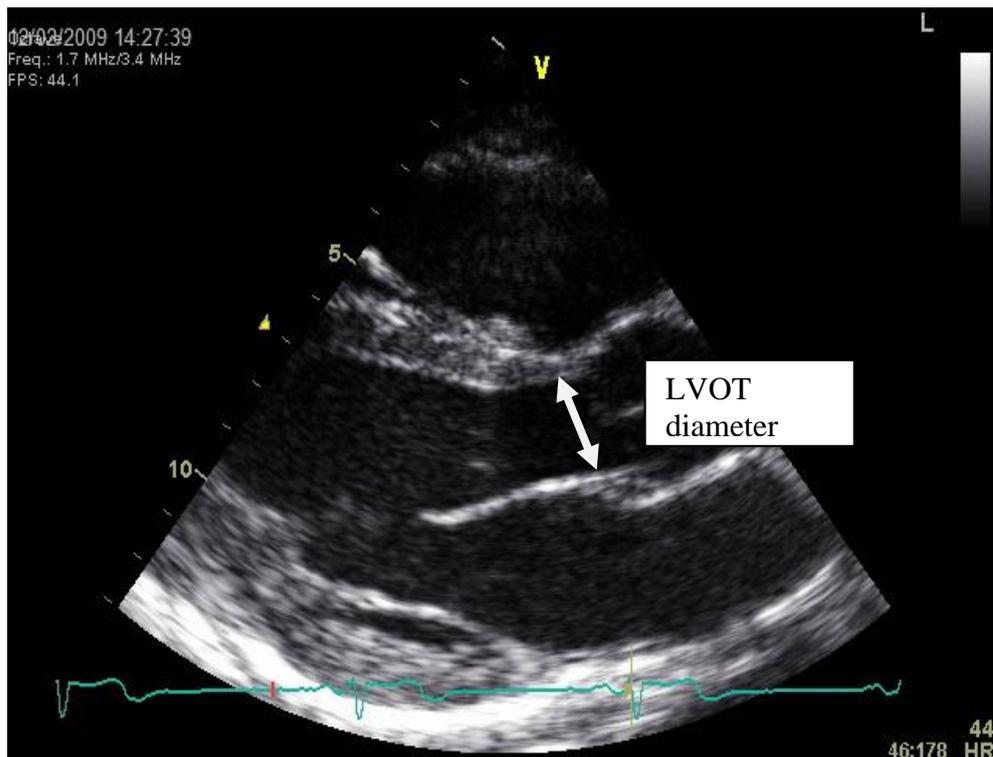
### ***3.9 Conventional Echocardiography Methods***

On Day 7, subjects returned to have echocardiography. Diabetic subjects were asked to have a light meal and the usual dose of insulin. Controls were also asked to have a light meal. A detailed baseline study was performed with the subject lying in a left lateral position, using a commercially available echocardiography machine (Vivid 7, GE Medical Systems, Horten, Norway) with a 2.5-MHz phased-array transducer.

Images were recorded in apical four-chamber, two-chamber, and five-chamber, and parasternal long-axis and short-axis, views. All imaging was performed by either of two experienced cardiologists who were blinded to whether the subjects were diabetic patients or controls. All analysis of baseline grey-scale echocardiographic images and myocardial velocity imaging were performed by myself, also without knowledge of the status of each subject.

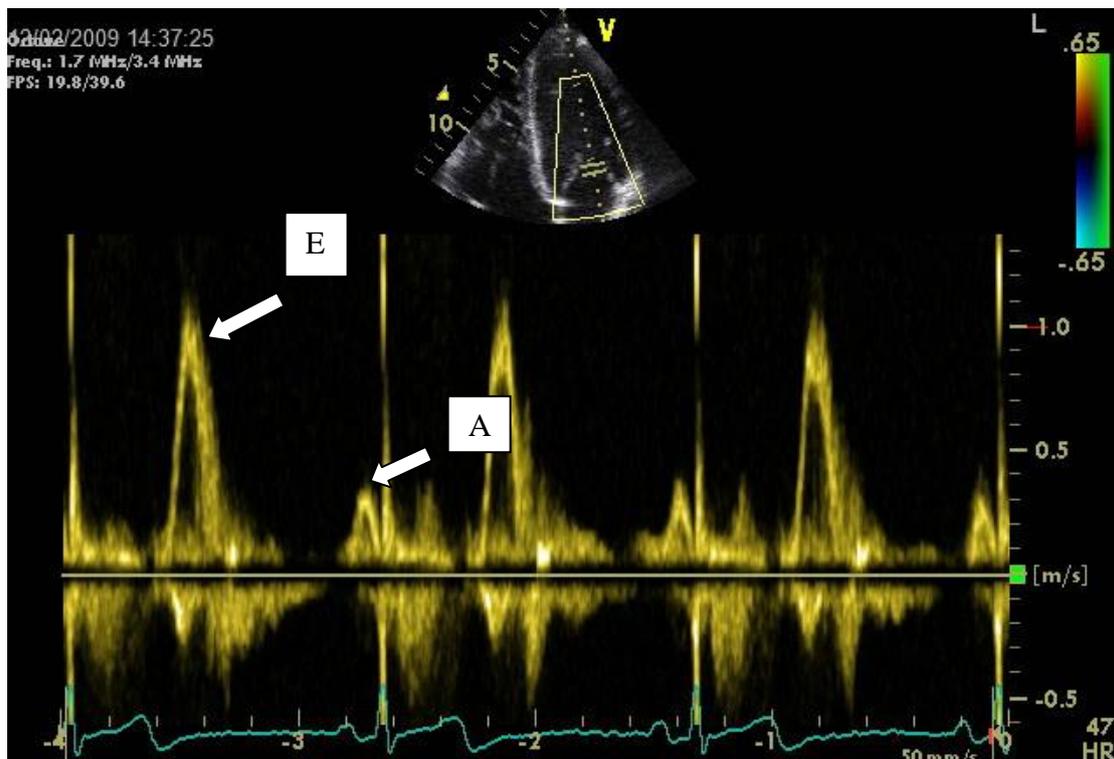
From the parasternal long-axis view (PLAX), the left ventricular outflow tract diameter was measured and cross-sectional area was calculated. (Figure 3.6)

**Figure 3.6: Parasternal long axis view (PLAX) for LVOT cross-sectional area calculation.**



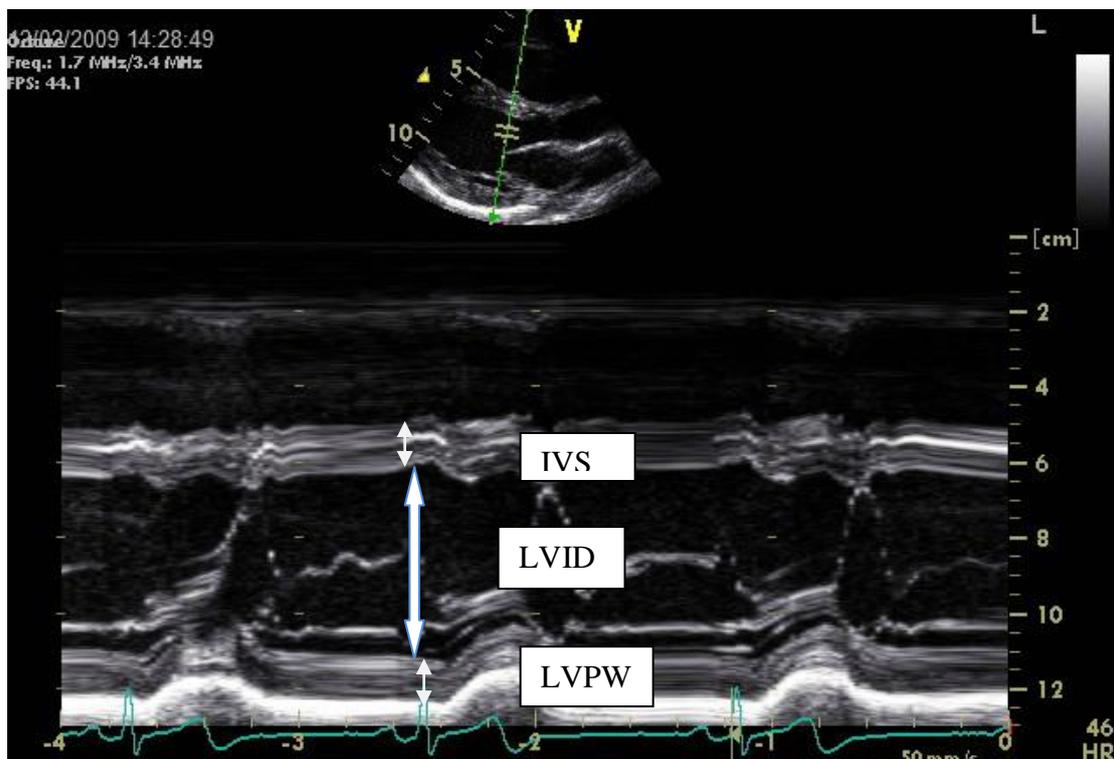
Mitral inflow velocities were recorded by using conventional pulsed-wave Doppler echocardiography, positioning the sample volume at the level of the tips of mitral valve leaflets in an apical four-chamber view. (figure 3.7)

**Figure 3.7: Pulsed Doppler for mitral inflow velocities**



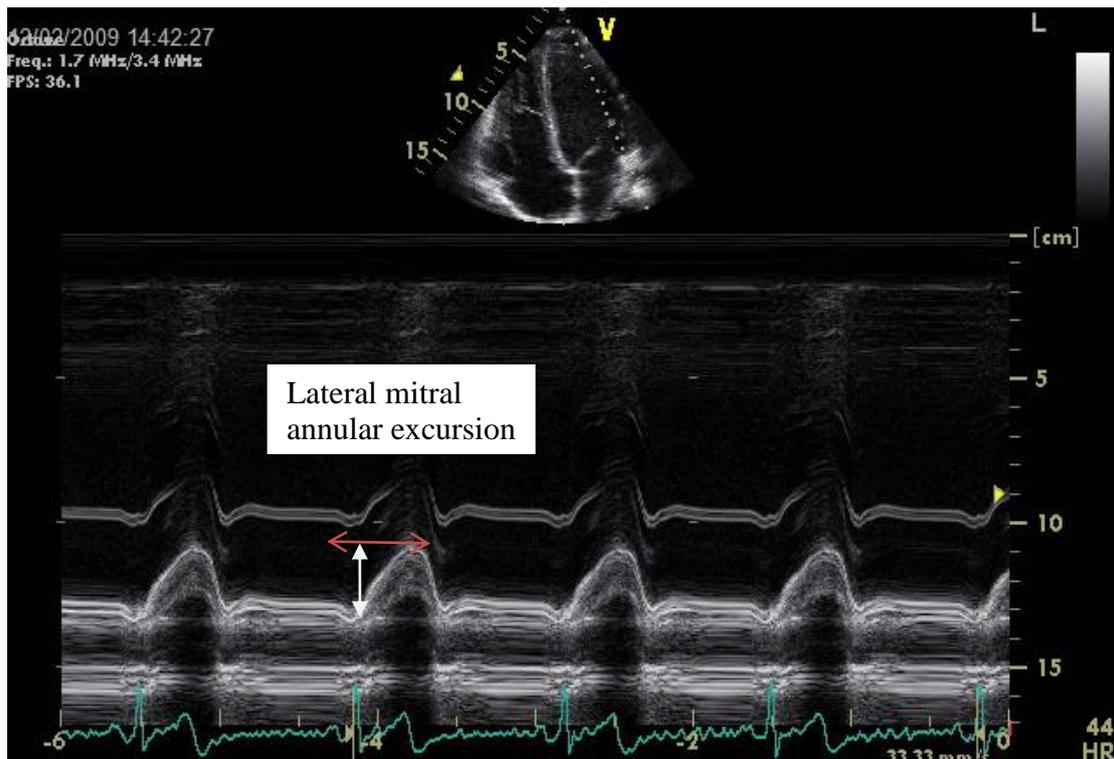
M-mode echocardiography was performed in accordance with the recommendation of the American Society of Echocardiography, in a parasternal long-axis view, to assess left ventricular dimensions. Indices of the left ventricular wall were measured.

**Figure 3.8: M-mode of left ventricle**



Lateral and medial mitral annular excursions and tricuspid annular excursions were measured from M-mode examinations. (figures 3.9, 3.10, and 3.11)

**Figure 3.9: M-mode examination showing lateral mitral annular excursion**



**Figure 3.10: M-mode examination showing medial mitral annular excursion**

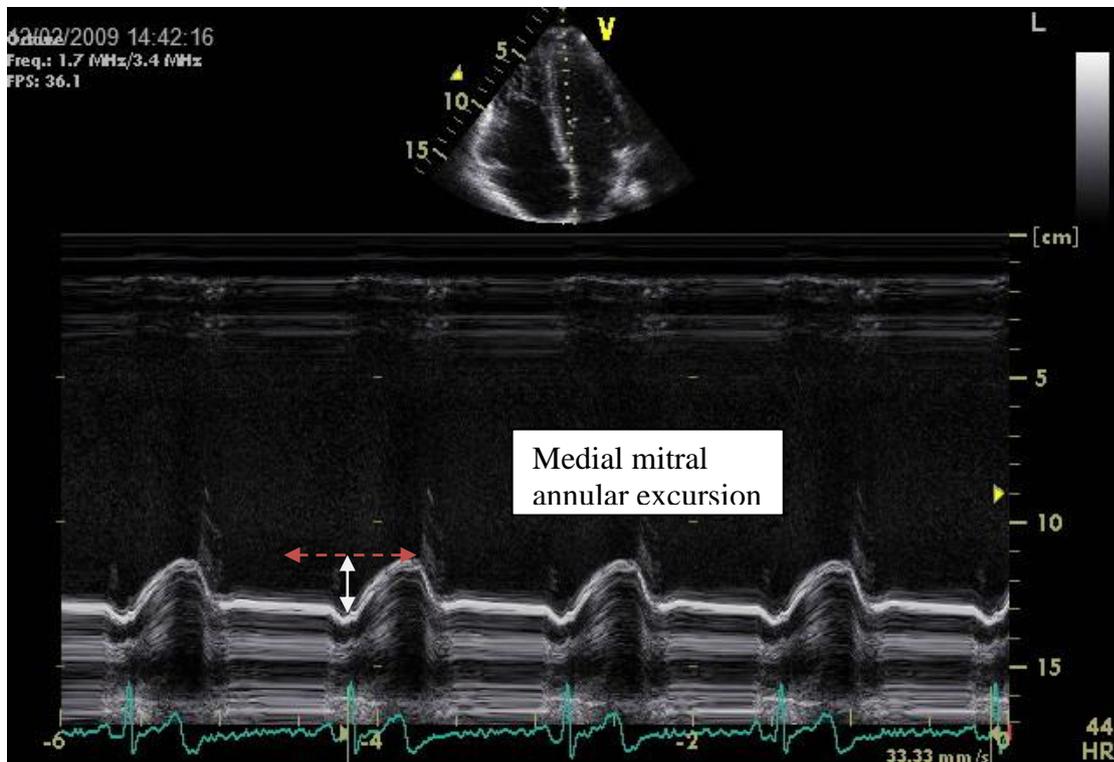
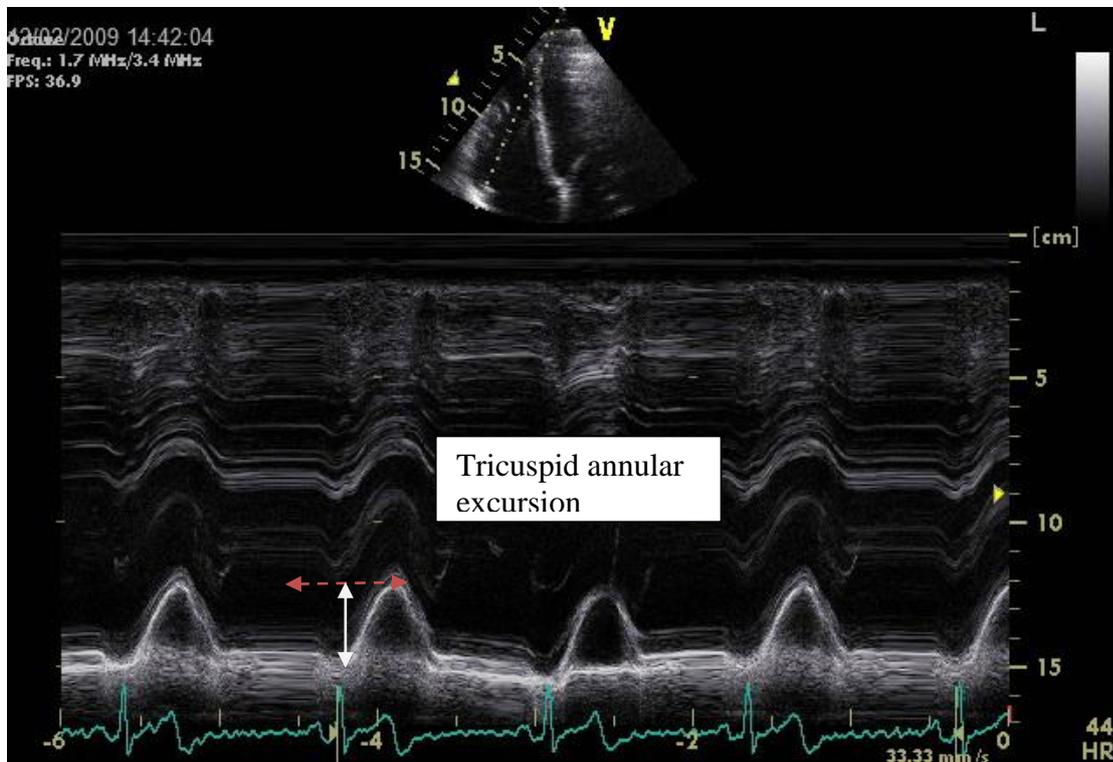
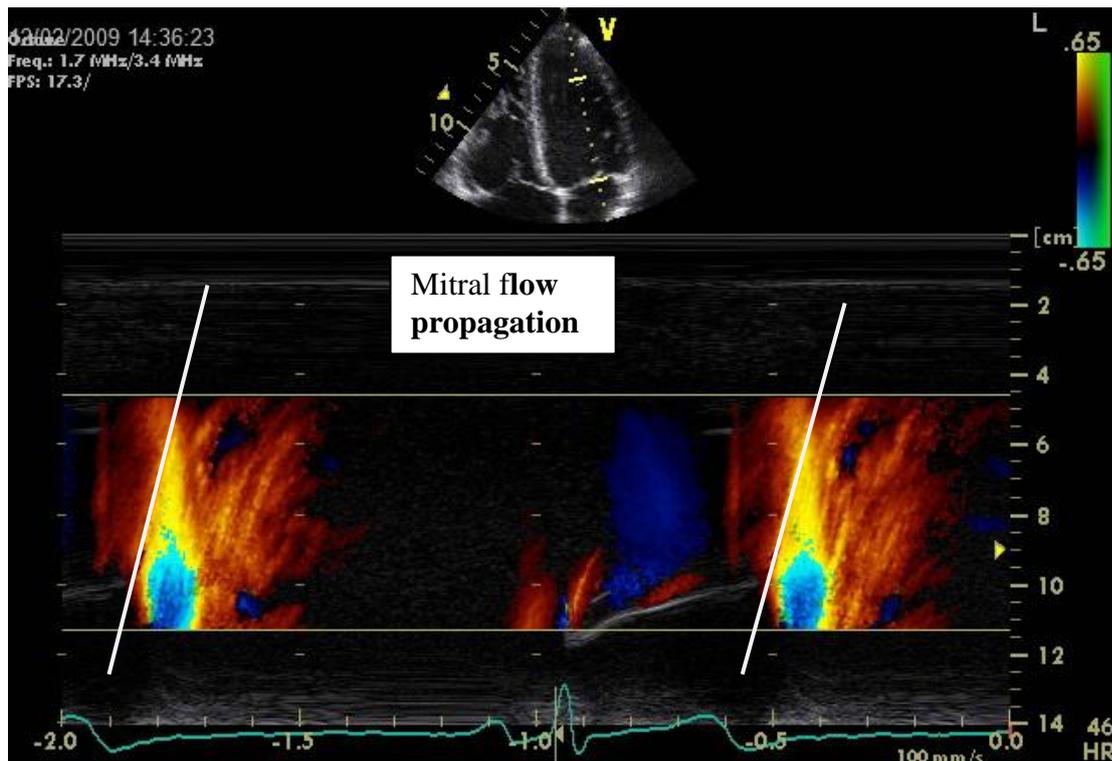


Figure 3.11: M-mode examination showing tricuspid annular excursion



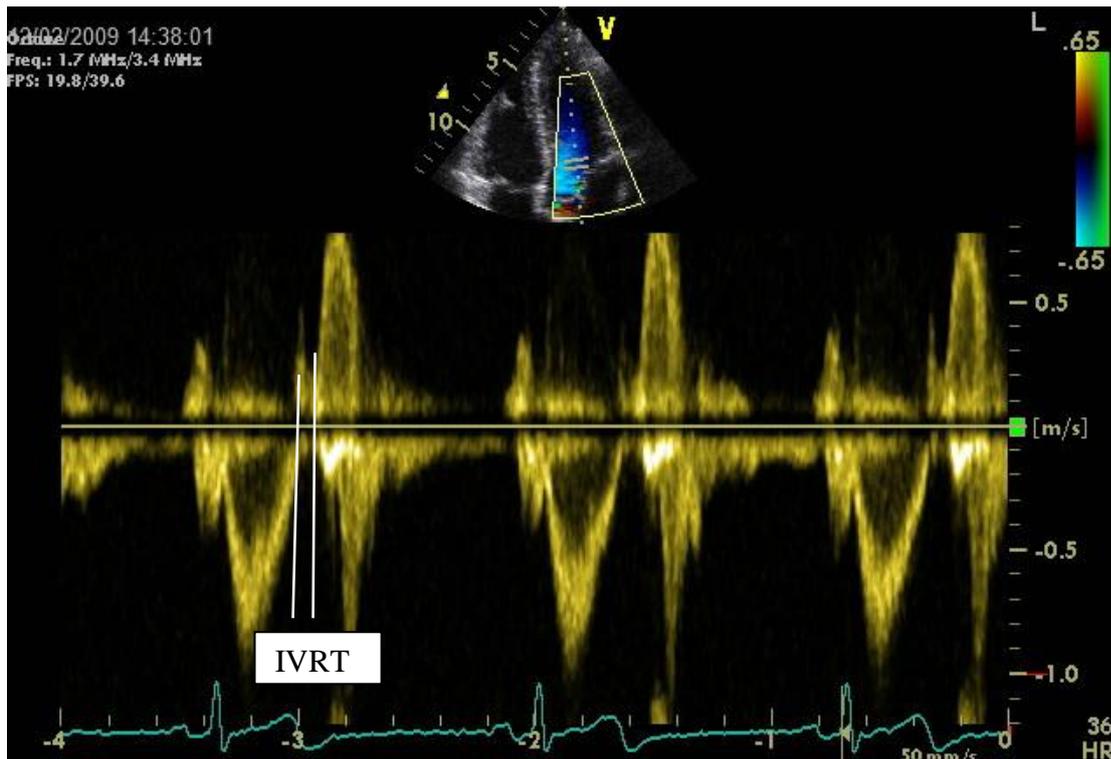
A colour M-mode recording of mitral flow propagation was performed in the apical long-axis view by placing the cursor parallel to the left ventricular inflow. (figure 3.12)

**Figure 3.12: Mitral flow propagation on colour M-mode**



Intraventricular relaxation time (IVRT) was measured from pulsed Doppler examination near the LVOT. (figure 3.13)

**Figure 3.13: IVRT measurement by placing cursor for pulsed Doppler near LVOT**



From pulsed Doppler for LVOT flow, the velocity time integral (VTI) was calculated. Stroke volume (SV) was calculated by multiplying the cross-sectional area of the left ventricular outflow tract (calculated from the dimension measured on the long-axis image) and the VTI. (figure 3.14)

Cardiac output was calculated by multiplying SV and heart rate (HR).

$$SV = VTI \times CSA \text{ of LVOT}$$

$$CO = SV \times HR$$

**Figure 3.14: measurement of velocity time integral (VTI) at LVOT**

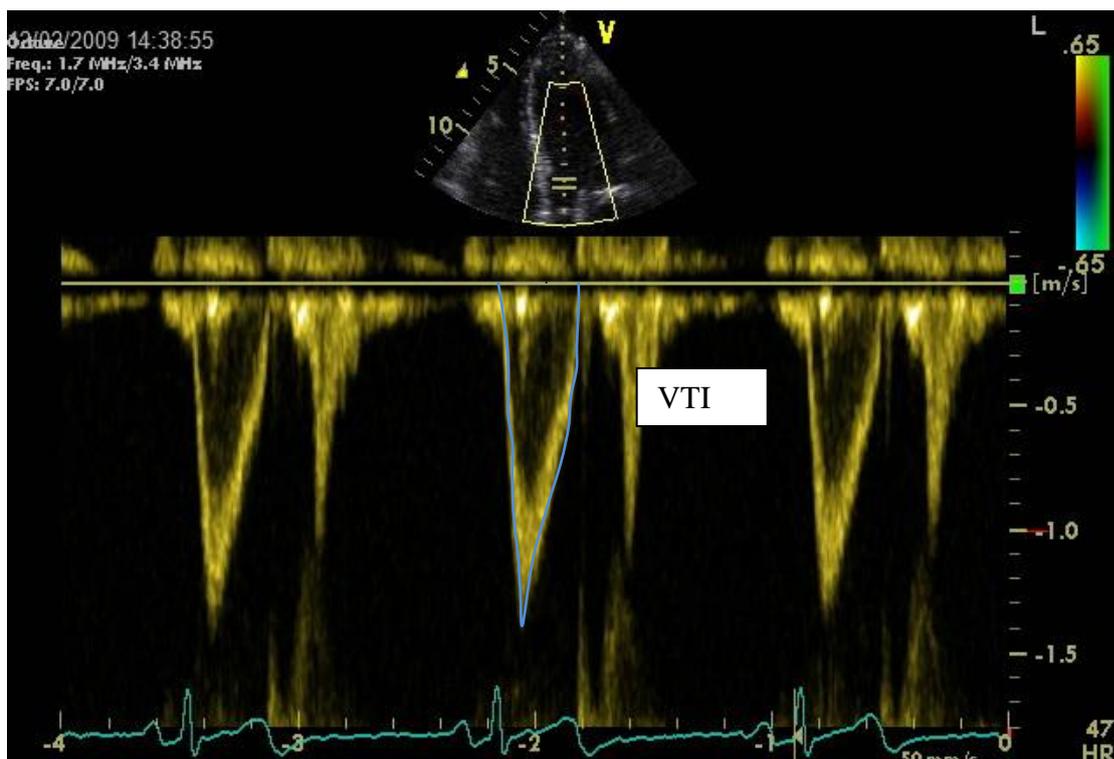
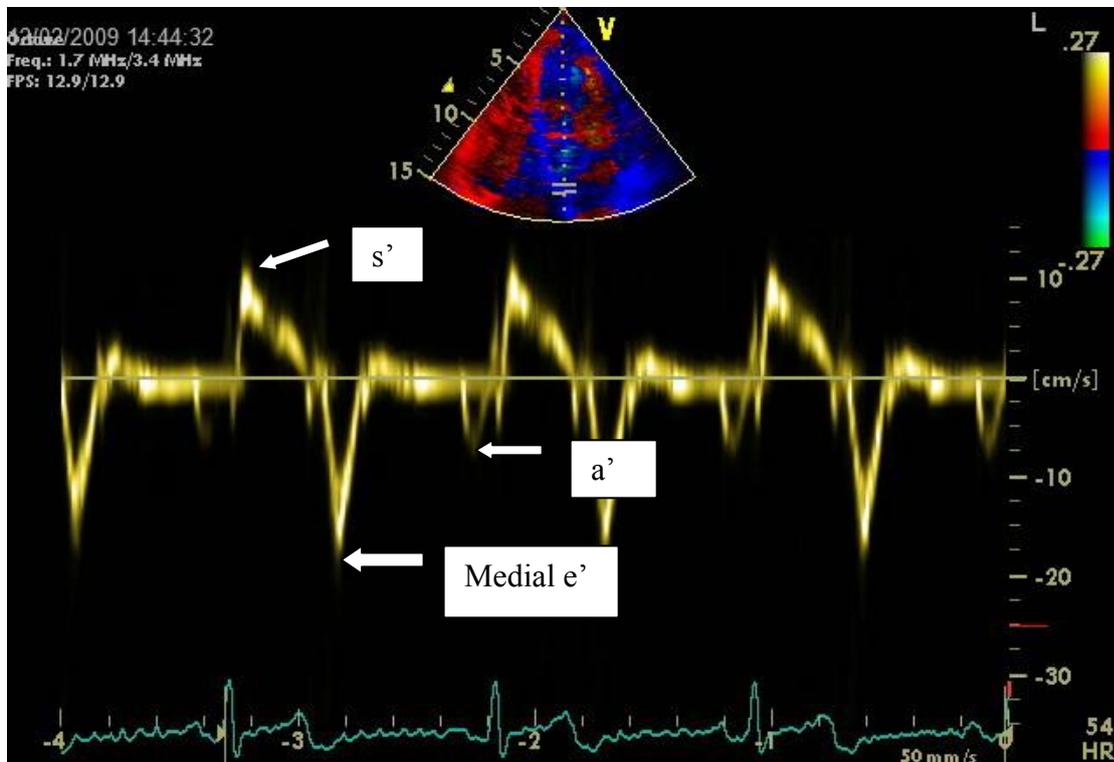
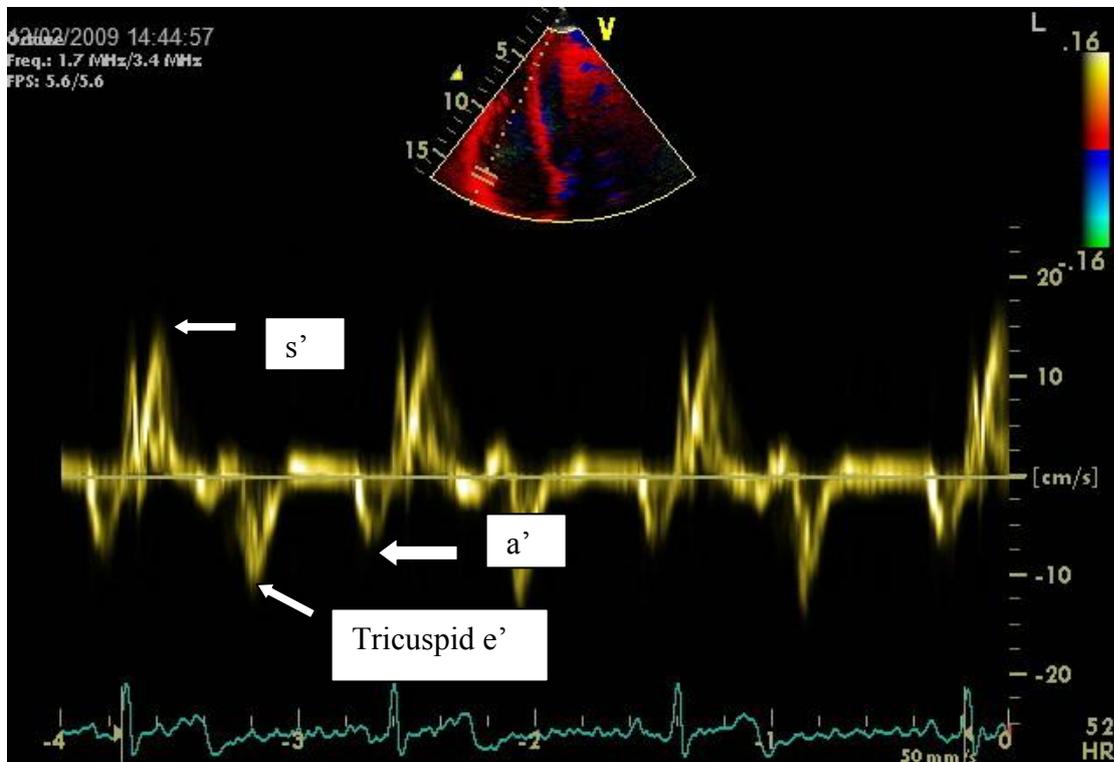




Figure 3.16: Pulsed Doppler of medial mitral annulus



**Figure 3.17: Pulsed Doppler of tricuspid annulus**



### ***3.10 Dobutamine Stress Echocardiography Methods***

After acquiring baseline echocardiography images, a standard dobutamine stress echocardiogram (DSE) combined with myocardial velocity image was performed according to the protocol. A cannula was inserted into a vein in the left antecubital fossa and a blood pressure cuff was applied to the right arm with the patient in the left lateral position. Myocardial velocity imaging was performed at each stage and images were stored for off-line analysis. Images were acquired in three apical imaging planes - a 4 chamber view, a 2 chamber view, and a long axis view – and in parasternal long axis and short axis views, so that myocardial velocity could be analysed off line for longitudinal myocardial function in basal septal (BS), basal lateral (BL), basal anterior (BA), basal anteroseptal (BAS), basal posterior (BP) and basal inferior (BI) segments, and for radial myocardial function in the basal posterior segment.

Images were acquired at the end of passive expiration with the patient holding his or her breath, in order to minimize disturbances from respiratory movement. Three cardiac cycles were recorded for each view. The depth and width of each image was optimized so that the frame rate recorded was more than 120 frames per second. Dobutamine was infused via a peripheral vein and increased every 3 minutes from a starting dose of 5  $\mu\text{g}/\text{kg}/\text{minute}$  to 10, 20, 30, and 40  $\mu\text{g}/\text{kg}/\text{minute}$  or until the subject reached the target heart rate of 80% (220 – patient's age in years). 12 lead ECG, pulse and blood pressure were monitored every 3 minutes throughout the procedure. Dobutamine infusion was stopped if the subject could not tolerate the infusion or when the target heart rate was achieved.

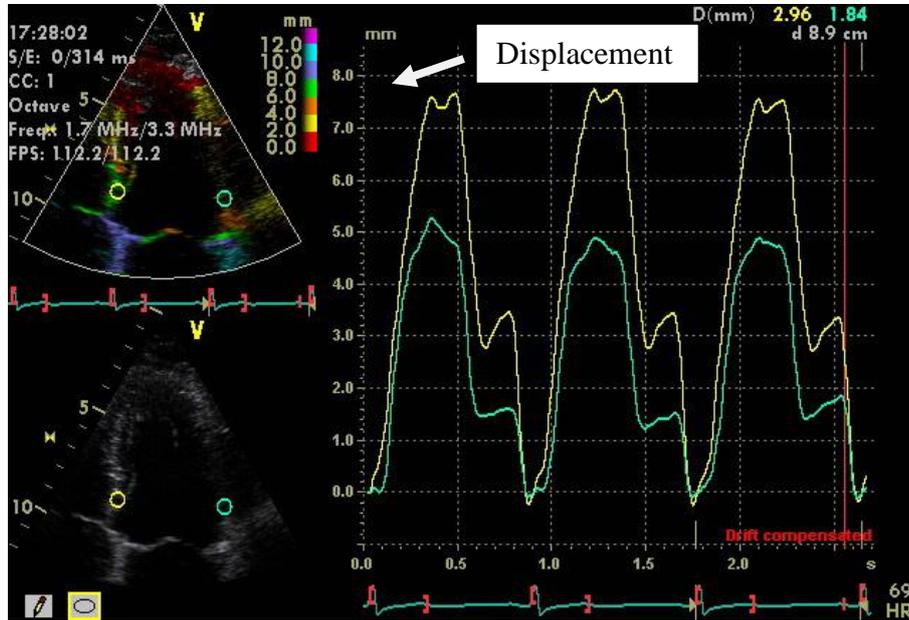
Images were analysed by myself off-line, for baseline echocardiographic characteristics, and longitudinal and radial myocardial function. The measurements were stored in an Excel spreadsheet.

### **3.11 Analysis**

*Baseline echocardiography:* Left ventricular dimensions were measured from a parasternal M-mode recording, and left ventricular mass index was calculated by Devereux's formula. [185] Left ventricular volumes and ejection fraction were calculated from apical 4- and 2-chamber views using Simpson's biplane method.

*Dobutamine stress echocardiography:* Images were analysed off-line using Echopac software (Echopac TV1, GE). Systolic velocity (figure 3.19) and displacement (figure 3.18) were measured in 6 basal myocardial segments (basal lateral, basal septal, basal anterior, basal anteroseptal, basal inferior and basal posterior) at rest and during the final minute of every stage of the dobutamine infusion, from 3 beats and averaged. Velocities of radial function were analysed from the basal posterior segment in a parasternal long-axis image. Systolic strain (figure 3.20), peak systolic strain rate and peak early diastolic strain rate (figure 3.21) were measured in basal septal and lateral segments at rest, at dobutamine dose of 20 $\mu$ g/kg/minute and 30 $\mu$ g/kg/minute respectively. All measurements were made by one observer.

**Figure: 3.18 Displacement in basal septal and basal lateral segments**



**Figure: 3.19 Regional myocardial velocities (S, E, and A) in basal septal segments (Velocities were measured off-line)**

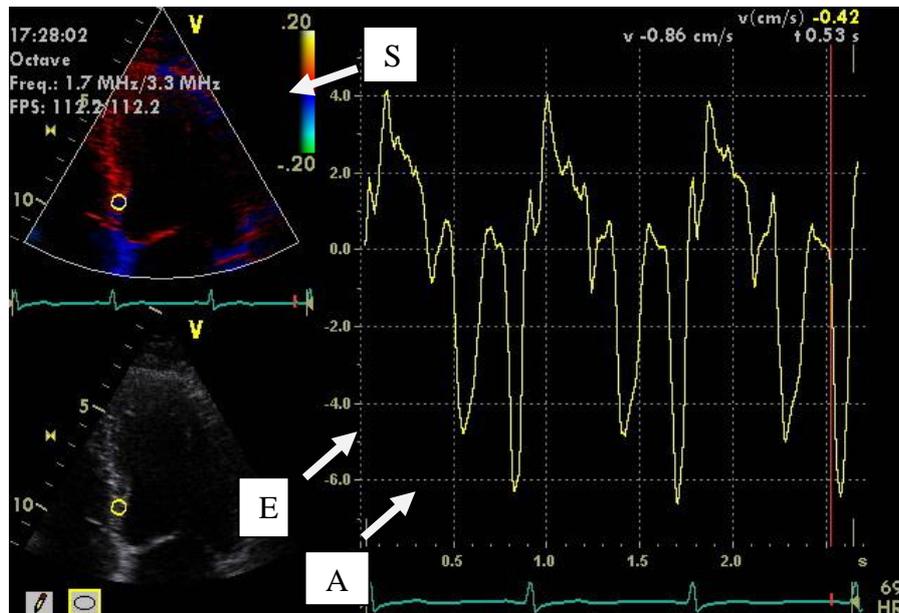


Figure 3.20 Measurement of strain in basal septal segment

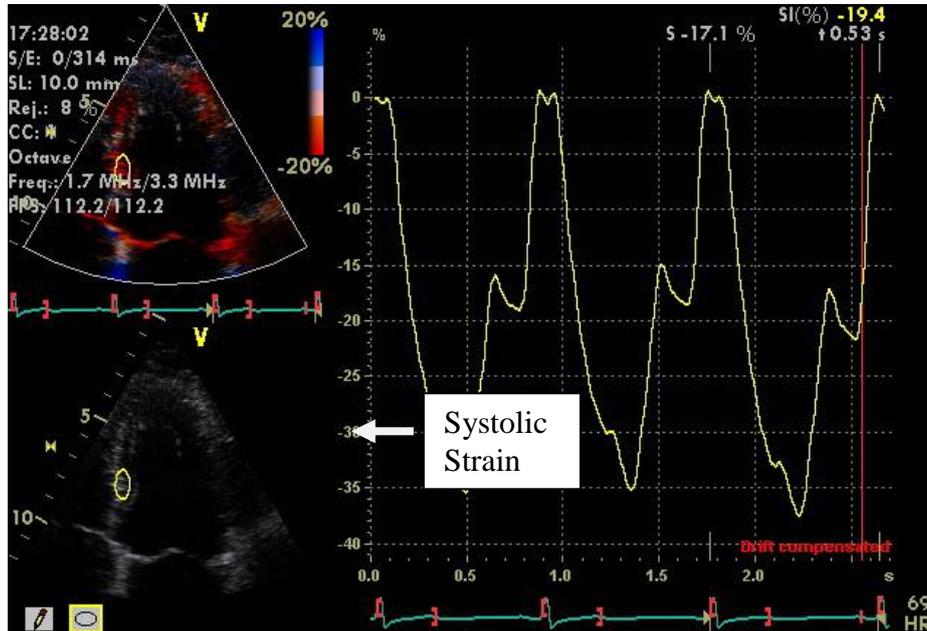
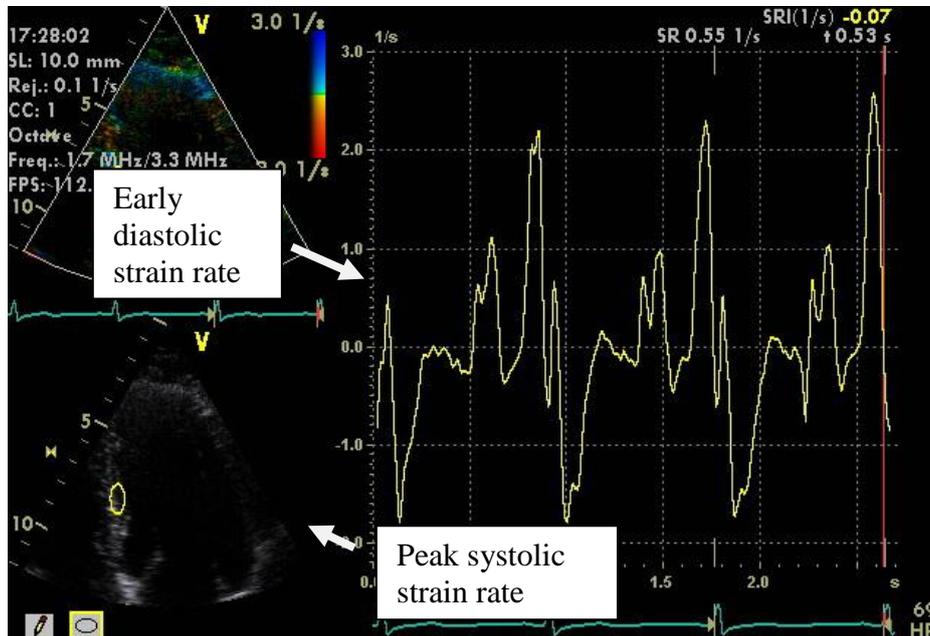


Figure 3.21 Measurement of strain rates in basal septal segment



### ***3.12 Reproducibility of myocardial function***

Intra-observer reproducibility was assessed by reanalysing the digitally stored studies after a minimum interval of 2 weeks, from a random selection of 10 diabetic subjects and 10 control subjects. Repeated measurements were made of myocardial systolic velocities in four basal segments, namely basal lateral (BL), basal septal (BS), basal posterior (BP), and basal anterior (BA) segments, at rest and at 10 and 30  $\mu\text{g}/\text{kg}/\text{min}$  of dobutamine. Repeated measurements of systolic displacement were made in three basal segments namely BL, BA and BP at the same stages of the protocol. Reproducibility of measurement was assessed by coefficient of variation and by Bland-Altman method.

### ***3.13 Reproducibility of vascular method***

Five healthy volunteers were recruited for studies of the reproducibility of the vascular method, and carotid ultrasound was performed twice in these subjects by myself, for cIMT measurement and measurement of stiffness parameters  $\beta$  and  $\epsilon$ . Measurements were repeated at least 2 weeks apart in the same subjects and reproducibility was assessed by coefficient of variation and by Bland-Altman method.

### ***3.14 Statistical Methods***

Data were analysed by using SPSS software 16 version (SPSS Inc, Chicago, IL, USA). Parametric data were compared by using independent t test and non-parametric data were compared by Chi- square test. Differences between groups were tested for statistical significance by using analysis of variance (ANOVA). Pearson correlations were used to test correlation between two parametric data. The results are presented as mean value  $\pm$  standard deviation. P value of  $<0.05$  in a two-tailed test was regarded as statistically significant.

Reproducibility of measurement was tested by both Bland-Altman test and coefficient of variation.

Pearson's correlation was used to assess bivariate correlations between myocardial or vascular function and baseline characteristics and biochemical results. Multivariate analysis on linear regression was used to assess for independent factors for myocardial and vascular function.

All analyses for displacement and velocities during dobutamine stress were performed by Repeated Measures Analysis of Variance (Linear Mixed Model) with subject treated as a Random effect and Dose/Diabetic Group as fixed effects. Dose level was the repeated element.

## CHAPTER 4- RESULTS - Baseline Characteristics

### 4.1 Baseline Characteristics (whole group)

Baseline characteristics of the subject in the whole group (n=53) are shown in Table

4.1

**Table 4.1 Baseline Characteristics of the whole group (n=53)**

	<b>Type 1 Diabetes Mellitus (n=19)</b>	<b>Controls (n=34)</b>	<b>P value</b>
<b>Age (years)</b>	21.1 ± 3.6 (17-28)	24.6 ± 3.0 (20-29)	0.001 *
<b>Height (m)</b>	1.71 ± 0.10 (1.53-1.93)	1.74 ± 0.09 (1.61-1.94)	0.23
<b>Weight (kg)</b>	73.7 ± 12.8 (50-106)	73.6 ± 13.1 (49-101)	0.98
<b>BMI (kg/m<sup>2</sup>)</b>	25.1 ± 3.1 (19.5-31.3)	23.9 ± 2.8 (18.2-30.9)	0.17
<b>Waist circumference (cm)</b>	84.4 ± 9.3 (68-106)	84.7 ± 10.9 (60-104)	0.91
<b>Waist Hip Ratio</b>	0.80 ± 0.07 (0.69-0.93)	0.81 ± 0.07 (0.61-0.94)	0.54
<b>Pulse (rate/min)</b>	72 ± 10 (53-90)	64 ± 11 (47-101)	0.02*
<b>SBP (mmHg)</b>	114 ± 9 (100-132)	115 ± 12 (94-147)	0.70
<b>DBP (mmHg)</b>	72 ± 6 (62-85)	71 ± 6 (60-84)	0.62
<b>Duration of diabetes mellitus (years)</b>	7.46 ± 6.24 (0.33-20)	NA	NA

\*Ranges of the data are presented in brackets.

34 controls and 19 type 1 diabetes mellitus patients participated in the whole study. All of them had baseline echocardiography. Patients in the T1DM group were about 3 years younger than the control group. ( $21.1 \pm 3.6$  vs.  $24.6 \pm 3.0$ ,  $p = 0.001$ ). However, BMIs of the two groups were matched. ( $25.1 \pm 3.1$  vs.  $23.9 \pm 2.8$ ,  $p = 0.17$ )

Subjects with diabetes mellitus were not on any other medications other than insulin and control subjects did not take any medicine. There was no history of peripheral neuropathy, and no recorded history of retinopathy or microalbuminuria in subjects with diabetes mellitus.

Resting pulse rate was significantly higher in type 1 diabetes mellitus in comparison with controls. ( $72 \pm 10$  vs.  $64 \pm 11$ ,  $p = 0.02$ )

There was no significant difference between the groups in waist hip ratio, and systolic or diastolic blood pressure.

#### 4.1.1 Female: Male Ratio (whole group)

The following table 4.2 shows participants in terms of gender and diabetes status.

**Table 4.2: Male Female Ratio (whole group) N=53**

	Sex		
	Male	Female	Total
<b>T1DM</b>	7	12	19
<b>Controls</b>	20	14	34
<b>Total</b>	27	26	53

On Chi Square test, there is no significant difference in sex ratio of the whole group. (i.e. all those patient who had baseline echocardiography and vascular scan) ( $p= 0.125$ )

#### **4.2 Exercise history**

All subjects gave a detailed exercise history including duration, frequency per week, type and intensity of exercise. The exercise activity is presented in metabolic equivalents (METs) as described by Jette et al. [186] There was no difference in the exercise activity in terms of METs per week between the two groups. ( $22.13 \pm 26.17$  vs.  $26.07 \pm 22.12$ ,  $p=0.61$ )

### **4.3 Discussion**

#### **Age**

Subjects aged between 16 years and 30 years were recruited in the study. Mean age was 21 years for type 1 diabetics and 23 years for control group. Type 1 diabetic subjects aged between 12 and 18 years were studied with tissue Doppler imaging by Salem et al. [187] They found evidence to suggest left ventricular diastolic dysfunction even at a younger age. The mean duration of diabetes in that study was  $7.6 \pm 3.1$  years and type 1 diabetic subjects had higher A, lower E/A ratio, lower Em and Em/Am. NT-pro-BNP was also found to be elevated in diabetic subjects.

The mean age for subjects in this study with type 1 diabetes mellitus was  $21.1 \pm 3.6$  years. In comparison with previous studies for myocardial dysfunction in type 1 diabetes mellitus, the subjects in this study were much younger. The mean ages for subjects with type 1 diabetes mellitus in Palmieri's studies were  $34 \pm 10$  years, in a study with low dose dobutamine infusion [104], and  $33 \pm 10$  years in another study . [105] In a study by Gul et al, the mean age for type 1 diabetes mellitus was  $27.7 \pm 6.9$  years. [188]

#### **Gender**

The protective effect of female gender on cardiovascular disease is lost in both type 1 and type 2 diabetes mellitus, and the reasons for this are poorly understood. [189] The rate of coronary artery disease was similar in both sexes in the Epidemiology of Diabetes Complications Pittsburgh follow up study. [190] In the Framingham study, the risk for heart failure was increased three folds for women with prior coronary artery disease.[31] Male: Female ratio in my study is not different either for the whole group or for the stress group.

### ***Heart rate***

Resting heart rate (RHR) is determined by circulating catecholamine levels, drug treatment such as beta-blockade, body temperature and autonomic function status. In physically fit athletes, vagal tone tends to be higher and it leads to slower heart rate. On the other hand, diabetic subjects are prone to autonomic neuropathy and when parasympathetic nervous system is involved, unopposed sympathetic drive could result in sinus tachycardia. The higher resting heart rate in type 1 diabetics in our study could be either due to early autonomic neuropathy or simply because control subjects were generally fitter than type 1 diabetics in my study. Autonomic neuropathy was not tested in my study. However, none of the subjects had any other microvascular complications of diabetes mellitus namely diabetic retinopathy or microalbuminuria. In the absence of other microvascular complications, it is unlikely that increased heart rate in subjects with diabetes is due to autonomic neuropathy.

Elevated resting heart rate is recognized to be a risk factor for cardiovascular mortality in the general population. [191]

In a follow up study of 523 diabetic patients including 221 type 1 diabetics, corrected QT interval (cQT) but not RHR was associated with cardiovascular mortality in T1DM whereas RHR but not cQT was associated with cardiovascular mortality in type 2 diabetes mellitus. [192] RHR was also associated with survival in a one-year follow-up study of diabetic patients with coronary artery disease. [193] During an average of 6.5 years of follow-up in the DCCT study, the percentage of subjects with abnormal autonomic nervous system function tests nearly doubled. Elevated resting heart rate may be due to increased sympathetic tone or impairment of vagal tone. RHR cannot be regarded as a reliable sign of autonomic neuropathy in the absence of other physical signs. [194] Autonomic neuropathy was shown to be associated with impaired coronary artery flow and myocardial dysfunction

in diabetes mellitus. [195] In the DCCT/EDIC study, RHR was lower in the intensive treatment group and the effect persisted during the follow up period. The mechanism was unknown. However, higher RHR was associated with higher glycated haemoglobin level. [196] In a recent prospective study of 1,088 type 2 diabetes mellitus patients resting heart rate was demonstrated to be an independent predictor of cardiovascular risk and all-cause mortality. [197]

However, as can be seen in the previous section, the amount of exercise activity as per metabolic equivalents (METs) does not seem to be statistically significantly different from that of control group, but the variability of exercise time was high.

### ***Blood pressure***

Normal blood pressure is defined by JNC7 as a systolic BP <120 mmHg and a diastolic BP <80 mmHg. [198] The mean values for diabetic subjects and controls in our study met these criteria, although 21% had higher systolic pressures on single measurements. There is no significant difference in both systolic and diastolic blood pressure between the two groups.

### ***Duration of diabetes***

Duration of diabetes mellitus is also a risk factor for cardiovascular disease. [199] [200] Generally, the longer the duration of diabetes mellitus, the higher is the risk of CVD. Nonetheless, there are subjects with T1DM who do not develop vascular complications despite long duration of diabetes mellitus. [201] Genetic factors via elevated HDL-cholesterol level have been suggested to be protective. There is a wide variation in duration of diabetes mellitus among patients with type 1 diabetes mellitus in this study.

## CHAPTER 5- RESULTS – biochemical results

### 5.1 Biochemical results

On the morning of the vascular study, subjects came in fasting from 22:00 pm overnight. Blood samples were taken for fasting glucose, HbA1c, lipid profiles, AST and high sensitivity C- reactive protein (hs CRP).

Fasting blood results for both type 1 diabetes mellitus and control group are shown in the table 5.1 below.

**Table 5.1 Biochemical results (whole group)**

	<b>Type 1 Diabetes Mellitus (n=19)</b>	<b>Controls (n=34)</b>	<b>P value</b>
<b>Fasting glucose (mmol/L)</b>	11.35 ±6.21	4.77 ±0.42	0.00*
<b>HbA1c (%)</b>	8.82 ±1.64	5.21 ±0.25	0.00*
<b>Creatinine (µmol/L)</b>	72.26 ±10.92	81.18 ±10.01	0.004*
<b>Total Cholesterol (mmol/L)</b>	4.17 ±0.66	4.22 ±0.82	0.83
<b>HDL (mmol/L)</b>	1.18 ±0.24	1.33 ± 0.23	0.042*
<b>LDL (mmol/L)</b>	2.47 ±0.79	2.52 ±0.73	0.83
<b>TG (mmol/L)</b>	1.18 ±0.90	0.84 ± 0.38	0.07
<b>AST (IU/L)</b>	16.06 ±5.53	20.16 ± 6.73	0.04*
<b>hsCRP (mg/dL)</b>	1.82 ±2.41	2.16 ± 4.49	0.76

Mean fasting blood glucose was 11.35 mmol/l with a standard deviation of  $\pm 6.21$  in type 1 diabetes mellitus. Mean HbA1c for diabetic group was 8.82 % with a standard deviation of  $\pm 1.64$ , indicating suboptimal glycaemic control in the study population.

However, apart from HDL cholesterol, no statistically different lipid levels were observed between the controls and type 1 diabetics. HDL was about 10% lower in subjects with type 1 diabetes mellitus. There was a trend towards triglyceride being higher in subjects with T1DM.

Serum creatinine level was higher in the control group. Serum creatinine levels were about 12% higher in the control group.

No difference in hsCRP level between the two groups was observed. There was no evidence of increased inflammatory reaction in type 1 diabetes mellitus.

## ***5.2 Discussion on biochemical results***

The current HbA1c target range set for young adults with type 1 diabetes mellitus is 7.5% according to NICE clinical guidelines CG 51. The cohort in the study had suboptimal control. Fasting blood glucose levels were high as well. No difference in the lipid levels was noted apart from serum HDL level which was higher in control group.

In a longitudinal study of young children with type 1 diabetes mellitus, serum total cholesterol correlated with the duration of diabetes mellitus and increasing HbA1c whereas HDL cholesterol fell with increasing age. [202] Dyslipidaemia was documented in subjects with type 1 diabetes mellitus and the rise in serum total cholesterol was associated with the increase in HbA1c. Nevertheless, lipid abnormalities seem to be more prevalent in young patients with type 2 diabetes mellitus than in those with type 1 diabetes mellitus. [203]

The relationship between coronary artery disease and serum HDL may be more complex as Costacou et al pointed out in their study. [204] Costacou et al studied populations from the Pittsburgh EDC study and observed that although there was an inverse relationship between serum HDL level and coronary artery disease in male subjects with type 1 diabetes mellitus, the relationship in female seemed more like a U-shape with higher incidences of coronary artery disease at both lower and higher HDL levels.

Serum creatinine level was higher in the control group than in the type 1 diabetes mellitus group. Higher creatinine level may be contributed by exercise activity or muscle bulk in the control group. In a population study, serum creatinine level was positively associated with upper arm circumference and physical activity. [205]

## CHAPTER 6- RESULTS - Baseline echocardiographic results

### 6.1 Baseline Echocardiographic results

Baseline echocardiographic results are shown in table 6.1 for the whole group (N=53), comparing 19 subjects with T1DM with 34 controls, and in table 6.2 for the stress group (N=39), comparing 18 subjects with T1DM and 21 controls.

**Table 6.1: Baseline echocardiographic data (whole group)**

	<b>Type 1 Diabetes Mellitus (n=19)</b>	<b>Controls (n=34)</b>	<b>P value</b>
<b>Pulmonary Artery Acceleration time (ms)</b>	151.41 ± 28.77	149.30 ± 12.12	0.76
<b>Left atrial area (cm<sup>2</sup>)</b>	13.84 ± 2.13	14.44 ± 2.93	0.47
<b>Flow Propagation Velocity (cm/s)</b>	6.55 ± 0.92	6.87 ± 0.99	0.28
<b>Deceleration time of mitral inflow (ms)</b>	202.74 ± 36.83	215.48 ± 39.27	0.25
<b>IVRT (ms)</b>	104.32 ± 16.47	110.00 ± 16.48	0.24
<b>LVOT VTI (cm/s)</b>	20.67 ± 4.46	22.54 ± 3.13	0.09
<b>SV (ml)</b>	69.43 ± 31.12	96.61 ± 22.55	0.001*
<b>HR (rate/min)</b>	75.18 ± 14.99	64.29 ± 10.31	0.004*
<b>CO (ml/min)</b>	4.83 ± 2.37	6.12 ± 1.35	0.015*
<b>LVEDV (ml)</b>	83.58 ± 22.52	105.73 ± 22.20	0.001*
<b>LVESV (ml)</b>	29.66 ± 11.02	35.86 ± 8.17	0.023*
<b>EF %</b>	66.27 ± 4.83	66.20 ± 3.51	0.95

**Table 6.2: Baseline grey scale and pulsed Doppler echocardiographic data (Stress group)**

	<b>Type 1 Diabetes Mellitus (n=18)</b>	<b>Controls (n=21)</b>	<b>P value</b>
<b>Pulmonary Artery Acceleration time (ms)</b>	148.34 ± 26.45	149.30 ± 12.12	0.88
<b>Left atrial area (cm<sup>2</sup>)</b>	13.80 ± 2.20	14.80 ± 2.57	0.23
<b>Flow Propagation Velocity (cm/s)</b>	6.61 ± 0.91	7.05 ± 1.14	0.22
<b>Deceleration time of mitral inflow (ms)</b>	201.54 ± 37.51	211.65 ± 35.92	0.25
<b>IVRT (ms)</b>	103.52 ± 16.61	110.32 ± 15.43	0.20
<b>VTI (cm/s)</b>	20.67 ± 4.46	22.54 ± 3.13	0.09
<b>SV (ml)</b>	69.36 ± 32.07	102.91 ± 22.54	0.001*
<b>HR (rate/min)</b>	75.81 ± 15.24	63.47 ± 9.77	0.005*
<b>CO (ml/min)</b>	4.85 ± 2.43	6.43 ± 1.27	0.014*
<b>LVEDV (ml)</b>	82.81 ± 22.91	110.15 ± 24.89	0.001*
<b>LVESV (ml)</b>	29.51 ± 11.31	37.91 ± 8.85	0.013*
<b>EF %</b>	66.24 ± 4.96	65.81 ± 2.99	0.74

As can be seen in table 6.1, there were no differences in pulmonary artery acceleration time, the left atrial area, the propagation velocity of mitral inflow, the isovolumic relaxation time and velocity time integral of left ventricular ejection, between the controls and the type 1 diabetes mellitus group.

Nevertheless, stroke volume was significantly higher in controls than in type 1 diabetes mellitus ( $96.61 \pm 22.55$  vs.  $69.43 \pm 31.12$ ,  $p = 0.001$ ), due to a small difference in the LVOT diameter. Heart rate was higher in type 1 diabetes mellitus with a statistical significance.

( $64.29 \pm 10.31$  vs.  $75.18 \pm 14.99$ ,  $p = 0.004$ )

Cardiac output was higher in the control group than in type 1 diabetes mellitus. Control groups also have bigger LVEDV and LVESV. However, overall global myocardial systolic function as indicated by EF was not different between the two groups.

## ***6.2 Discussion on baseline echocardiographic results***

The PAT (pulmonary artery acceleration time) was defined as the time in milliseconds from the onset of right ventricular ejection to the time of the peak systolic velocity of forward pulmonary flow. [206] In normal individuals, the pulmonary acceleration time (PAT) exceeds 110 ms and it progressively shortens with increasing in pulmonary hypertension (PH).

There have been few studies of right ventricular function in diabetes mellitus. Karamitsos et al studied 66 patients with type 1 diabetes mellitus and reported right ventricular diastolic dysfunction as indicated by reduction in tricuspid annular diastolic tissue velocities with normal right ventricular systolic function. [102] The pulmonary artery acceleration time is not a sensitive marker to detect early changes in right ventricular

dysfunction. In my study, pulmonary acceleration time in both type 1 diabetes mellitus and control were within normal range and they were not different between the two groups.

Left atrial remodelling may be an indicator of chronic burden of cardiovascular disease. [207] [208] Larger left atrial volume in type 2 diabetes mellitus subjects was reported by Poulsen et al. [209] Left atrial enlargement was thought to be due to pressure overload and it was associated with increased NT-proBNP, increased LV mass index, reduced total arterial compliance, and valvulo-arterial impedance. The subjects with LA enlargement were older than those without ( $63.0 \pm 10$  years vs.  $56.4 \pm 11.3$ ,  $p < 0.001$ ). Similarly, left atrial volume index in type 2 diabetes mellitus was also demonstrated to be higher in comparison with a normal population and when compared with subjects with hypertension. [210]

However, the left atrial volume in this study was not different between type 1 diabetes mellitus and controls. Left atrial volume increases if there is a chronic increase in left ventricular filling pressure. Unless diastolic function is markedly abnormal, then with a relatively short duration of diabetes mellitus, left atrial volume is unlikely to be changed in young subjects with type 1 diabetes mellitus.

Control subjects have bigger left ventricular volumes as evident by higher LV EDV and LV ESV from biplane measurements using Simpson's method. As a result of bigger EDV, stroke volume in control subjects is higher than that in subjects with type 1 diabetes mellitus. Nevertheless, cardiac output was higher in the control group secondary to bigger stroke volume. LV EDV is influenced by a number of factors such as venous return, intrathoracic pressure, the duration of diastole, which in turn is determined by heart rate, and ventricular stiffness. [211]

Assessment of diastolic dysfunction demonstrated that IVRT and the deceleration time (DT) of mitral inflow were not significantly longer in type 1 diabetes mellitus. This is in contrast to the findings by Suys et. al [80], who reported that young male type 1 diabetic subjects had longer IVRT in comparison to controls, and by Schannwell et al [76], who also demonstrated increased IVRT and DT in subjects with type 1 diabetes mellitus. Also, in my study, the flow propagation velocity of mitral inflow, which is an indicator of early diastolic suction of the left ventricle, was not reduced. All of these are global (“blood pool”) indices of left ventricular diastolic function, and they become abnormal once a substantial percentage of the myocardium shows similar regional changes.

### 6.3: M- mode data

M-mode findings are shown in the table 6.3.

**Table 6.3: M- mode echocardiographic data (N=53)**

	<b>Type 1 Diabetes Mellitus (N=18)</b>	<b>Controls (N=21)</b>	<b>P value</b>
<b>Lateral mitral annulus excursion (mm)</b>	1.49 (± 0.24)	1.56 (± 0.26)	0.36
<b>Medial mitral annular excursion (mm)</b>	1.24 (± 0.17)	1.40 (± 0.21)	0.008*
<b>Tricuspid annulus excursion (mm)</b>	1.98 (± 0.46)	2.19 (± 0.40)	0.10
<b>LVOT diameter (cm)</b>	2.11 (± 0.31)	2.33 (± 0.22)	0.005*
<b>LVOT CSA (cm<sup>2</sup>)</b>	3.57 (± 1.12)	4.29 (± 0.81)	0.01*
<b>IVSd (mm)</b>	0.82 (± 0.16)	0.79 (± 0.13)	0.44
<b>LVIDd (mm)</b>	4.63 (± 0.67)	5.04 (± 0.52)	0.017*
<b>LVPWd (mm)</b>	1.05 (± 0.25)	0.88 (± 0.15)	0.004*
<b>IVSs (mm)</b>	1.06 (± 0.23)	1.10 (± 0.24)	0.61
<b>LVIDs (mm)</b>	3.13 (± 0.64)	3.50 (± 0.50)	0.02*
<b>LVPWs (mm)</b>	1.50 (± 0.23)	1.36 (± 0.30)	0.08
<b>FS %</b>	32.53 (± 7.52)	30.54 (± 6.19)	0.31
<b>LVM (g)</b>	169.03 (± 50.26)	171.56 (± 54.16)	0.87
<b>LVMI (g/m<sup>2</sup>)</b>	92.32 (± 22.62)	90.74 (± 21.35)	0.80

LVOT= left ventricular outflow tract, IVS= interventricular septum, LVID=left ventricular internal diameter, LVPW= left ventricular posterior wall, FS= fractional shortening, LVM= left ventricular mass, LVMI= left ventricular mass index, d=diastole, s=systole

On M-mode echocardiographic analysis, medial mitral annular excursion was lower in type 1 diabetes mellitus in comparison with the control group whereas no difference was observed between the two groups for lateral mitral annular and tricuspid annular excursions.

Left ventricular outflow tract diameter and left ventricular outflow tract cross-sectional area were higher in controls than in type 1 diabetes mellitus by 9.4%, and by 17% with a p value of 0.005, and 0.01 respectively.

M-mode measurements of left ventricular diameters showed that the control group had bigger left ventricular internal diameters in both systole and diastole, whereas LVPW during diastole was thicker in type 1 diabetes mellitus. However, overall left ventricular mass and left ventricular mass index showed no statistical difference between the two groups.

#### ***6.4 Discussion on M-mode results***

LVOT diameter and cross sectional area were bigger in controls than those in type 1 diabetes mellitus. The reasons why this might be the case is not clear. LVOT diameter may be used to calculate LVOT cross-sectional area. LVOT diameter was shown to be correlated to body surface area independent of sex.[212]

LVOT diameter is measured from the parasternal long axis view. Potential errors in measurement may occur from two sources. Slight angulation or lateral displacement of the transducer could result in slightly oblong or oval section of LVOT which could lead to underestimation of LVOT diameter. Inaccurate identification of tissue-blood interface may also result in over or underestimation of LVOT diameter. [213]

LVOT CSA (LVOT cross-sectional area) was calculated by using the formula:

$$\text{LVOT CSA} = \pi \times (d/2)^2 \text{ where } d = \text{LVOT diameter.}$$

Since LVOT diameter is halved and squared in the formula, a slight error in the measurement of LVOT d could lead to bigger error in LVOT CSA measurement and hence cardiac output (CO).

LVID both during systole and diastole was bigger in controls than in type 1 diabetes mellitus. However, LVPW was thicker in type 1 diabetes mellitus. Overall, left ventricular mass is similar. Left ventricular hypertrophy is a risk factor for the development of heart failure. Two previous studies reported that patients with type 1 diabetes had increased LV mass. [119] [122] Both used magnetic resonance imaging, which is the most accurate non-invasive method to measure this, but the mean ages of patients in those studies were  $47 \pm 2$  years and  $50 \pm 9$  years respectively. In the study by Astrup et al, the patients also had diabetic nephropathy and some had hypertension. [122] Epidemiological studies have reported that left ventricular mass is increased in diabetes mellitus, but they too had possible confounding factors. In the Strong Heart Study, diabetic subjects had higher BMI and systolic blood pressure, and patients with hypertension were not excluded.[32] In the Framingham study, about 15 % of the participants were already diagnosed to have hypertension or were taking antihypertensive drugs. [214] Hypertensive subjects were included in the Cardiovascular Health Study. [215] In my study, left ventricular mass index was not different between type 1 diabetic patients and controls, and it was within the normal range. Unlike the previous studies, our patients were young and normotensive, and they had no other cardiovascular co-morbidities. My data suggest that LV mass index may not be increased in young subjects with diabetes mellitus without other risk factors, and that the higher LVMI observed in other studies may be related to hypertension with increasing age.

## **CHAPTER 7 – RESULTS – Reproducibility of myocardial function assessment**

### ***7.1 Reproducibility of myocardial displacement and velocities***

Peak systolic velocities at 0, 10 and 30  $\mu\text{g}/\text{kg}/\text{minute}$  of dobutamine in BL, BS, BA and BP myocardial segments and displacement at 0, 10, and 30  $\mu\text{g}/\text{kg}/\text{minute}$  of dobutamine in BL, BA and BP myocardial segments were analysed from 10 randomly selected subjects. Their digitally stored studies were re-analysed after an interval of at least 2 weeks. Intra-observer reproducibility calculated by Bland-Altman methods and coefficients of variation are shown in the tables (7.1 & 7.2) and figures (7.1, 7.2, 7.4, 7.5, and 7.6. & 7.7).

Similarly, mitral inflow early diastolic velocity (E), and lateral and medial tissue velocity  $e'$ , were measured in 10 subjects and reanalysed after at least 2 weeks . (See Figure 7.3)

Intra-observer variability (coefficients of variation) was reported to be ranging from 6-11% and inter-observer variability from 5-9% in basal segments. [216] [217] Therefore, my reproducibility results are comparable with other studies.

**Table 7.1: Intra-observer reproducibility of PSV at basal segments (Bland-Altman Analysis)**

		Bias	SD of Bias	95% Limit of agreement	
				From	To
BL	0 $\mu$ g/kg/min	0.12	0.31	-0.50	0.73
	10 $\mu$ g/kg/min	0.57	1.20	-1.78	2.92
	30 $\mu$ g/kg/min	-0.24	1.37	-2.94	2.45
BS	0 $\mu$ g/kg/min	-0.39	0.30	-0.97	0.19
	10 $\mu$ g/kg/min	-0.03	0.26	-0.53	0.47
	30 $\mu$ g/kg/min	-0.49	0.49	-1.45	0.48
BA	0 $\mu$ g/kg/min	0.09	0.28	-0.46	0.64
	10 $\mu$ g/kg/min	-0.05	0.45	-0.93	0.84
	30 $\mu$ g/kg/min	0.12	0.59	-1.04	1.29
BP	0 $\mu$ g/kg/min	0.02	0.54	-1.04	1.07
	10 $\mu$ g/kg/min	-0.19	0.34	-0.86	0.47
	30 $\mu$ g/kg/min	-0.34	0.82	-1.93	1.26

**Table 7.2: Intra-observer reproducibility of displacement at basal segments (Bland-Altman Analysis)**

		Bias	SD of Bias	95% Limit of agreement	
				From	To
BL	0 $\mu$ g/kg/min	0.27	0.45	-0.60	1.15
	10 $\mu$ g/kg/min	0.17	0.51	-0.81	1.17
	30 $\mu$ g/kg/min	-0.23	0.90	-2.00	1.54
BA	0 $\mu$ g/kg/min	-0.01	0.89	-1.75	1.73
	10 $\mu$ g/kg/min	0.02	0.69	-1.34	1.38
	30 $\mu$ g/kg/min	0.22	0.83	-1.40	1.84
BP	0 $\mu$ g/kg/min	-0.14	0.59	-1.31	1.02
	10 $\mu$ g/kg/min	-0.14	0.92	-1.95	1.67
	30 $\mu$ g/kg/min	-0.52	0.75	-1.98	0.94

Figure 7.1: Intra-observer variability for peak systolic velocity

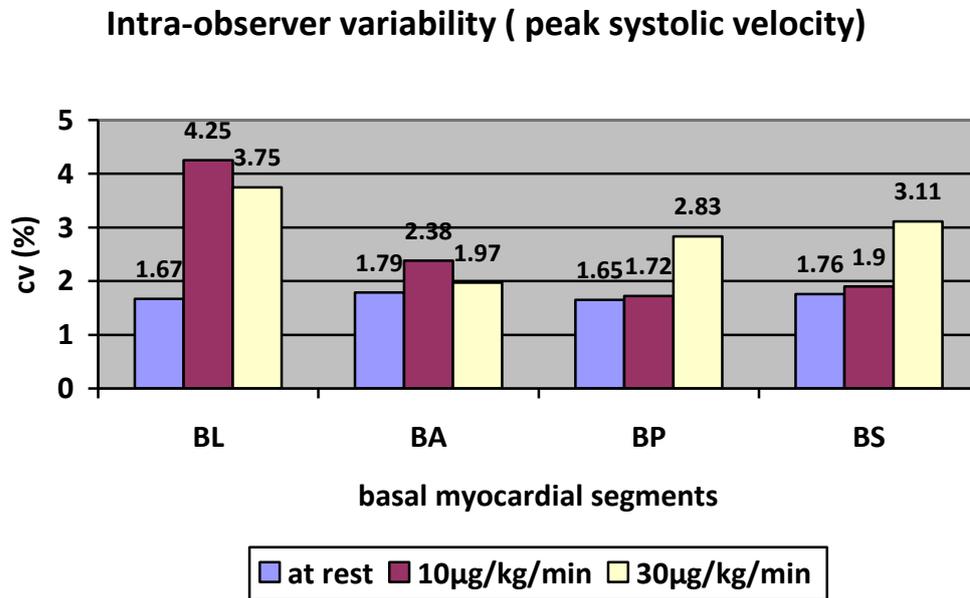


Figure 7.2: Intra-observer variability for basal displacement analysis

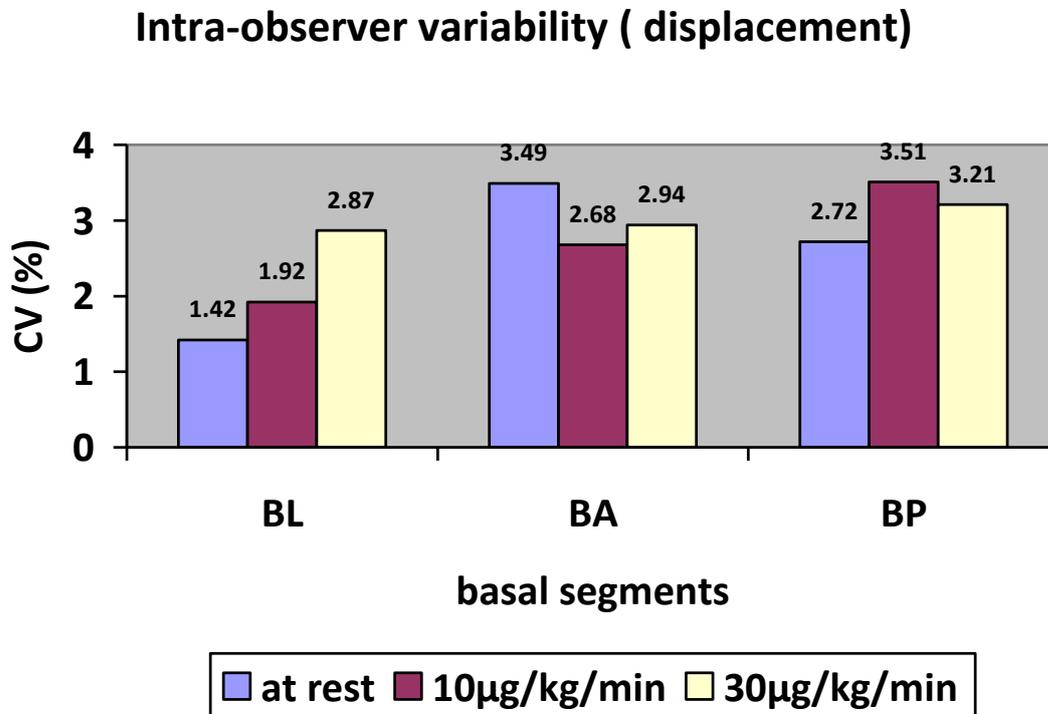


Figure 7.3: Intra-observer variability for diastolic velocities

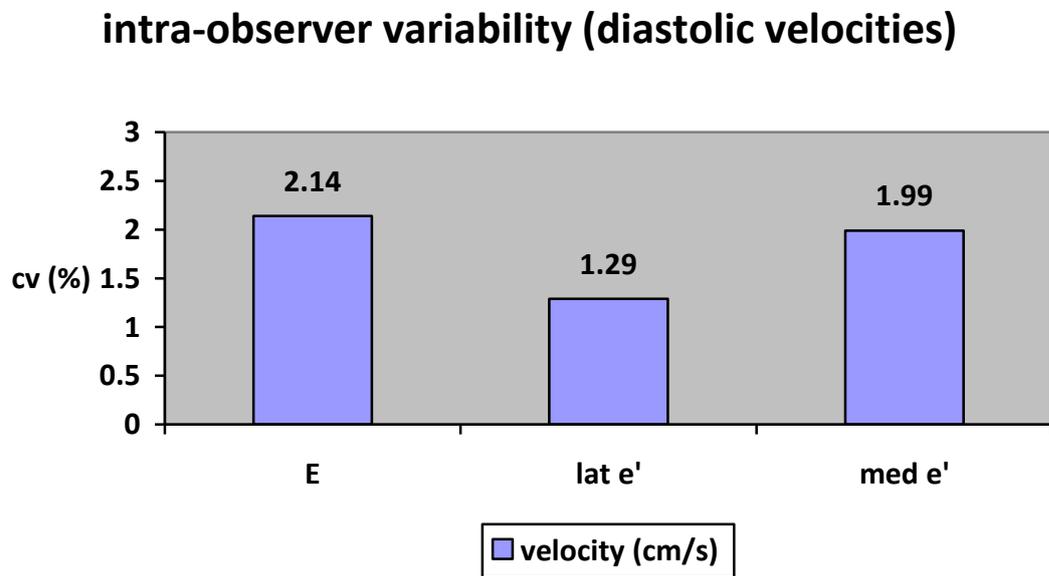
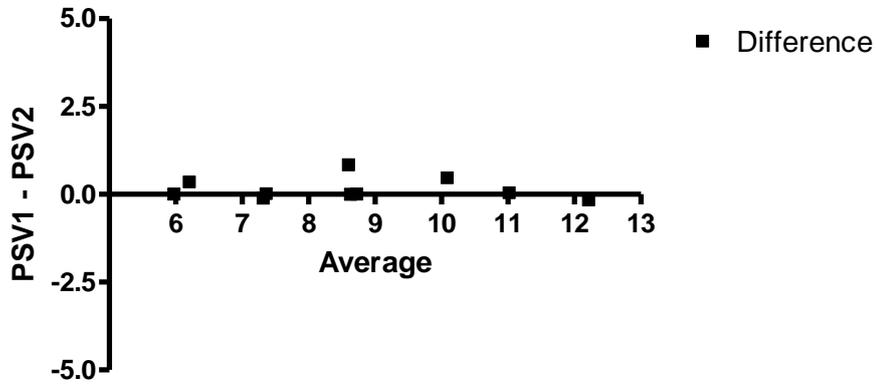
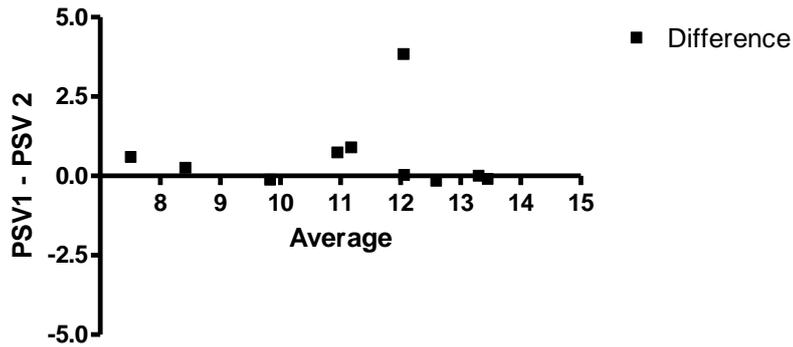


Figure 7.4: Bland-Altman analysis PSV at Basal Lateral (BL) segments

**Bland-Altman of PSV BL at rest: Difference vs average**



**Bland-Altman of PSV BL 10 $\mu$ g/kg/min : Difference vs average**



**Bland-Altman of PSV BL 30 $\mu$ g/kg/min: Difference vs average**

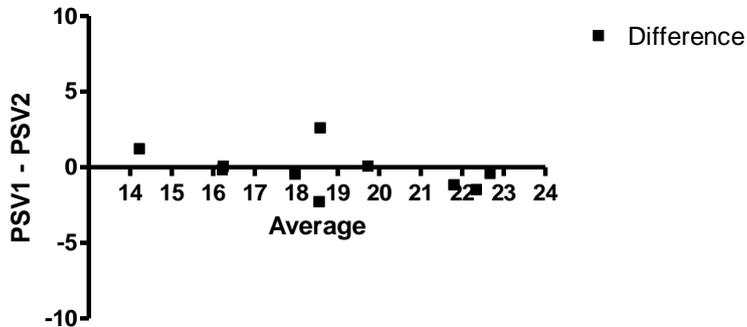
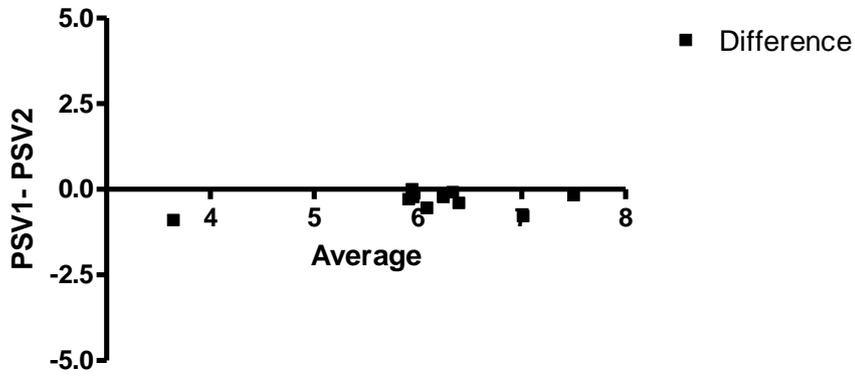
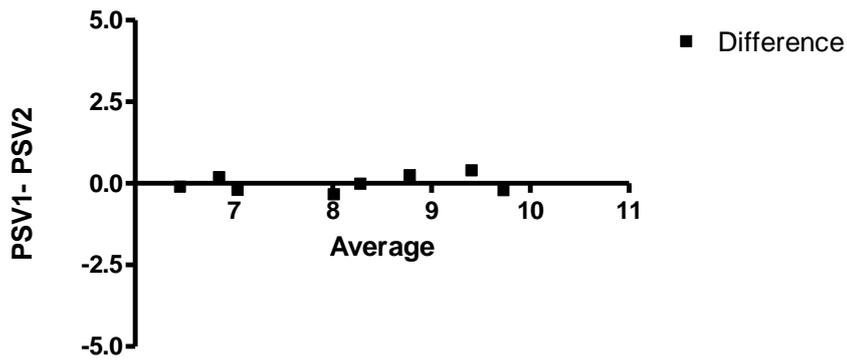


Figure 7.5: Bland-Altman analysis of PSV in Basal Septal (BS) segments

**Bland-Altman of PSV BS at rest: Difference vs average**



**Bland-Altman of PSV BS 10 $\mu$ g/kg/min: Difference vs average**



**Bland-Altman of PSV BS 30 $\mu$ g/kg/min: Difference vs average**

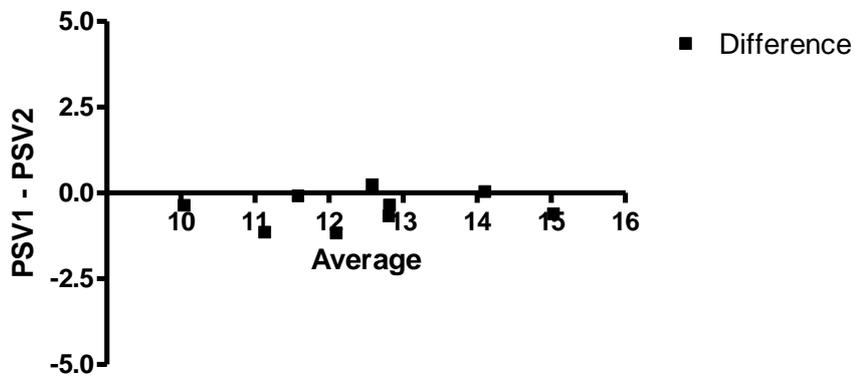
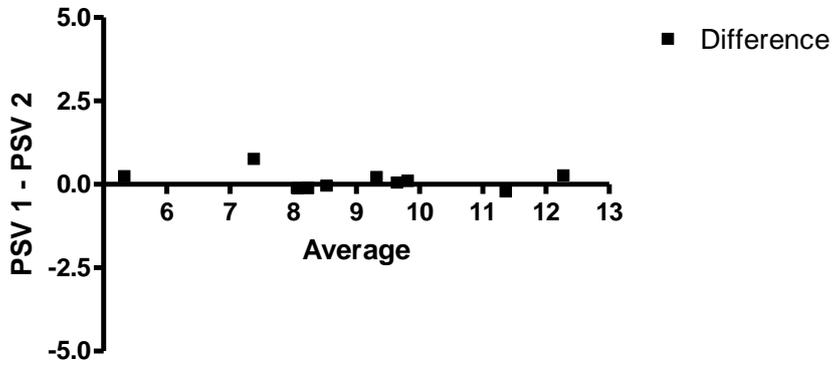
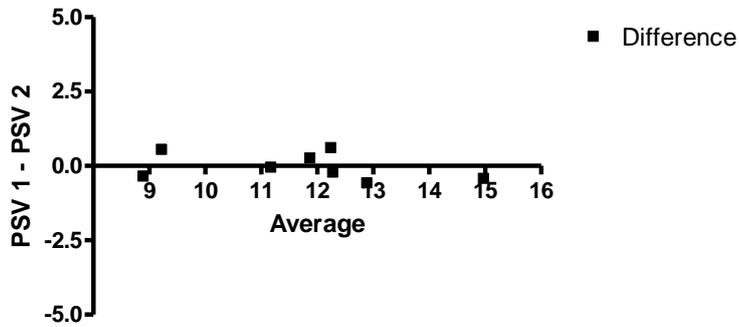


Figure 7.6: Bland-Altman Analysis of PSV in Basal Anterior (BA) segments

**Bland-Altman of PSV BA at rest: Difference vs average**



**Bland-Altman of Data PSV BA 10 $\mu$ g/kg/min: Difference vs average**



**Bland-Altman of PSV BA 30 $\mu$ g/kg/min: Difference vs average**

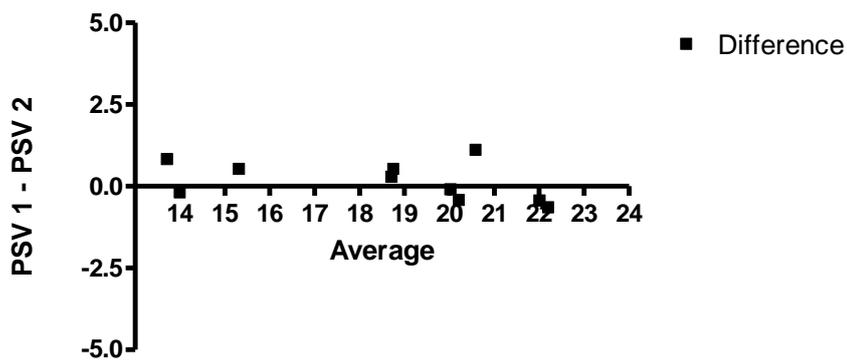
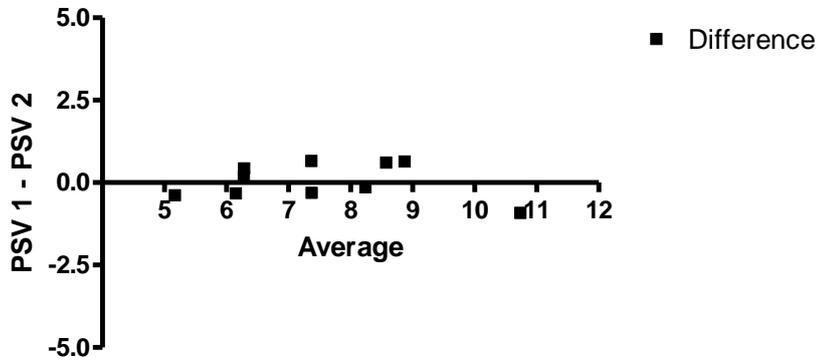
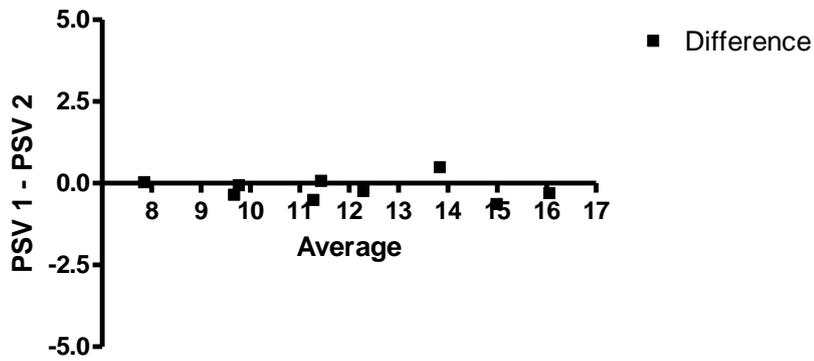


Figure: 7.7: Bland-Altman Analysis of PSV in Basal Posterior (BP) segments

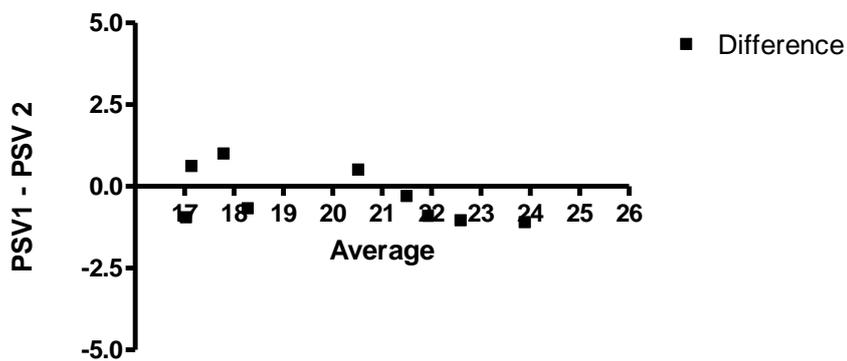
Bland-Altman of PSV BP 0  $\mu\text{g}/\text{kg}/\text{min}$ :Difference vs average



Bland-Altman of PSV BP 10  $\mu\text{g}/\text{kg}/\text{min}$ :Difference vs average



Bland-Altman of PSV BP 30  $\mu\text{g}/\text{kg}/\text{min}$ :Difference vs average



## CHAPTER 8- RESULTS -Diastolic function

### 8.1 Diastolic function results

Diastolic function in those patients who had stress echocardiography (N=39) is shown in table 8.1.

**Table 8.1: Diastolic function (type 1 diabetes mellitus vs. controls)**

	<b>Type 1 Diabetes Mellitus (n=18)</b>	<b>Controls (n=21)</b>	<b>P value</b>
<b>Mitral early diastolic velocity (E) (cm/s)</b>	82.6 ± 16.9	83.7 ± 10.8	0.80
<b>Mitral late diastolic velocity (A) (cm/s)</b>	58.6 ± 18.4	48.3 ± 10.0	0.03*
<b>E/A ratio</b>	1.5 ± 0.4	1.8 ± 0.4	0.02*
<b>Lateral mitral annular early diastolic velocity (cm/s)</b>	16.9 ± 3.4	18.7 ± 2.9	0.07
<b>Medial mitral annular early diastolic velocity (cm/s)</b>	13.0 ± 2.7	14.8 ± 2.0	0.02*
<b>E/e' ratio</b>	5.2 ± 1.1	5.0 ± 0.8	0.52

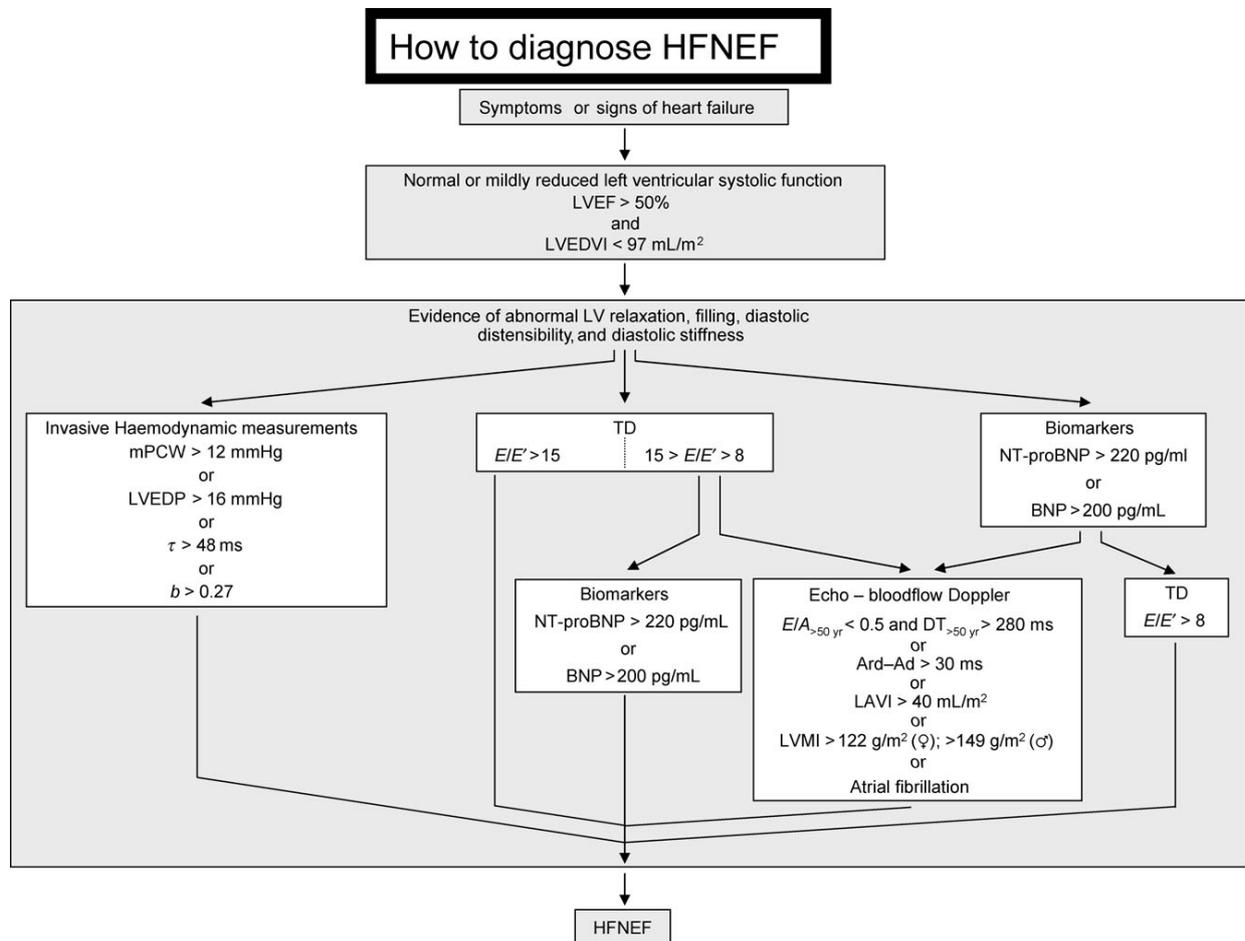
Early diastolic velocity (E) as measured by pulsed Doppler at the mitral valve showed no difference between controls and type 1 diabetes mellitus. However, the late diastolic velocity (A) of mitral inflow was observed to be higher in type 1 diabetes mellitus than in controls. The E/A ratio was lower in type 1 diabetes mellitus than in controls.

On assessment of mitral annular velocities by tissue Doppler, there was a trend for the lateral mitral annular tissue early diastolic velocity to be lower in type 1 diabetes mellitus. The medial mitral annular tissue early diastolic velocity was significantly lower in type 1 diabetes mellitus than in the controls. There was no statistically significant difference in e/e' ratio between type 1 diabetes mellitus and controls.

## ***8.2 Discussion on diastolic function***

Diastolic heart failure or heart failure with normal ejection fraction (HFNEF) may occur as a manifestation of diabetic heart muscle disease. Whether DHF or HFNEF exists as a single syndrome independent of systolic heart failure remains controversial. [218] The Heart Failure and Echocardiography Associations of the European Society of Cardiology published a consensus recommendation on the diagnosis of diastolic heart failure or HFNEF in 2007. The summary flow chart is shown in figure 8.1

**Figure 8.1: Diagnostic flowchart for the diagnosis of HFNEF**



**Figure: Diagnostic flowchart on ‘How to diagnose HFNEF’ in a patient suspected of HFNEF.** LVEDVI, left ventricular end-diastolic volume index; mPCW, mean pulmonary capillary wedge pressure; LVEDP, left ventricular end-diastolic pressure;  $\tau$ , time constant of left ventricular relaxation;  $b$ , constant of left ventricular chamber stiffness; TD, tissue Doppler;  $E$ , early mitral valve flow velocity;  $E_0$ , early TD lengthening velocity; NT-proBNP, N-terminal-pro brain natriuretic peptide; BNP, brain natriuretic peptide;  $E/A$ , ratio of early ( $E$ ) to late ( $A$ ) mitral valve flow velocity;  $DT$ , deceleration time; LVMI, left ventricular mass index; LAVI, left atrial volume index;  $Ard$ , duration of reverse pulmonary vein atrial systole flow;  $Ad$ , duration of mitral valve atrial wave flow. (reproduced from Eur Heart J 2007;28: 2539-2550.) [218]

Left ventricular diastole consists of four phases (1) isovolumetric relaxation, (2) early diastolic filling, (3) diastasis, and, (4) atrial contraction. Diastolic function of the left ventricle may be assessed using pulsed wave Doppler ultrasound most simply by measuring

the mitral inflow pattern: early diastolic velocity (E), late diastolic velocity (A), and E/A ratio. From the E wave, the deceleration time (DT) of early mitral inflow can be derived. Under normal circumstances, early filling wave E is larger than late filling wave A, which is contributed by atrial contraction. In the presence of left ventricular diastolic dysfunction, with impaired relaxation of the left ventricle, the E velocity tends to be reduced whereas the A velocity is increased, leading to reversal of E/A ratio. The utility of measurement of mitral velocities may be limited, however, because the E velocity is load- dependent, being affected by changes in preload. With impairment of diastolic function, the left ventricle relaxes slower, and it may also become stiffer. Both DT and the IVRT may be prolonged in diastolic heart failure.

Three patterns of mitral inflow related to abnormal relaxation of the left ventricle have been described. The first is abnormal relaxation with reversal of the E/A ratio. The second has been called pseudo-normalization, where the E/A ratio looks similar to a normal ratio because a compensatory increase in left atrial pressure restores the transmitral gradient to the normal range. The third pattern is termed a restrictive pattern, with a greatly increased E velocity, a short DT and a high E/A ratio in advanced diastolic failure. [219]

The early diastolic velocity of the mitral annulus, and the flow propagation velocity of mitral inflow as measured by colour M-mode ultrasound, are less load-dependent than the mitral E velocity and they are not affected by pseudo-normalization.

Many early studies reported that patients with diabetes mellitus had left ventricular diastolic dysfunction, using several different markers including abnormal mitral inflow patterns.

Regan et al conducted an invasive study involving 17 patients with type 2 diabetes mellitus but no coronary artery disease. They reported that subjects with diabetes mellitus had elevated left ventricular end-diastolic pressure volume ratio. [220]

It has been suggested that diastolic dysfunction may be one of the early manifestations of diabetic cardiomyopathy. Raev studied 157 asymptomatic young (mean 26.6 years) subjects with type 1 diabetes mellitus by M-mode echocardiography and suggested that left ventricular diastolic function was impaired before systolic function. [78]

In my study, the peak diastolic velocity during atrial contraction, (A) was higher in type 1 diabetes mellitus. As a result, the E/A ratio was lower in type 1 diabetes mellitus, indicating mild diastolic abnormality compared with the controls (although still within normal range). This is in accordance with many other studies. Zarich et al showed that E/A was significantly lower in type 1 diabetes mellitus than in controls in a study of 21 type 1 diabetics and 21 controls.[79] Similar findings were found in other studies involving type 1 diabetes mellitus. [221] [222] [223] In a few studies, the E/A ratio was reported to be normal. [96] [88]

Limitations of using the mitral inflow pattern to assess diastolic function are its load-dependency, and inability to differentiate between a normal pattern and pseudo-normalization. These limitations may be overcome by measuring other parameters of diastolic function which are less load-dependent such as mitral flow propagation and the velocities of the mitral annulus.

In my study, mitral inflow propagation was similar between type 1 diabetes mellitus and controls. Other measures of diastolic function such as IVRT and DT of E wave were also similar between the two groups. None of the subjects fulfilled criteria for the diagnosis for HFNEF. (figure 8.1)

Mean left ventricular filling pressure, as estimated by the E/e' ratio, and chronically by left atrial dimensions, was normal in the diabetic subjects and similar to the controls. These global left ventricular changes are affected once substantial amount of regional myocardium show similar changes.

Slower relaxation of the myocardium was noted at the medial mitral annulus, as the early diastolic tissue velocity was reduced in comparison with controls. The early diastolic velocity of long-axis lengthening of the left ventricle may be a more sensitive regional marker of relaxation.

These results, suggesting that subtle reductions in regional early diastolic function may be the most sensitive markers of myocardial dysfunction in patients with diabetes, are in agreement with previous reports

## CHAPTER 9- RESULTS – Stress echocardiography results

### 9.1 Baseline Characteristics (Stress Group)

Baseline characteristics of all subjects who had dobutamine stress echocardiography are shown in table 9.1.

**Table 9.1 Baseline Characteristics of the stress group (N=39)**

	<b>Type 1 Diabetes Mellitus</b>	<b>Controls</b>	<b>P value</b>
<b>Number</b>	18 ( F= 11)	21 ( F=8)	0.205
<b>Age (years)</b>	21.3 ± 3.6 (17-28)	23.0 ± 2.4 (20-28)	0.90
<b>Height (m)</b>	1.71 ± 0.10 (1.53-1.93)	1.76 ± 0.09 (1.61-1.89)	0.14
<b>Weight (kg)</b>	74.8 ± 12.4 (50-106)	73.8 ± 12.2 (52-93)	0.81
<b>BMI (kg/m<sup>2</sup>)</b>	25.4 ± 2.9 (19.5-31.3)	23.5 ± 2.2 (19.3-26.7)	0.04*
<b>Waist circumference (cm)</b>	85.2 ± 8.9 (68-106)	84.6 ± 9.6 (60-99)	0.84
<b>Waist Hip ratio (cm)</b>	0.80 ±0.07 (0.69-0.93)	0.80 ±0.07 (0.61-0.94)	0.95
<b>Pulse (beats/minute)</b>	72 ±10 (53-90)	63 ±9 (47-83)	0.005*
<b>SBP (mmHg)</b>	114 ± 9 (100-132)	116 ±10 (101-147)	0.52
<b>DBP (mmHg)</b>	72 ± 6 (62-85)	71 ± 5 (62-82)	0.52

\*Ranges of the data are presented in brackets.

There was no difference in age between type 1 diabetes mellitus and controls. However, BMI was 7.28 % higher in type 1 diabetes mellitus than controls. ( $25.4 \pm 2.9$  vs.  $23.5 \pm 2.2$ ,  $P=0.04$ ).

Pulse rate was about 12% faster in type 1 diabetes mellitus whereas blood pressure, both systolic and diastolic was similar between the two groups.

## 9.2 Biochemical Results

Biochemical results in the stress group are shown in table 9.2.

**Table 9.2 Biochemical results (stress group) (N=39)**

	<b>Type 1 Diabetes Mellitus (n=18)</b>	<b>Controls (n=21)</b>	<b>P value</b>
<b>Fasting glucose (mmol/L)</b>	11.3 ± 6.4	4.6 ± 0.4	0.00**
<b>HbA1c (%)</b>	8.8 ± 1.7	5.2 ± 0.3	0.00**
<b>Creatinine (µmol/L)</b>	72.0 ± 11.2	82.3 ± 9.6	0.004**
<b>Total Cholesterol (mmol/L)</b>	4.2 ± 0.7	3.8 ± 0.5	0.08
<b>HDL (mmol/L)</b>	1.2 ± 0.2	1.3 ± 0.2	0.06
<b>LDL (mmol/L)</b>	2.4 ± 0.8	2.2 ± 0.5	0.23
<b>TG (mmol/L)</b>	1.2 ± 0.9	0.7 ± 0.3	0.03*
<b>AST (IU/L)</b>	16.3 ± 5.7	18.3 ± 3.8	0.22
<b>hsCRP (mg/dL)</b>	1.9 ± 2.5	1.2 ± 2.3	0.36

As expected , the diabetic subjects had higher fasting glucose and HbA1c level.

Serum creatinine was higher in controls than in type 1 diabetes mellitus. There were trends for serum cholesterol to be higher in type 1 diabetes mellitus, and for serum HDL to be higher in control. Fasting serum triglycerides were higher in subjects with type 1 diabetes mellitus.

### 9.3. Myocardial Displacement

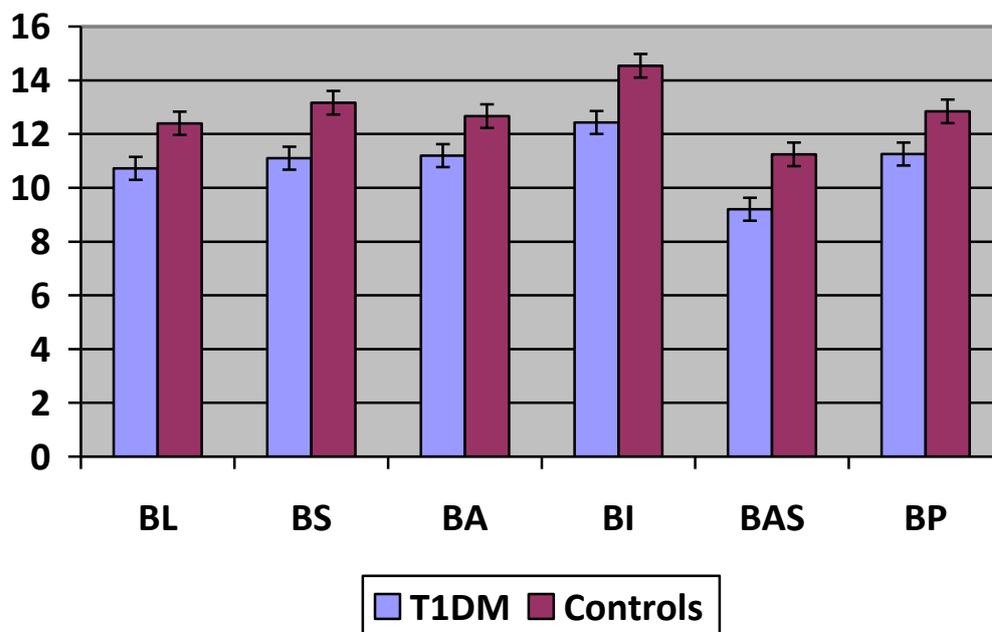
#### 9.3.1 Myocardial Displacement at rest

Myocardial displacement in mm as measured by tissue Doppler imaging or myocardial velocity imaging, at rest is shown in table 9.3. It was reduced significantly in all 6 basal segments in subjects with type 1 diabetes mellitus, compared with controls.

**Table 9.3: Myocardial displacement (mm) of basal segments at rest**

	Type 1 Diabetes Mellitus (n=18)	Controls (n=21)	P value
Basal lateral	10.72 ± 1.84	12.40 ± 2.35	0.02*
Basal septal	11.10 ± 2.17	13.16 ± 1.83	0.003*
Basal anterior	11.20 ± 2.15	12.67 ± 2.19	0.04*
Basal inferior	12.43 ± 2.53	14.54 ± 1.85	0.005*
Basal anteroseptal	9.20 ± 2.04	11.24 ± 1.81	0.002*
Basal posterior	11.26 ± 1.56	12.84 ± 2.57	0.03*

**Figure 9.1: Myocardial displacement (mm) in basal segments at rest**



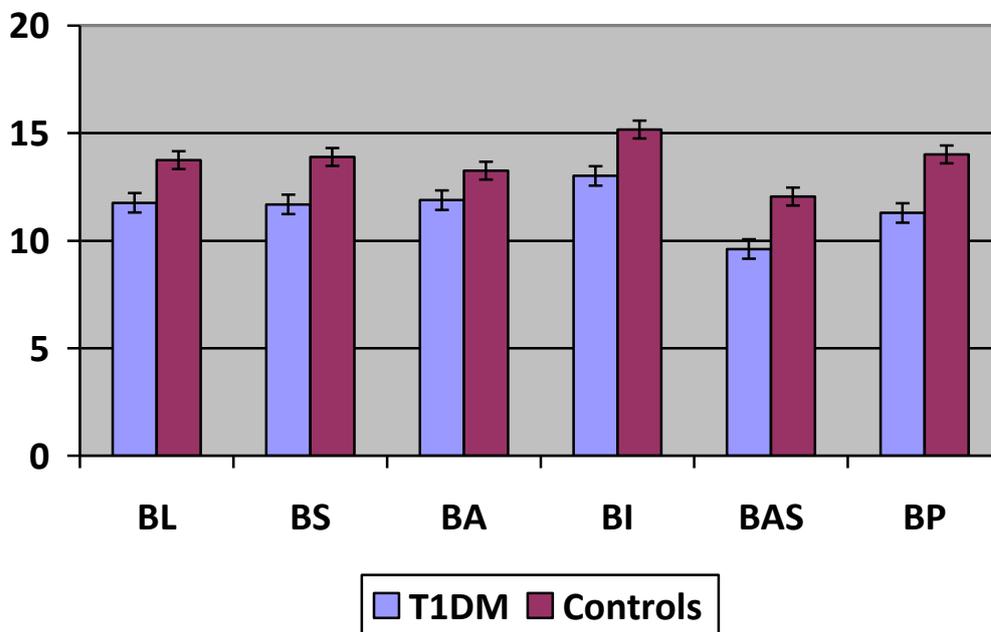
### 9.3.2. Myocardial Displacement at dobutamine dose of 5 µg/kg/min

Similar reduction of myocardial displacement in basal segment was noted in the early stage of dobutamine stress (table 9.4 and figure 9.4)

**Table 9.4: Myocardial displacement (mm) of basal segments at dobutamine dose of 5µg/kg/min**

	Type 1 Diabetes Mellitus (n=18)	Controls (n=21)	P value
Basal lateral	11.76 ± 1.96	13.75 ± 2.07	0.004*
Basal septal	11.69 ± 2.37	13.89 ± 2.13	0.004*
Basal anterior	11.89 ± 2.12	13.26 ± 2.10	0.05*
Basal inferior	13.01 ± 2.36	15.16 ± 1.90	0.003*
Basal anteroseptal	9.61 ± 1.93	12.05 ± 2.34	0.001*
Basal posterior	11.29 ± 1.85	14.01 ± 2.26	0.000*

**Figure 9.2: Myocardial displacement (mm) of basal segments at 5µg/kg/min**



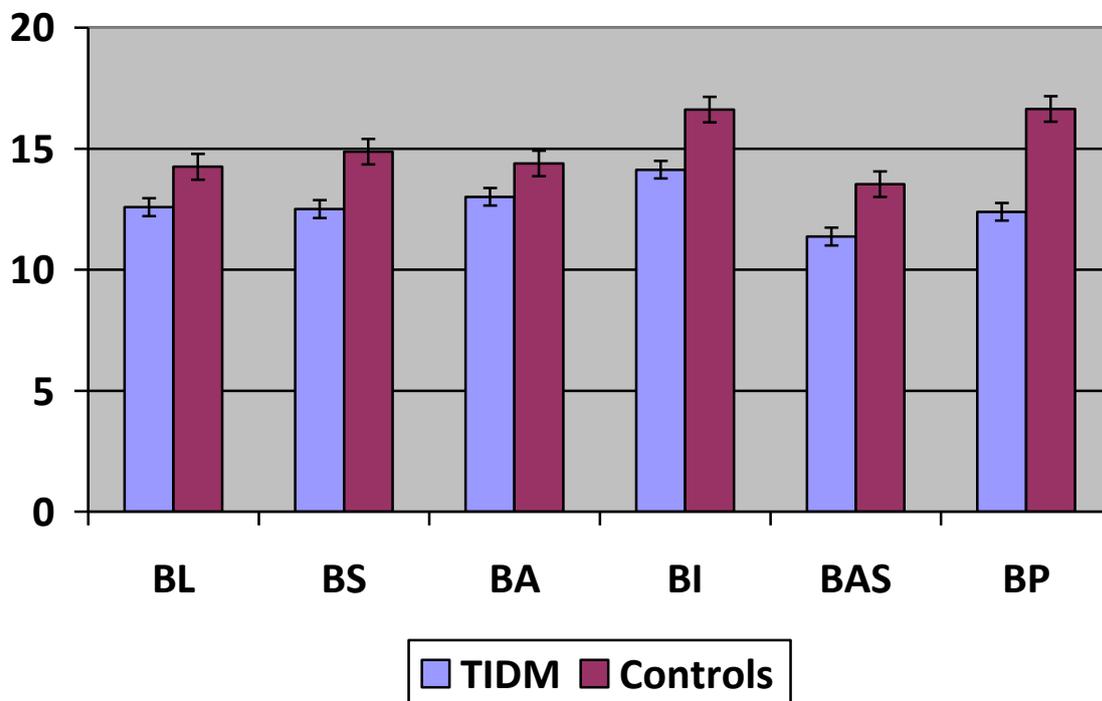
### 9.3.3 Displacement at dobutamine dose of 10 $\mu$ g/kg/min

Similar reductions in myocardial displacement were observed to be maintained at a higher dose of dobutamine infusion except in the basal anterior and basal lateral segment where trend was present but, the differences were not significant. (table 9.5 and figure 9.5)

**Table 9.5: Myocardial displacement (mm) of basal segments at 10 $\mu$ g/kg/min**

	Type 1 Diabetes Mellitus (n=18)	Controls (n=21)	P value
Basal lateral	12.58 $\pm$ 3.07	14.25 $\pm$ 2.63	0.08
Basal septal	12.50 $\pm$ 2.46	14.88 $\pm$ 2.56	0.006*
Basal anterior	13.01 $\pm$ 2.21	14.39 $\pm$ 2.40	0.07
Basal inferior	14.13 $\pm$ 2.96	16.61 $\pm$ 2.50	0.007*
Basal anteroseptal	11.37 $\pm$ 2.63	13.53 $\pm$ 2.87	0.02*
Basal posterior	12.39 $\pm$ 4.06	16.64 $\pm$ 2.95	0.001*

**Figure 9.3: Myocardial displacement (mm) of basal segments at 10 $\mu$ g/kg/min**



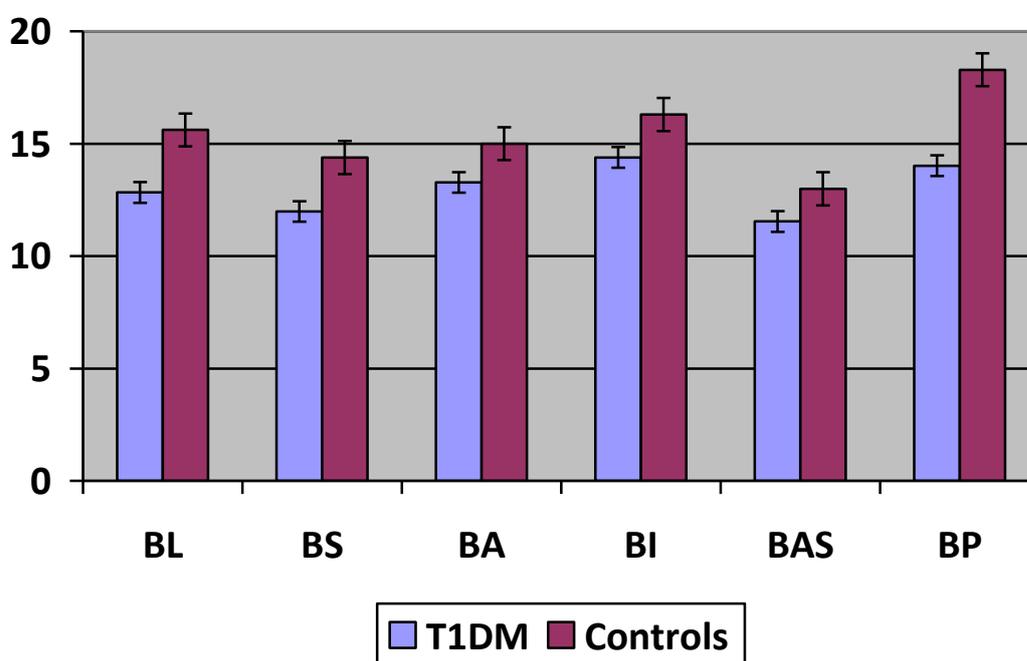
### 9.3.4 Displacement at dobutamine dose of 20 $\mu$ g/kg/min

At dobutamine dose of 20µg/kg/min, myocardial displacements at basal segments were again reduced in type 1 diabetes mellitus except for the basal anteroseptal segment where displacement was lower in type 1 diabetes mellitus but it was not statistically significant. (table 9.6 and figure 9.6)

**Table 9.6 Myocardial displacement (mm) of basal segments at 20µg/kg/min**

	<b>Type 1 Diabetes Mellitus (n=18)</b>	<b>Control (n=21)</b>	<b>P value</b>
<b>Basal lateral</b>	12.83 ± 2.93	15.61 ± 2.43	0.003*
<b>Basal septal</b>	11.99 ± 1.91	14.38 ± 2.52	0.002*
<b>Basal anterior</b>	13.28 ± 2.72	15.00 ± 2.29	0.041*
<b>Basal inferior</b>	14.39 ± 2.95	16.30 ± 2.57	0.037*
<b>Basal anteroseptal</b>	11.54 ± 2.90	12.99 ± 3.02	0.14
<b>Basal posterior</b>	14.02 ± 3.27	18.29 ± 2.32	0.000*

**Figure 9.4: Myocardial displacement (mm) of basal segments at 20µg/kg/min**



### 9.3.5 Displacement at dobutamine dose of 30µg/kg/min

Myocardial displacement in most basal segments remained lower in type 1 diabetes mellitus except in two, namely basal anterior and basal anteroseptal segments. The number of

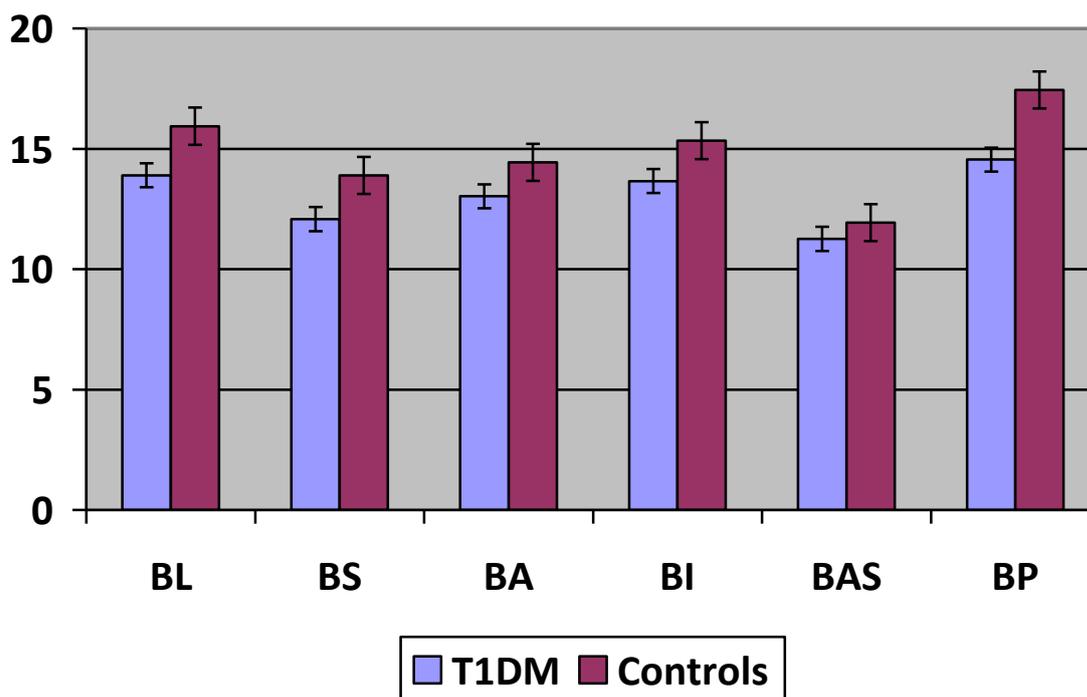
subjects reduced in both groups because of the subjects' inability to tolerate dobutamine.

(table 9.7 and figure 9.7)

**Table 9.7: Myocardial displacement (mm) of basal segments at dobutamine dose of 30µg/kg/min**

	Type 1 Diabetes Mellitus (n=16)	Control (n=20)	P value
Basal lateral	13.90 ± 2.45	15.94 ± 2.63	0.026*
Basal septal	12.08 ± 2.57	13.89 ± 1.90	0.020*
Basal anterior	13.03 ± 2.03	14.44 ± 2.82	0.10
Basal inferior	13.66 ± 2.38	15.34 ± 2.46	0.045*
Basal anteroseptal	11.26 ± 2.15	11.93 ± 2.75	0.43
Basal posterior	14.55 ± 1.45	17.44 ± 2.93	0.001*

**Figure 9.5: Myocardial displacement (mm) of basal segments at 30µg/kg/min**



### 9.3.6 Displacement at dobutamine dose of 40µg/kg/min

Many subjects and patients could not tolerate higher doses of dobutamine. (table 9.8 and figure 9.8)

**Table 9.8: Myocardial displacement (mm) at basal segments at dobutamine dose of 40µg/kg/min**

	Type 1 Diabetes Mellitus (n=10)	Control (n=13)	P value
Basal lateral	14.41 ± 3.00	15.99 ± 2.98	0.23
Basal septal	12.03 ± 1.54	13.35 ± 1.77	0.08
Basal anterior	13.21 ± 3.48	14.33 ± 2.87	0.40
Basal inferior	13.70 ± 1.95	14.43 ± 1.63	0.34
Basal anteroseptal	11.50 ± 2.12	13.01 ± 2.78	0.17
Basal posterior	15.77 ± 1.72	17.21 ± 2.66	0.15

**Figure 9.6: Myocardial displacement (mm) in basal segments at 40µg/kg/min**

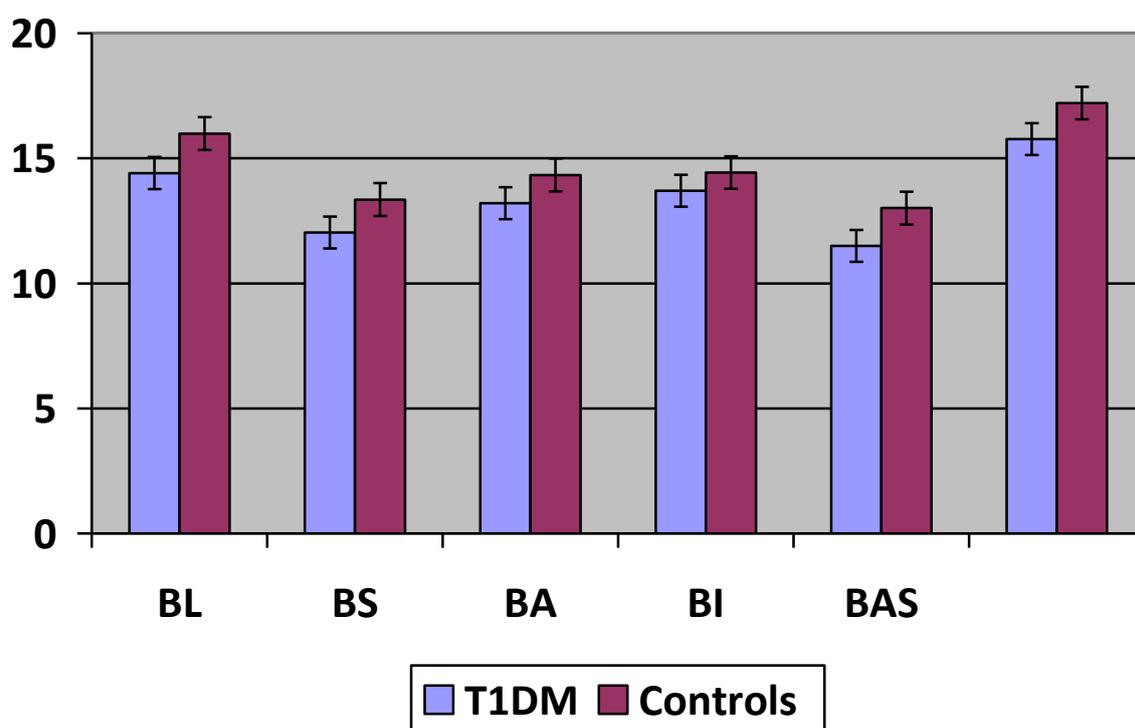
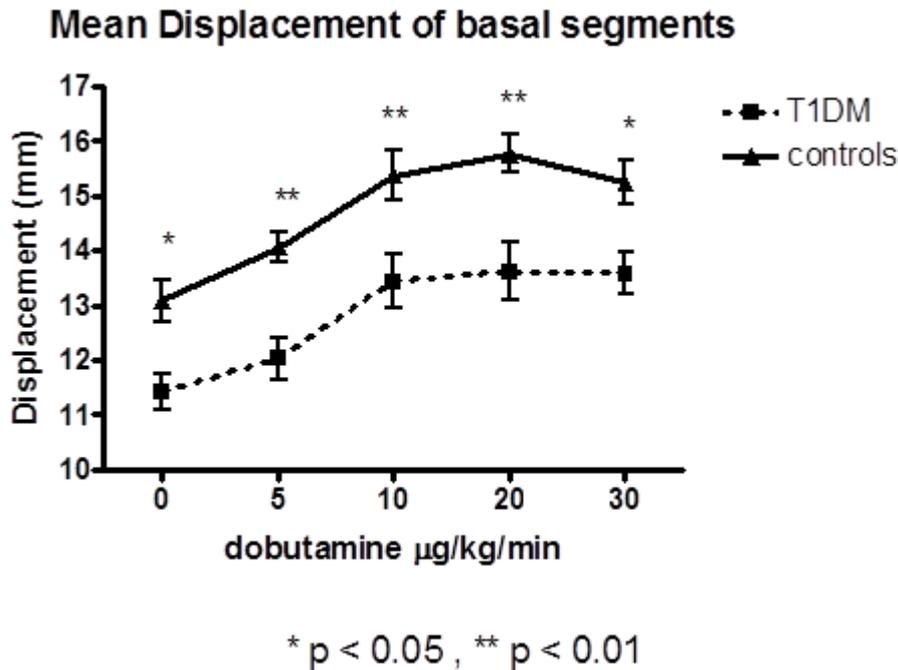


Figure 9.7: Mean basal displacement during dobutamine stress



All analyses for displacement and velocities during dobutamine stress were performed by a repeated measures analysis of variance (Linear Mixed Model) with subject treated as a random effect and dose/diabetic group as fixed effects. Dose level is the repeated element.

Mean myocardial displacement at the basal segments of myocardium at rest was lower in type 1 diabetes mellitus than in controls. Displacement increased both in subjects with type 1 diabetes mellitus and in controls, with increasing doses of dobutamine and it peaked at 20µg/kg/min of dobutamine. Thereafter displacement started to plateau in patients with type 1 diabetes mellitus and to decline in controls. Nevertheless, throughout the course of dobutamine infusion, displacements in type 1 diabetes mellitus were lower than displacements in controls. There were significant differences for both the dose (p<0.001) and between type 1 diabetes and control group (p < 0.001).

#### ***9.4 Longitudinal myocardial velocity***

Longitudinal myocardial velocities (peak systolic velocity) of all 6 basal segments at different stages of dobutamine are shown in tables 8.7-8.11 and figure 8.8-8.12) Overall, PSV was not different between the two groups in most segments during dobutamine infusion, apart from the stage of dobutamine 10 $\mu$ g/kg/min where PSV was lower in the subjects with type 1 diabetes mellitus in the basal inferior, basal anteroseptal and basal posterior segments. (table 9.11)

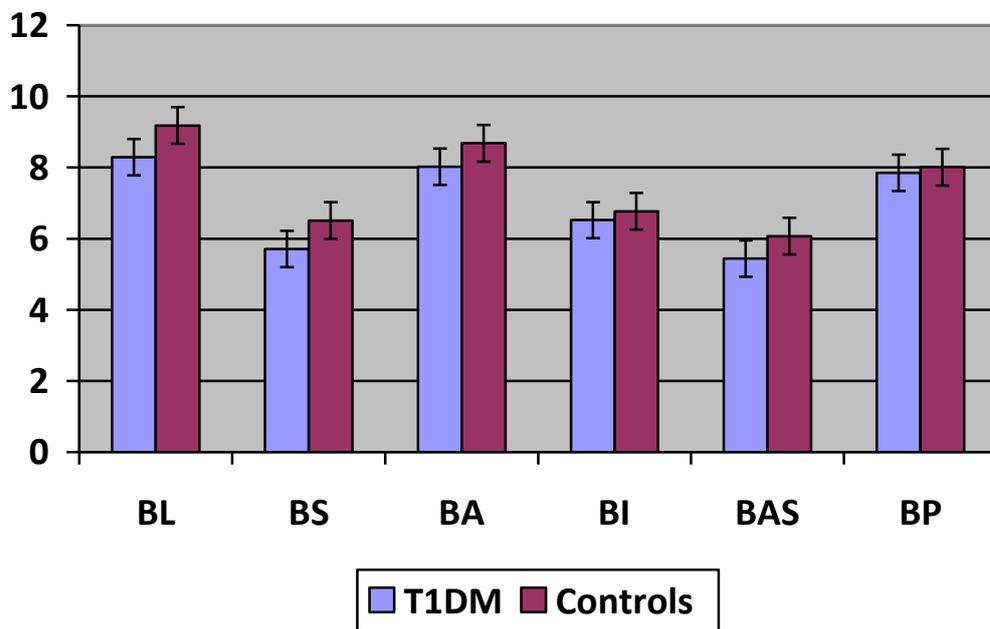
9.4.1 Longitudinal myocardial velocity (Systolic velocity, S) at rest

**Table 9.9: Peak Systolic Velocity (PSV) (cm/s) of Basal Segments at Rest**

	Type 1 Diabetes Mellitus (n=18)	Control (n=21)	P value
<b>Basal lateral</b>	8.29 ± 1.55	9.18 ± 2.04	0.14
<b>Basal septal</b>	5.71 ± 1.11	6.51 ± 1.15	0.04*
<b>Basal anterior</b>	8.02 ± 1.57	8.68 ± 2.26	0.31
<b>Basal inferior</b>	6.52 ± 1.01	6.77 ± 0.79	0.39
<b>Basal anteroseptal</b>	5.44 ± 0.93	6.07 ± 1.24	0.09
<b>Basal posterior</b>	7.85 ± 1.51	8.01 ± 1.79	0.76

Apart from basal septal segment no other statistical significant was found for peak systolic velocity (S) at rest for basal segments.

**Figure 9.8: Peak Systolic Velocity (PSV) (cm/s) of Basal Segments at Rest**

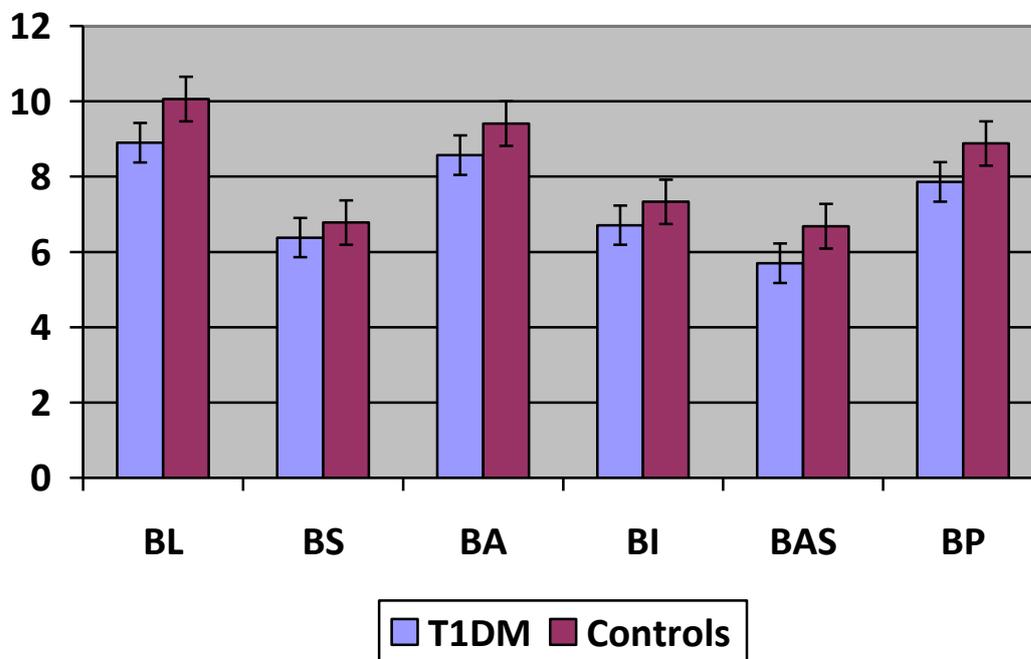


9.4.2 Longitudinal myocardial velocity (Systolic velocity, S) at dobutamine 5µg/kg/min

**Table 9.10: Peak Systolic Velocity (PSV) (cm/s) of Basal Segments at dobutamine 5µg/kg/min**

	<b>Type 1 Diabetes Mellitus (n=18)</b>	<b>Control (n=21)</b>	<b>P value</b>
<b>Basal lateral</b>	8.90 ± 2.14	10.06 ± 1.92	0.08
<b>Basal septal</b>	6.38 ± 1.24	6.78 ± 0.74	0.22
<b>Basal anterior</b>	8.57 ± 2.06	9.41 ± 2.11	0.22
<b>Basal inferior</b>	6.71 ± 1.16	7.33 ± 1.00	0.08
<b>Basal anteroseptal</b>	5.70 ± 0.82	6.68 ± 1.31	0.009*
<b>Basal posterior</b>	7.86 ± 1.94	8.88 ± 1.74	0.09

**Figure 9.9: Peak Systolic Velocity (PSV) (cm/s) of Basal Segments at dobutamine 5µg/kg/min**

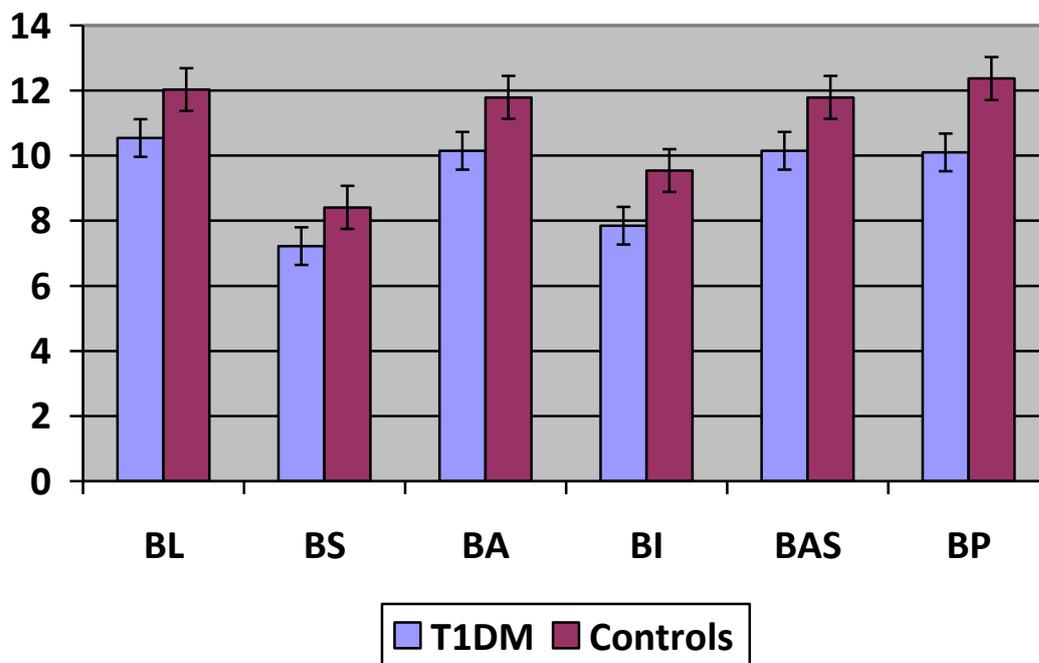


9.4.3 Longitudinal myocardial velocity (Systolic velocity, S) at dobutamine 10µg/kg/min

**Table 9.11: Peak Systolic Velocity (PSV) (cm/s) of Basal Segments at dobutamine 10µg/kg/min**

	<b>Type 1 Diabetes Mellitus (n=18)</b>	<b>Control (n=21)</b>	<b>P value</b>
<b>Basal lateral</b>	10.54 ± 3.05	12.03 ± 2.63	0.11
<b>Basal septal</b>	7.22 ± 1.98	8.41 ± 2.02	0.07
<b>Basal anterior</b>	10.15 ± 2.67	11.79 ± 2.80	0.07
<b>Basal inferior</b>	7.85 ± 2.06	9.54 ± 2.39	0.02*
<b>Basal anteroseptal</b>	10.15 ± 2.67	11.79 ± 2.80	0.04*
<b>Basal posterior</b>	10.10 ± 3.49	12.37 ± 2.59	0.03*

**Figure 9.10: Peak Systolic Velocity (PSV) (cm/s) in Basal Segments at dobutamine 10µg/kg/min**

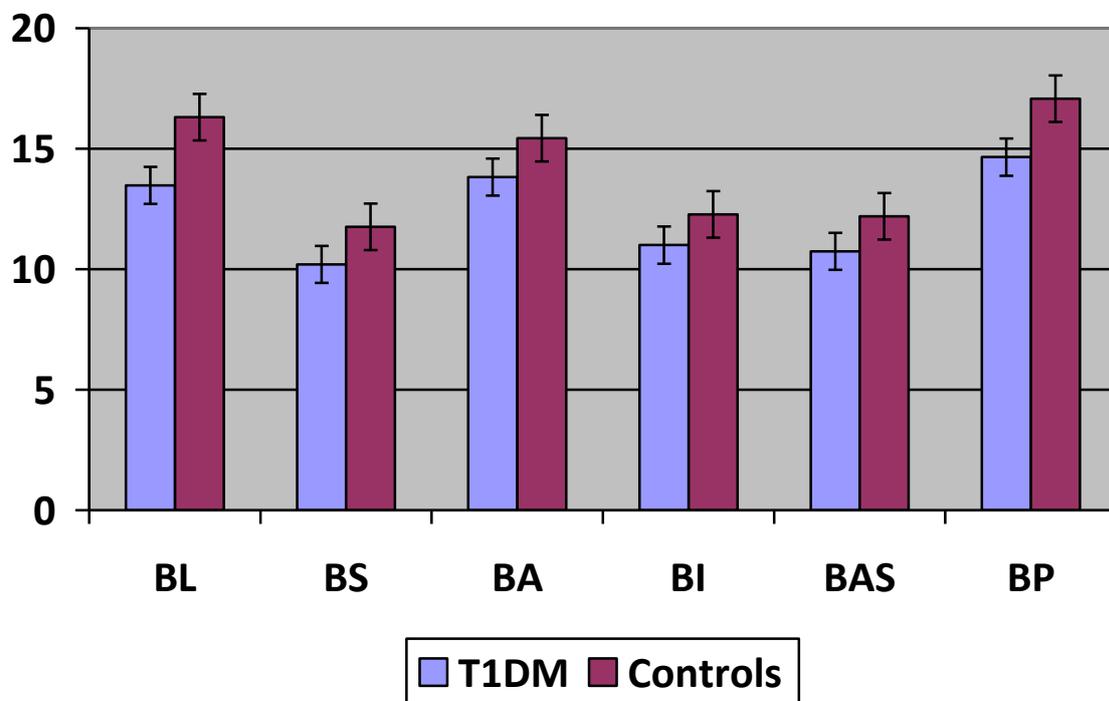


9.4.4 Longitudinal myocardial velocity (Systolic velocity, S) at dobutamine 20µg/kg/min

**Table 9.12: Peak Systolic Velocity (PSV) (cm/s) of Basal Segments at dobutamine 20µg/kg/min**

	Type 1 Diabetes Mellitus (n=18)	Control (n=21)	P value
<b>Basal lateral</b>	13.48 ± 3.10	16.31 ± 2.67	0.005*
<b>Basal septal</b>	10.20 ± 2.70	11.76 ± 2.63	0.08
<b>Basal anterior</b>	13.82 ± 3.70	15.44 ± 2.59	0.13
<b>Basal inferior</b>	11.00 ± 2.83	12.27 ± 2.59	0.15
<b>Basal anteroseptal</b>	10.74 ± 2.90	12.20 ± 3.04	0.14
<b>Basal posterior</b>	14.65 ± 3.05	17.08 ± 2.76	0.02*

**Figure 9.11: Peak Systolic Velocity (PSV) (cm/s) in Basal Segments at dobutamine 20µg/kg/min**

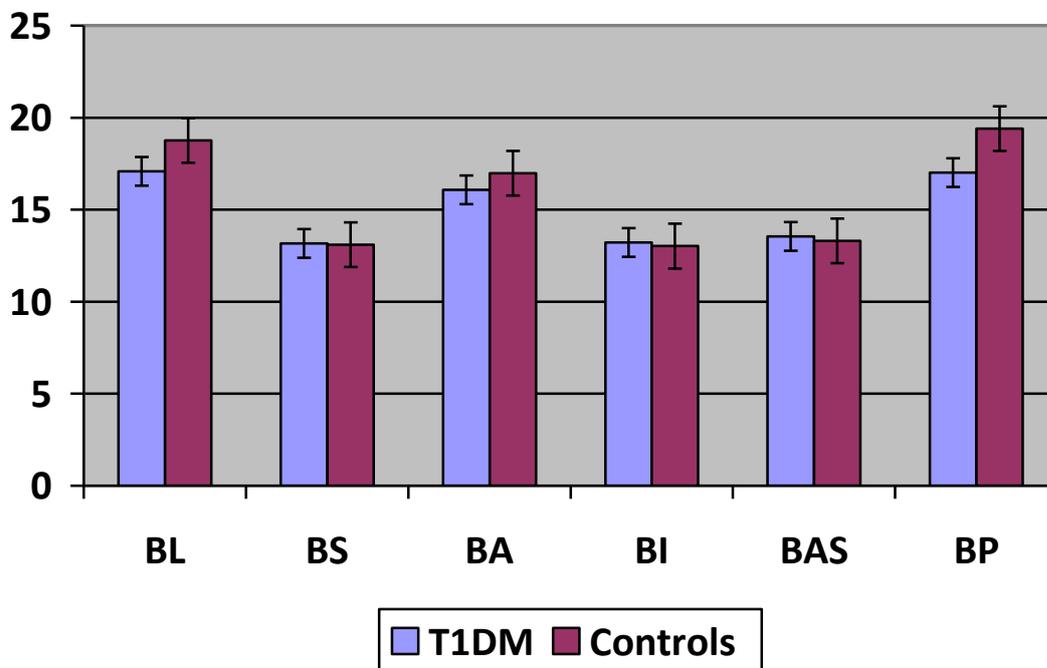


9.4.5 Longitudinal myocardial velocity (systolic velocity, S) at dobutamine 30µg/kg/min

**Table 9.13: Peak Systolic Velocity (PSV) (cm/s) of Basal Segments at dobutamine 30µg/kg/min**

	Type 1 Diabetes Mellitus (n=15)	Control (n=20)	P value
<b>Basal lateral</b>	17.08 ± 2.96	18.76 ± 2.69	0.09
<b>Basal septal</b>	13.16 ± 2.41	13.10 ± 2.37	0.95
<b>Basal anterior</b>	16.08 ± 3.00	16.98 ± 3.12	0.40
<b>Basal inferior</b>	13.22 ± 1.95	13.02 ± 2.19	0.78
<b>Basal anteroseptal</b>	13.55 ± 1.99	13.31 ± 2.57	0.77
<b>Basal posterior</b>	17.01 ± 3.5	19.40 ± 3.04	0.04*

**Figure 9.12: Peak Systolic Velocity (PSV) (cm/s) of Basal Segments at dobutamine 30µg/kg/min**



9.4.6 Longitudinal myocardial velocity (systolic velocity, S) at dobutamine 40µg/kg/min

**Table 9.14: Peak Systolic Velocity (PSV) (cm/s) of Basal Segments at dobutamine 40µg/kg/min**

	<b>Type 1 Diabetes Mellitus (n=10)</b>	<b>Control (n=14)</b>	<b>P value</b>
<b>Basal lateral</b>	18.01 ± 3.86	20.22 ± 2.52	0.10
<b>Basal septal</b>	14.04 ± 1.61	13.48 ± 2.35	0.52
<b>Basal anterior</b>	17.23 ± 3.96	17.86 ± 2.46	0.64
<b>Basal inferior</b>	14.34 ± 2.23	13.76 ± 1.94	0.50
<b>Basal anteroseptal</b>	15.29 ± 1.94	14.65 ± 3.51	0.61
<b>Basal posterior</b>	19.97 ± 4.29	20.69 ± 2.99	0.64

**Figure 9.13: Peak Systolic Velocity (PSV) (cm/s) of Basal Segments at dobutamine 40µg/kg/min**

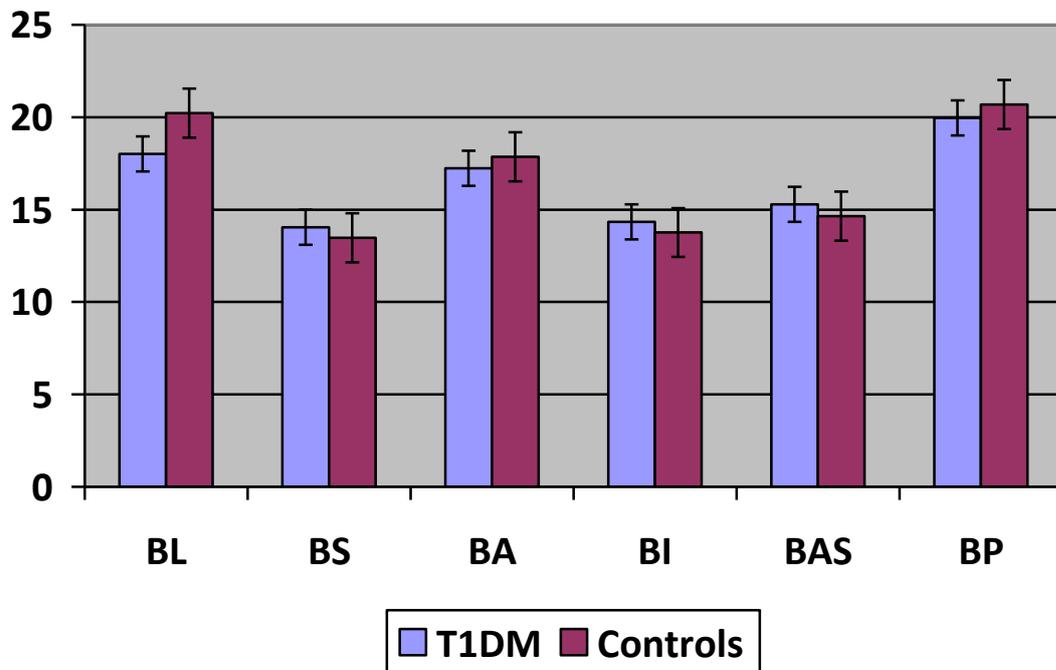
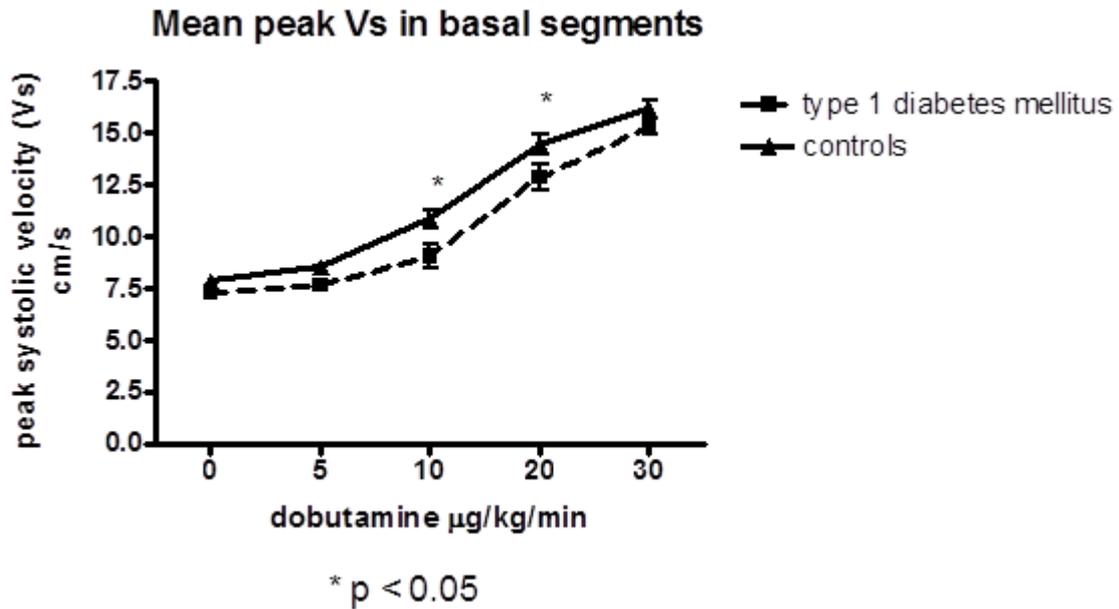


Figure 9.14: Mean peak systolic velocity during dobutamine stress



The peak systolic velocity of long-axis shortening, averaged from 6 basal myocardial segments, was similar at rest in the diabetic subjects to the controls but it increased more slowly during the infusion of dobutamine with significantly lower values at 10 and 20 µg/kg/minute. The velocities at higher doses (30 µg/kg/minute) were similar. Similar to displacement, there was no interaction and there were significant differences for dose ( $p < 0.001$ ) and between type 1 diabetes mellitus and control groups ( $p = 0.003$ ).

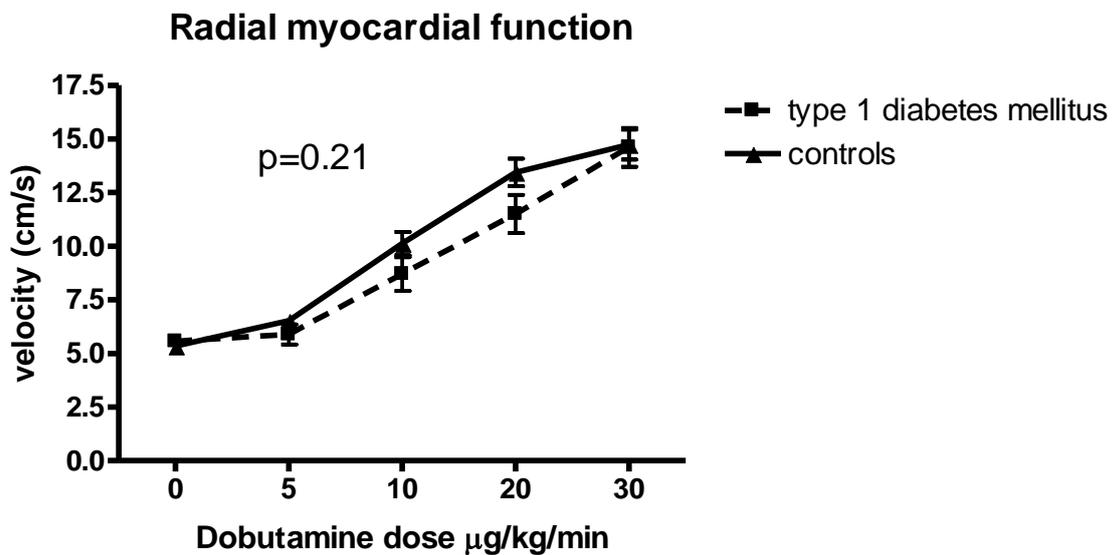
### 9.5 Radial myocardial function

Radial myocardial function as measured in the basal posterior segment in the posterior wall in a parasternal long-axis view is shown in table 9.15 and figure 9.17.

**Table 9.15: Radial myocardial function (systolic velocity) (cm/s) during dobutamine stress**

	Type 1 Diabetes Mellitus	Control	P value
at rest	5.56 ± 0.99	5.02 ± 1.41	0.15
5µg/kg/min	5.91 ± 1.54	6.51 ± 1.42	0.22
10µg/kg/min	9.22 ± 2.89	9.92 ± 2.50	0.43
20µg/kg/min	11.97 ± 3.53	13.37 ± 2.73	0.18
30µg/kg/min	14.88 ± 3.40	14.58 ± 2.95	0.78
40µg/kg/min	16.06 ± 2.65	14.69 ± 2.35	0.22

**Figure 9.15: Radial myocardial function during dobutamine stress**



There were no differences in radial function between controls and diabetics at rest or during dobutamine (two way ANOVA,  $p = 0.21$ ).

### ***9.6 Discussions on left ventricular systolic function and radial myocardial function***

Global left ventricular systolic function, as indicated by ejection fraction, was similar in the type 1 diabetic subjects to controls and both were within the normal range, but longitudinal systolic function as indicated by excursion of the medial mitral annulus was reduced by an average of 11%. This site, at the base of the ventricular septum, is the one which shows the earliest signs of ageing and of subclinical myocardial disease in many conditions, perhaps because the basal septal segment has the largest radius of curvature and therefore the greatest regional wall stress.

Longitudinal displacements of the myocardium in the basal segments are persistently lower in type 1 diabetes mellitus both at rest and during dobutamine stress even though systolic velocities are similar between groups. This might indicate that displacement may be a more sensitive marker than peak systolic velocity in detecting early subtle changes in subclinical myocardial dysfunction in type 1 diabetes mellitus.

A reduction of longitudinal function with preservation of global function usually implies that there has been a change in shape of the left ventricle so that it becomes more spherical. In earlier studies of type 2 diabetic subjects, reduced longitudinal function was associated with an increase in radial function [85] [89] perhaps because a more spherical end-diastolic shape increases the end-diastolic length of the midwall myocardial fibres which then contract more as a result of the Frank Starling relationship. Nevertheless, in a study of type 2 diabetes mellitus patients by Ernande et al, systolic radial function was reduced in type 2 diabetes mellitus on echocardiography by speckle tracking method. [90] This may just be a

reflection of different stages in the natural history of the disease in the subjects studied in those studies or it might be related to technical factors related to different imaging modalities.

In the type 1 diabetic subjects in my present study, however, there was no significant increase in radial function, measured either as fractional shortening (which was 6% higher in the diabetics, n.s.) or as the systolic velocity of inward motion of the basal posterior wall in a parasternal image. Given their relatively short duration of diabetes and younger age, the diabetic subjects in this study may be at an earlier stage in the subclinical progression of myocardial dysfunction.

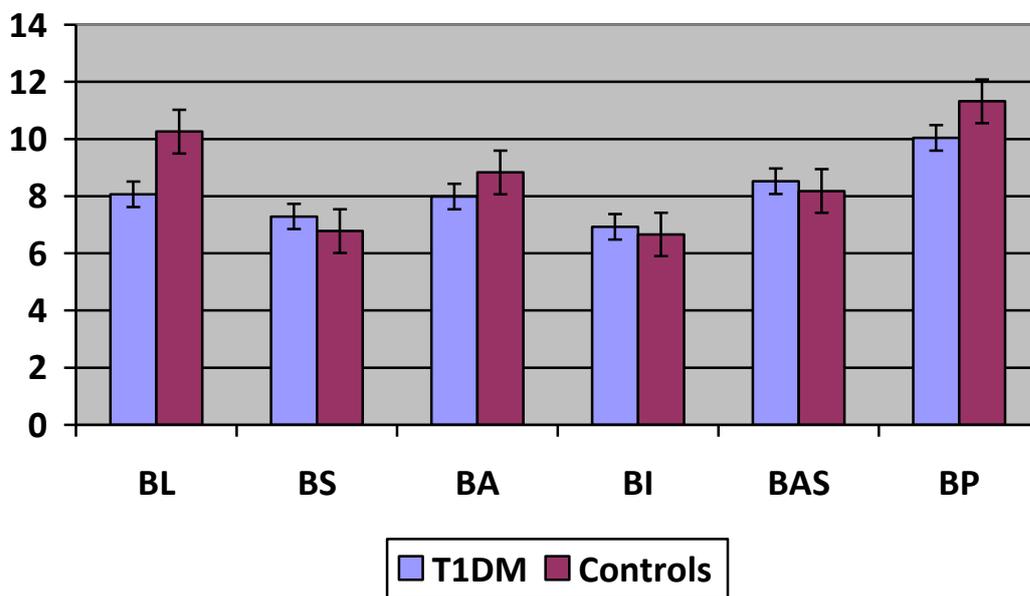
### 9.7 Myocardial functional reserve

Myocardial functional reserve, which is the absolute increment of PSV at maximal dobutamine dose from PSV at rest, is shown in table 9.16 and figure 9.18.

**Table 9.16: Myocardial functional reserve (cm/s) in basal segments**

	Type 1 Diabetes Mellitus	Control	P value
Basal lateral	8.07 ± 3.06	10.26 ± 2.36	0.02*
Basal septal	7.29 ± 2.45	6.78 ± 1.85	0.47
Basal anterior	7.99 ± 3.27	8.83 ± 2.93	0.41
Basal inferior	6.93 ± 2.84	6.66 ± 1.79	0.73
Basal anteroseptal	8.52 ± 2.57	8.18 ± 2.86	0.71
Basal posterior	10.04 ± 4.38	11.32 ± 4.58	0.40

**Figure 9.16: Myocardial function reserve (cm/s) in six basal segments**



Myocardial functional reserve was not different between subjects with type 1 diabetes and controls, except in the basal lateral segment where it was 21% lower in the diabetics (p = 0.018)

## ***9.8 Discussions on myocardial functional reserve***

Palmieri et al studied 20 patients with type 1 diabetes mellitus by giving low dose dobutamine with a peak dose of 7.5 $\mu$ g/kg/minute and reported that peak systolic lateral mitral annular velocity was lower in type 1 diabetes mellitus at rest in comparison with controls. [104] The control groups in their study did not receive dobutamine and therefore, functional reserve were not compared between the groups in that study. In a different study by Palmieri et al, they reported that stroke volume index and cardiac index (measured by bioimpedance) were the same in patients with type 1 diabetes compared with normotensive and hypertensive controls at rest, but they were reduced at peak exercise. [105]

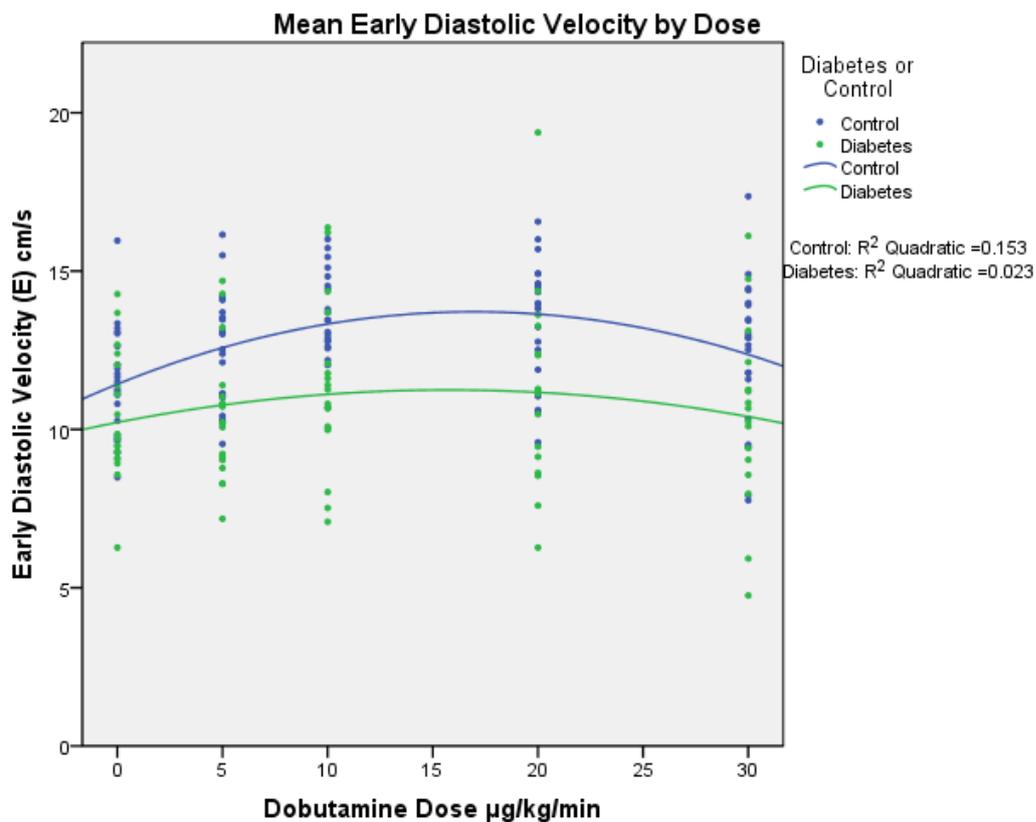
In previous studies of patients with type 2 diabetes mellitus, the peak systolic velocity of long-axis shortening of the left ventricle ( $V_s$ ) was reduced, both at rest and at peak stress with dobutamine [89] or only during peak stress. [86] A reduction in left ventricular functional reserve in type 2 diabetes mellitus was also reported by Von Bibra et al. [95] In our study of subjects with type 1 diabetes, however, peak systolic velocity ( $V_s$ ) did not show any difference between patients and controls at rest or at peak doses; it was significantly lower only at intermediate doses of dobutamine (10  $\mu$ g/kg/minute and 20  $\mu$ g/kg/minute).

The hypothesis that myocardial response to dobutamine would be blunted in type 1 diabetes mellitus, therefore, was not confirmed.

### 9.9 Early diastolic velocity during dobutamine stress

Mean early diastolic velocity responses to dobutamine are shown in figure 9.19.

**Figure 9.17: Early diastolic velocity during dobutamine stress**



There is no significant interaction ( $p=0.996$ ). Mean early diastolic velocity (tissue) response to dobutamine is not different between type 1 diabetes mellitus and control ( $p=0.60$ )

However, as can be observed from the figure, early diastolic velocity response in type 1 diabetes mellitus is somewhat flat whereas in control it increases incrementally from rest to dobutamine dose of 10 µg/kg/minute.

### 9.10 Strain and strain rate

Systolic strain, peak systolic strain and early diastolic strain rates are shown in table

9.17.

**Table 9.17: Strain and strain rate in basal septal and basal lateral segments**

			<b>Type 1 Diabetes Mellitus (N=18)</b>	<b>Controls (N=21)</b>	<b>P-value</b>
<b>Basal Septal segment</b>	<b>Systolic strain (%)</b>	<b>at rest</b>	-19.8 ± 6.1	-24.5 ± 5.2	0.014*
		<b>20µg/kg/min</b>	-26.3 ± 5.8	-24.8 ± 6.2	0.44
		<b>30µg/kg/min</b>	-26.8 ± 7.9	26.7 ± 6.9	0.97
	<b>Peak systolic strain rate (l/s)</b>	<b>at rest</b>	-1.5 ± 1.0	-1.9 ± 1.1	0.31
		<b>20µg/kg/min</b>	-2.3 ± 0.66	-2.5 ± 0.71	0.38
		<b>30µg/kg/min</b>	-2.8 ± 0.99	-3.0 ± 1.4	0.63
	<b>Early diastolic strain rate (l/s)</b>	<b>at rest</b>	2.2 ± 0.86	2.5 ± 0.9	0.37
		<b>20µg/kg/min</b>	2.7 ± 1.05	2.9 ± 1.05	0.59
		<b>30µg/kg/min</b>	3.5 ± 1.6	3.6 ± 1.3	0.76
<b>Basal Lateral segment</b>	<b>Systolic strain (%)</b>	<b>at rest</b>	-14.3 ± 6.9	-17.0 ± 6.7	0.23
		<b>20µg/kg/min</b>	-15.8 ± 5.8	-17.4 ± 5.8	0.44
		<b>30µg/kg/min</b>	-14.8 ± 5.9	-16.5 ± 5.9	0.40
	<b>Peak systolic strain rate (l/s)</b>	<b>at rest</b>	-1.4 ± 0.61	-1.4 ± 0.63	0.86
		<b>20µg/kg/min</b>	-1.8 ± 1.1	-2.1 ± 0.71	0.25
		<b>30µg/kg/min</b>	-1.9 ± 0.93	-2.3 ± 1.3	0.27
	<b>Early diastolic strain rate (l/s)</b>	<b>at rest</b>	1.8 ± 0.7	2.03 ± 0.9	0.42
		<b>20µg/kg/min</b>	2.5 ± 1.6	2.4 ± 1.0	0.77
		<b>30µg/kg/min</b>	2.3 ± 1.3	2.9 ± 1.1	0.17

Apart from systolic strain at rest in the basal segment which was lower in type 1 diabetes mellitus, measurements of strain, and strain rates were similar between the two groups.

### ***9.11 Discussion on strain and strain rate***

The majority of studies of myocardial function in diabetes mellitus used myocardial velocity rather than strain and strain rate. Two studies [98] [90] did investigate strain and strain rate in diabetes mellitus, at rest. Di Cori et al [98] reported lower peak systolic strain in type 1 diabetes mellitus. Ernande et al [90] showed lower longitudinal strain on speckle tracking in basal and mid segments of the septal and lateral walls in type 2 diabetes mellitus. No study in type 1 diabetes mellitus using strain and strain rate during stress was found. Even though a difference in displacement during dobutamine infusion was observed in this study, no difference was found for systolic strain, peak systolic strain rate and early diastolic strain rate.

### 9.12 Heart rate, systolic and diastolic blood pressure responses during dobutamine stress

Changes in heart rate, systolic blood pressure, and diastolic blood pressure in response to dobutamine infusion are shown in table 9.18-9.20.

**Table 9.18: Heart rate (beats/minute) response to dobutamine infusion**

	Type 1 diabetes mellitus (n=18)	Control (n=21)	P-value
at rest	75 ± 11	62 ± 10	0.001*
5µg/kg/min	77 ± 13	63 ± 10	0.000*
10µg/kg/min	78 ± 12	70 ± 11	0.034*
20µg/kg/min	98 ± 24	93 ± 16	0.42
30µg/kg/min	120 ± 20	114 ± 19	0.37

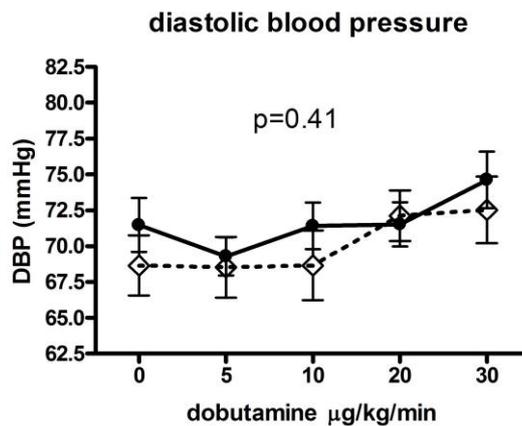
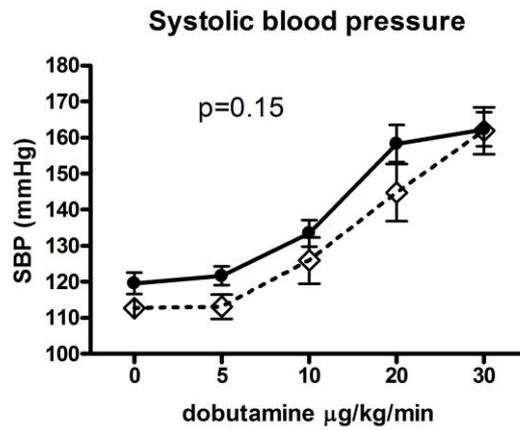
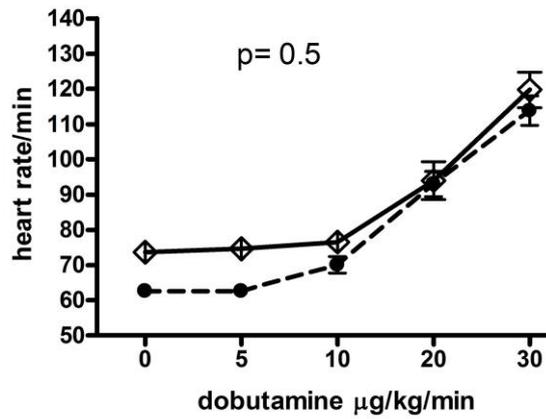
**Table 9.19: Systolic blood pressure (mmHg) response to dobutamine infusion**

	Type 1 diabetes mellitus (n=18)	Control (n=21)	P-value
at rest	114 ± 9	120 ± 14	0.12
5µg/kg/min	114 ± 13	122 ± 12	0.06
10µg/kg/min	127 ± 26	133 ± 17	0.32
20µg/kg/min	144 ± 29	158 ± 24	0.10
30µg/kg/min	159 ± 27	162 ± 21	0.70

**Table 9.20: Diastolic blood pressure (mmHg) response to dobutamine infusion**

	Type 1 diabetes mellitus (n=18)	Control (n=21)	P-value
at rest	70 ± 9	71 ± 9	0.57
5µg/kg/min	70 ± 11	69 ± 6	0.71
10µg/kg/min	70 ± 11	69 ± 6	0.54
20µg/kg/min	72 ± 8	72 ± 7	0.61
30µg/kg/min	73 ± 9	75 ± 9	0.50

Figure 9.18: changes in heart rate, SBP and DBP during dobutamine stress



◇ = type 1 diabetes mellitus; ● = control

As can be seen the figure 9.20, heart rate was higher in type 1 diabetes mellitus at rest. Heart rate in controls increased incrementally from a dobutamine dose of 5 µg/kg/minute and

was similar to that of the subjects with type 1 diabetes mellitus at dobutamine doses  $\geq 10\mu\text{g}/\text{kg}/\text{minute}$ . The heart rate in type 1 diabetes mellitus was higher at rest and the response to dobutamine was somewhat blunted when compared to controls between dobutamine doses of  $5\mu\text{g}/\text{kg}/\text{minute}$  and  $10\mu\text{g}/\text{kg}/\text{minute}$ .

No significance differences were observed in terms of SBP and DBP responses to dobutamine infusion in type 1 diabetes mellitus and controls.

### ***9.13 Discussion on heart rate and blood pressure response to dobutamine***

Dobutamine exerts its inotropic effect mainly via  $\beta_1$  receptor in the myocardium even though it has some actions via  $\beta_2$  and  $\alpha_1$  receptors. [224] Vasodilatory effects of  $\beta_2$  are normally countered by vasoconstrictive effects of  $\alpha_1$  and net changes in blood pressure are minimal. It is well recognized that heart rate and systolic blood pressure increases with dobutamine, but diastolic blood pressure normally does not increase significantly or may even decrease with dobutamine infusion. [225] [226] [227]

Therefore, changes in blood pressure observed in my study are consistent with normal responses to dobutamine infusion in both type 1 diabetes mellitus and controls. Nevertheless, blunted heart rate responses in type 1 diabetes mellitus after dobutamine dose of  $10\mu\text{g}/\text{kg}/\text{minute}$  may be due to denervation of receptors in the myocardium in type 1 diabetes mellitus. In animal studies, there is some in-vitro evidence of  $\beta_2$  receptor attenuation in cardiomyocytes in the hyperglycaemic state. [228] Moreover, treatment with the  $\beta$  receptor antagonist, metoprolol, prevents cardiomyocyte apoptosis in streptozotocin-induced diabetic mice. [229]

### ***9.14 Effect of exercise on myocardial function***

The effect of exercise on myocardial function was determined by stratifying subjects into three groups based on their exercise history in terms of metabolic equivalent per week. All analyses were performed by Repeated Measures Analysis of Variance (Linear Mixed Model) with subject treated as a Random effect and Dose/Diabetic Group as fixed effects. Dose level is the repeated element.

There was no significant interaction between diagnostic group and changes in myocardial displacement and early diastolic velocity during stress echocardiography, related to the previous exercise history of the subjects. ( $p=0.12$  and  $p=0.56$  respectively) There was a weak interaction between diagnostic group and the responses of myocardial systolic velocity, according to exercise history ( $p=0.039$ ).

### ***9.15 Discussion on the effect of exercise***

The effect of exercise of myocardial function is well-recognized. Most studies on myocardial function in diabetes mellitus failed to include exercise history which is a potential confounder in assessing longitudinal myocardial function.

In my study, detailed exercise history was taken and the effects of exercise on myocardial function in type 1 diabetes mellitus during dobutamine stress were further analysed. The findings imply that those changes in longitudinal myocardial function observed in my study are unlikely to be due to physical inactivity of the subjects with diabetes mellitus, or conversely due to increased responses because of greater physical fitness in the controls.

## CHAPTER 10- RESULTS - Correlates of myocardial function

### 10.1 Correlates of myocardial function (stress group)

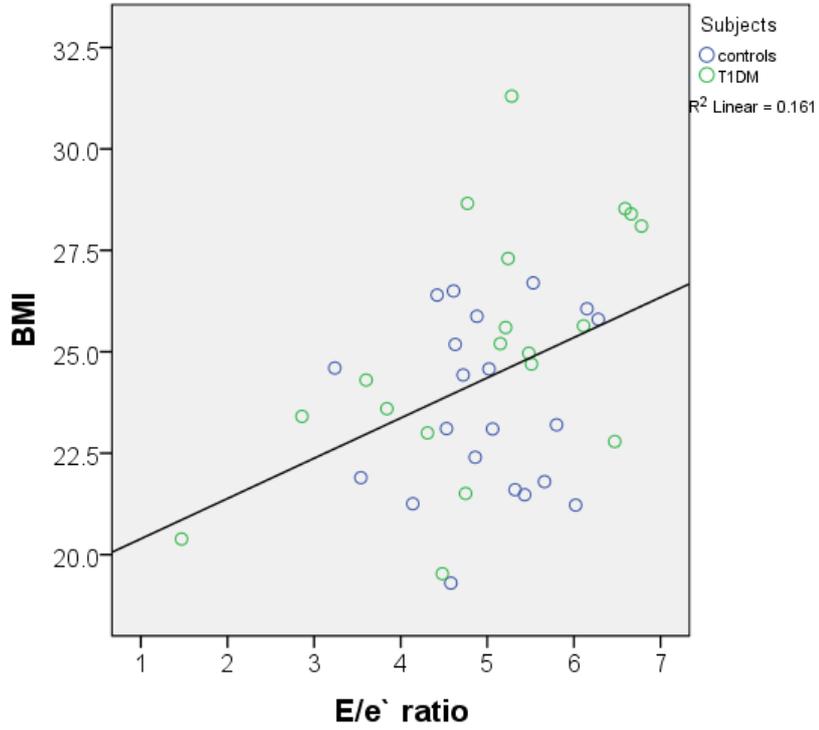
Correlates of diastolic function in all the subjects who had stress echocardiography are shown in Table 10.1

**Table 10.1: Pearson correlations between anthropometric measures/biochemical results and myocardial function (n=40)**

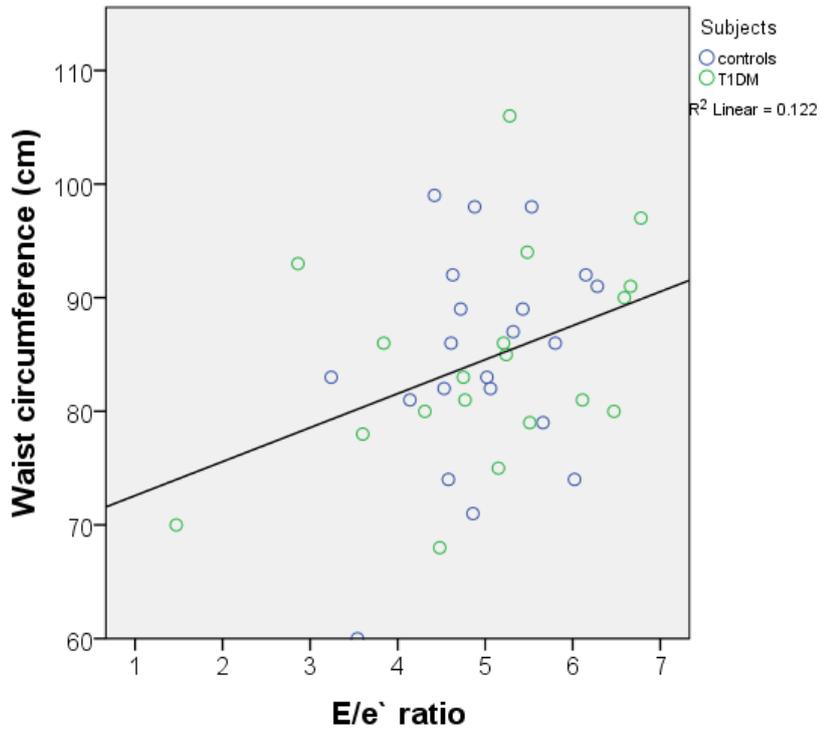
	<b>E</b>	<b>A</b>	<b>E/A</b>	<b>Lateral e'</b>	<b>Medial e'</b>	<b>E/e'</b>
	<b>r</b>	<b>r</b>	<b>r</b>	<b>r</b>	<b>r</b>	<b>r</b>
	<b>p</b>	<b>p</b>	<b>p</b>	<b>p</b>	<b>p</b>	<b>p</b>
<b>Age</b>	-0.14 0.39	-0.23 0.15	-0.23 0.16	-0.06 0.70	0.09 0.58	0.30 0.86
<b>BMI</b>	0.13 0.42	0.1 0.53	-0.23 0.14	-0.03 0.86	0.08 0.61	<b>0.40</b> <b>0.01*</b>
<b>Waist circumference</b>	-0.04 0.83	-0.05 0.78	-0.24 0.14	-0.27 0.09	0.04 0.79	<b>0.35</b> <b>0.03*</b>
<b>Waist hip ratio</b>	-0.19 0.24	-0.07 0.65	-0.12 0.48	<b>-0.41</b> <b>0.009**</b>	-0.07 0.67	0.14 0.41
<b>Total cholesterol</b>	-0.17 0.31	0.24 0.14	0.06 0.72	-0.17 0.31	-0.17 0.30	-0.09 0.60
<b>HDL</b>	0.16 0.32	-0.004 0.98	-0.23 0.15	<b>0.42</b> <b>0.007*</b>	<b>0.44</b> <b>0.005*</b>	-0.21 0.20
<b>LDL</b>	-0.09 0.56	0.25 0.12	0.19 0.23	-0.13 0.42	-0.23 0.15	-0.11 0.52
<b>TG</b>	-0.18 0.26	-0.12 0.47	-0.09 0.58	<b>-0.38</b> <b>0.02*</b>	-0.21 0.20	0.29 0.07
<b>hsCRP</b>	0.13 0.43	<b>0.40</b> <b>0.01**</b>	-0.09 0.58	-0.09 0.58	-0.18 0.91	0.23 0.15

In these subjects, BMI and waist circumference correlated positively with E/e' ratio, waist circumference with E/e' ratio. Waist-hip ratio correlated inversely with lateral e'. These relationships are displayed in figures 10.1, 10.2 and 10.3.

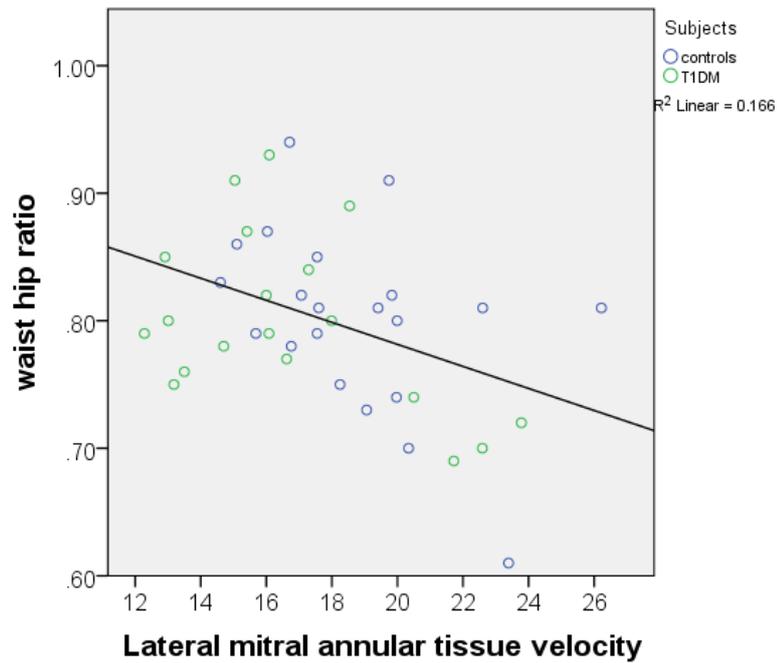
**Figure 10.1: Correlation between BMI and E/e' (n=40)**



**Figure 10.2: Correlation between waist circumference and E/e' (n=40)**



**Figure 10.3: Correlation between waist hip ratio and lateral e' (n=40)**



**Summary (correlates of myocardial function on stress group analysis)**

When myocardial function was analysed as a whole group including both type 1 diabetes mellitus and controls, E/e' increases with BMI and waist circumference whereas lateral e' decreases with increasing waist hip ratio, increasing serum triglycerides and decreasing HDL.

## 10.2 Correlates of Myocardial function in type 1 diabetes mellitus

Correlates of diastolic myocardial function in 19 subjects with type 1 diabetes mellitus are shown in table 10.3.

**Table 10.2: Pearson correlations between anthropometric measures/biochemical results and myocardial function in subjects with type 1 diabetes mellitus (n=19)**

	<b>E (r,p)</b>	<b>A (r,p)</b>	<b>E/A (r,p)</b>	<b>Lateral e' (r,p)</b>	<b>Medial e' (r,p)</b>	<b>E/e' (r,p)</b>
<b>BMI</b>	0.23 0.35	0.24 0.32	-0.38 0.11	0.21 0.38	0.37 0.12	<b>0.57</b> <b>0.01**</b>
<b>Waist circumference</b>	-0.12 0.64	0.03 0.90	-0.37 0.11	-0.16 0.50	0.14 0.57	0.40 0.09
<b>Waist hip ratio</b>	-0.28 0.25	0.02 0.92	-0.18 0.35	<b>-0.45</b> <b>0.05*</b>	-0.15 0.54	0.17 0.49
<b>Total cholesterol</b>	-0.001 1.0	0.34 0.18	0.04 0.88	-0.13 0.60	-0.13 0.59	-0.02 0.93
<b>HDL</b>	0.19 0.44	0.24 0.32	-0.31 0.19	0.33 0.17	0.36 0.13	-0.13 0.59
<b>LDL</b>	0.07 0.78	0.33 0.17	0.22 0.37	-0.05 0.85	-0.23 0.35	-0.1 0.68
<b>TG</b>	-0.19 0.44	-0.29 0.22	-0.14 0.56	-0.35 0.14	-0.07 0.79	0.33 0.017
<b>hsCRP</b>	0.12 0.62	<b>0.55</b> <b>0.02*</b>	-0.16 0.53	-0.14 0.58	0.002 0.99	0.38 0.11
<b>Fasting glucose</b>	0.03 0.91	0.13 0.60	0.03 0.92	-0.47 0.051	-0.18 0.48	0.03 0.89
<b>HbA1c</b>	-0.25 0.31	-0.04 0.89	0.04 0.88	-0.31 0.22	-0.19 0.46	0.06 0.82

Lateral e' correlates negatively with waist hip ratio, and E/e' correlates positively with BMI. Lateral mitral inflow diastolic velocity (A) positively correlates with hsCRP. No correlations were observed between diastolic velocities and HbA1c in type 1 diabetes mellitus. In the diabetic subjects, the indices of diastolic function at rest were not significantly related to HbA1c; correlations (Pearson) with HbA1c were -0.25 (p=0.31) for E, -0.04 (p =0.89) for the E/A ratio, and -0.27 (p = 0.28) for the mean early diastolic velocity of



### Multiple regression analysis

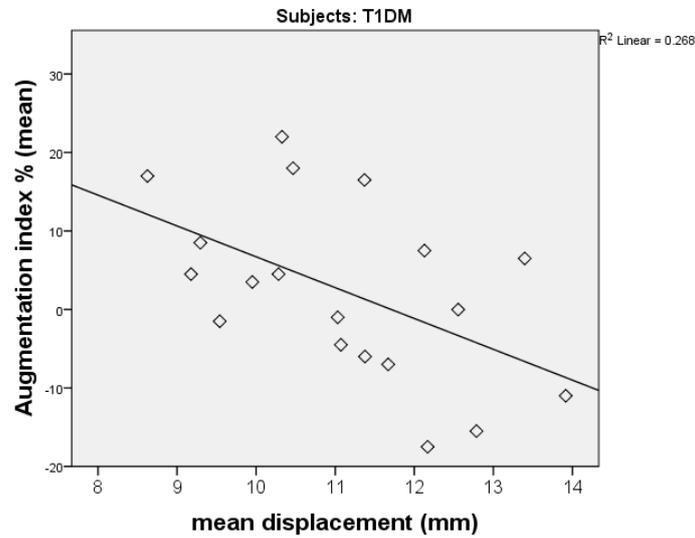
On multi-linear regression analysis, in a model which consists of BMI, waist hip ratio, total cholesterol, fasting glucose and HbA1c as predictors was used. BMI and total cholesterol were independent predictors for mean  $e'$  ( $\beta$  coefficient = 0.79,  $p=0.01$ ; and  $\beta$  coefficient = -0.68,  $p=0.02$ , respectively) and for medial  $e'$  ( $\beta$  coefficient = 0.84,  $p=0.02$ ; and  $\beta$  coefficient = -0.65,  $p=0.04$ , respectively) in type 1 diabetes mellitus

Similarly, in the same model for type 1 diabetes mellitus, waist-hip ratio and total cholesterol were independent predictors for lateral mitral annular tissue velocity. ( $\beta$  coefficient = -0.51,  $p=0.02$ ; and  $\beta$  coefficient = - 0.56,  $p=0.04$ , respectively). Mitral inflow early diastolic velocity (E) was independently predicted by BMI ( $\beta$  coefficient = 0.74,  $p = 0.05$ ) in type 1 diabetes mellitus. Fasting blood glucose and HbA1c were not selected by the model and therefore did not predict any of the indices of myocardial function (lateral, medial and mean mitral annular velocities and mitral inflow early diastolic velocity (E).) Correlates of myocardial velocities in T1DM are shown in table 10.3.

**Table 10.3: Pearson's correlation of myocardial velocities and displacement, with age, HbA1c and vascular parameters, in type 1 diabetes mellitus (n=19)**

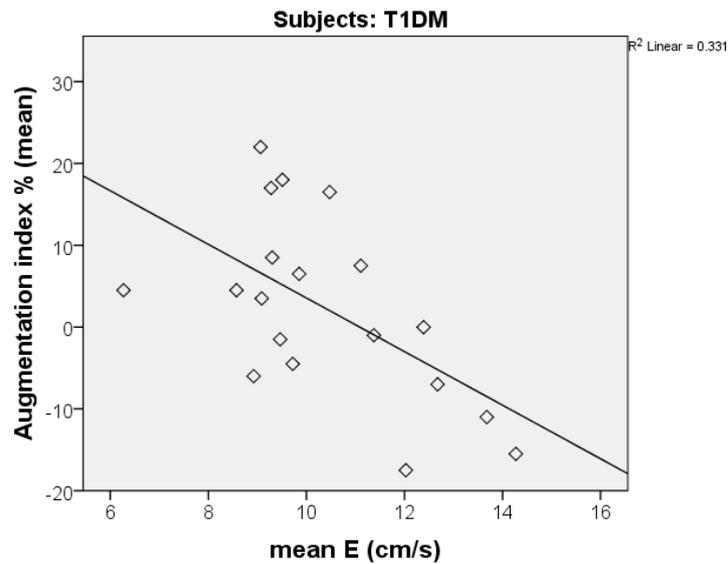
	<b>Age</b>	<b>WHR</b>	<b>HbA1c</b>	<b>cIMT</b>	<b>AIx</b>
	<b>r</b>	<b>r</b>	<b>r</b>	<b>r</b>	<b>r</b>
	<b>p</b>	<b>p</b>	<b>p</b>	<b>p</b>	<b>p</b>
<b>Displacement</b>	-0.14 0.55	-0.21 0.39	-0.14 0.5	-0.27 0.27	<b>-0.52</b> <b>0.02*</b>
<b>PSV</b>	-0.23 0.34	-0.2 0.42	<b>-0.50</b> <b>0.04</b>	-0.36 0.13	-0.37 0.12
<b>E (tissue)</b>	-0.29 0.23	0.29 0.24	-0.36 0.14	-0.24 0.33	<b>-0.58</b> <b>0.01*</b>
<b>A (tissue)</b>	<b>0.53</b> <b>0.02*</b>	<b>0.47</b> <b>0.05*</b>	-0.19 0.45	<b>0.47</b> <b>0.04*</b>	0.22 0.37

**Figure 10.6: Correlation between augmentation index and displacement**



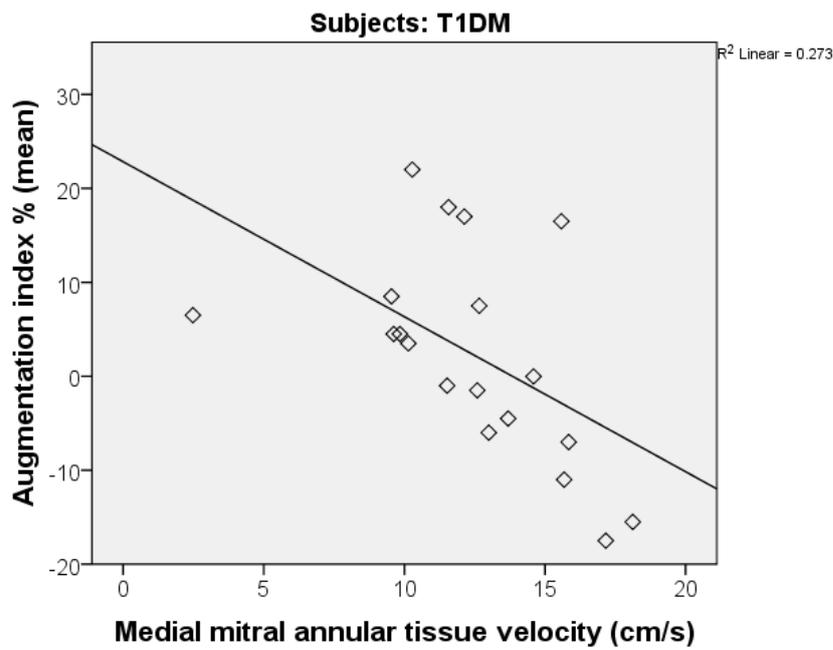
Displacement negatively correlates with augmentation index in type 1 diabetes mellitus. ( $r = -0.52$ ,  $p = 0.02$ ) (figure 10.6)

**Figure 10.7: Correlation between augmentation index and early diastolic velocity E (tissue velocity)**



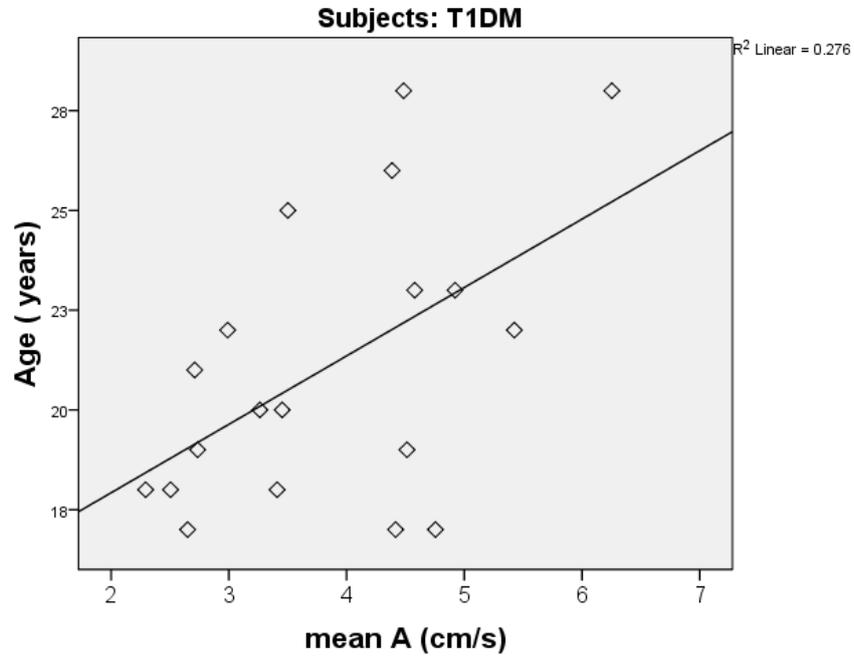
Early diastolic velocity E also negatively correlates with augmentation index in type 1 diabetes mellitus. ( $r = -0.58$ ,  $p = 0.01$ ) (figure 10.7)

**Figure 10.8: Correlation between augmentation index and medial mitral annular tissue velocity (medial e') in type 1 diabetes mellitus (n=19)**

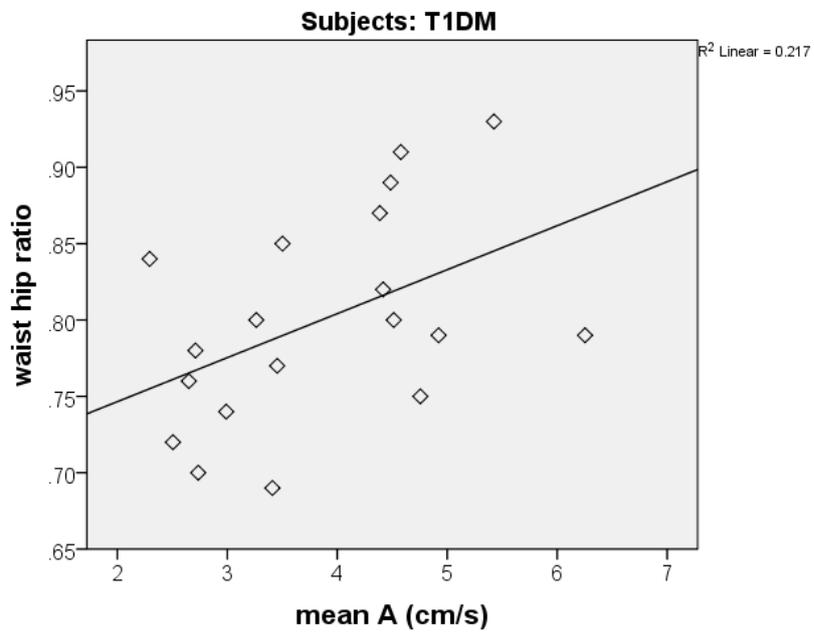


Augmentation index correlates inversely with medial e' in subjects with type 1 diabetes mellitus. ( $r=-0.52$ ,  $p=0.02$ ) (Figure 10.8)

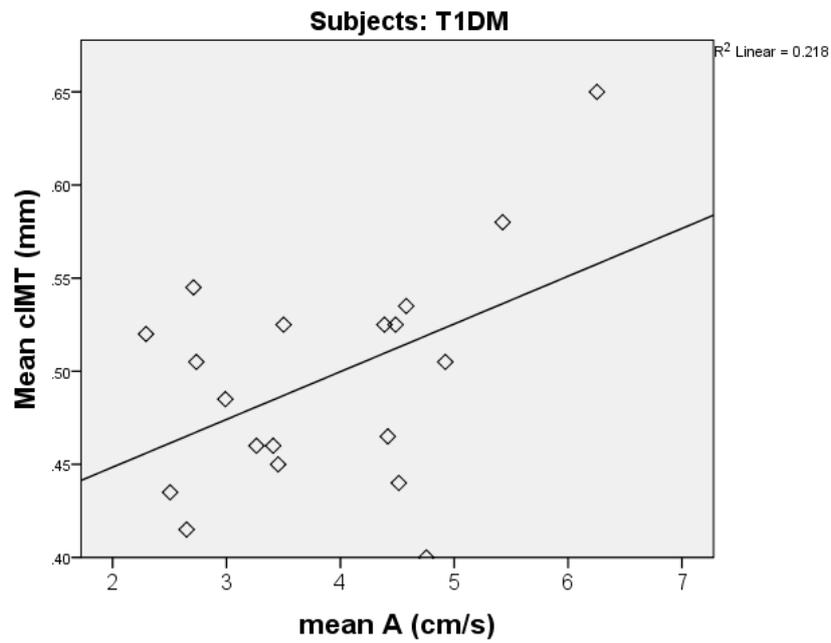
**Figure 10.9: Correlation between age and late diastolic tissue velocity A**



**Figure 10.10: Correlation between waist hip ratio and late diastolic tissue velocity (A)**

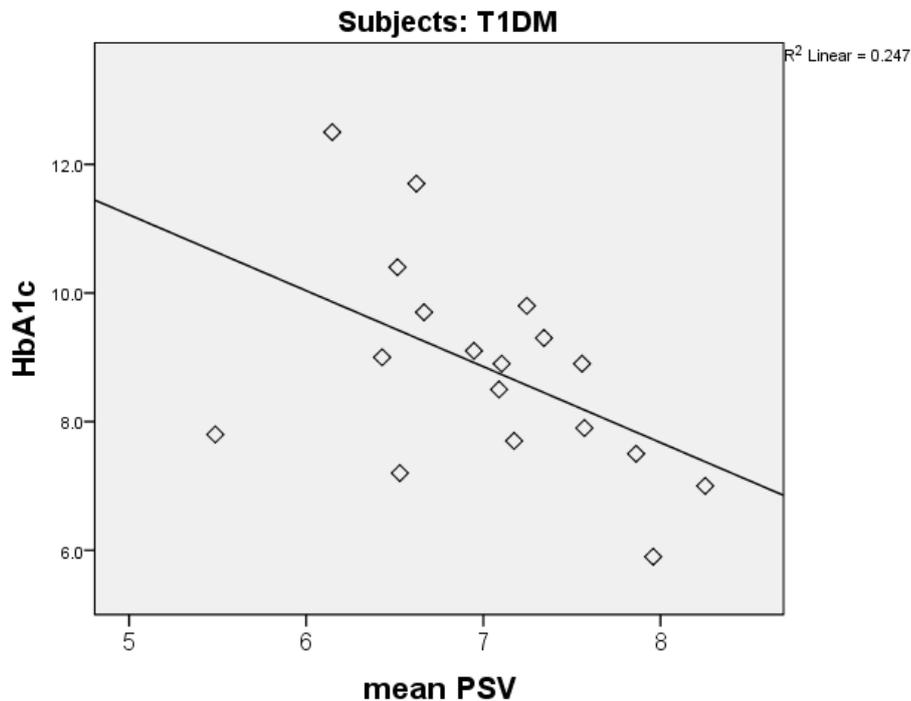


**Figure 10.11: Correlation between mean cIMT and late diastolic tissue velocity**



Late diastolic tissue velocity A positively correlates with age ( $r=0.53$ ,  $p=0.02$ ), waist hip ratio ( $r=0.47$ ,  $p=0.05$ ) and cIMT ( $r=0.47$ ,  $p=0.04$ ) in subjects with type 1 diabetes mellitus. (figures 10.9, 10.10, and 10.11)

**Figure 10.12: Correlation between mean peak systolic velocity and HbA1c**



A negative correlation between peak systolic velocity and HbA1c was noted in type 1 diabetes mellitus. ( $r = -0.5$ ,  $p = 0.04$ ) (figure 10.13)

### Multiple regression analysis

On multiple regression analysis, in a model consisting age, waist hip ratio, SBP, serum triglycerides, stiffness index  $\beta$ , cIMT, and HbA1c, WHR predicted displacement ( $\beta$  coefficient =  $-0.5$ ,  $p = 0.04$ ) in the control group.

In a model consisting age, waist-hip ratio, SBP, serum triglycerides, stiffness index  $\beta$ , cIMT, HbA1c and total cholesterol, WHR predicts mean E in controls ( $\beta$  coefficient =  $-0.73$ ,  $p = 0.009$ ) and in type 1 diabetes mellitus ( $\beta$  coefficient =  $-0.66$ ,  $p = 0.05$ ). In the same model, stiffness index  $\beta$  also predicts mean E. ( $\beta$  coefficient =  $0.67$ ,  $p = 0.03$ )

In a model consisting of age, waist hip ratio, serum triglycerides, stiffness index  $\beta$ , cIMT, HDL, HbA1c and augmentation index, peak systolic velocity is predicted by HbA1c ( $\beta$  coefficient =  $-0.72$ ,  $p = 0.02$ ) and cIMT ( $\beta$  coefficient =  $-0.88$ ,  $p = 0.05$ ).

### **Summary (Correlates of myocardial function in type 1 diabetes mellitus)**

Early tissue diastolic velocity E and mean displacement decrease with increasing AIX whereas late tissue diastolic velocity A increases with increasing age, waist hip ratio, and cIMT. Peak systolic velocity (PSV) decreases with high HbA1c. Left ventricular filling pressure increases with raised BMI. Lateral  $e'$  decreases with increasing waist hip ratio.

### 10.3 Correlates of myocardial function in control group

Pearson correlations of diastolic myocardial function for control group is shown table

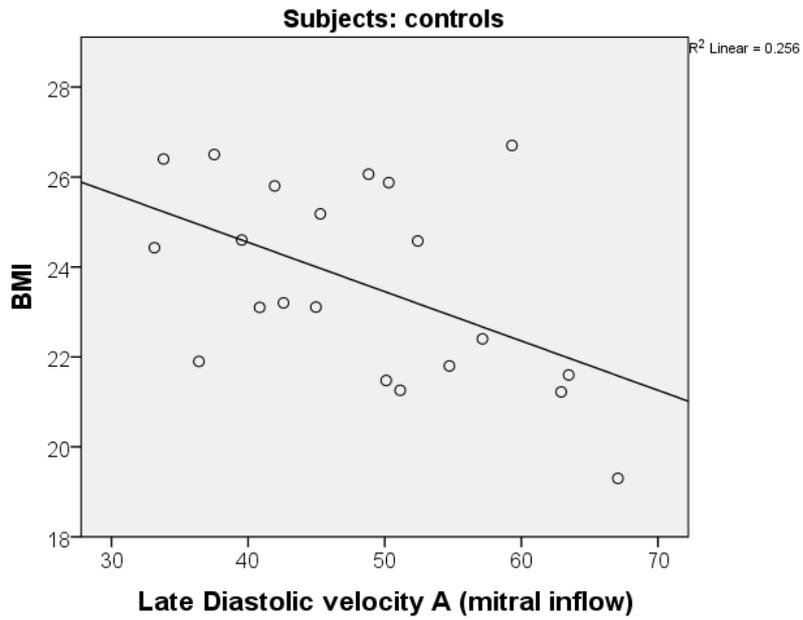
10.5.

**Table 10.4: Pearson correlations between anthropometric measures and biochemical results, with myocardial function in control subjects (n=21)**

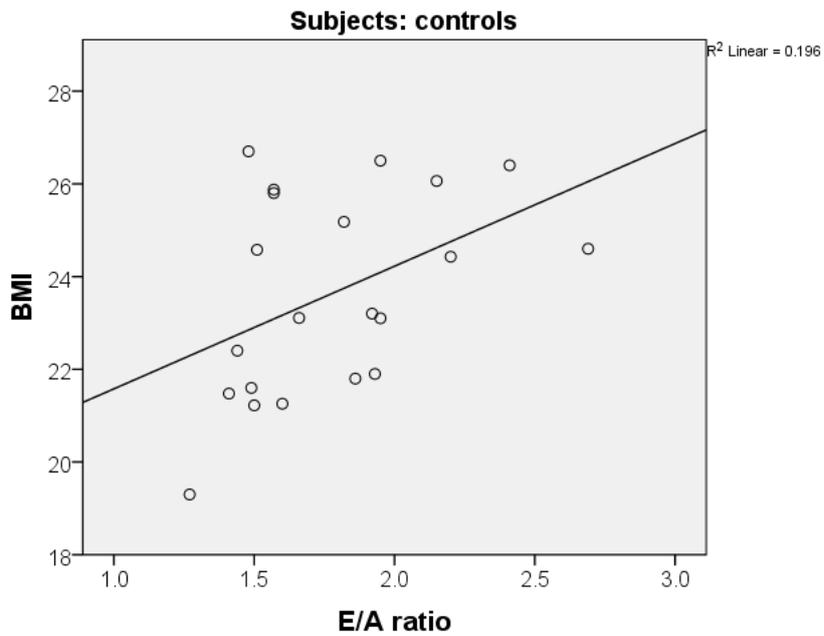
	<b>E (r,p)</b>	<b>A (r,p)</b>	<b>E/A (r,p)</b>	<b>lateral e' (r,p)</b>	<b>medial e' (r,p)</b>	<b>E/e' (r,p)</b>
<b>BMI</b>	0.01 0.96	<b>-0.51</b> <b>0.02*</b>	<b>0.44</b> <b>0.04*</b>	-0.16 0.48	-0.13 0.59	0.09 0.72
<b>Waist circumference</b>	0.07 0.77	-0.17 0.45	0.18 0.45	-0.42 0.06	-0.11 0.65	0.31 0.17
<b>Waist hip ratio</b>	-0.09 0.70	-0.16 0.48	0.09 0.71	-0.42 0.06	0.02 0.95	0.1 0.67
<b>total cholesterol</b>	<b>-0.46</b> <b>0.03*</b>	-0.28 0.22	0.001 0.99	-0.24 0.30	-0.01 0.96	0.09 0.70
<b>HDL</b>	0.09 0.71	-0.12 0.60	0.32 0.16	0.37 0.10	0.35 0.12	-0.39 0.08
<b>LDL</b>	-0.42 0.06	-0.19 0.42	-0.11 0.65	-0.09 0.69	0.03 0.91	-0.13 0.58
<b>TG</b>	-0.12 0.59	-0.09 0.71	-0.05 0.82	-0.27 0.25	-0.24 0.29	0.25 0.28
<b>hsCRP</b>	0.17 0.46	0.11 0.63	0.03 0.91	0.05 0.83	0.11 0.65	0.01 0.96

BMI negatively correlates with late diastolic velocity (A) and positively correlates with E/A ratio in control subjects. (figure 10.13 and 10.14)

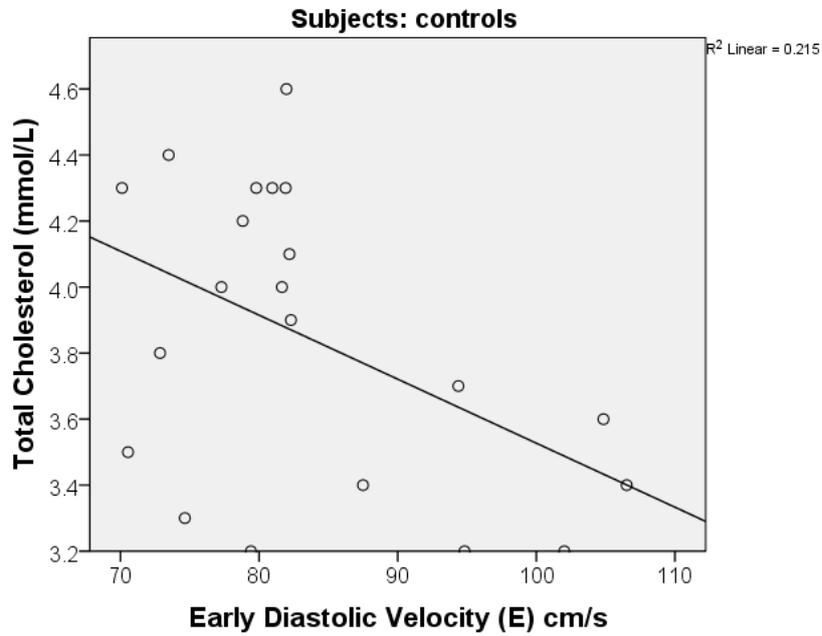
**Figure 10.13: Correlations between BMI and late diastolic (mitral inflow) velocity (A) in control subjects (n=21)**



**Figure 10.14: Correlations between BMI and E/A ratio in control subjects (n=21)**



**Figure 10.15: Correlation between total cholesterol and early diastolic velocity (mitral inflow) (E) in control subjects (n=21)**



Early diastolic velocity (E) decreases with increasing level of total cholesterol in control subjects. (figure 10.15)

**Summary (correlates of myocardial function in control group)**

Early diastolic E and late diastolic A velocities decrease with increasing BMI and cholesterol, respectively whereas E/A ratio increases with increasing BMI.

#### ***10.4 Discussion on correlates of myocardial function in type 1 diabetes mellitus***

When data were analysed from the subjects with type 1 diabetes mellitus and the controls together, estimated mean left ventricular filling pressure, using E/e' ratio, increased with BMI and waist circumference, and lateral mitral annular early diastolic velocity decreased with increasing waist-hip ratio. These results imply that irrespective of diabetic status, left ventricular diastolic function is adversely affected by increasing obesity and in particular central obesity.

On subgroup analysis, in type 1 diabetes mellitus, the early diastolic tissue velocity and the mean displacement of the basal myocardial segments decreased with increasing central systolic blood pressure (estimated from the augmentation index). The late diastolic tissue velocity A increased with increasing age, waist-hip ratio, and cIMT. Peak systolic velocity (PSV) decreased with high HbA1c. Left ventricular filling pressure increased with raised BMI. Lateral e' decreased with increasing waist hip ratio. These results suggest that myocardial function may be adversely affected by conduit artery stiffness, and early atherosclerosis, as well as obesity.

A modest correlation between PSV and HbA1c was observed, but no other correlations were found between HbA1c and myocardial function in this study. Most significant correlations were between myocardial function and features of adiposity. It may imply that the influence of glycaemia on myocardial function in type 1 diabetes mellitus may not be as strong as that of adiposity. In fact, the link between HbA1c and cardiovascular disease in type 1 diabetes mellitus is not consistent.

The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) showed that elevated glycosylated haemoglobin level was associated with both all cause and cardiovascular mortality in T1DM. [230] However, some major epidemiological studies such as the Pittsburgh Epidemiology of Diabetes Complications (EDC) study and the EURODIAB study did not demonstrate a link between hyperglycaemia and cardiovascular disease or mortality in T1DM. [231] [232] Kilpatrick et al , after analysing data from the DCCT trial, argued that it is mean blood glucose rather than HbA1c that has better predictive value for cardiovascular disease in type 1 diabetes mellitus. [233] A meta-analysis based on three prospective studies of glycosylated haemoglobin and coronary heart disease in T1DM concluded that there is a moderate increase in risk of cardiovascular disease with increasing levels of glycosylated haemoglobin. [234]

In an observational study , 20, 985 patients with type 1 diabetes mellitus with mean age of 38.6 years were followed up for nine years. [235] The hazard ratio for development of heart failure was 3.98 (95% CI 2.23 –7.14) in those patients whose HbA1c was more than 10.5 % in comparison with a control group of patients who had lower HbA1c levels (mean 6.5%) Similar changes were noted in a study of 48,858 patients, of whom the majority had type 2 diabetes mellitus. It was observed that for every 1% increase in HbA1c there was an 8% increase in the risk of heart failure or death related to heart failure. [236] Even in those patients who were not previously diagnosed with diabetes mellitus or those who were not on any treatment for diabetes mellitus, HbA1c seem to be a strong predictor of mortality in a group of older patients with impaired left ventricular systolic function. [237] Good et al studied a group of patients with a median age of 72 years and they found that mortality in those patients with left ventricular systolic dysfunction increased sharply when HbA1c was higher than 6.7%.

The relationship between HbA1c and cardiovascular mortality may not be straightforward or linear. The strategy of bringing HbA1c closer to normal did not work in type 2 diabetes mellitus patients in the ACCORD study. [238] In fact, in an observational study of ambulatory patients with established heart failure, the relationship between HbA1c and overall mortality was found to be U- shaped.

There is evidence to suggest that poor glycaemic control or higher HbA1c is associated with development of heart failure in type 1 diabetes mellitus.

Nevertheless, the link between HbA1c or glycaemic control and more subtle, subclinical myocardial dysfunction in type 1 diabetes mellitus in particular is not consistently observed. In a study of 25 patients with type 1 diabetes mellitus and 26 healthy controls, HbA1c was found to be correlated with indexes of increased left ventricular filling pressure such  $E/e'$  and  $E/Vp$  ( mitral inflow velocity). [81] Similarly, HbA1c was negatively correlated with features of diastolic dysfunction such as  $E/A$  ratio,  $E_m$  and  $E_m/A_m$  in study of 50 type 2 diabetes mellitus and 50 controls. [121] A similar negative correlation between HbA1c and diastolic  $E/A$  ratio was reported even in children and adolescents with type 1 diabetes mellitus. [187] No correlation between HbA1c and echocardiographic data was found in a study of 40 type 1 diabetes mellitus subjects and 40 controls. [98]

In my study, only one association between myocardial function and HbA1c was observed – the peak systolic velocity of long-axis shortening of the left ventricle (PSV) was inversely related to HbA1c. However, it was observed that estimated mean left ventricular filling pressure ( $E/e'$ ) correlated positively with BMI and waist circumference, and lateral mitral annular early diastolic velocity  $e'$  correlated negatively with waist-hip ratio. Moreover, lateral and medial mitral annular velocities were positively correlated with serum HDL, and lateral mitral annular velocity was negatively correlated with serum triglycerides, and waist-

hip ratio, when data were analysed as a whole group. Further subgroup analysis showed that in the control group, BMI correlated negatively with late diastolic velocity (A) and positively with E/A ratio, whereas in the diabetic group, BMI, waist-hip ratio and hsCRP correlated positively with E/e' ratio, lateral e' and late diastolic velocity (A).

These findings suggest that even in young subjects and irrespective of their diabetic status, myocardial function seems to be more related to features of metabolic syndrome and obesity rather than actual blood glucose level. In the Pittsburgh Epidemiology of Diabetes Complications (EDC) study, after 10 years of follow up of 603 patients with type 1 diabetes mellitus, it was concluded that factors such as insulin resistance, HDL cholesterol, and non-HDL cholesterol, rather than glycaemia, predict cardiovascular disease in type 1 diabetes mellitus. [231] In the same study, coronary artery calcification was reported to be related to features of adiposity such as visceral adipose tissue, subcutaneous adipose tissue, BMI and waist circumference, again in type 1 diabetes mellitus. [239]

With changes in life style, the prevalence of obesity is increasing in young subjects with T1DM. Insulin sensitivity has been found to be reduced in T1DM children and adolescents. The Finn Diane Study proved that the prevalence of metabolic syndrome was as high as 35-40% in T1DM. Insulin sensitivity was lower in pubertal and post pubertal adolescents and in those with high BMI, and waist circumference. [240]

Elevated insulin resistance is a known risk factor for macrovascular complications in type 2 diabetes mellitus. Insulin resistance and metabolic syndrome are also associated with both micro and macrovascular complications in T1DM. [241] In the DCCT study, the intensive therapy group gained more weight than the conventional treatment group, and had changes similar to those seen in the metabolic syndrome such as high blood pressure, high triglycerides, and high total and LDL cholesterol.[242] Many epidemiological studies

showed that high total cholesterol,[200] high LDL, high triglycerides, and low HDL[199] [107] are associated with cardiovascular disease in type 1 diabetes mellitus.

Moreover, myocardial steatosis or higher myocardial triglycerides contents on <sup>1</sup>H-magnetic resonance spectroscopy have been reported in type 2 diabetes mellitus. [243] Ng et al demonstrated that myocardial triglycerides contents correlated with global LV longitudinal strain, global LV longitudinal systolic strain rate and diastolic strain rate. [244] Rijzewijk et al also demonstrated the link between diastolic dysfunction and myocardial triglyceride contents in type 2 diabetes mellitus. [245]

However, the presence of triglycerides in the myocardium in itself is not thought to be pathological currently. It may be a mere reflection of increased exposure of the myocardium to high lipid environment.[246]

In patients with diabetes mellitus, the myocardium uses free fatty acids as its main energy source because of reduced glucose transport into the cells secondary to impaired transcription of glucose transport such as GLUT-4 and increased availability of free fatty acids because of hypertriglyceridemia. [247] Increased fatty acid utilization by cardiac muscle has been described in both type 1 and type 2 diabetes mellitus. [248] This increase in free fatty acid oxidation, and altered substrate metabolism, increases oxygen consumption in the myocardium. Transgenic mouse models showed that exposure to free fatty acids even in the absence of hyperglycaemia could cause lipotoxicity in cardiac muscles.[249] [250]

Obesity is a known independent risk factor for heart failure. [251] Increase in waist circumference and waist-hip ratio has been shown to link with the risk of heart failure. [252]

In this study, I have described an association between features of adiposity such as high waist-hip ratio, dyslipidaemia and adverse diastolic function in a group of young subjects irrespective of their diabetic status; the mean age was  $21.1 \pm 3.6$  years in T1DM and

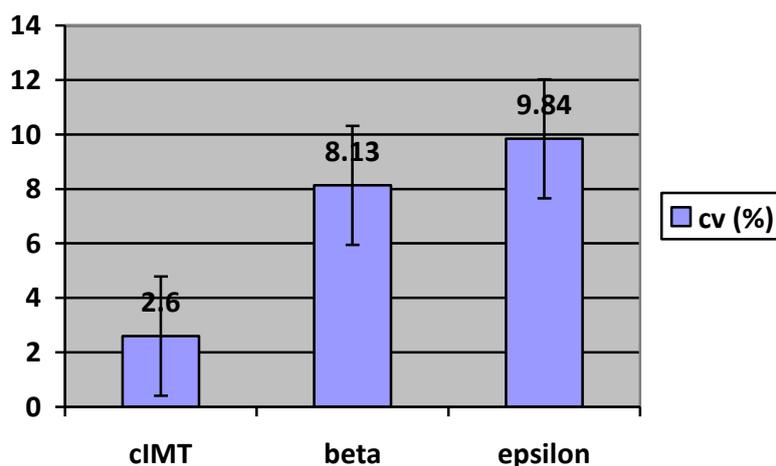
24.2 ± 2.9 years in controls, and the mean BMI 25.1 ± 3.1 in type 1 diabetes mellitus and 24.2 ± 2.7 in controls. Only a modest correlation was found between glycaemia and peak longitudinal systolic function. In order to delay or prevent the development of overt heart failure or cardiovascular disease in young subjects with type 1 diabetes mellitus, it may be prudent that other risk factors such as lipid profiles are controlled in the early stages of diabetes mellitus as well as glycaemic control.

## CHAPTER 11- RESULTS - Vascular structure and function

### 11.1 Reproducibility of vascular measurements

Five volunteers were recruited and high-resolution ultrasound scans were performed on both sides of the neck, for cIMT measurement, and stiffness parameters  $\beta$  and  $\epsilon$ . Images of the carotid artery were acquired 1 cm below the carotid bulb. Images were analysed off-line using Vascular Image Laboratory Software, and cIMT was measured on the far wall within 1cm of carotid bulb. A mean value from the two sides was taken. Stiffness parameters  $\beta$  and  $\epsilon$  were acquired at the same time and average values were taken. The same procedures, both scanning and analysis, were repeated in the same subjects after 2 weeks. Reproducibility was calculated as shown in Figure 11.1 and Table 11.1.

Figure 11.1: Coefficient of variation (CV) of vascular measurements



**Table 11.1: Intra-observer reproducibility of vascular measurements (Bland-Altman Analysis)**

	Bias	SD of Bias	95% limit of agreement	
			From	To
<b>cIMT</b>	<b>-0.006</b>	<b>0.029</b>	<b>-0.062</b>	<b>0.05</b>
<b>Stiffness parameter <math>\beta</math></b>	<b>-0.59</b>	<b>0.98</b>	<b>-2.52</b>	<b>1.34</b>
<b>Stiffness parameter <math>\epsilon</math></b>	<b>-6.3</b>	<b>17.95</b>	<b>-41.48</b>	<b>28.88</b>

### ***11.2 Discussion on vascular reproducibility***

Intra-observer variability of cIMT was reported to be range from 2.4% to 10.6%. [253] [254] Reproducibility of near wall and far wall cIMT are similar. However, ultrasonic far wall measurements more accurately reflect the true thickness. [255] The intra-observer coefficient of variation of 2.6% found in my study is therefore comparable with other studies.

In a study of 10 healthy subjects, Niki et al reported that coefficient of variation for  $\beta$  is less than 15%. [256] Intra-observer coefficient of variation (CV) was reported to range from 15.5% to 18.6% for  $\epsilon$  and from 15.9 % to 19.2% for stiffness index  $\beta$  by Swampillai in her thesis. [257] Therefore, the CVs for stiffness index  $\beta$  and  $\epsilon$  in my study compare favourably to previous reports. The numbers of paired analyses were small, however.

### ***11.3 Vascular stiffness and cIMT***

Table 11.2 shows the results of tests of vascular structure and function in the subjects with type 1 diabetes mellitus and the controls.

**Table 11.2: Markers of vascular stiffness and cIMT (T1DM vs. controls)**

	<b>Type 1 Diabetes Mellitus (n=19)</b>	<b>Controls (n=34)</b>	<b>P value</b>
<b>Augmentation index (AIx) %</b>	2.34 ± 11.22	3.39 ± 12.61	0.77
<b>cIMT (mm)</b>	0.50 ± 0.60	0.48 ± 0.50	0.39
<b>Beta index <math>\beta</math></b>	4.44 ± 0.84	4.90 ± 1.07	0.11
<b>Epsilon <math>\epsilon</math></b>	52.29 ± 12.23	59.47 ± 14.55	0.07

There was no significant difference between controls (n = 18) and T1DM (n=13) in carotid-femoral pulse wave velocity but there was a trend for it to be higher in the diabetic subjects ( $5.53 \pm 0.74$  m/s in controls vs.  $5.73 \pm 1.13$  m/s in the diabetics,  $p = 0.06$ ). Pulse wave velocity was added to the protocol after the study started, and so it was assessed only in 31 subjects (13 T1DM and 18 controls).

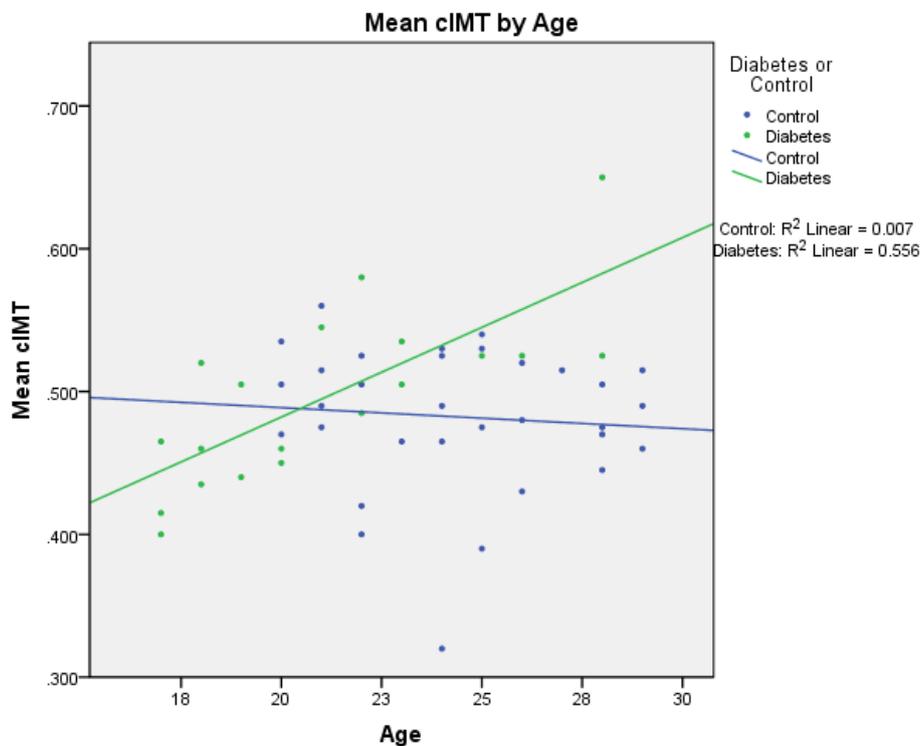
### 11.4 Univariate Analysis of Variance of vascular function

Vascular measurements such as AIx had a wide range, and may be influenced by many factors.. Therefore, differences between groups in cIMT, augmentation index,  $\beta$  and  $\epsilon$  were analysed further after being corrected for age, sex, BMI, waist-hip ratio and blood pressure.

#### 11.4.1 Relationship between age and diabetes/controls for cIMT

The following figure shows the relationship between age and mean cIMT in type 1 diabetes mellitus and control group.

**Figure 11.2: Relationship between age (years) and mean cIMT (mm)**

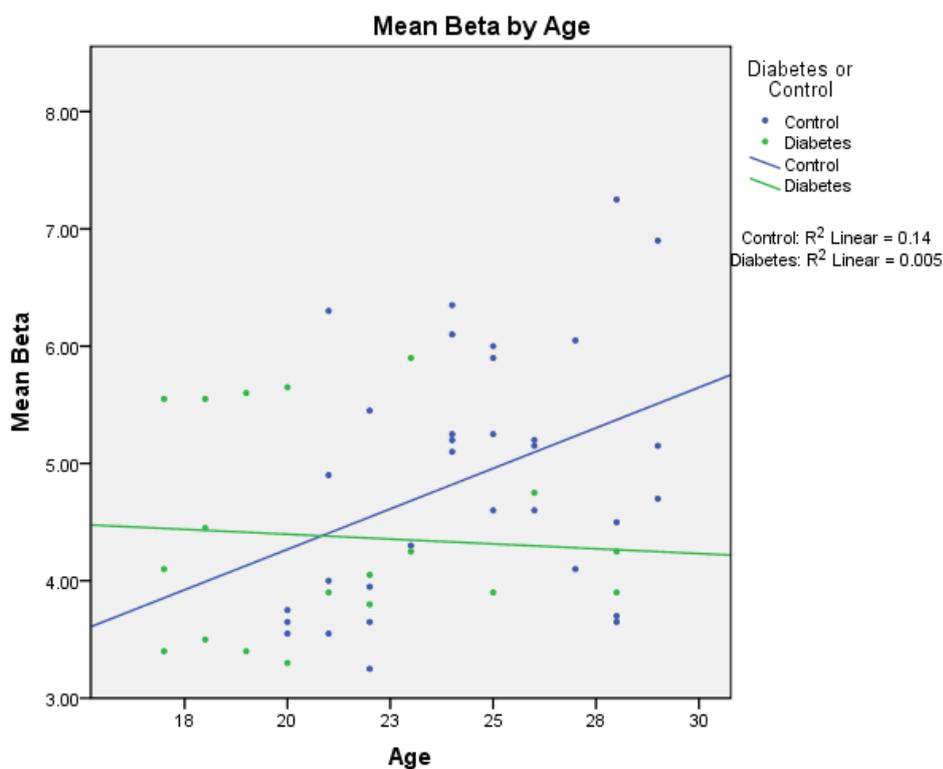


There is strong interaction between age and diagnostic group for this variable. In this small sample, age did not appear to influence mean cIMT in the control group, whereas there was a clear relationship in the diabetic group – with cIMT increasing with age in type 1 diabetes mellitus ( $R^2 = 0.56$ ,  $p = 0.002$ ).

#### 11.4.2 Relationship between age and stiffness parameter $\beta$

The following figure shows the relationship between mean  $\beta$  and age in both type 1 diabetes mellitus and control group.

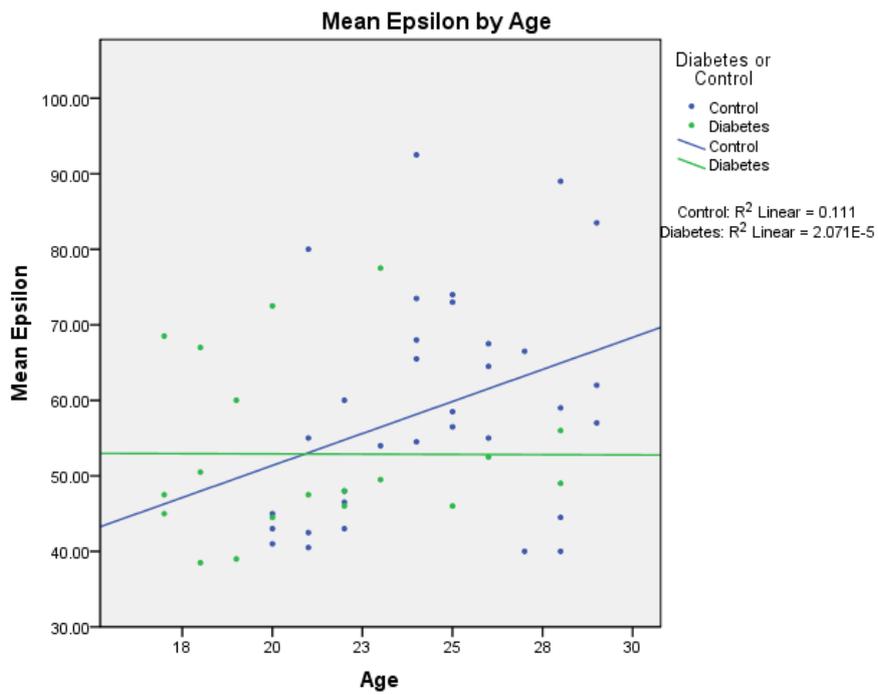
**Figure 11.3: Relationship between age (years) and mean  $\beta$**



Mean  $\beta$  correlates with age stronger in control group ( $R^2=0.14$ ,  $p=0.04$ ).

### 11.4.3 Relationship between age and stiffness parameter $\epsilon$

**Figure 11.4 Relationship between age and stiffness parameter  $\epsilon$**

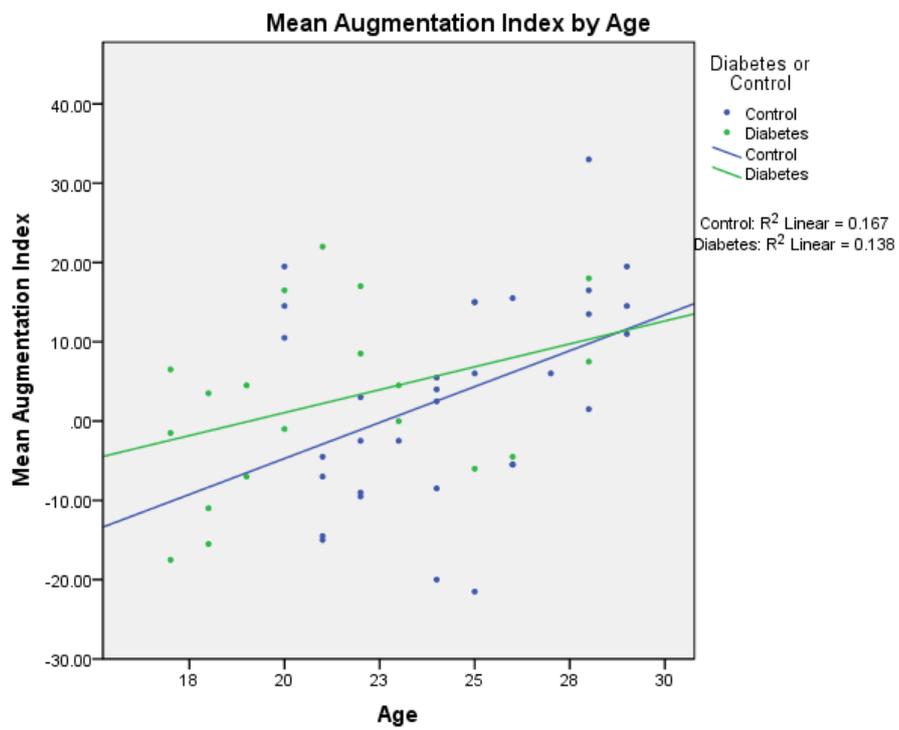


In the control group, mean  $\epsilon$  correlated with age. ( $R^2=0.11$ ,  $p=0.01$ )

#### 11.4.4 Relationship between age and augmentation index

Augmentation index increased both in subjects with type 1 diabetes mellitus and in controls. (figure 11.5)

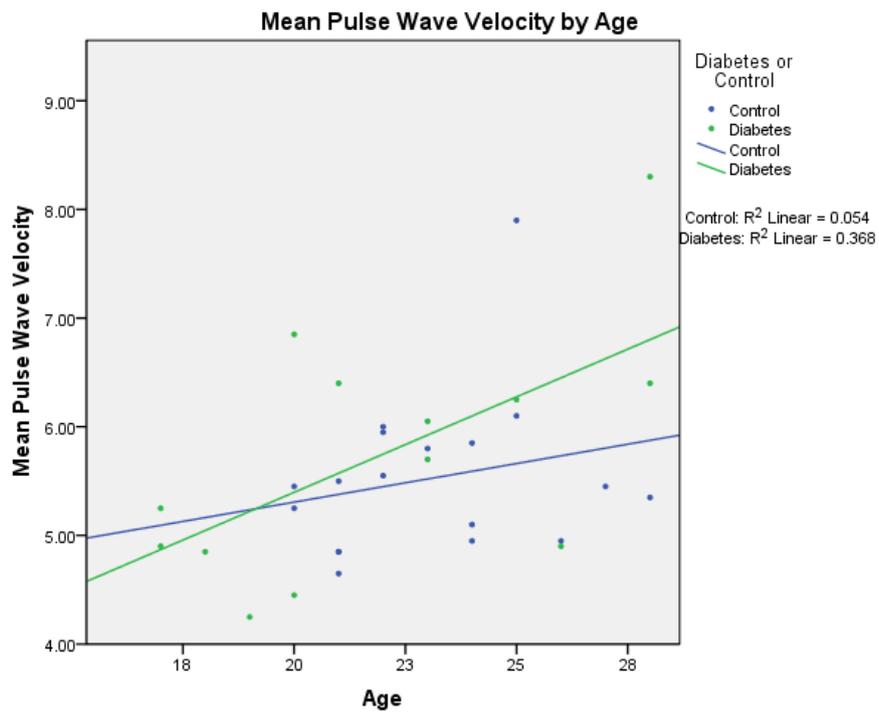
**Figure 11.5: Relationship between age and augmentation index**



### 11.4.5 Relationship between age and pulse wave velocity

The following diagram shows the relationship between mean PWV and age.

**Figure 11.6: relationship between mean pulse wave velocity (PWV) (m/s) and age (years)**



### ***11.5 Wave Intensity Results***

Data were analysed by wave intensity methods. Net wave intensity was separated into forward and backward compression waves by applying water-hammer equation to pressure-volume loop, using an automated software. Timing, duration, amplitude and net energy of individual waves were measured. All wave intensity data set were assessed statistically for quality, using published methods, and those with noisy data and abnormal quality indices were excluded. Results are therefore presented for the remaining 50 subjects (19 type 1 diabetes mellitus and 31 controls). Table 11.3.

**Table 11.3: Wave intensity analyses**

	<b>Type 1 diabetes mellitus (n =19)</b>	<b>Controls (n=31)</b>	<b>P-value</b>
<b>Local wave speed (cm/s)</b>	2.53 ± 2.21	2.71 ± 0.95	0.70
<b>Mean arterial BP (mmHg)</b>	85.63 ± 5.68	84.97 ± 6.2	0.71
<b>Stiffness index <math>\beta</math></b>	4.49 ± 1.25	4.81 ± 1.26	0.39
<b>Pressure-strain elastic modulus <math>\epsilon</math> (kPa)</b>	54.47 ± 15.78	57.29 ± 16.29	0.55
<b>AIx (%)</b>	3.37 ± 15.56	2.87 ± 19.67	0.93
<b>AIx 4<sup>th</sup> derivative</b>	-1.75 ± 6.37	-1.13 ± 10.99	0.82

Those data with skewed distribution were log transformed and analysed. (Table 11.4)

**Table 11.4: Log transformed wave intensity results (all data were log transformed)**

	<b>Type 1 diabetes mellitus</b>	<b>Controls</b>	<b>P value</b>
<b>FCW peak</b>	1.13 ± 0.15	1.06 ± 0.19	0.23
<b>FCW integral</b>	2.76 ± 0.16	2.74 ± 0.20	0.71
<b>Duration FCW</b>	2.00 ± 0.06	2.04 ± 0.04	0.005*
<b>BCW peak</b>	0.73 ± 0.38	0.63 ± 0.28	0.28
<b>BCW integral</b>	2.44 ± 0.34	2.37 ± 0.26	0.39
<b>Duration BCW</b>	2.08 ± 0.06	2.10 ± 0.07	0.44
<b>FEW peak</b>	0.41 ± 0.20	0.38 ± 0.23	0.67
<b>FEW integral</b>	1.91 ± 0.21	2.85 ± 0.23	0.35
<b>Duration FEW</b>	2.87 ± 0.18	1.81 ± 0.12	0.17
<b>FCWP peak</b>	1.75 ± 0.30	1.77 ± 0.11	0.79
<b>FCWP integral</b>	3.54 ± 0.13	3.54 ± 0.11	0.93
<b>BCWP peak</b>	1.58 ± 0.16	1.52 ± 0.13	0.16
<b>BCWP integral</b>	3.4 ± 0.14	3.38 ± 0.12	0.44
<b>BCWP/FCWP peak</b>	1.83 ± 0.39	1.76 ± 0.10	0.31
<b>BCWP/FCWP integral</b>	1.85 ± 0.04	1.84 ± 0.05	0.41

FCW = forward compression wave, BCW= backward compression wave, FEW= forward expansion wave, FCWP= forward compression wave pressure, BCWP= backward compression wave pressure.

## ***11.6 Discussion on vascular structure and function***

There were no significant differences between the diabetic group and the controls, in augmentation index (AIx), pulse wave velocity, carotid intima-media thickness, and the indices of conduit arterial stiffness  $\beta$  and  $\epsilon$ . Also, no differences were noted after adjustments for age, sex, waist-hip ratio and blood pressure.

The finding that AIx is not different between T1DM and control is contrary to many other studies. Heilman et al studied 30 children (ages 4.7 to 18.6 years) with type 1 diabetes mellitus, with a mean duration of diabetes mellitus of  $5.4 \pm 3.4$  years and mean HbA1c of 9.8%, and 30 healthy controls, and they demonstrated that children with T1DM had increased AIx @75 (augmentation index adjusted for heart rate at 75/minute) and increased cIMT. [258] Similarly, Haller et al demonstrated increased AIx @ 75 in 43 children who were 10 - 18 years old. However, in these studies, the wide age range could potentially be a confounding factor, if the results are influenced by growth and rapidly changing arterial structure such as diameter. [259]

Many studies showed an increased AIx in type 1 diabetes mellitus. Wilkinson et al reported increased AIx and PWV in 35 patients with T1DM aged 30 years who had had diabetes mellitus for a minimum of 10 years. [171] Lacy et al studied a mixture of type 1 and type 2 diabetes mellitus subjects and found that PWV but not AIx was increased in diabetes mellitus. [168]

In a larger study involving 535 subjects with type 1 diabetes mellitus (age  $14.6 \pm 3.3$  years) and 241 controls (age  $21.4 \pm 2.5$  years), AIx @ 75 was higher in type 1 diabetes mellitus and the presence of diabetes mellitus, male sex and increased mean arterial pressure were independent determinants of arterial stiffness. [260] BMI, SBP and DBP were higher in

subjects with type 1 diabetes mellitus in this study. However, Yu et al reported normal arterial stiffness in young subjects with type 1 diabetes mellitus. [261] In the SEARCH for Diabetes in Youth study, Wadwa et al [262] studied arterial stiffness, PWV and AIx75 in young subjects with type 1 and type 2 diabetes mellitus and demonstrated that subjects with type 2 diabetes mellitus had a higher AIx and PWV. They also reported that arterial stiffness was associated with waist circumference and blood pressure, irrespective of diabetes type.

Increased cIMT in type 1 diabetes mellitus has been reported in many studies. [148] [263] The differences between the findings in my study and other studies might be explained if I was studying a different cohort of subjects with type 1 diabetes mellitus; in my study, subjects with T1DM had a relatively short duration of diabetes mellitus. However, Atabek et al studied 45 patients with type 1 diabetes mellitus aged  $14.5 \pm 2.5$  years who had had diabetes mellitus for  $4.4 \pm 2.5$  years and they reported that cIMT was thicker in type 1 diabetes mellitus and that it was positively associated with the duration of diabetes mellitus. [148] Vastagh et al demonstrated a thicker cIMT in 42 patients with type 1 diabetes mellitus (aged  $34 \pm 10$  years with an average duration of diabetes mellitus  $15 \pm 10$  years) when compared to age-matched controls. [263]

Brachetta et al reported increased arterial stiffness in a group of type 1 diabetes mellitus patients with diabetes mellitus for more than 10 years, related to their duration of disease. [264] cIMT changes with time and treatment, and it may regress or progress with time or treatment. [153] [154] [155] Assessing cIMT at one particular time-point and interpreting it may be difficult.

Using analysis of variance, no differences between T1DM and controls were observed for any of the measurements of vascular function, namely cIMT, augmentation index, pulse wave velocity,  $\beta$  and  $\epsilon$ . The main consistent finding was the association between age and

vascular function. A strong interaction between age and vascular function was observed both in type 1 diabetes mellitus and in controls. In T1DM group, age was a significant factor for cIMT, AIx and PWV whereas in the control age was a significant factor for  $\beta$  and  $\epsilon$ , AIx and pwv.

It is well-recognized that conduit arteries becomes stiffer as age progresses. McEniery et al showed that both augmentation index and PWV increases with age. [265] Pulse wave velocity (PWV) increases gradually until about 60 years of age. [266] [267] Interactions between age and cIMT, AIx and PWV in subjects with T1DM with a narrow age range of  $21.1 \pm 3.6$  years, may be a sign that diabetes mellitus is accelerating the vascular aging process. No other studies were found concerning  $\beta$  and  $\epsilon$  in subjects with type 1 diabetes mellitus.

Wave intensity measurement at the carotid artery by echo-tracking technique is not widely used in clinical practice. Wave separation could provide further information about interactions between the heart and arterial system but acquiring the data is technically challenging and the variability of the data is relatively high; hence it is now appreciated that its power to show differences is low unless sample sizes are large

The duration of the forward compression wave (FCW) was shorter in subjects with type 1 diabetes mellitus than in the controls. This shorter duration of FCW may merely be a reflection of the faster heart rate in type 1 diabetes mellitus. Apart from duration of FCW, no other differences between type 1 diabetes mellitus and controls were noted; for example the diabetic subjects did not demonstrate increased backwards wave reflections. Therefore, there was no evidence in this study of changes in wave intensity or abnormalities of local wave travel in the carotid artery, in type 1 diabetes mellitus subjects when compared with controls.

## 11.7 Correlates of vascular function

### 11.7.1 Correlates of vascular function (whole group)

Correlates of vascular function for the whole group are shown in table 11.5.

**Table 11.5: Correlates of vascular function (n=53)**

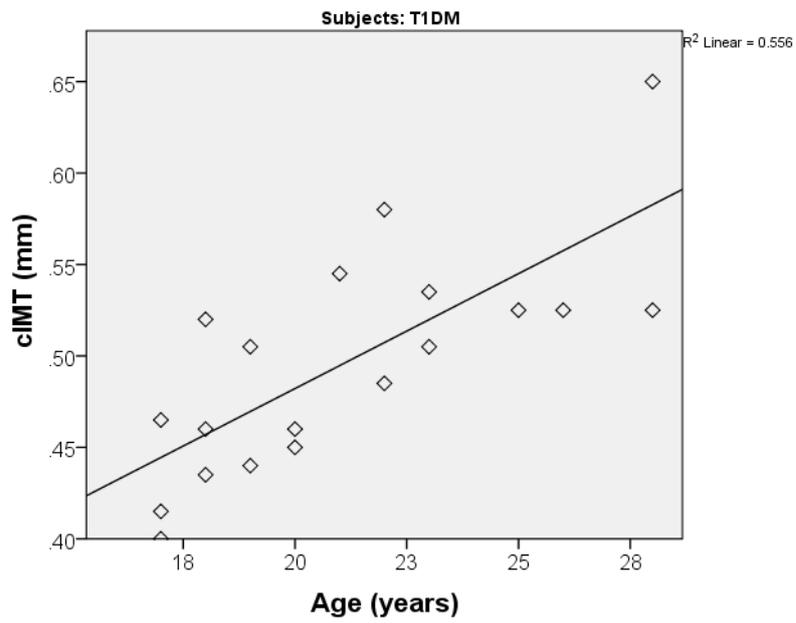
	$\epsilon$ r p	Age r p	Weight r p	Waist Hip Ratio r p
$\beta$ r p	0.9 0.000*	0.28 0.05*	0.32 0.02*	0.22 0.13
$\epsilon$ r p	-	0.33 0.02*	0.41 0.003*	0.37 0.008*

Pressure-strain elastic modulus  $\epsilon$  and stiffness parameter  $\beta$  positively correlated with age and weight, when data were analysed as one group including the diabetics and controls. Carotid  $\epsilon$  also correlates positively with waist-hip ratio. Irrespective of the diabetes status, the carotid artery stiffens with increasing age and body weight.

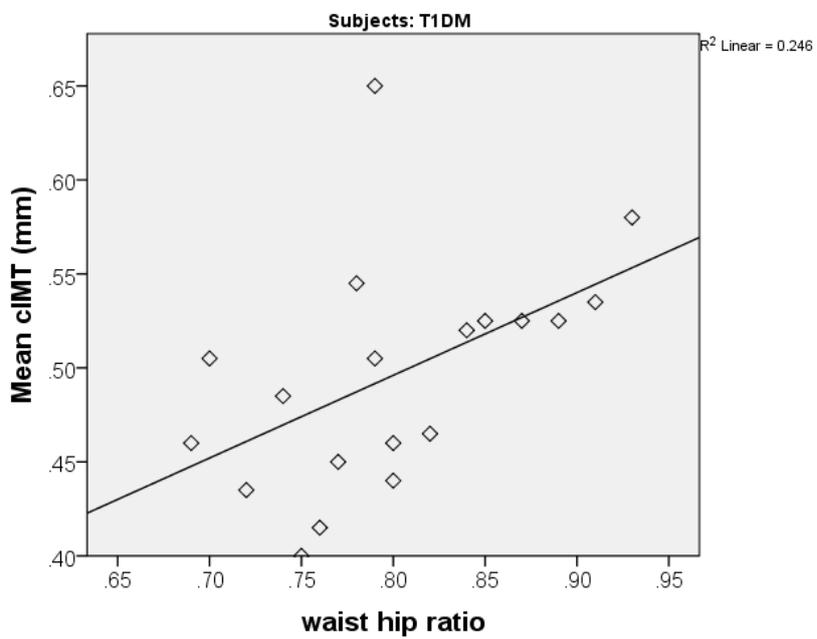
### 11.7.2 Correlates of vascular structure and function in type 1 diabetes mellitus (n=19)

Only cIMT is observed to correlate positively with age ( $r = 0.75$ ,  $p = 0.000$ ) (shown in Figure 11.7) and waist-hip ratio ( $r = 0.5$ ,  $p = 0.31$ ) (shown in Figure 11.8) in type 1 diabetes mellitus.

**Figure 11.7: Correlation between cIMT and Age**



**Figure 11.8: Correlation between cIMT and waist hip ratio**



### ***11.8 Discussion on correlates of vascular function***

Stiffness parameter  $\beta$  and pressure-strain elastic modulus  $\epsilon$  increases with age. Kawasaki et al demonstrated that  $\beta$  increases with advancing age as the vessel diameter increases and the percentage of cyclical change in diameter decreases. [268] Mitchell et al reported increased arterial stiffness with ageing in healthy men and women. [266] The findings in this study are consistent with other studies.

Stiffness parameter  $\beta$  and pressure-strain modulus  $\epsilon$  also increase with features of adiposity such as increasing weight and waist-hip ratio. In a prospective study involving 14 non-obese men who were followed up over 6 to 8 weeks, stiffness index  $\beta$  was found to be increased with weight gain and arterial stiffness was associated with total abdominal fat, abdominal visceral fat and waist circumference. [269]

In this study, similar changes were observed for cIMT in subjects with type 1 diabetes mellitus. cIMT increased with increasing age and waist-hip ratio, more clearly in type 1 diabetes mellitus than in controls.

Age was associated with cIMT in many studies in type 1 diabetes mellitus. [270] [263] cIMT was also reported to be associated with markers of obesity such as increased BMI in type 1 diabetes mellitus. Therefore, central adiposity may play an important role in progression of subclinical atherosclerosis.

## CHAPTER 12 – SUMMARY AND CONCLUSIONS

### *12.1 General discussion*

Main findings in this thesis are:

1. Subjects with T1DM have normal global systolic left ventricular function and normal left ventricular mass index when compared with age-matched controls, but they have reduced medial mitral annular excursion on M-mode echocardiography, indicating a reduction in longitudinal function of the left ventricle. (Chapter 6.1 and 6.3)
2. Subjects with T1DM have a lower mitral E/A ratio, related to a lower early diastolic velocity (E) and an increased late diastolic velocity (A), indicating impaired ventricular relaxation or diastolic dysfunction. (Chapter 8.1)
3. Subjects with T1DM have reduced longitudinal displacement on myocardial velocity imaging at rest and during dobutamine stress. (Chapter 9.3)
4. Subjects with T1DM, however, have normal longitudinal systolic velocity at rest and during dobutamine stress. (Chapter 9.4)
5. Subjects with T1DM, have normal radial myocardial systolic function. (Chapter 9.5)
6. Subjects with T1DM showed incremental increases in peak systolic velocity in response to dobutamine, and thus, they have normal myocardial functional reserve. (Chapter 9.7)
7. Subjects with T1DM showed normal systolic strain, peak early diastolic strain rate and peak systolic strain rate at rest and during dobutamine stress. (Chapter 9.10)
8. Observed differences in longitudinal function are not explained by the effects of exercise. (Chapter 9.14)

9. Features of adiposity and adverse lipid levels correlate with adverse diastolic function in young subjects (including type 1 diabetes mellitus and controls). (Chapter 10.1)
10. Similarly, increased BMI and waist hip ratio in type 1 diabetes mellitus correlate with reduced left ventricular diastolic function. (Chapter 10.2)
11. In young subjects with type 1 diabetes mellitus, mean displacement and mean early diastolic velocity are inversely related to central aortic blood pressure (augmentation index) .(Chapter 10.2)
12. In young subjects with type 1 diabetes mellitus, only mean peak systolic velocity in the basal segments is inversely related to HbA1c. (Chapter 10.2)
13. HbA1c and cIMT are independent predictors for peak systolic velocity (PSV) whereas BMI and total cholesterol are independent predictors for diastolic velocities. (Mean e' and medial mitral annular e'). (Chapter 10.2)
14. Young normotensive subjects with type 1 diabetes mellitus showed no evidence of early atherosclerosis and conduit artery stiffness when compared with controls. (Chapter 12.1)
15. In type 1 diabetes mellitus patients and controls analysed as one group, conduit arterial stiffness (beta and epsilon in the common carotid artery) is related to features of adiposity such as weight and waist-hip ratio. (Chapter 13.1)

I have demonstrated that in young subjects with type 1 diabetes mellitus who do not have any other complications, there is evidence of reduced longitudinal myocardial function of the left ventricle, both systolic and diastolic. This finding is in agreement with other studies in type 1 diabetes mellitus. Di Cori et al reported reduction in systolic strain and strain rate in 40 subjects with type 1 diabetes mellitus ( $28 \pm 4.2$  years). [98] Similarly, Palmieri et al

demonstrated lower systolic strain and strain rate in 20 patients with type 1 diabetes mellitus. (aged  $34 \pm 10$  years) [104] Apart from an M-mode echocardiography study by Raev et al [78], the largest study using tissue Doppler in type 1 diabetes mellitus was carried out by Konduracka et al, and they reported normal left ventricular function in 185 subjects with type 1 diabetes mellitus (age  $34.8 \pm 7.9$  years). [88] However, in Konduracka's study, systolic velocity was not reported. The findings in my study showed that there is reduced longitudinal movement of the myocardium (displacement) on myocardial velocity imaging in type 1 diabetes mellitus and this was supported by the finding of reduced medial mitral annular excursion. Nevertheless, PSV remains similar between the two groups, implying that the duration of systole must be shorter in the subjects with diabetes mellitus. In fact, in the Copenhagen City Heart Study, it was noted that even though longitudinal displacement was significantly lower in diabetic subjects, peak systolic velocity was not. [271] It suggests that displacement is a more sensitive parameter than PSV in detecting subclinical myocardial dysfunction.

This reduction in myocardial displacement was found throughout the dobutamine infusion. However, myocardial functional reserve was normal in my study. This is the first study to demonstrate myocardial function reserve in terms of incremental change in peak systolic velocity but myocardial functional reserve in response to dobutamine has been reported in type 2 diabetes mellitus. Vinereanu et al reported a reduction in myocardial functional reserve [85] whereas Fang et al [86] reported a normal functional reserve. Studies in type 2 diabetes mellitus usually involve older patients and subjects invariably tend to have confounders such as hypertension.

In my study, I have demonstrated in young subjects with type 1 diabetes mellitus who do not have any other complications and who are normotensive, that myocardial functional

reserve is preserved. In my study, peak systolic velocity (PSV) did not show any difference between patients and controls at rest or at peak doses of dobutamine, but the response was significantly lower at intermediate doses of dobutamine (10  $\mu\text{g}/\text{kg}/\text{minute}$  and 20  $\mu\text{g}/\text{kg}/\text{minute}$ ).

Normal myocardial functional reserve indicates that subjects with type 1 diabetes mellitus have preserved metabolic capability to cope with increased work load of the myocardium. In other words, small vessel disease is unlikely as one would expect reduction in myocardial functional reserve with any form of vascular disease in the event of increased oxygen demand. In a magnetic resonance spectroscopy and stress magnetic resonance imaging study in type 1 diabetes mellitus, it was shown that myocardial energetics in type 1 diabetes mellitus was impaired as indicated by reduced phosphocreatinine/ $\gamma$ -ATP ratio and that this impairment was independent of coronary microvascular function. Therefore, early changes in myocardial dysfunction may be due to metabolic changes. [272]

A few studies in type 2 diabetes mellitus have shown that there was reduction in longitudinal myocardial function with an increase in radial function. [89] [85] It was hypothesized that increased radial function may be a “compensatory” response to reduction in longitudinal function as longitudinal fibres are more prone to the effects of ischaemia. However, Ernande et al reported reduction in radial function in type 2 diabetes mellitus. [90] In my study, I did not find any reduction in radial myocardial function. The inconsistencies in these findings of increased radial function may be due to the technical difficulty in obtaining radial velocities on myocardial velocity imaging as myocardial velocity obtained by TDI are angle dependent and prone to the motion of the heart. Moreover, it may be due to the differences in the pathophysiology between type 1 and type 2 diabetes mellitus. Subjects in type 2 diabetes mellitus tend to have other associated confounding factors such as

hypertension. Lastly, the results may vary depending on the clinical stage in the natural history of the disease.

Contrary to many other studies, I did not find any evidence of vascular disease in the study group. The subjects with type 1 diabetes mellitus in the study had had diabetes mellitus for a relatively shorter duration of diabetes mellitus and none of the subjects had any other complications of diabetes mellitus such as retinopathy and microalbuminuria. However, it is noted that conduit artery stiffness correlates with features of adiposity and abnormal lipid profile.

In fact, no strong correlations between changes in myocardial function and glycaemia were observed apart from the association between peak systolic velocity (PSV) and mean HbA1c. This finding is consistent with finding by Vinereanu et al. [85] Nevertheless, adverse myocardial function correlates with features of adiposity such as increased waist circumference and adverse lipid levels. In the diabetic heart, glucose uptake is reduced and there is increased utilization of free fatty acids (FFA). [273] The use of FFA for energy is less energy efficient. This increased utilization of FFA may be due to increased availability of FFA in the circulation. Reduced glucose uptake, increased FFA uptake and reduced diastolic function were reported in 78 patients with type 2 diabetes mellitus. [274] Obesity itself is known to be associated with myocardial dysfunction or heart failure. [275]

Similarly, although vascular function in young subjects with type diabetes mellitus did not differ statistically from those of controls, factors which correlated with vascular structure and function were similar to those of myocardial function in my study. Body weight, higher BMI and abnormal lipids seem to be more significant determinants of both myocardial and vascular function than are glycaemia or blood glucose level.

Patients with diabetes mellitus have a poorer prognosis when they have heart failure. [276] The prevalence of subclinical diastolic dysfunction in type 2 diabetes mellitus was reported to be as high as 40%. [82] [277] The prevalence of clinical heart failure in type 2 diabetes mellitus has been reported to be about 12%. [278] Early detection of subclinical disease may be helpful in preventing heart failure, if it can lead to earlier interventions that have been proved to be effective.

In my study, reduced myocardial function was observed in young subjects with type 1 diabetes mellitus despite them having comparable vascular function and structure to controls. It is possible that metabolic changes in diabetes mellitus affect both myocardium and blood vessels at the same time. Later in the evolution of the disease, other risk factors such as hypertension and dyslipidaemia might exacerbate conduit arterial stiffness which in turn might worsen myocardial dysfunction. Obesity is observed to be independently associated with the prevalence of heart failure with preserved ejection fraction in diabetes mellitus.[279] Co-existing hypertension worsens left ventricular diastolic function in patients with type 2 diabetes mellitus. [280]

In my study, it was also noted that augmentation index negatively correlates with displacement and early diastolic tissue velocity E whereas cIMT positively correlates with late diastolic velocity A in type 1 diabetes mellitus. There are not many studies which studied both myocardial function and vascular function at the same time, particularly in type 1 diabetes mellitus. Endothelial dysfunction as indicated by flow mediated dilation was associated with the number of left ventricular segments with myocardial dysfunction in type 1 diabetes mellitus. [100] Loimaala et al found that pulse wave velocity negatively correlated with mitral annular early diastolic velocity. [116] The association between vascular function and myocardial function is not observed to be strong in my study. This may be due to the fact

that subjects have not developed detectable vascular dysfunction yet or may be due to small number of subjects in the study.

## ***12.2 Clinical Implications***

Tissue Doppler imaging or myocardial velocity imaging may be useful in detecting early myocardial dysfunction in young subjects with type 1 diabetes mellitus. Longitudinal displacement may be the most sensitive parameter to detect early subclinical dysfunction in type 1 diabetes mellitus. However, in the absence of long-term clinical studies, its use should currently be on an individual basis.

In fact, early changes in the myocardium may be functional or metabolic. If that is the case, early treatment of cardiovascular risk factors might halt progression into overt heart failure. DCCT/EDIC study has proved that intensive glycaemic control reduced cardiovascular disease and mortality in type 1 diabetes mellitus.[109] Intensive treatment was associated with weight gain and metabolic syndrome and its consequences. [241] However, benefit of intensive treatment still outweighs the risks of metabolic syndrome.

In my study, it appears that even in young subjects who have normal BMI and normal lipid profiles, body weight and lipid level seem to play a significant role in determining subclinical myocardial and vascular function. It is possible that the same metabolic abnormalities or pathophysiological process affect both myocardial and vascular function simultaneously at an early stage in the disease process. It is imperative that a multiple risk control approach is used in the management of type 1 diabetes mellitus so that long-term cardiovascular complications can be reduced, delayed or prevented. It implies that early management in type 1 diabetes mellitus should also concentrate on multiple risk factors management even in a younger age group on top of good glycaemic control.

### ***12.3 Study limitations***

Sample size is a major limitation in my study. Despite my regular efforts of recruiting from diabetic clinics in Cardiff and Vale NHS trust hospitals, only 19 type 1 diabetes mellitus patients were recruited. However, I managed to recruit 34 controls. Excellent reproducibility in myocardial velocity imaging should limit type 2 error.

In my study, pseudonormalization of mitral inflow velocity pattern was not excluded as Valsalva manoeuvre was not performed during echocardiography and pulmonary vein flow was not measured. However, it is very unlikely that diastolic dysfunction in young subjects with type 1 diabetes mellitus would be that advanced without other complications.

Another limitation of my study was that autonomic function was not tested as the study was mainly designed to detect subclinical myocardial and vascular dysfunction and myocardial functional reserve. Again, in the absence of other microvascular complications, it is very unlikely that subjects would have significant autonomic neuropathy.

This study is a cross-sectional study and therefore, it is not possible to define causal relationship in any of the associations observed in the study.

### ***12.4 Conclusions***

Subjects with type 1 diabetes mellitus have reduced longitudinal myocardial function but normal myocardial functional reserve. Features of adiposity such as increased waist circumference, dyslipidaemia and hyperglycaemia associate with adverse myocardial function in young subjects with type 1 diabetes mellitus. Controlling lipid levels and maintaining body weight may be as important as glycaemic control in type 1 diabetes mellitus.

### ***12.5 Future directions***

A longitudinal follow-up study with myocardial velocity imaging may increase current understanding of the natural history of diabetic cardiomyopathy.

An intervention study to observe the effects of lipid and weight control on myocardial function in type 1 diabetes mellitus may help to reduce cardiovascular mortality in type 1 diabetes mellitus.

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