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Disturbances in myocardial diastolic and vascular function with the emphasis on type 2 diabetes

Diagnostic and therapeutic opportunities

by

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Att våga
är att förlora fotfästet en liten stund
Att inte våga
är att förlora sig själv

Søren Kierkegaard (1813-1855)

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ABSTRACT

Background and aims
Cardiovascular involvement is common in diabetes and diastolic myocardial and endothelial dysfunction are early signs. The prognosis is serious and tools for early detection and a search for improved management strategies are important. This thesis explores tools for the early detection of myocardial involvement and examines whether intensive glucose control could improve diastolic and endothelial dysfunction in patients with type 2 diabetes mellitus.

Study I
In comparison with mitral pulse wave Doppler, Tissue Doppler Imaging (TDI), a relatively pre-load independent technique, may improve the early identification of patients with diastolic dysfunction. Fifteen controls without heart failure, 30 patients with heart failure, were studied, 15 patients with diastolic left ventricular dysfunction and 15 with systolic left ventricular dysfunction. All the patients with diastolic heart failure were identified by mitral pulse wave Doppler or TDI, but only 11 were identified by atrio-ventricular plane displacement. The number of false positive patients were eight, ten and nine, respectively ($p<0.01$, $p<0.05$ and NS) for each of the three methods.

Study II
Eighty-seven patients with type 2 diabetes classified as having no (n=60), mild (n=13) or moderate (n=14) left ventricular diastolic dysfunction by Doppler echocardiography and TDI were investigated with Velocity Vector Imaging (VVI) which evaluates myocardial deformation (strain). Left atrial volume was larger in patients with moderate diastolic dysfunction compared with mild or no diastolic dysfunction (p=0.01). Left atrial roof strain distinguished no diastolic dysfunction from mild and moderate diastolic dysfunction (p=0.0073). Systolic left atrial strain correlated to total emptying fraction (r=0.70, p<0.0001) and inversely to left atrial volume (r=-0.35, p=0.0009).

Studies III-IV
Thirty-nine patients with type 2 diabetes and signs of diastolic dysfunction but no other cardiovascular disease manifestations were randomly assigned to glucose normalisation by insulin (I-group; n=21) or oral glucose-lowering agents (O-group; n=18). Myocardial diastolic dysfunction and coronary flow reserve were studied with Doppler echocardiography, including TDI and myocardial contrast-enhanced echocardiography. Fasting glucose and HbA_{1c} were normalised in both groups, but this did not significantly influence myocardial diastolic dysfunction in either group (p=0.65). There was no difference in coronary flow reserve before and after improved glycaemic control. Twenty-two of the patients (I-group n=10; O-group n=12) were also investigated in terms of endothelial function and skin microcirculation by brachial artery flow-mediated dilatation (FMD) and laser Doppler fluxmetry respectively. Glycaemic normalisation did not improve microcirculation. A reduction in FMD from 6.0 ± 2.2 to 4.7 ± 3.0% was observed in the I-group (p=0.037), but there was no change in the O-group (4.3±2.3 to 4.7±3.3%; p=0.76). The between-group difference was not significant (p=0.12).

Conclusions
TDI is useful for diagnosing diastolic myocardial dysfunction, with accuracy similar to that of conventional echocardiography including mitral pulse wave Doppler flow.
Left atrial deformation, measured as regional and overall systolic strain, is impaired in patients with type 2 diabetes mellitus and mild to moderate left ventricular diastolic dysfunction and offers new information on regional LA function and LA volumes. Further, it may add to traditional Doppler echocardiography measurements for diagnosis of diastolic dysfunction.
The hypothesis that improved glycaemic control would reverse early signs of myocardial diastolic and endothelial dysfunction in patients with type 2 diabetes was not proven. Whether it is possible to influence more pronounced dysfunction, particularly in patients with less well-controlled and long-standing diabetes, remains to be further explored in controlled clinical trials.

SAMMANFATTNING

Bakgrund och syften
Kardiovaskulära komplikationer är vanliga vid diabetes. Tidiga tecken är diastolisk myokardiell liksom endotelial dysfunktion. Eftersom prognosen är allvarlig är instrument för tidig upptäckt och förbättrad behandling viktiga. Denna avhandling undersöker metoder för tidig upptäckt av myokardiell påverkan och testar hypotesen att intensiv glukos kontroll förbättrar diastolisk och endotel dysfunktion hos patienter med typ 2 diabetes mellitus.

Studie I
Tissue Doppler Imaging (TDI) är en teknik som är relativt oberoende av fyllnadstryck (preload) och kan, jämfört med mitral puls våg Doppler-teknik, kan, förbättra tidig identifiering av patienter med diastolisk dysfunktion. Femton kontroller utan hjärtsvikt och 15 patienter med diastolisk och 15 med systolisk vänster kammar dysfunktion och hjärtsvikt studerades. Alla patienter med diastolisk hjärtsvikt identifierades genom mitral puls våg Doppler eller TDI men endast 11 genom förskjutning i det atrio-ventrikulära hjärtplanet. Antalet falskt positiva patienter var 8, 10 och 9 ($P < 0,01$, $P < 0,05$ och NS) för respektive metod.

Studie II
Åttiosju patienter med typ 2 diabetes utan (n = 60) och med mild (n = 13) eller måttlig (n = 14) vänster kammar diastolisk dysfunktion vid Doppler ekokardiografi eller TDI undersöktes med Velocity Vector Imaging (VVI), en teknik som utvärderar myokardiell deformatering (strain). Vänster förmaksvolym var större hos patienter med måttlig än hos de med lindrig eller ingen påvisad diastolisk dysfunktion ($p = 0,01$). Strain i vänster förmaks tak separerade normal från lindrig och måttlig diastolisk dysfunktion ($p = 0,0073$). Systolisk strain i vänster förmak korrelerade till den totala tömningsfraktionen ($r = 0,70$, $p < 0,0001$) och omvänt till vänster förmaks volym ($r = -0,35$, $p = 0,0009$).

Studie III-IV
Trettionio patienter med typ 2 diabetes och tecken på diastolisk dysfunktion men utan andra tecken till hjärt-och kärlsjukdom randomiserades till normalisering av blodsockret med hjälp av insulin (I-gruppen, n = 21) eller orala glukossänkande medel (O-gruppen; n = 18). Myokardiell diastolisk dysfunktion och koronar flödesreserv studerades med hjälp av Doppler-ekokardiografi inklusive TDI samt myokardiell kontrast-förstärkt ekokardiografi. Fasteblodsocker och HbA_{1c} normaliserades i båda grupperna, men detta påverkade inte nämnvärt den myokardiella diastoliska dysfunktion i respektive grupp ($p = 0,65$). Det förelåg ingen skillnad vad gäller koronar flödes reserv före eller efter förbättrad glykemisk kontroll. Tjugo två av patienterna (I-grupp n = 10, O-grupp n = 12) undersöktes med avseende på endotelfunktion och mikrocirkulation i huden genom flödes-medierad dilatation (FMD) i en armartär och laser Doppler fluxmetry. Normalisering av blodsockret förbättrade inte mikrocirkulationen. En minskning av FMD från 6,0 ± 2,2 till 4,7 ± 3,0% observerades i I-gruppen ($p = 0,037$) men inte i O-gruppen (4,3 ± 2,3 till 4,7 ± 3,3%, $p = 0,76$). Skillnaden mellan de två grupperna var inte signifikant ($p = 0,12$).

Konklusioner
TDI är en användbar teknik för att diagnostisera diastolisk myokardiell dysfunktion med en noggrannhet motsvarande den med konventionell ekokardiografi inklusive mitralpulsvågsDoppler flöde.
Deformation av vänster förmak, mätt som regional och övergripande systolisk strain, är nedsatt hos patienter med typ 2 diabetes och mild till måttlig vänsterkammar diastolisk dysfunktion och erbjuder ny information om regional funktion och volymer i vänster förmak. Metoden kan därmed ersätta eller komplettera en del traditionella Doppler ekokardiografiska mätningar.

Hypotesen att förbättrad glukoskontroll skulle kunna normalisera tidiga tecken på myokardiell diastolisk och endotel dysfunktion hos patienter med typ 2 diabetes bekräftades inte. Huruvida det är möjligt att påverka mer uttalad dysfunktion, särskilt hos patienter med mindre väl kontrollerad och mer långvarig diabetes återstår att utredas i kontrollerade kliniska prövningar.

LIST OF ORIGINAL PAPERS

This thesis is based on the following studies, which will be referred to by their Roman numerals.

I

Jarnert C, Mejhert M, Ring M, Persson H, Edner M. Doppler tissue imaging in congestive heart failure patients due to diastolic or systolic dysfunction: a comparison with Doppler echocardiography and the atrio-ventricular plane displacement technique. European Journal of Heart Failure 2000;2:151-160

II

Jarnert C, Melcher A, Caidahl K, Persson H, Rydén L, Eriksson M J. Left atrial velocity vector imaging for the detection and quantification of left ventricular diastolic function in type 2 diabetes. European Journal of Heart Failure 2008; 10:1080-1087.

III

Jarnert C, Landstedt-Hallin L, Malmberg K, Melcher A, Öhrvik J, Persson H, Rydén L. A randomized trial on the impact of strict glycaemic control on myocardial diastolic function and perfusion reserve. A report from the DADD (Diabetes mellitus And Diastolic Dysfunction) study. European Journal of Heart Failure 2009; 11:39-47.

IV

Jarnert C, Kalani M, Rydén L, Böhm F. The impact of strict glycaemic control on endothelial function and skin microcirculation in patients with type 2 diabetes. In manuscript.

List of Abbreviations

A	Atrial transmitral peak flow velocity
ACE	Angiotensin Converting Enzyme
AVPD	Atrio Ventricular Plane Displacement
DADD	Diabetes mellitus And Diastolic Dysfunction
E	Early transmitral peak flow velocity
E/A	Ratio of peak mitral flow velocities
E/E´	Ratio of diastolic peak annular velocities
EF	Ejection Fraction
FPG	Fasting Plasma Glucose
GAD	Glutamic Acid Decarboxylase antibodies
HbA_{1c}	Glycosylated haemoglobin A_{1c}
LA	Left Atrial
LDF	Laser Doppler Fluxmetry
MBFI	Myocardial Blood Flow Index
MBVI	Myocardial Blood Volume Index
MiDD	Mild Diastolic Dysfunction
MoDD	Moderate Diastolic Dysfunction
MPWD	Mitral Pulse Wave Doppler
NoDD	No Diastolic Dysfunction
NYHA	New York Heart Association classification
ROI	Region Of Interest
SI	Signal Intensity
TDI	Tissue Doppler Imaging
WHO	World Health Organisation
WMI	Wall Motion Index
VVI	Velocity Vector Imaging

INTRODUCTION

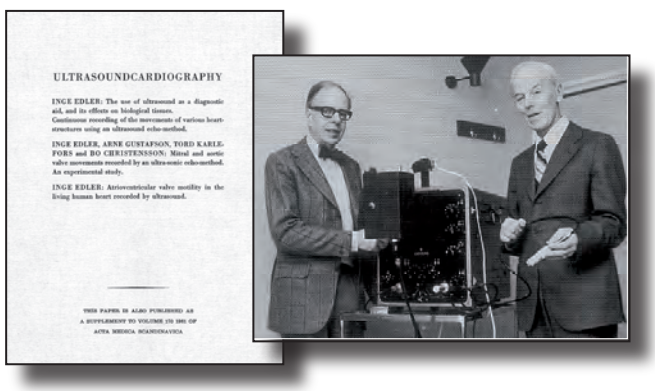
History

Myocardial dysfunction and heart failure

Descriptions of the clinical manifestations of compromised myocardial function, heart failure, could already be found in ancient Egypt, Greece and India. The Romans used digitalis glycosides, an extract from Scilla Maritima, for the symptoms caused by this disease. It was not until 1628, however, when William Harvey (1578-1657) published what was at that time a revolutionary book, *Exercitatio anatomica de motu cordis et sanguinis in animalibus*, that the first modern view of the circulatory system, a prerequisite for understanding the pathophysiology of myocardial dysfunction, became available. Questioning Galenos' ancient view that blood streamed to the tissues and was consumed there, Harvey demonstrated the presence of a cardiovascular system through which blood was pumped to the tissues by the heart and was then transported back through the venous system.

Diagnosis

An important step towards understanding heart failure was taken in 1895 when Wilhelm Röntgen (1845-1923) discovered that it was possible to use X-rays to study the interior of the body. His technique allowed a more detailed investigation of heart enlargement and fluid accumulation in the lungs and pleural cavities, characteristic features of compromised myocardial function. Cardiac catheterisation, introduced by Werner Forssman (1904-1979) in 1929 and further developed by André F Cournand (1895-1988) and Dickinson Richards (1895-1973), led to the haemodynamic hallmarks of the failing myocardium, reductions in cardiac output and increased filling pressures. Another very important tool enabling detailed non-invasive studies of myocardial function in patients with heart failure was the invention of echocardiography, presented in 1954 by the cardiologist Inge Edler (1911-2001) and engineer Hellmuth Hertz (1920-1970) [1].


ULTRASOUNDCARDIOGRAPHY

INGE EDLER: The use of ultrasound as a diagnostic aid, and its effects on biological tissues. Continuous recording of the movements of various heart-structures using an ultrasound echo-method.

INGE EDLER, ARNE GUSTAFSON, TORD KARLEFORS and BO CHRISTENSSON: Mitral and aortic valve movements recorded by an ultra-sonic echo-method. An experimental study.

INGE EDLER: Atrioventricular valve motility in the living human heart recorded by ultrasound.

THIS PAPER IS ALSO PUBLISHED AS A SUPPLEMENT TO VOLUME 170 1961 OF ACTA MEDICA SCANDINAVICA

Figure 1. Inge Edler (to the right) and Hellmuth Hertz and the frontpage of the thesis written by Dr Edler in 1961, named Ultrasoundcardiography.

Since then, even more advanced techniques for the study of myocardial function and dysfunction have been introduced; they include coronary arteriography, myocardial scintigraphy and cardiac magnetic resonance imaging.

Pathophysiology

The original concept of heart failure stated that it was merely related to a decrease in myocardial systolic function, reducing the ability of the heart to pump blood to the extent that was needed for the various tissues in a given situation to preserve diastolic filling pressures [2]. Accordingly, mild forms of failing function caused symptoms only as a response to increased circulatory demands, such as physical activity, while symptoms were already apparent at rest in patients with severely depressed systolic function, as reflected by the well-known New York Heart Association (NYHA) classification of various stages of this syndrome[3]. Important progress was made following the insight that an initially only modest reduction in myocardial function triggers a compensatory enhancement of the activity in the sympathetic nervous and renin-angiotensin systems. Although this may have short-term benefits, long-lasting activation increases the burden on the failing heart, thereby inducing remodeling of the myocardial tissue. A vicious circle, as depicted in Figure 2, is initiated and it further compromises myocardial function and thereby the clinical condition [4, 5].

It is only during the last decade that it has become apparent that many patients presenting with a classical picture of heart failure have preserved systolic myocardial function. This caused Dougherty et al. to suggest the term “diastolic heart failure” to cover this patient category [6, 7].

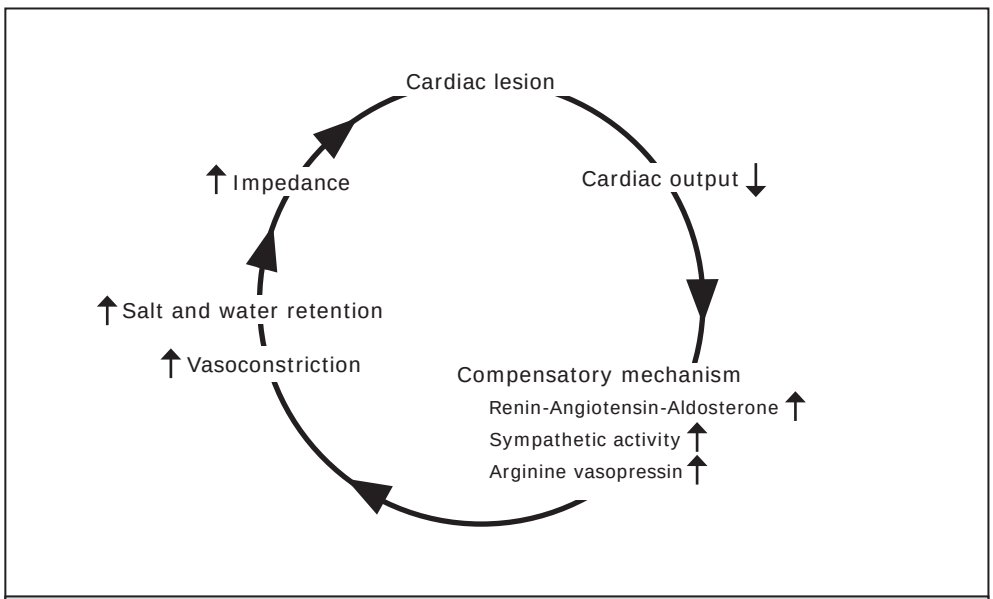


Figure 2. Neuro-hormonal activation caused by depressed myocardial function leads to a vicious circle, further compromising the already reduced myocardial function.

Therapy

Extracts from the plant known as Sea Squill, Scilla Maritima (Figure 3), were probably the first treatment for heart failure known 300 years B.C. and used by Greek and Roman physicians [8]. The active compounds are glycosides with heart-stimulating and diuretic properties [9]. Apart from the use of Scilla, the only available treatment for heart failure for hundreds of years was blood letting and leeches. Other herbs that were used to provoke diuresis in ancient times were parsley and Common Bugloss (Anchusa Officinalis, Figure 4). The active compound in them is allantoin. In 1785, William Withering (1741-1799) discovered that extracts made from the flower of the foxglove (Figure 5), containing digitalis purpurea, were effective against dropsy, as heart failure was known at that time. This initiated the long-lasting use of digitalis as the primary treatment for heart failure. It is still being used, even if its use is less frequent since modern clinical trials demonstrated that the effect of digitalis is fairly limited, at least in the presence of other more effective drugs [10]. In the 19th and early 20th centuries heart failure, associated with peripheral fluid retention was treated with Southey's tubes, which were inserted into oedematous parts of the body, allowing some drainage of fluid [9]. Other ways of eliminating excess fluid and relieving patients from symptoms were pleural and abdominal punctures. Diuretics, originally based on mercury and subsequently in the form of thiazides, were not introduced until 1920 and 1958 respectively. In particular, the introduction of thiazide diuretics and subsequently loop diuretics [11-13] made the treatment of the symptoms of congestion much more effective, to the great benefit of patients.

Understanding the need to counteract neuro-hormonal activation revolutionized the therapy of patients with heart failure due to compromised myocardial function. One of the first studies to demonstrate improved effects not only on symptoms but also on survival was the first Consensus study[14]. This was followed by similar trials with the same outcome. The introduction of beta-blockers and angiotensin-receptor blockers was made somewhat later [15-18].

Figure 3. Scilla Maritima- drawn by Mrs Elisabeth Blackwell (1700-1758), and published in *"The Curious Herbal"*.

Reprinted with permission from the library of the Society of Apothecaries.

Figure 4. Common Bugloss (Anchusa Officinalis).

Figure 5. Foxglove from *"An account of the Foxglove and some of its medical uses with practical remarks on dropsy and other diseases"* by Dr. William Withering.

Reprinted with permission from the Hagströmer medico-historical library at Karolinska Institutet.

Diabetes mellitus

The first known notes on diabetes are those made by the Egyptian physician Hesy-Radates in the Ebers Papyrus dated about 1552 B.C., who reported on a condition in which the patient was "passing too much urine". The word diabetes appeared for the first time in the English language in 1425. It was not until the 16th century, however, that Paracelsus identified diabetes as a serious, general disorder. In 1797, the Scottish physician John Rollo (†1809) created the first medical therapy for diabetes, prescribing an 'animal diet' for his patients of 'plain blood puddings' and 'fat and rancid meat' in an attempt to manage them with a diet. Approximately 100 years later in 1869, the German medical student Paul Langerhans (1847-1888) discovered the islet cells of the pancreas but was unable to explain their function. Further research led to the extraction of a pancreatic hormone, insulin, studied in several series of animal experiments by Frederick G Banting (1891-1941) and John Macleod (1876-1935) among others. Insulin was given to a patient with diabetes, the 14-year-old boy Leonard Thompson, for the first time in 1922. He was a 'charity patient' at the Toronto General Hospital and he lived for 13 years before dying of pneumonia at the age of 27. Another 13-year-old patient, Elizabeth Evans Hughes, the daughter of the US Secretary of State Charles Evans Hughes, arrived in Toronto in the same year to be treated by Banting for her diabetes. Upon arrival, she was in very poor physical condition, but she responded immediately to insulin and went on to live a productive life until she died in 1981 at the age of 73 years. In appreciation of their discovery of insulin, Frederick Banting and John Macleod were awarded the Nobel Prize in Medicine in 1923 [19].

Heart failure

Definition and classification

According to the European management guidelines, the diagnosis of the clinical syndrome of heart failure is based on a combination of typical symptoms and objective signs of functional abnormalities of the heart. Furthermore, it is stated that heart failure is not a diagnosis in itself and that the underlying reason must be searched for [6]. The most frequently used classification of the severity of heart failure is that issued by the New York Heart Association as presented in Table 1 [3].

Table 1. New York Heart Association (NYHA) classification of heart failure.

NYHA Class 1
No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation or dyspnoea.

NYHA Class 1I
Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation or dyspnoea.

NYHA Class 1II
Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity results in fatigue, palpitation or dyspnoea.

NYHA Class 1V
Unable to carry on any physical activity without discomfort. Symptoms at rest. If any physical activity is undertaken, discomfort is increased.

Heart failure is commonly referred to as a clinical condition related to either systolic or diastolic myocardial dysfunction. Systolic dysfunction, the best defined entity, relates to the impaired capacity of the heart to eject blood from the left ventricle, usually quantified as a low ejection fraction (EF). Diastolic dysfunction has been more recently defined. In a new European consensus document, it is, however, stated that the definition of isolated diastolic heart failure should be seen as a combination of clinical symptoms, normal left ventricular ejection fraction and objective signs of abnormal filling or relaxation of the left ventricle as documented by cardiac catheterisation, echocardiographic methods and elevated levels of natriuretic peptides BNP and/or NTproBNP [20]. There is still no consensus concerning the cut-off value for a preserved EF [6]. One explanation for the discrepancy in the definitions is that the EF is defined by stroke volume divided by the end-diastolic volume for the relevant ventricular chamber of the heart. It is therefore closely related to the increase in the end-systolic and end-diastolic volumes.

Epidemiology

The prevalence of heart failure is increasing in western society for several reasons, including ageing populations and the increased survival of patients with ischaemic and valvular heart disease. The improved care of patients with heart failure resulting in increased longevity is also contributing [21]. According to the most recent European guidelines, presented in 2008, the prevalence of heart failure and asymptomatic left ventricular dysfunction is about 4% of the population [6, 22]. The prevalence rises steeply with age to reach 10-20% in the 70- to 80-year age group [23]. In younger age groups, heart failure is more frequent in men due to their higher prevalence of coronary heart disease, a difference that levels out with increasing age [6, 24]. In addition to ischaemic heart disease, the most common reasons for heart failure are hypertension, valvular dysfunction and cardiomyopathy [6, 25, 26]. Symptoms of heart failure in patients with preserved myocardial systolic function are more common in women, the elderly and patients with hypertension or diabetes mellitus [6]. The actual prevalence varies in different populations, but it has been estimated that it may be about 50% of all patients with heart failure [27]. Compromised diastolic myocardial function appears to be particularly common in patients with diabetes. Boyer et al.[28] reported that, among 61 normotensive diabetic patients, all without symptoms of heart failure, signs of diastolic myocardial dysfunction were detected in as many as 74%. The presence of diabetes is highly predictive of diastolic dysfunction and BNP and NT-proBNP can predict the echocardiographic severity of diastolic dysfunction [29].

Diabetes mellitus

Definition and classification

Diabetes mellitus is a metabolic disorder of multiple aetiology characterised by hyperglycaemia due to decreased insulin secretion, insulin action or a combination [30]. The defective insulin action influences not only carbohydrate metabolism but also fat and protein metabolism. Diabetes is associated with microvascular complications causing organ damage including retinopathy, neuropathy, nephropathy and autonomic dysfunction. Patients with diabetes also run a considerable risk of developing macrovascular complications, including cardiovascular, cerebrovascular and peripheral artery disease.

The first unified classification of diabetes was published in 1979 by the National Diabetes Data Group [31] and in 1980 by the World Health Organisation [32]. Since then, a few modifications have been presented by the WHO and by the American Diabetes Association (ADA) [33]. The criteria established by the WHO are presented in Table 2.

Table 2. Values for the diagnosis of diabetes mellitus and other categories of glucose abnormalities according to WHO in 2006.

	Glucose concentration (mmol/l)	
	Plasma Venous	**Whole blood** Capillary
Diabetes mellitus		
Fasting	≥ 7.0	≥ 6.1
or		
two hours post glucose load	≥ 11.1	≥ 11.1
Impaired Glucose Tolerance (IGT)		
Fasting	< 7.0	< 6.1
and		
two hours post glucose load	≥ 7.8	≥ 7.8
Impaired Fasting Glucose (IFG)		
Fasting	6.1 to 6.9	5.6 to 6.1
and		
two hour post glucose load	< 7.8	< 7.8

Diabetes is classified into two major types; type 1 with no remaining insulin production and type 2 predominantly dependent on a combination of insulin deficiency and resistance. Type 1 diabetes is characterised by a deficiency of insulin production due to an autoimmune process leading to pancreatic islet β-cell destruction. The onset of this disease takes place at a young age and the first symptoms may be sudden and quite severe, including polyuria, thirst, tiredness and weight loss. Late Autoimmune Diabetes in Adults (LADA) is a similar type of diabetes. This type has its onset in older patients and in this case the progress is slower compared with type 1 diabetes mellitus. Patients with type 1 diabetes and LADA have antibodies against pancreatic β-cells such as glutamic-acid-decarboxylase (GAD).

Type 2 diabetes is the most common and accounts for 85-90% of all diabetes. It is caused by insufficient insulin secretion, combined in most cases with reduced insulin sensitivity. The onset takes place typically during middle age and frequently in patients who are overweight and physically inactive.

Epidemiology

The prevalence of diabetes is 4-5% in western society and it increases with age [6]. From a global perspective, the disease is rapidly increasing, especially in the developing countries. The vast majority of the increase relates to type 2 diabetes. The most recent predictions by the International Federation of Diabetes [34] estimate that diabetes affected 246 million

people in 2007 (6% of the global population) and that an increase to approximately 380 million people can be expected by 2025 (7% of the global population). There are several explanations, but obesity due to a changing lifestyle, with a decreasing demand for physical activity and excessive food intake, is regarded as the main reason, in combination with ageing populations [35].

Diabetes and heart failure

Prevalence and incidence

A relationship between diabetes and heart failure has been discussed for many years. Epidemiological evidence indicates that diabetes increases the risk of future heart failure [22, 36-38]. In 2001 and 2004, Nichols et al. reported on the close link between diabetes and heart failure [39, 40]. In a hospital-based registry, they identified 9,591 individuals diagnosed with type 2 diabetes and an age- and gender-matched control group without diabetes for the diagnosis of heart failure. The prevalence was 11.8% among those with diabetes and 4.5% among those without diabetes. The incidence when examined 2.5 years later was 7.7 and 3.4% in those free from heart failure at baseline. The heart failure incidence/1,000 patient years was almost three times higher in the diabetic group. The difference was independent of age, diabetes duration, presence of ischaemic heart disease and hypertension. Notably, the patients with diabetes were ten years younger on average at the time of the heart failure diagnosis and there were no gender differences. Bertoni et al. [41] studied the prevalence and incidence of heart failure in older populations using a national 5% sample of Medicare claims from 1994-1999 to create a population-based cohort of 151,738 untreated beneficiaries with diabetes at ≥ 65 years. The prevalence was high, 22%, as was the incidence, 12.6/100 patient years. There were no gender differences and both prevalence and incidence increased with age and diabetes co-morbidities. In the Reykjavik population-based study conducted by Thrainsdottir et al. [22], the prevalence of the combination of heart failure and diabetes was 0.5% in men and 0.4% in women, increasing with age. Heart failure was identified in 12% of patients with diabetes compared with 3% of those without.

Pathophysiology

The exact mechanisms underlying the association between diabetes and heart failure are still the subject of debate, although there are several possibilities. Coronary artery disease is evidently an important contributor and hypertension and obesity, frequent among patients with type 2 diabetes, may also contribute [42, 43]. However, Lundbaeck suggested the possibility of a diabetes-specific cardiomyopathy back in 1954 [44]. Other researchers followed this path and, two decades later, Rubler et al. published supporting data concluding that myocardial disease may be a complication of diabetes in itself and may not only relate to co-existence with coronary artery disease [45]. In 2001, Iribarren et al. [46] investigated the relationship between glycaemic control and various manifestations of cardiovascular disease. They showed that an increase in glycosylated haemoglobin A_{1c} ($HbA1_c$) of 1% was associated with an 8% greater likelihood of heart failure. These results lent support to the assumption that poor glycaemic control may enhance the risk of developing heart failure, but there are few if any data that support the hypothesis that improved glycaemic control reduces this risk.

It seems as though diabetes increases the severity of heart failure at any given level of left

ventricular systolic dysfunction [47]. Likewise, the link between diabetes and myocardial dysfunction may exist independent of coronary artery disease [48, 49]. Diabetic cardiomyopathy has been related to an increase in left ventricular wall thickness and myocardial mass and, early in the process, signs of myocardial diastolic dysfunction [50, 51]. Another characteristic feature of diabetic cardiomyopathy is a lack of response to injury [52]. Evidently, the impact of the metabolic disease and hypertension appears in some respects to act synergistically. This may explain why hypertension carries a higher risk of cardiovascular events and mortality in patients with diabetes than those without it. It may also explain why meticulous blood pressure-lowering treatment appears to be particularly effective in the diabetic subject.

After the onset of diastolic dysfunction, progressive myocardial dysfunction occurs, with structural and functional abnormalities such as myocyte hypertrophy, deposition of PAS- positive glycoproteins, collagen [53], abnormalities in calcium handling [54], interstitial oedema, intramyocardial collagen accumulation [53], interstitial fibrosis and intramyocardial microangiopathy [55]. Moreover, there is experimental and clinical evidence of enhanced cellular apoptosis with myocyte loss [56-58]. Several of these changes are believed to be a consequence of oxidative stress induced by hyperglycaemia. This suggests a causative link between hyperglycaemia, oxidative stress, cardiomyocyte apoptosis and diabetic cardiomyopathy. In spite of this, attempts to improve the early signs of myocardial dysfunction by means of glucose normalisation have so far not been successful, although uncontrolled observations in more severe diabetes seem promising[59].

Prognostic implications

In general, the prognosis of heart failure is serious, despite modern treatment [60, 61]. The opportunity to treat the underlying reason is naturally crucial, as is the individual response to specific treatment. Approximately 40% of all patients admitted to hospital because of heart failure are dead or re-admitted within one year. The prognosis of heart failure due to diastolic dysfunction is similar to that of systolic heart failure [62].

A patient with diabetes has higher mortality for cardiovascular reasons rather than because of the diabetes itself [63]. Bertoni et al.[41]followed a population of elderly patients with diabetes for 60 months. Those who developed heart failure had a mortality of 32.7/100 person years, considerably higher than the 3.7/100 person years among those who remained free from heart failure. Thrainsdottir et al. reported in a follow-up of the Reykjavik study that the all-cause and cardiovascular mortality after 10 years increased with increasing glucose abnormality with and without heart failure. The lowest survival was found in those with the combination of diabetes and heart failure [26].

To summarise; heart failure and diabetes are two increasingly common conditions. The combination carries a serious prognosis, causing Bell and colleagues to state that heart failure is a frequent, forgotten and often fatal complication of diabetes [64]. It is therefore important to improve the early detection and treatment of this connection.

Diagnosis of diastolic dysfunction

Myocardial diastolic dysfunction is difficult to evaluate, as it comprises abnormalities in left ventricular relaxation, distensibility and filling. There are three main types of abnormal

echocardiographic filling patterns [6]. The first, seen in the early stages, is impaired relaxation in the early phase of diastole, with a decrease in the early transmitral peak flow (E) velocity and a compensatory increase in the atrial transmitral peak flow (A) velocity, resulting in a decrease in the ratio of peak mitral flow velocities (E/A ratio). These changes, which mostly exist in the presence of normal or low left ventricular filling pressures, are common in patients with hypertension and in normal elderly persons. The second stage, seen in patients with intermediately advanced diastolic dysfunction, is characterised by a normalised E/A ratio, a normal deceleration time and a normal performance of the E- and A-wave. This is called pseudonormalisation, due to the concomitant elevation of diastolic filling pressures, and can be disclosed by other Doppler parameters such as pulmonary venous flow or Tissue Doppler Imaging (TDI). The third and most severe stage is the restrictive phase. These patients present with elevated filling pressures, a short deceleration time and a markedly increased E/A ratio. This means that, apart from clinical signs of heart failure, the diagnosis and detailed grading of diastolic dysfunction is dependent on reliable diagnostic methods. Relaxation is disturbed

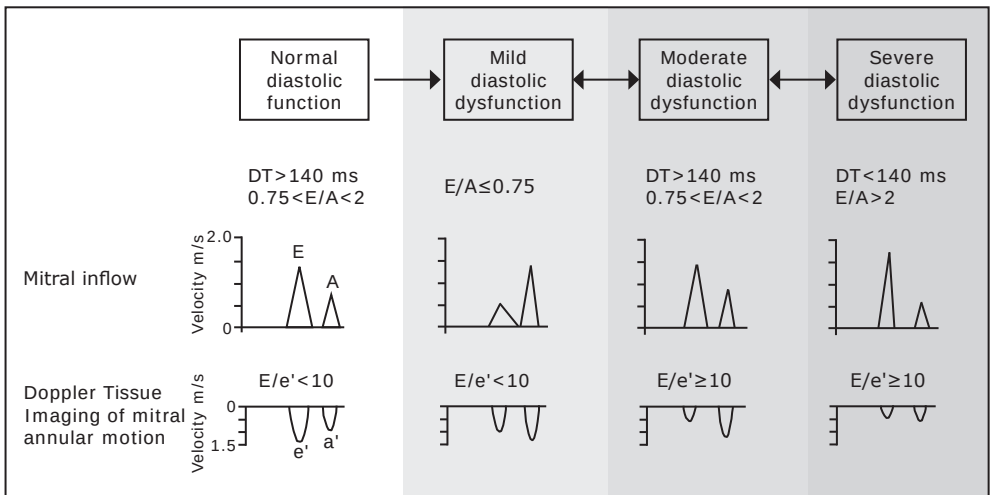


Figure 6. Echocardiography-Doppler criteria for Assessment of Diastolic Function. Modified from Bursi et al [89]. See text for further explanation.

in all stages, whereas compliance is lower in intermediate and severe conditions [65].

Diastolic dysfunction may be influenced by several confounding factors such as heart rate, age, blood pressure, left ventricular hypertrophy, left atrial pressure, myocardial fibrosis and contractility. The most reliable technique for assessing diastolic function is invasive and this method is still the golden standard [66]. Vasan et al. suggested criteria for the diagnosis of diastolic dysfunction. Patients should be categorised according to the degree of diagnostic certainty and have to fulfil three criteria: signs of heart failure, objective evidence of normal systolic function in combination with diastolic left ventricular dysfunction. The first two criteria require clinical observation, laboratory tests, X-rays and echocardiography, while the third criteria necessitates cardiac catheterisation to demonstrate an influence on left ventricular filling pressures. Cardiac catheterisation is, however, not generally accessible and is inconvenient for both the investigator and the patient. For this reason, echocardiographic and Doppler techniques are more useful in clinical practice. In their consensus document,

Paulus et al. [20] underlined the importance of tissue Doppler and analyses of natriuretic hormones as important complements to previous diagnostic tools.

Echocardiographic assessment of diastolic function is a patient-safe, relatively effective, non-invasive tool. A complementary method is TDI, which estimates myocardial systolic and diastolic tissue velocities more directly, thereby providing a relatively load-independent measurement of left ventricular relaxation [67, 68]. In particular, the ratio of diastolic peak annular velocities (E/E´ ratio) correlates closely with left ventricular filling pressures [69]. The addition of maximum left atrial (LA) volume may improve the diagnostic accuracy [70] and LA volume index to body surface area has also been shown to correlate with the severity of diastolic dysfunction [71].

Velocity vector imaging (VVI) is a novel method based on two-dimensional B-mode images. This method involves tracking ultrasonic speckles permitting the angle-independent measurement of tissue velocity and deformation [72]. Another method that might become useful in terms of non-invasive tools for estimating diastolic dysfunction is strain, a dimensionless parameter representing object deformation, and strain rate, representing deformation per unit time [73, 74].

Management of heart failure in the diabetic patient

General aspects

The purpose of heart failure treatment in the diabetic patient is like that for any other patient, namely to reduce mortality and morbidity. The evidence-based medication of heart failure is based on a combination of angiotensin converting enzyme (ACE) inhibitors and/or angiotensin II-receptor blockers, β-blockers, diuretics and aldosterone antagonists. This treatment improves ventricular function, reduces symptoms and the rate of hospital admissions and results in increased longevity [6]. Unfortunately few, if any, clinical trials on heart failure specifically address diabetic patients. Information on the efficacy of various drugs is therefore based on diabetic subgroups included in various heart failure trials (CONSENSUS, Merit-HF, SOLVD, CHARM)[14, 75-77]. One disadvantage is that these subgroups are not always well defined in terms of the diabetic state and treatment. In spite of this, it appears that the impact of this treatment is similar in patients with and without diabetes. Since diabetic patients have a considerably higher absolute mortality and morbidity, the relative impact of therapy is more pronounced in this patient cohort [25].

Diastolic dysfunction

When it comes to the management of patients with heart failure symptoms and a preserved systolic left ventricular ejection fraction, the treatment recommendations are considerably more vague. For this large group of patients, there is still no specific, evidence-based therapy. In a few small studies, ACE inhibitors and calcium antagonists appeared to have a favourable influence [78, 79]. The beta-blocker carvedilol was tested in the Swedic trial [80] recruting patients with echocardiographic signs of diastolic relaxation disturbances but relatively normal levels of natriuretic peptides, i.e. mild diastolic heart failure. There were indications that the drug had a positive effect on diastolic function but no clinical effects. Three large trials

investigated the effects of blockers of the renin-angiotensin-aldosterone system (RAAS), all with negative results (CHARM, CHF-PEP, I-PRESERVE)[77, 81, 82]. In Charm Preserved[83], 3,000 patients with clinical symptoms of heart failure and an EF of > 40% were included. The patients received regular cardiovascular medication and, in addition, the angiotensin-receptor blocker candesartan or placebo. There were no significant changes in mortality but a reduction in hospital admissions for heart failure. In the CHF-PEP study, perindopril was studied in a population of elderly patients previously treated with diuretics. Many patients stopped the study medication and received ACE inhibitors, which reduced the strength of the study. A reduction was seen in the need for hospital admissions, but there were no conclusive results concerning mortality. The most recent study, I-PRESERVE, enrolled 4,128 patients above the age of 60 years, in NYHA Class II–IV and with an EF of ≥ 45%, were enrolled. They were randomised to 300 mg/day of irbesartan or placebo and follow up lasted for a median of 49.5 months. Irbesartan did not cause any significant change in the primary endpoint of death or hospitalisation for cardiovascular reasons.

Heart failure in the diabetic patient

It has been suggested that strict glucose control would be beneficial in this group of patients [84], but, as has already been emphasised, there is still no strong evidence in favour this assumption.

A search for novel treatment modalities is ongoing and one of them is metabolic modulation. Attention has been paid to compounds that shift energy production from the beta-oxidation of free fatty acids towards the energetically more efficient glucose oxidation in conditions such as heart failure, but as yet there is no evidence of success [85, 86].

Unresolved issues

A better understanding of the mechanisms underlying diastolic heart failure in patients with diabetes, including additional potential targets for treatment, is needed. There is also a need for further refinement of the diagnostic opportunities. The methods that are currently available are still not sufficiently specific and demanding, in particular when it comes to the screening of early signs of diastolic dysfunction in diabetic patients. Moreover, early uncontrolled observations [59] relating to the opportunity to improve diastolic function by means of meticulous glucose control still have to be confirmed. It would then be of particular importance to see whether it is possible to influence the signs of this kind of dysfunction without cardiovascular symptoms, thereby hopefully creating an opportunity to counteract progression towards advanced symptomatic heart failure.

AIMS

To search for improved diagnostic methods for the assessment of left ventricular diastolic function (Studies I-II).

To explore the possibility of applying VVI as a method to quantify and detect early signs of left ventricular diastolic dysfunction and left atrial function in patients with type 2 diabetes mellitus (Study II).

To test the hypothesis that intensive glucose control, in particular if based on insulin, improves the signs of diastolic dysfunction and compromised myocardial blood flow reserve in patients with type 2 diabetes (Study III).

To explore the influence of glucose normalisation on flow-mediated dilatation and skin microcirculation comparing insulin with oral glucose-lowering therapy (Study IV).

PATIENTS AND METHODS

Patients and study protocol

Study I

The objective of Study I was to identify characteristic DTI patterns in healthy controls and in patients with clinical signs of heart failure and diastolic or systolic left ventricular dysfunction. A secondary aim was to compare the DTI method with conventional Doppler echocardiography, i.e. mitral pulsed wave Doppler flow and the atrio-ventricular plane displacement technique, to examine the potential of DTI to identify patients with diastolic heart failure. In Study I, DTI is synonymous with the subsequently used abbreviation TDI.

Definitions

Systolic heart failure was defined as a combination of at least two clinical symptoms/signs (dyspnea, tiredness or ankle oedema) and a left ventricular EF of <35% [87].
Diastolic heart failure was defined as clinical signs of heart failure in the absence of echocardiographic signs of compromised left ventricular systolic function (left ventricular EF >45%) [88].

Patients

The material consisted of 30 patients, while 15 age-matched healthy subjects without clinical and electrocardiographic evidence of cardiovascular disease served as controls. Fifteen patients with clinical signs of heart failure and a left ventricular EF of >45% made up a group with diastolic heart failure, while the group with systolic heart failure comprised 15 patients with a left ventricular EF of <35%.
Each attendee was investigated on one occasion, following the stabilisation of his/her clinical symptoms about seven days after hospital admission. Following the collection of information relating to previous diseases, NYHA class and current medication, all the participants underwent a thorough medical examination. In the diastolic group, three patients had a history of prior myocardial infarction (Q-wave = 2). Nine patients in the systolic group had had a prior myocardial infarction (Q-wave = 5). The region of the DTI registration was outside the infarction area.

Pertinent patient characteristics are presented in Table 3. The percentage of patients with angina pectoris, diabetes mellitus and prior congestive heart failure was similar in the two groups, while hypertension and prior myocardial infarction were more prevalent in patients with systolic heart failure, as was the use of digitalis, angiotensin-receptor blockers and nitrates. Patients with diastolic heart failure were more frequently prescribed beta-blockers. NYHA grade I was more frequent among patients with diastolic heart failure, while grades II or III were more common in those with systolic heart failure.

Table 3. Baseline characteristics of the patients in the study. Values presented are % or mean±SD unless otherwise stated.

Variable	**Healthy controls** n=15	**Diastolic heart failure** n=15	**Systolic heart failure** n=15
Male/female (n/n)	6/9	6/9	9/6
Age (range)	76±3 (72-81)	78±4 (74-86)	77±4 (70-81)
Hypertension	0	27	40
Angina pectoris	0	53	40
Diabetes mellitus	0	13	13
Prior heart failure	0	60	67
Prior myocardial infarction	0	20	60
Physical examination			
Heart rate (range)	63±7 (56-78)	64±7 (52-80)	69±14 (47-92)
Mean systolic blood pressure (mmHg)	150±21	144±23	130±20
Mean diastolic blood pressure (mmHg)	77±7	76±14	71±10
Cardiovascular treatment			
Aspirin	0	40	60
Diuretics	0	93	93
ACE-inhibitors	0	40	87
Digitalis	0	20	53
Beta-blockers	0	53	40
Nitrates	0	47	73

Studies II-IV

Shared definitions

Diastolic dysfunction was defined as an abnormality in diastolic filling or relaxation of the left ventricle based on specific echocardiographic criteria as outlined below (page 28-29). In Study II, diastolic function was divided into four categories according to Bursi et al. [89]: no diastolic dysfunction and mild, moderate or severe dysfunction. In Studies III and IV, diastolic dysfunction was categorised as present or absent according to criteria from the Mayo Clinic [27, 90, 91] without further grading (see Table 4).

Type 2 diabetes mellitus was defined according to the World Health Organisation from 1999 [92] and categorised as previously known or newly detected. The previous or ongoing prescription of glucose-lowering treatment (diet or oral drugs) was allowed, but present or preceding insulin treatment was not.

Hypertension was defined as a resting supine blood pressure of >160/95 or a history of hypertension in combination with ongoing blood pressure-lowering therapy.

Ischaemic heart disease was defined as a previous myocardial infarction, angina pectoris, stable or unstable, a previous percutaneous coronary intervention or coronary artery bypass surgery.

General aspects of the patient material

Studies II to IV were conducted on one patient population recruited as outlined in Figure 7. Patients were invited to participate in the study by advertisements in the local press, at the diabetes day care clinic at Karolinska University Hospital and primary care clinics with a special interest in diabetes run by the Stockholm County Council and within the Stockholm Association for Patients with Diabetes. In a structured nurse-based telephone interview, 146 volunteers were asked about their previous medical history with the emphasis on cardiovascular manifestations and blood glucose-lowering therapy. Patients with type 2 diabetes mellitus, aged between 40 and 70 years, with a fasting plasma glucose (FPG) of ≥7.0 mmol/l or HbA_{1c} >5.5 %, were eligible for the studies. Hypertension was accepted if it was effectively treated prior to enrolment. Of the interviewed patients, 121 were selected, according to the given criteria, for a more detailed screening procedure as detailed below and in Figure 7.

Specific characteristics of Study II

The objective of Study II was to explore the possibility of applying VVI as a method to quantify and detect early signs of left ventricular diastolic dysfunction and LA function in patients with type 2 diabetes mellitus.

Patients and protocol

Following a more thorough case history, physical examination, laboratory tests including FPG and HbA_{1c} and transthoracic Doppler echocardiography and TDI, 34 patients were excluded due to reasons outlined in Figure 7. The final population in Study III therefore consisted of 87 of the 121 screened patients.

Specific characteristics of Study III

The objective of Study III, the DADD (Diabetes mellitus And Diastolic Dysfunction) study, was to test the hypothesis that strict, in particular insulin-based, glucose control would improve diastolic function and myocardial flow reserve in patients with type 2 diabetes and early signs of diastolic dysfunction.

Patients

The patient material in Study III comprised 40 of the 121 screened patients (Figure 7). The screening process included a thorough case history, physical and laboratory examination, as well as transthoracic Doppler echocardiography and TDI, to find early diastolic dysfunction and preserved systolic left ventricular function. Any ongoing treatment with beta blockade, diuretics, statins, ACE inhibitors or angiotensin II-receptor blockers should have been stable for at least three months preceding enrolment. Thirty-nine of the 40 randomised patients completed the study according to the protocol. One patient from the O-group, who stopped taking all glucose-lowering drugs 20 weeks after randomisation, was excluded as a protocol violator.

Protocol

Using a Prospective, Randomised, Open, Blind Evaluation (PROBE; Figure 8) trial design, patients were randomly allocated to insulin or oral glucose-lowering agents titrated to achieve

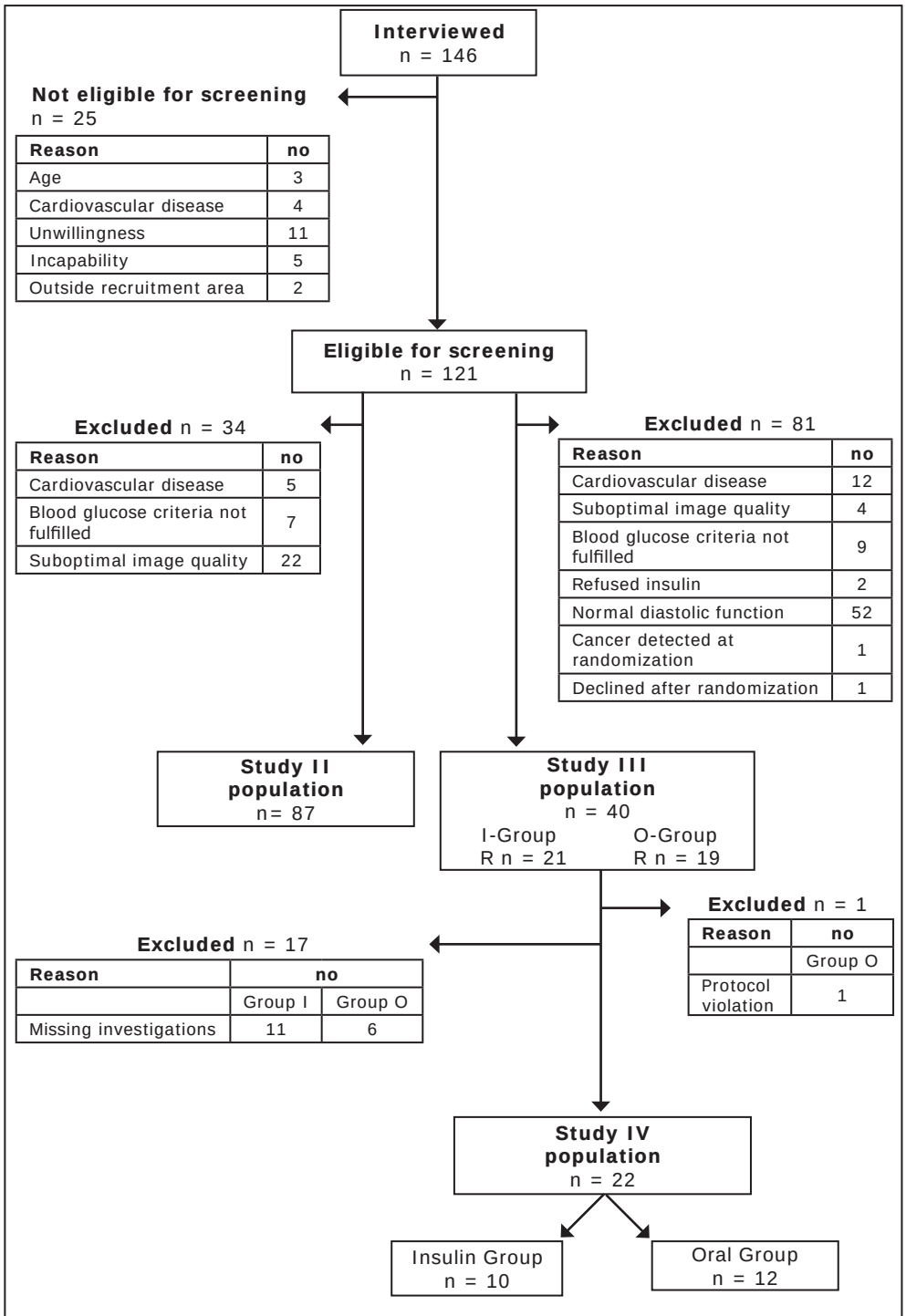


Figure 7. Flow chart for the patient populations in study II-IV.

strict glucose control (FPG <5.0 mmol/l, HbA_{1c} <5.5%). All patients received structured lifestyle advice and were trained in the self-monitoring of blood glucose and to enter their FPG values in a diary. Patients in group I were started on a long-lasting insulin analogue, glargine (Lantus®, Aventis Pharma, Stockholm, Sweden), administered once daily. Those not reaching the glycaemic target were also prescribed a rapid-acting insulin analogue, aspart (Novo Rapid®, Novo Nordisk, Copenhagen, Denmark), with meals. Patients in group O were started on metformin (Metformin®, Meda) and repaglinide (Novonorm®, Novo Nordisk, Copenhagen, Denmark) was added if postprandial values were still high after three weeks. The four-month study period began when a patient had reached the glycaemic target of a self-monitored FPG of <5.0 mmol/L for at least three consecutive days or the best achieved glucose level after a maximum titration period of eight weeks. The treatment regimen was not changed during these months, apart from minor adjustments related to hypoglycaemic episodes or gastrointestinal side-effects. Each patient visited the research clinic at least four times and was contacted by a study nurse bi-weekly to discuss the self-monitored glucose values.

An echocardiographic investigation including TDI and myocardial perfusion studies before and after dipyridamole infusion was performed before randomisation and after four months of intensified glucose-lowering treatment.

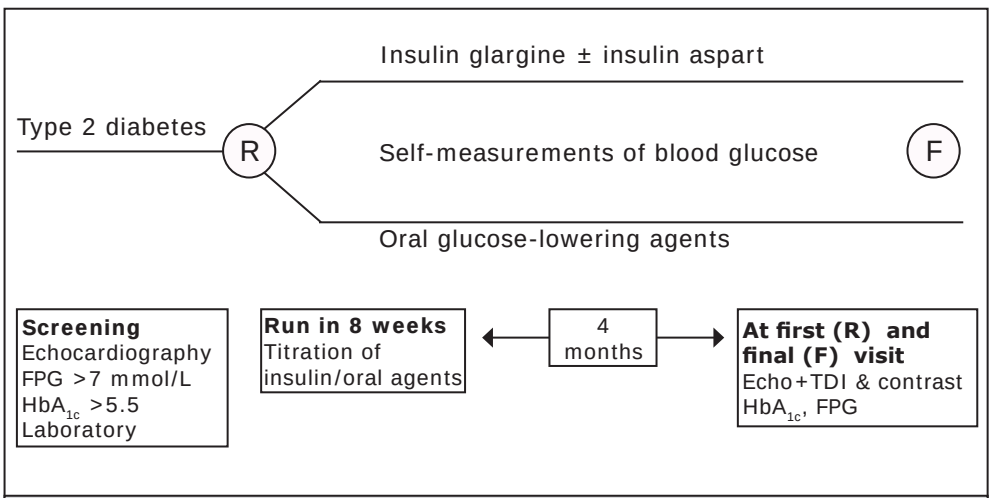


Figure 8. Study design in the DADD study.

Specific characteristics of Study IV

The objective of Study IV was to explore the influence of glucose normalisation on flow-mediated dilatation and skin microcirculation comparing insulin with oral glucose-lowering therapy.

Patients and protocol

The patient material in Study IV comprised 22 of the 40 patients in Study III (Figure 7). The patients in this study, 10 from the insulin group and 12 from the oral group, underwent vascular investigations in the form of flow-mediated dilatation and skin microcirculation at randomisation and by the end of study in combination with a more extensive laboratory investigation.

Methods

Ultrasound investigations (Study I)

Echocardiography, DTI and AVPD

All measurements were made on one occasion with the patient in the left semi-lateral position. All recordings of the left ventricular dimensions, volumes and ejection fraction were performed according to recommendations by the American Society of Echocardiography [93]. Biplane volumes were calculated from area tracings using the disc-summation method (modified Simpson's rule). Ejection fractions were calculated as (diastolic-systolic/diastolic) volumes using dedicated equipment and software (TomTec Imaging Systems Inc., CO, USA).

Conventional Doppler echocardiography with Mitral Pulse Wave Doppler (MPWD) flow was measured by positioning the sampling volume between the tips of the open mitral leaflets in the apical four-chamber view. Early and atrial transmitral peak flow velocities, the E/A ratio and the deceleration time of E were registered. The intraventricular relaxation time was measured using the continuous wave Doppler technique.

Pulsed Doppler DTI was performed with the 4-mm sampling volume in the middle of the interventricular septum, 5-10 mm below the mitral annulus in the apical four-chamber view. As described in Figure 9, the registrations comprised maximum systolic velocity, the duration of the systolic movement, regional intraventricular relaxation time, maximum velocity during the early filling phase and atrial contraction filling phase, the e-wave deceleration time and the regional isovolumic contraction time.

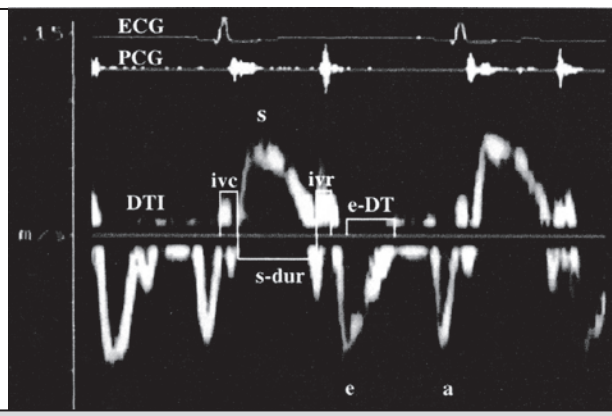


Figure 9. Example of a DTI registration from Study I.
Abbreviations: s = maximum systolic velocity, s-dur = duration of the systolic movement, ivr = regional intraventricular relaxation time, e = maximum velocity during the early filling phase, a = atrial contraction filling phase, e-DT = e-wave deceleration time, ivc = regional isovolumic contraction time.

The atrio-ventricular plane displacement (AVPD) towards and away from the cardiac apex was calculated using M-mode echocardiography from an apical window. The septal and lateral AVPD was measured in the four-chamber view and the anterior and posterior AVPD in the two-chamber view. The total AVPD was measured from the lowest to the highest point

of contraction. A mean value of the AVPD expressed in mm was calculated according to Höglund et al. [94]. The contribution of AVPD during late diastole (AV-A) was calculated from the ratio of the magnitude of motion due to atrial systole to the total AVPD (AV-A/AV-mean) according to Alam et al. [95](Figure 10).

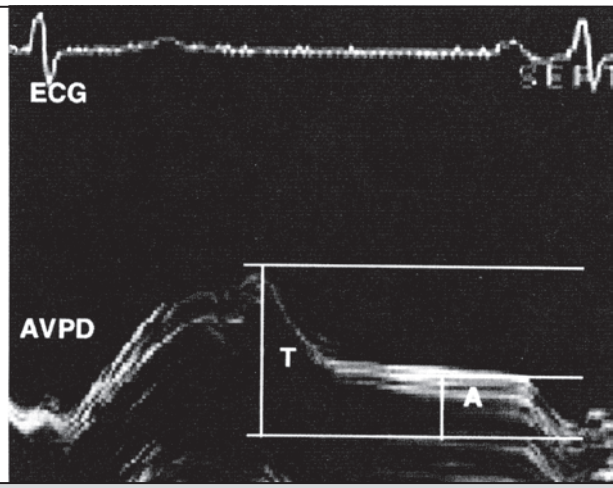


Figure 10. Example of Atrio Ventricular Plane Displacement (AVPD) recorded by M-mode echocardiography illustrating the total (T) movement during systole and during atrial contraction (A) from Study I.

Ultrasound examinations (Studies II-IV)

Echocardiography

The echocardiograms in Studies II-IV were obtained at the same time of the day and with the patient in the supine left lateral position during quiet respiration. The equipment, a Siemens Sequoia c512, rev 8.0 (Siemens Medical Systems, Mountain View, CA), was designed for TDI and real-time echo contrast imaging (Contrast Pulse Sequencing) and equipped with a 4V1C transducer. The Doppler-echocardiographic measurements were performed according to standards outlined by the American Society of Echocardiography [93, 96, 97]. The data presented are the average of three representative cardiac beats in sinus rhythm.

Left atrial volume was calculated in the apical four-chamber view using the single plane area length method [98]. In Study II, the outline of the atrial endocardium was traced manually at the end of ventricular systole at the point of the largest LA volume as estimated visually. The LA maximum volume was indexed for body surface area.

Left ventricular systolic function was assessed by a Wall Motion Index (WMI) applying a 17-segment model, registered in the parasternal longitudinal, the short-axis and the apical four- and two-chamber views respectively [99]. The systolic function was considered normal if WMI was ≤ 1.1 [93].

From the apical four-chamber view, left ventricular diastolic function was assessed by pulsed Doppler recordings of mitral inflow and TDI recordings of velocities in the mitral

annulus. The transmitral peak E-wave velocity (cm/s) and peak A-wave velocity (cm/s) were recorded during quiet breathing. The ratio of maximum mitral flow velocities (the E/A ratio, including a Valsalva manoeuvre) was calculated. Longitudinal left ventricular myocardial velocities were measured with low gain pulsed tissue Doppler in the apical four-chamber view with the sampling gate (3 mm) at the septal and lateral parts of the mitral annulus to be expressed as an average of the septal and lateral recordings. Systolic mitral annular velocity, early diastolic and late diastolic mitral annular peak velocities were determined, together with the E/É and É/Á ratios.

In Study II, left ventricular diastolic function was classified as no diastolic dysfunction (NoDD: deceleration time >140 ms, E/A>0.75 but <2 and E/E´<10), mild dysfunction (MiDD: E/A≤0.75 and E/E´<10), moderate dysfunction (MoDD: deceleration time >140 ms, E/A>0.75 but <2 and E/E´≥10) and severe dysfunction (deceleration time <140 ms, E/A>2 and E/E´≥10) [27, 68, 89].

All the patients in Study III had normal systolic function, while 16 of the 21 patients in the I-group fulfilled one and five two of the echocardiographic criteria for impaired diastolic function (Table 4). Among the 18 patients in the O-group, nine fulfilled one, seven two and two three of these criteria.

Table 4. Myocardial systolic and diastolic function at randomisation by group belonging as defined by echocardiography and TDI.

	Group I	**Group O**
Myocardial systolic function		
Wall motion index (WMI)≤1.1	21	18
Myocardial diastolic dysfunction criteria		
E/A<0.75	6	6
E/A decreasing by 0.5 after Valsalva manoeuvre	6	5
E´<8 cm/s recorded at the septal wall	7	11
E/E´>15	0	1
E/A 0.75-1.5 combined with LA volume index >32ml/m^2	6	5
E/E´ 8-15 in combination with E/A>1.5 and LA volume index >32ml/m^2	1	1

Velocity Vector Imaging

In study II tissue velocity and deformation, evaluated by a 2-D based quantitative technique originally developed for the left ventricular myocardium, was applied on the routine grey-scale echocardiographic images of the left atrial myocardium. The VVI technique uses the combination of speckle tracking (a series of unique B-mode pixel tracking algorithms), mitral annulus motion and tissue-blood border detection. An endocardial tracing of a single frame was manually derived from a routine digital cine loop. The periodic displacement of the pixels within this region was tracked in subsequent frames. The movement was estimated by a fast Fourier transformation process. The velocity vector of every point in the border was calculated by adding the border motion to the relative velocity of the tissue.

The strain and strain rate were obtained by comparing the displacement of the speckles in relation to each other along the endocardial contour throughout the cardiac cycle [72]. The 2D sequences were stored as standard Digital Imaging and Communication in Medicine (DICOM) format images and the analyses were performed offline with Syngo VVI version 2 (Siemens Medical Solutions, Mountain View, CA, USA). The subendocardium of the left atrium was traced at the end of ventricular systole and two to three cardiac cycles were averaged to obtain a velocity vector profile of atrial myocardial motion. Strain and strain rate measurements were performed in the myocardium of three LA walls, septal, lateral and the roof, as depicted in Figure 11. Peak systolic LA strain was measured at left ventricular systole, while LA strain rates were obtained as peak systolic, peak diastolic (early left ventricular filling) and late diastolic (atrial contraction) values.
An LA volume curve was automatically generated by the VVI software through the continuous tracing of the LA myocardium, calculating LA volumes frame by frame using the single-plane Simpson method. The following volume indices were analysed: maximum (LA vol max), minimum (LA vol min) and pre-atrial contraction (LA vol preA). The LA total emptying fraction (LA-EmF) was calculated as LA-EmF = (LA vol max −LA vol min)/LA vol max ×100 and the LA active emptying fraction was derived as (LA vol preA − LA vol min)/LA vol preA×100.

Myocardial contrast echocardiography

In Study III, low mechanical index myocardial contrast echocardiography was performed at rest and during maximum dipyridamole (0.84 mg/kg)-induced vasodilatation. The contrast agent (SonoVue, Bracco, Milan, Italy) was given intravenously at a constant rate of 0.8-1.0 ml/min. Imaging in the apical two- and four-chamber views was initiated after at least two minutes of infusion to be stored digitally pending analysis. Gain was adjusted for the optimal visualization of contrast echoes in the myocardium. The signal intensity (SI) was measured offline at a work station (Research-Arena™ 1.0, TomTec Imaging Systems GmbH, Germany) with dedicated software (Axius Auto Tracking Contrast Quantification, Siemens Medical Systems, Mountain View, CA as shown in Figure 12). A region of interest (ROI) was outlined by hand from the four-chamber view. End systolic frames were analysed and SI expressed as log compressed data during contrast replenishment fitted to an exponential function of the primary components of myocardial flow; the initial slope provides a measure of flow velocity and $SI_{plateau}$ that correlates to myocardial capillary blood volume [100, 101], the product of which constitutes the myocardial blood flow index (MBFI). Another ROI was placed in the ventricular cavity, close to the septum, to measure blood pool SI. After log decompression, the $SI_{plateau}$ was normalised for the blood pool SI to obtain a myocardial blood volume index MBVI).

Vascular investigations

Study IV

The participants in Study IV were allowed a light breakfast but refrained from tobacco and caffeine-containing products for at least eight hours preceding the investigations. These investigations were performed in a quiet, dimly lit room with the temperature kept between 22-24°C and with the patients in the supine position.

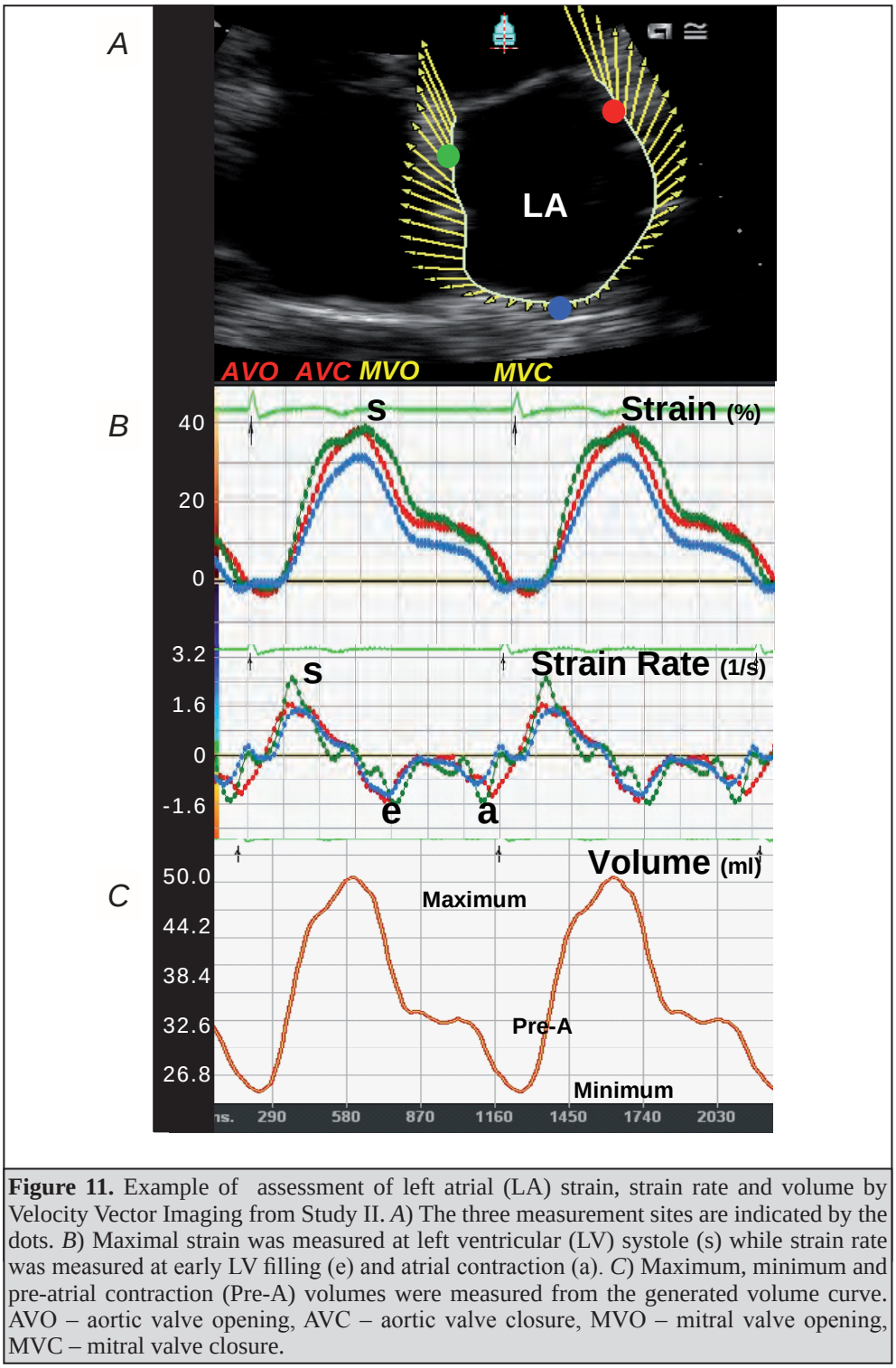


Figure 11. Example of assessment of left atrial (LA) strain, strain rate and volume by Velocity Vector Imaging from Study II. *A*) The three measurement sites are indicated by the dots. *B*) Maximal strain was measured at left ventricular (LV) systole (s) while strain rate was measured at early LV filling (e) and atrial contraction (a). *C*) Maximum, minimum and pre-atrial contraction (Pre-A) volumes were measured from the generated volume curve. AVO – aortic valve opening, AVC – aortic valve closure, MVO – mitral valve opening, MVC – mitral valve closure.

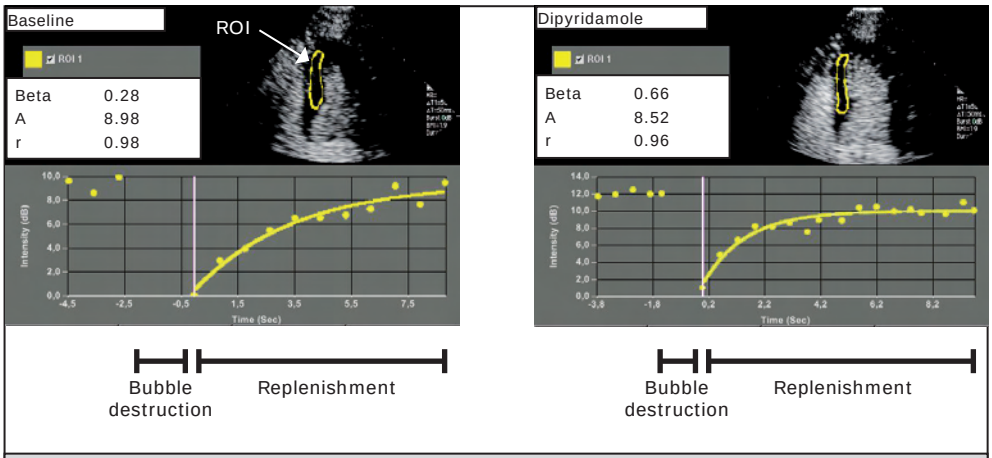


Figure 12. Myocardial contrast echo from Study III showing replenishment after bubble destruction in a region of interest (ROI), corresponding to the interventricular septum at baseline (left panel) and after dipyridamole (right panel).
Beta denotes the slope of the curve corresponding to inflow velocity (x s-1).
A denotes maximal video intensity (dB). R denotes correlation coefficient.

Flow-mediated dilatation

Non-invasive examination of the brachial artery of the non-dominant arm [102] was performed using an 8 MHz linear-array transducer connected to a Siemens Sequoia® (Siemens Medical Systems, Mountain View, CA, USA, Figure 13). Baseline images were saved every three seconds for one minute and a mean value was calculated from these recordings. A blood pressure cuff, positioned below the elbow, was then inflated to 260 mmHg for five minutes. Endothelium-dependent vasodilatation was determined by continuously imaging the artery during three minutes of hyperaemia following cuff release. A mean value was calculated from three recordings at maximum dilatation. Endothelium-independent vasodilatation was determined following the sublingual administration of nitroglycerine (0.4 mg). All the images were analysed using proprietary software (Brachial analyzer®, Medical Imaging Applications, Iowa City, IA, USA) by a technician blinded to treatment allocation. The maximum diameter was measured beat to beat by an automated contour detection system with the lumen diameter defined as the distance between the intima of the far and near vessel walls. Dilatation was calculated as maximum lumen diameter after ischaemia or nitroglycerine minus the lumen diameter at baseline divided by the lumen diameter at baseline.

Skin microcirculation

The total skin microcirculation was measured by Laser Doppler Fluxmetry (LDF; Periflux, 4001 Master, Perimed®, Stockholm, Sweden) on the volar side of the forearm. The LD output signal was continuously recorded on a PC (Perisoft software, Perimed®, Järfälla, Sweden). The LDF signal was recorded as perfusion units during three minutes of rest and calculated as the computer integrated mean. The LDF was also registered during hyperaemia following five minutes of local heating (44°C) of the skin. The following variables were determined: resting LDF, peak LDF and percentage increase in LDF following local heating.

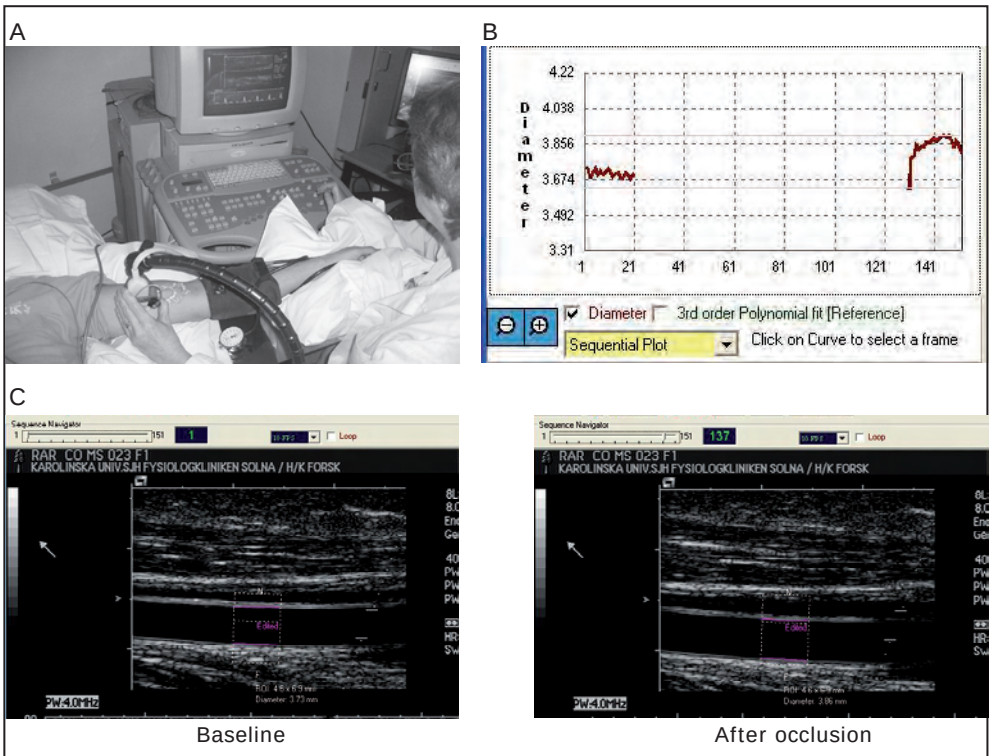


Figure 13 A-C. Example of registration of flow-mediated dilatation from Study IV. *A)* Non-invasive assessment of the forearm using an 8 MHz linear-array transducer connected to a Siemens Sequoia®. *B)* Example of registration of the vessel diameter before and after occlusion. *C)* Images of the brachial artery at baseline and after occlusion.

Biochemical analyses

Studies II-IV

All the samples in Studies II-IV were analysed according to normal laboratory routines at the central laboratory department at Karolinska University Hospital. In Study II, venous blood was sampled on one occasion following 10 minutes of supine rest for the determination of FPG, HbA_{1c} and serum creatinine. In Studies III and IV, the following tests were made at the time of randomisation and on the final visit: total serum cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerides, GAD antibodies, FPG, HbA_{1c} and albumin-creatinine ratio. HbA_{1c}, analysed by high performance liquid chromatography, was presented as Mono S with a measurement interval of 2.9-17.2% and a reference value of <5.3%. Swedish HbA_{1c} = 0.989 x International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) HbA_{1c} + 0.88%; $r^2 = 0.996$. [103].

In Study IV, the following laboratory examinations were added to those already mentioned: high sensitive C-reactive protein, free fatty acids, serum amyloid A protein and fibrinogen. In addition, plasma endothelin-1 immunoreactivity was analysed by radioimmunoassay using commercially available antiserum (rabbit anti-ET-1, IHC 6901, Peninsula, Merseyside, UK) and tracer (ET-1, NEX259, PerkinElmer, Boston) following ethanol extraction [104].

Statistical analysis

Study I

Continuous variables are summarised as mean ± SD, unless otherwise stated. Analysis of variance (ANOVA) was used to test for group differences. Categorical data were analysed using Fisher's exact test. A two-sided *p*-value of <0.05 was considered statistically significant.

Studies II-IV

In Studies III and IV, statistical analyses was performed on the per protocol population, i.e. randomised patients without major protocol violations. Continuous variables were summarised as mean ± SD in Studies II-IV. In Study III, they were also summarised as the median and range and, in Study IV, as range or quartiles. Categorical variables were presented as counts and percentages (%). Overall differences between groups of continuous variables were assessed using the Kruskal-Wallis non-parametric one-way analysis of variance. In Study II, in the event of a significant overall difference (p-value<0.05), pair-wise comparisons were performed using the Wilcoxon Mann-Whitney rank sum test. In Study III, associations between echocardiographic and glucometabolic variables were analysed using linear regression models.

In Study II, overall differences between groups of categorical variables were assessed using the chi-square test. Spearman's rank correlation coefficient was used to calculate the associations between echocardiographic and VVI variables. A two-sided p-value of < 0.05 was considered statistically significant.

The sample size in Study III was based on observational data from our laboratory [59] in which E´ increased by approximately 1.0 cm/s for an insulin induced decrease in fasting glucose of 3.8±2.6 mmol/L but was essentially unchanged when glycaemic control was intensified by means of oral glucose lowering drugs. It was assumed that a difference in E´ by at least 1.0 cm/s between the two treatment arms would be of clinical relevance. Based on the observational study, the standard deviation of the change in E´ among patients before and after improved treatment was assumed to be 0.9 cm/s. With these assumptions, a power of 80% and a significance level of 5% approximately 15 patients would be needed in each treatment arm assuming a normal shift model and performing a t-test. To allow for dropouts and the use of non-parametric tests it was judged reasonable to aim at a total material of 40 patients.

Ethical considerations

Study I

The study was approved by the ethics committee at Karolinska Hospital. The nature and the purpose of the investigation were explained to the patients, who gave their informed consent. The investigation was conducted according to the revised Declaration of Helsinki [105].

Studies II-IV

The studies were performed according to the principles outlined in the Declaration of Helsinki. The study protocols were approved by the ethics committee of the Ethics Committee North, Karolinska Hospital. All patients gave their oral and written informed consent to participate in the study.

RESULTS

Study I

Echocardiographic parameters

Left ventricular end-diastolic and end-systolic dimensions and wall thickness expressed in millimetres, end-diastolic and end-systolic volumes expressed in millilitres and ejection fractions in the three groups are listed in Table 5. There were no statistically significant differences between the controls and the patients in the diastolic heart failure group, whereas the left ventricles in the systolic heart failure group were dilated and had a depressed ejection fraction.

Table 5. Basic echocardiographic parameters.

	Controls n = 15	**Diastolic heart failure** n = 15	**p-value** C vs. DHF	**Systolic heart failure** n = 15	**p-value** All groups
Male/ female	6/9	6/9		9/6	
LVEDd (mm) (range)	48.2±5.3 (38.6-55.4)	46.6±4.3 (39.6-53.7)	NS	64.4±8.8 (48.9-77.1)	<0.001
LVESd (mm)	30.4±5.2 (20.1-38.6)	30.9±4.1 (21.2-38.5)	NS	54.4±12.4 (32.9-71.6)	<0.001
IVS (mm)	10.9±2.1 (8-14.1)	12.6±3.1 (8.8-18.9)	NS	11.0±3.5 (6.7-18.8)	NS
PW (mm)	9.0±1.2 (7.4-10.6)	9.6±1.8 (6.8-12.7)	NS	10.2±2.1 (5.9-14.9)	NS
LVEDv (ml)	93.5±22 (58.1-127.3)	74.4±24 (46.3-116.8)	NS	193.4±58 (109.7-295.2)	<0.001
LVESv (ml)	39.2±10 (20.1-55.9)	33.2±14 (15.8-60.1)	NS	144±45 (79.3-220.1)	<0.001
EF (%)	58±5.3 (51.6-68.3)	56.9±7 (47.8-69.4)	NS	25.9±4.3 (19.2-34.6)	<0.001

Abbreviations; EF, ejection fraction; IVS, inter-ventricular septum; LVEDd, left ventricular end-diastolic diameter; LVESd, left ventricular end-systolic diameter; LVEDv, left ventricular end-diastolic volume; LVESv, left ventricular end-systolic volume; PW, posterior wall. Mean ± S.D. (range).

The MPWD, DTI and AVPD findings in the three groups are summarised in Table 6. The only MPWD variable that differed significantly between the control and diastolic heart failure groups was the deceleration time. The maximum velocity of atrial filling and the pulmonary vein systolic/diastolic ratio were lower in patients with systolic heart failure. Turning to DTI, the duration of the systolic contraction time was shorter in diastolic heart failure patients than in controls and even shorter in patients belonging to the systolic heart failure group. Patients with diastolic heart failure had a lower maximum velocity during atrial filling and a longer isovolumetric contraction time than controls. Compared with the findings among controls and diastolic heart failure patients, all DTI variables, apart from the e/a ratio, were significantly different in the systolic heart failure group. The mean AVPD values during both systole and diastole were lower in the diastolic heart failure group than in controls and even lower in the systolic heart failure group.

Table 6. MPWD, DTI and AVPD in controls, patients with diastolic heart failure and systolic heart failure.

	Controls n = 15	**Diastolic heart failure** n = 15	**p-value** Controls vs. diastolic heart failure	**Systolic heart failure** n = 15	**p-value** All groups
Male/female	6/9	6/9		9/6	
MPWD					
E velocity (cm/s) (range)	64.3±14.0 (40-87)	62.8±19.5 (35-100)	NS	62.7±25.8 (32-131)	NS
A velocity (cm/s)	78.4±11.4 (58-103)	85.5±27.5 (35-149)	NS	63.8±23.6 (34-98)	<0.05
E/A	0.82±0.1 (0.63-1.15)	0.78±0.3 (0.52-1.42)	NS	1.15±0.7 (0.48-2.62)	NS
E-DT (ms)	234±41 (170-311)	272±39 (198-313)	<0.05	218±74 (149-400)	<0.05
IVR (ms)	120±16 (100-155)	127±24 (83-171)	NS	118±23 (83-170)	NS
PV-s/d	1.52±0.4 (0.66-2.31)	1.57±0.6 (0.47-2.62)	NS	1.02±0.6 (0.28-1.94)	<0.05
DTI					
s velocity (cm/s)	6.6±0.8 (5.7-8)	6.1±1.1 (4-8)	NS	4.2±0.7 (2.8-5.5)	<0.001
s dur (ms)	320±20 (285-355)	283±42 (173-330)	<0.01	211±37 (131-280)	<0.001
ivr (ms)	104±19 (76-137)	126±52 (58-257)	NS	209±61 (112-368)	<0.001
e velocity (cm/s)	6.2±1.2 (4.7-8.3)	5.8±2.1 (3-11.5)	NS	3.9±0.9 (3-6.3)	<0.001
a velocity (cm/s)	9.9±1.5 (7.8-13.3)	8.6±1.7 (6.3-12.5)	<0.05	5.6±2.5 (2.3-11)	<0.001
e/a	0.63±0.1 (0.43-0.78)	0.69±0.3 (0.34-1.47)	NS	0.82±0.4 (0.27-1.74)	NS
e-DT (ms)	117±28 (80-170)	100±37 (53-183)	NS	63±14 (43-86)	<0.001
ivc (ms)	63±18 (37-96)	85±27 (46-144)	<0.05	127±49 (32-168)	<0.001
AVPD					
AV mean (mm)	12.3±1.2 (10.5-14.3)	10.8±1.7 (7.4-13.4)	<0.01	6.6±1.4 (4.2-9.2)	<0.001
AV-A (mm)	6.2±0.6 (5.1-7.3)	5.4±1.0 (3.3-6.7)	<0.05	3.7±1.2 (1.8-5.8)	<0.001
AV-A/mean (%)	50±5 (44-60)	52±12 (34-75)	NS	56±11 (43-77)	NS

[a] Abbreviations: A and a, atrial; AV, atrio-ventricular; DT, deceleration time; E and e, early; IVR and ivr, intraventricular relaxation time; ivc, isovolumetric contraction time; PV, pulmonary vein; s, systolic. Mean ± S.D. (range).

Identification of patients with diastolic heart failure

Patients with diastolic heart failure could not be differentiated from controls using M-mode and regular two-dimensional echocardiography (Table 5; Figures 14 and 15). The MPWD E/A decreased with age and a cut-off of <0.7 was used as abnormal in Study I [106, 107]. At that level, 10 of the 15 diastolic heart failure patients were correctly identified, with four false-positive controls (NS). If an E-DT of >260 ms or an E/A ratio of <0.7 was used [108, 109], 14 of the 15 diastolic heart failure patients were identified, with seven false-positive controls (p<0.05). After adding a pulmonary vein flow systolic/diastolic ratio of <1.0 to the detection criteria, all 15 diastolic heart failure patients were identified, with eight false-positive controls (p<0.01).

A pulsed Doppler DTI s duration of <302 ms identified nine of 15 patients with diastolic heart failure, with only two false-positive controls (p<0.05). If the s duration or an isovolumetric contraction time of >70 ms was applied, 12 of 15 diastolic heart failure patients were identified, with five false-positive controls (p<0.05). If a maximum a velocity of <9.3 cm/s was used as an indicator, all 15 diastolic heart failure patients were identified but with 10 false-positive controls (p<0.05). Using the AVPD technique, an AV mean of <11.5 mm identified 10 of the 15 diastolic heart failure patients, with five false-positive controls (NS). If the AV mean of <11.5 was combined with the AV-A/AV mean of >51%, 11 patients of 15 were identified but with nine false-positive controls (NS).

MPWD
E/A

	Group C	Group DHF
Yes	4	10
No	11	5

N.S.

DTI
s-dur

	Group C	Group DHF
Yes	2	9
No	13	6

p<0.05

AVPD
AV-mean

	Group C	Group DHF
Yes	5	10
No	10	5

N.S.

Figure 14. Identification of patients with diastolic heart failure patients (Group DHF) in comparison with controls (group C) by MPWD using a low E/A ratio(<0.7) (left), pulsed TDI a short s-duration (<302ms) (middle) and AVPD a low AV-mean (<11.5 mm) (right).

MPWD
E/A or E-DT or PVs/d

	Group C	Group DHF
Yes	8	15
No	7	0

p<0.01

DTI
s-dur or a-vel or ivc

	Group C	Group DHF
Yes	10	15
No	5	0

p<0.05

AVPD
AV-mean or AV-LA/AV-mean

	Group C	Group DHF
Yes	9	11
No	6	4

N.S.

Figure 15. Identification of patients with diastolic heart failure (Group DHF) in comparison with controls (Group C) by MPWD using a low E/A ratio (<0.7) or a long E-DT (>260 ms) or a low PV s/d (<1.0) (left), pulsed DTI a short s-duration (<302 ms) or a low maximal a velocity (<9.3 cm/s) or a long ivc (>70 ms) (middle) and AVPD a low AV-mean (<11.5) or a high AV-LA/AV-mean ratio (>51%) (right).

Co-variation

There was a weak correlation between the MPWD maximum A velocity and the ejection fraction (r=0.35; p<0.05) and the latter variable was inversely correlated to the E/A ratio, (r= -0.36; p<0.05). The PV s/d correlated weakly with the ejection fraction (r=0.42; p<0.05). All pulsed Doppler DTI parameters with the exception of the e/a ratio correlated to the ejection fraction, with the strongest correlation to the s-duration (r=0.81; p<0.001).
The DTI ivr and ivc were inversely correlated to the ejection fraction (r=-0.73 and r= -0.62 respectively; p<0.001). The DTI-derived e-DT correlated to the ejection fraction (r=0.66; p<0.001), while E-DT obtained by MPWD showed a weak inverse correlation to this variable (r= -0.35; p<0.05). The AVPD AV mean also correlated to the ejection fraction (r=0.83; p<0.001).
Heart rate (65±10; range 47-92 beats/min) correlated to both MPWD E/A and DTI e/a (r=0.45 and 0.41 respectively; p<0.01). The E-DT showed an inverse correlation to heart rate (r=-0.37; p<0.05), but there was no relationship between heart rate and DTI e-DT. The AVPD AV mean and AV-A were both inversely correlated to heart rate (-0.41 and -0.47 respectively; p<0.01).

Study II

Clinical data

The baseline characteristics of the 87 patients included in Study II are shown in Table 7. According to the previously described criteria, they were divided into three groups: no (NoDD = 69%), mild (MiDD = 15%) and moderate diastolic dysfunction (MoDD = 16%).

Echocardiographic parameters

Doppler echocardiographic data are presented in Table 8. Left ventricular diastolic and systolic dimensions did not differ between the three groups. In accordance with the classification, there were clear cut differences in mitral inflow and TDI parameters, with a graded increase in the E/E´ ratio and a biphasic pattern in the E/A ratio and deceleration time with increasing diastolic dysfunction. Of the classification-independent measures, LA volume and LA volume index were significantly higher in the MoDD than in the NoDD groups. The septal systolic mitral annular velocity was significantly lower in the MiDD and MoDD groups than among patients belonging to the NoDD group, despite a similar WMI (data not shown) in all groups.

LA deformation and volumes by VVI

Data from VVI are summarised in Table 9. Maximum LA volume measured by VVI correlated well with that obtained using the single-plane area-length method (r =0.89; p<0.0001). The maximum and minimum LA volumes and the LA pre-atrial contraction volume were larger in the MoDD patients than in those belonging to the NoDD and MiDD groups. The overall group comparison did, however, reveal a significant difference only for the pre-A volume. The total LA emptying fraction was lowest in patients with MoDD, while the LA active emptying fraction showed a biphasic response (Table 8), with the highest values in patients with impaired left ventricular relaxation (MiDD). Maximum LA strain at the time of left ventricular end systole was highest among patients with NoDD. LA systolic strain rate did not differ between the diastolic function groups. The LA strain rate at atrial contraction was

Table 7. Pertinent patient clinical characteristics in the total patient material and by group allocation. Values presented are n (%), mean and SD unless otherwise stated.

Variable		Diastolic dysfunction			
	All n= 87	**No** n=60	**Mild** n=13	**Moderate** n=14	**p=** overall
Age [years]	60.3±7.2	58.8±7.5	63.1±5.9	63.7±5.6*	0.035
Males	43 (49)	32 (53)	4 (31)	7 (50)	0.33
Body Mass Index [kg/m2]	28±5	28±5	27±4	30±7	0.38
Diabetes duration [years]	4.4±3.7	4.1±3.4	6.1±5.5	4.1±2.6	0.63
Hypertension	36 (42)	22 (38)	7 (54)	7 (50)	0.47
Laboratory findings					
HbA1c [%]	6.0±1.4	6.0±1.6	6.1±1.0	6.1±0.9	0.21
F-plasma glucose [mmol/L]	8.1±2.8	8.1±3.2	8.5±1.0**	8.0±1.9	0.031
S-creatinine [µmol/L]	71.2±12.9	72.3±13.5	67.5±13.3	72.8±10.2	0.35
Glucose-lowering treatment					
Lifestyle only	46 (54)	32 (55)	6 (46)	8 (57)	0.81
Sulphonylurea	17 (20)	11 (19)	3 (23)	3 (21)	0.93
Metformin	35 (41)	22 (38)	6 (46)	7 (50)	0.66
Thiazolidinediones	1 (1)	1 (2)	0	0	NA
Pharmacological treatment[1]					
β-blockers	12 (14)	5 (9)	3 (23)	4 (28)	0.11
ACE-inhibitors/AII-receptor blockers	27 (32)	15 (25)	6 (46)	6 (43)	0.23
Calcium channel blockers	9 (11)	5 (9)	1 (8)	3 (21)	0.41
Statins	22 (26)	13 (22)	6 (46)	3 (21)	0.22
Diuretics	12 (14)	6 (10)	2 (15)	4 (29)	0.25

1: Information complete in 85 (96%) of the patients.
* Significant difference between NoDD and MoDD, p-value=0.039
** Significant difference between NoDD and MiDD P- value= 0.01

lower in patients belonging to the MoDD group than those in the NoDD and MiDD groups, suggesting a decrease in left atrial contractile function.

Relationship between LA deformation and parameters of diastolic function

There was a relatively strong positive correlation between the mean systolic LA strain and LA-EmF (r= 0.70; p<0.0001). Systolic LA strain in the septal, lateral and roof positions correlated significantly with LA-EmF (r= 0.64, r= 0.58, r=0.63; p<0.0001 respectively). Moreover, there were inverse relationships between LA strain and LA volumes (strongest for the minimum LA volume). Left atrial volume pre-A showed a slightly higher correlation with E/E´(r= 0. 28; p= 0.0081) as compared with LA maximum volume (r= 0.21; p=0.050).

Table 8. Pertinent Doppler- echocardiographic data in all patients and by group allocation. Values presented are mean and SD unless otherwise stated.

	Diastolic dysfunction				p-values=		
Variables	**No** n= 60	**Mild** n = 13	**Moderate** n= 14	**p-value=** overall	**No/ mild**	**No/ moderate**	**Mild/ moderate**
Included in the classification*							
E velocity (cm/s)	73±15	58±8	86±14	NA			
A velocity (cm/s)	65±13	85±14	83±17	NA			
Deceleration time (msec)	176±27	197±45	168±22	NA			
E/A ratio	1.2±0.3	0.7±0.1	1.1±0.4	NA			
E´ velocity (cm/s)	10.0±1.8	7.3±1.4	7.8±1.1	NA			
E/E´ ratio	7.4±1.4	8.2±1.8	11.1±1.2	NA			
Further variables							
Left ventricular dimension							
end diastole (mm)	46±4	44±5	48±7	0.080			
end systole (mm)	30±4	26±8	30±6	0.41			
Interventricular septal thickness (mm)	10.6±1.5	11.3±1.5	11.6±1.7	0.064			
Left atrial volume (ml)	57.9±16.0	58.8±16.1	72.3±22.4	0.015	0.94	0.004	0.090
Left atrial volume index (ml/m^2)	29.5±6.0	30.0±6.9	35.7±9.7	0.071			
A´ velocity (cm/s)	12.0±1.8	11.0±1.4	11.0±2.5	0.52			
S´ velocity (cm/s)	8.5±1.0	8.0±1.5	7.7±1.1	0.012	0.035	0.019	0.75

*Within the definition. NA = not applicable. S´= systolic mitral annular velocity, A´= late diastolic mitral annular peak velocity

A cross-validated discriminant analysis of normal versus mild/moderate diastolic dysfunction as expressed by LA strain alone revealed a misclassification rate of 33%, with a sensitivity of 74% (20 of 27) and a specificity of 63% (38 of 60). To answer the question regarding what proportion of patients with abnormal (normal) LA strain has mild/moderate (no) DD as assessed by echocardiography, the positive and negative predictive values were calculated. Of 42 patients with abnormal LA strain, 20 had mild/moderate dysfunction (positive predictive value = 48%) and, of the 45 patients with normal LA strain, 38 had NoDD (negative predictive value = 84%).

Table 9. Descriptive analysis of the VVI variables by group. Values presented are mean and SD unless otherwise stated.

Variable	**Diastolic dysfunction**				**p-value**		
	No n = 60	**Mild** n = 13	**Moderate** n =14	**p-values** overall	**No/ mild**	**No/ moderate**	**Mild/ moderate**
Left atrial volume (ml)							
Maximum	62.7±15.0	63.7±13.1	74.9±19.7	0.061			
Pre-A	42.9±13.7	47.7±11.8	54.5±16.7	0.033	0.21	0.018	0.24
Minimum	31.1±10.6	30.6±8.2	40.1±14.3	0.059			
Total emptying fraction (%)	51.2±8.5	52.1±5.8	45.9±6.5	0.044	0.72	0.028	0.021
Active emptying fraction (%)	27.8±7.4	35.6±7.1	26.2±7.3	0.0040	0.0033	0.55	0.0054
LA strain at left ventricular systole (%)							
Septum	29.9±9.3	25.9±7.4	23.7±4.9	0.037	0.14	0.023	0.66
Roof	29.2±7.2	23.9±8.2	23.7±4.9	0.0073	0.039	0.0095	0.81
Lateral	30.9±8.7	26.7±7.2	25.0±4.7	0.029	0.14	0.017	0.55
Mean	30.0±7.6	25.5±6.9	24.1±4.4	0.0094	0.089	0.0065	0.59
LA strain rate systole mean (s^{-1})	1.2±0.4	1.9±3.3	1.1±0.3	0.16			
LA E strain rate diastole mean (s^{-1})	-1.2±0.6	-0.8±0.3	-0.9±0.4	0.037	0.020	0.16	0.58
LA A strain rate diastole mean (s^{-1})	-1.0±0.5	-1.1±0.5	-0.7±0.3	0.016	0.42	0.012	0.019

Study III

Clinical data

Pertinent clinical characteristics at baseline are presented in Table 10. Age, gender and risk factors for cardiovascular disease were comparable between the two groups, while hypertension and treatment with ACE inhibitors/angiotensin II-receptor blockers was slightly more common among patients in the O-group.

Results related to diabetes treatment

Details regarding the diabetes state are presented in Table 11. By the end of the study, all the patients in the I-group were being treated with insulin. Eight of them continued with

Table 10. Pertinent patient characteristics by group allocation. Values presented are n (%) or mean±SD unless otherwise stated .

Variable	Group I n = 21	Group O n = 18
Age (years)	59±8	62±7
Females	12 (57)	11 (61)
Risk factors		
Treated hyperlipidemia	7 (33)	9 (50)
Treated hypertension	7 (33)	11 (61)
Smoker active/ex-smoker	9 (43)	3 (17)
Cardiovascular treatment		
β-blockers	4 (19)	4 (22)
ACE inhibitors/AII-receptor blockers	5 (24)	9 (50)
Calcium channel blockers	2 (9)	3 (17)
Diuretics	5 (24)	6 (33)
ASA	4 (19)	5 (27)
Physical examination		
BMI (kg/m^2; median and range)	28 (21-44)	26 (18-48)
Heart rate (beats/min)	68±8	65±9
Blood pressure (mm Hg)		
Systolic	141±13	146±18
Diastolic	80±6	80±9

metformin, while this drug was discontinued in one patient due to low glucose values. Patients randomised to the O-group were all given metformin and 12 required additional repaglinide to achieve the glucose target. This resulted in a comparable decrease in fasting glucose (I-group = – 2.2±2.1 and O-group -1.5 ±0.8 mmol/L; p=0.083) and HbA_{1c} (I-group -0.6±0.4 % and O-group -0.7±0.4 %; p= 0.690) between the two groups. During the study period, body weight increased (mean±SD 1.0±2.3 kg) in the I-group, while it decreased (3.0±4.4 kg) in the O-group (p=0.015 between the two groups).

Echo-Doppler and TDI data

Doppler-echocardiographic and TDI data are shown in Table 12. Left ventricular systolic and diastolic dimensions (data not presented) and mitral inflow parameters did not differ between the two groups at the time of randomisation. The mean LA volume index was above normal [110, 111] in both groups (I-group=31±6.0 ml/m^2 and O-group=33±7.0 ml/m^2). Improved glycaemic control did not significantly influence the variables expressing compromised diastolic function in either of the treatment groups.

Myocardial perfusion data

Due to suboptimal image quality, information on MBFI and MBVI was lost in some patients,

Table 11. Patient characteristics related to diabetes. Values presented are n (%) or mean±SD unless otherwise stated.

Variable	Group I n=21	Group O n=18	p =
Diabetes duration, (years; median and range)	5 (0-17)	6 (0-14)	-
Glucose-lowering treatment			
At randomisation			
Metformin	9 (43)	7 (39)	-
Glimepiride/glipizide	1 (5)	3 (17)	-
At end of study			
Metformin n (%; mean dose in g/day)	8 (38; 2.0)	18 (100; 2.3)	-
Glimepiride/glipizide	0	0	-
Repaglinide n (%; mean dose in mg/day)	0	12 (67;2.1)	-
Insulin glargine (U/day; median dose and range)	30 (10-56)	-	-
Insulin aspart (U/day; median dose and range)	10 (0-30)	-	-
Laboratory findings			
FPG (mmol/L)			
Study start	8.3±1.8	7.8±1.6	-
End of study	6.0±1.5	6.3±1.6	-
Δ FPG	-2.2±2.1	-1.5±0.8	0.083
HbA_{1c} (%)			
Study start	6.0±0.8	5.9±0.8	-
End of study	5.3±0.8	5.1±0.9	-
Δ HbA_{1c}	-0.6±0.4	-0.7±0.4	0.690
Patients with HbA_{1c} <5.5% (n; %)	15 (71)	15 (83)	
Adverse events			
Patients with hypoglycaemic symptoms	5 (24)	3 (16)	-

as detailed in Table 12. The myocardial blood flow index showed a numerical increase from rest to stress in the two groups (Table 12). The MBFI during stress improved somewhat following improved glycaemic control in both groups, but this change did not reach statistical significance. In the same way, there was a numerical increase in MBVI from rest to stress in the total population, a change apparent among patients in the I-group but not in the O-group. The difference between the two groups was, however, statistically insignificant (p=0.10).
There was no correlation between the actual change in glycaemic control, expressed as $HbA_{1c,}$ and the change in E/E´, as an expression of diastolic function, when investigated with regression plots (Figure 16).

Table 12. Diastolic endpoints in the total patient material and by group allocation. p-values refer to the differences before and after treatment between groups.

	All		Group I		Group O		p =
Variables	Rand	Final	Rand	Final	Rand	Final	
	n=39		n=21		n=18		
E velocity (cm/s)	74±18	73±18	75±16	69±13	73±20	77±22	0.038
A velocity (cm/s)	73±16	73±17	77±16	76±16	67±15	70±17	0.13
E/A	1.0±0.3	1.1±0.4	1.0±0.2	0.9±0.3	1.1±0.4	1.2±0.4	0.45
E´ velocity (cm/s)	10.3±1.9	10.0±1.7	10.3±2.4	10.2±1.5	10.3±1.9	9.9±2.1	0.65
A´ velocity (cm/s)	12.2±2.2	12.1±2.1	12.2±2.3	12.1±2.0	12.1±2.2	12.2±2.3	0.55
E/E´	7.4±2.2	7.6±2.2	7.6±2.4	7.3±2.1	7.3±2.0	7.9±2.3	0.10
E´/A´	0.9±0.2	0.8±0.3	0.9±0.2	0.8±0.2	0.9±0.2	0.8±0.3	0.84
MBFI* rest	1.6±1.2	1.7±1.2	1.6±1.06	1.7±1.2	1.6±1.3	1.7±1.3	0.50
stress	3.3±2.2	4.3±3.7	3.05±1.8	3.7±2.7	3.6±2.5	4.7±4.5	1.00
MBVI** rest	0.14±0.06	0.14±0.07	0.13±0.06	0.15±0.07	0.15±0.06	0.13±0.07	0.23
stress	0.17±0.06	0.19±0.06	0.17±0.04	0.20±0.06	0.16±0.07	0.16±0.06	0.10

Available data in * I-group 16 patients, O-group 17 patients
** I-group 16 patients, O-group 16 patients

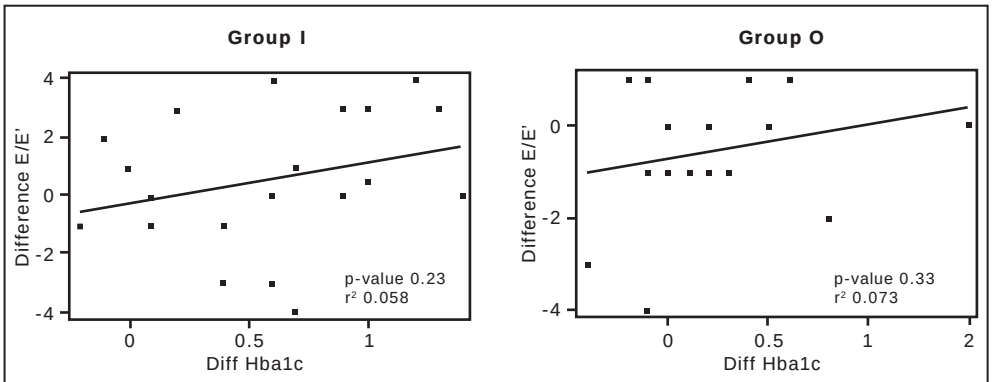


Figure 16. Regression plots showing correlation between actual change in glycaemic control expressed as Hba1c and diastolic function showed as E/E´ in group I and O respectively.

Table 13. Pertinent patient clinical characteristics by group. Values presented are n (%) and mean±SD unless otherwise stated.

Variable	**Group I** n = 10	**Group O** n = 12
Age (years)	60±5	63±7
Females	6 (60)	7 (58)
Diabetes duration, (years; median and range)	4 (1-10)	6 (0-14)
Risk factors		
Hyperlipidemia	4 (40)	9 (75)
Hypertension	4 (40)	9 (75)
Active-/ex-/non-smoker (n)	2/2/6	0/1/11
Glucose-lowering treatment		
At randomisation		
Insulin/oral (n)	0/5	0/6
Metformin	5 (50)	4 (33)
Glimepiride/glipizide	-	2 (17)
At end of study		
Insulin/oral (n)	10/0	0/12
Metformin n (%; mean dose in g/day)	-	12 (100; 2.3)
Glimepiride/glipizide	-	-
Repaglinide n (%; mean dose in mg/day)	-	8 (67;2.25)
Insulin glargine (U/day; median dose and range)	33 (12-56)	-
Insulin aspart (U/day; median dose and range)	-	-
Cardiovascular treatment		
β-blockers	2 (20)	3 (25)
ACE inhibitors/AII-receptor blockers	3 (30)	8 (67)
Calcium channel blockers	1 (10)	1 (8)
Diuretics	3 (30)	5 (42)
ASA	3 (30)	4 (33)
Statins	4 (40)	6 (50)
Physical examination		
BMI (kg/m2; median and range)	30 (23-39)	28 (18-48)
Heart rate (beats/min)	68±9	63±9
Blood pressure (mm Hg)		
Systolic	145±12	146±20
Diastolic	80±6	80±10
Ankle pressure (mmHg)	155±14	157±27

Study IV

Clinical data

The clinical characteristics of the participants in Study IV are presented in Table 13. The two groups were well balanced apart from a somewhat higher percentage of patients with hyperlipidemia, hypertension and receiving renin-angiotensin receptor blockers in the O-group. Diabetes duration and the prescription of glucose-lowering treatment were comparable at randomisation. By the end of the study, all the patients in the I-group were treated with insulin. Patients randomised to the O-group were all given metformin and, in eight of them, repaglinide had to be added to achieve the glucose target.

Data biochemical analyses and inflammatory markers

Laboratory data are presented in Table 14. There was a comparable improvement in glycaemic control in both groups, as reflected by a decrease in HbA_{1c} of 0.6 and 0.5% in the I- and O-groups respectively. By the end of the study, eight patients (80%) in the I-group and ten (83%) in the O-group had an HbA_{1c} of ≤5.5%. Patients in the I-group had higher values for high sensitive CRP throughout the study (I- vs. O-group p=0.01) and higher serum amyloid A levels by the end of the study (I- vs. O-group p=0.02).

Table 14. Pertinent biochemical analyses at randomisation and end of study by group. Values presented are median and quartile (1 and 3). p-values refer to the differences before and after treatment between groups using the Kruskal-Wallis test.

Variable	**Group I**		**Group O**		**p - value**
	Randomisation	End of study	Randomisation	End of study	
FPG (mmol/L)	7.6 (6.9, 7.6)	5.4 (5.2, 5.7)	7.7 (7.1, 8.4)	6.1 (5.3, 7.0)	0.59
HbA_{1c} (%)	5.8 (5.7, 6.3)	5.2 (4.7, 5.5)	5.5 (5.1, 6.2)	5.0 (4.6, 5.3)	0.46
Plasma cholesterol (mmol/L)					
Total	4.6 (4.4, 5.2)	4.7 (4.3, 5.9)	4.4 (4.0, 5.2)	5.2 (4.5, 5.9)	0.86
HDL	1.1 (0.9, 1.6)	1.0 (0.9, 1.5)	1.2 (0.9, 1.4)	1.1 (1.0, 1.6)	0.11
LDL	2.5 (2.2, 3.0)	3.1 (2.4, 4.0)	2.4 (1.9, 3.3)	3.2 (2.5, 3.8)	0.31
P-triglycerides (mmol/L)	1.3 (0.9, 1.4)	1.1 (0.8, 1.9)	1.6 (1.2, 2.0)	1.4 (0.9, 2.3)	0.72
hsCRP (mg/L)	2.4 (0.8, 3.1)	2.4 (1.4, 2.5)	0.9 (0.3, 1.3)	0.6 (0.4, 1.2)	0.62
Non-esterified fatty acids (mmol/L)	0.48 (0.35, 0.63)	0.48 (0.33, 0.57)	0.57 (0.5, 0.8)	0.65 (0.5, 0.8)	0.77
Endothelin-1 (pmol/L)	2.4 (2.3, 3.7)	3.0 (2.4, 3.5)	3.2 (2.6, 4.5)	3.1 (2.6, 4.6)	0.61
Serum amyloid A protein (mg/L)	2.7 (1.9, 4.1)	3.7 (2.4, 5.8)	1.9 (1.7, 2.8)	2.3 (1.7, 2.9)	0.84
Fibrinogen (g/L)	3.9 (3.7, 4.8)	3.8 (3.4, 4.5)	3.8 (3.0, 4.2)	3.9 (3.2, 4.3)	0.79

Flow-mediated dilatation

The brachial artery diameter was similar in the two groups at baseline (I-group = 3.3±0.6 mm; O-group = 3.3±0.7 mm) and did not change during the study period. Flow-mediated dilatation (Figure 17 panel A) was reduced in the I-group from 6.0 ± 2.2% to 4.7 ± 3.0% (p=0.037) and remained essentially unchanged in the O-group (4.3±2.3 and 4.7±3.3% respectively; p=0.76). The change in flow-mediated dilatation following improved glycaemic control did not differ significantly between the two treatment groups (p=0.12). The intensified glycaemic control did not significantly influence the endothelium-independent vasodilatation (Figure 17 panel B) in either of the two groups.

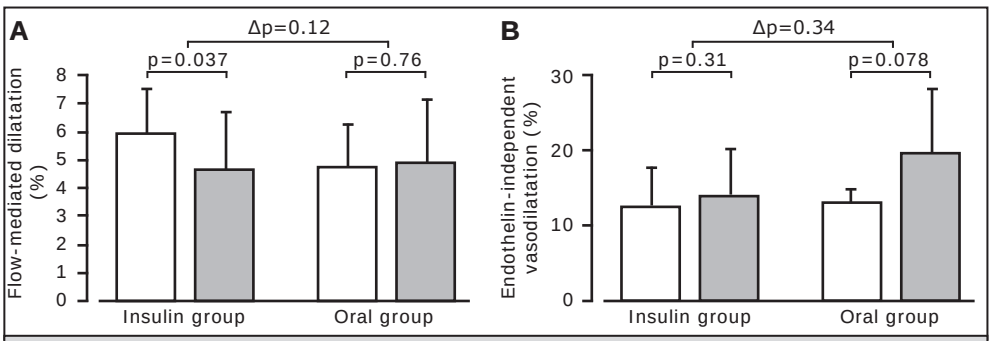


Figure 17.

Panel A: Flow mediated dilatation, presented as %, by treatment group before (open bars) and after (filled bars) glucose normalization.

Panel B: Endothelium independent vasodilatation (after Nitroglycerine), presented as %, by treatment group before (open bars) and after (filled bars) glucose normalization.

Values are presented as mean and 95% CI. Δ*P* refers to the in between group comparison before and after intensified glucose control.

Skin microcirculation

Ankle blood pressure (Table 13) was comparable in the two groups at randomisation and did not change during the study period. There were minor numerical differences between the two groups with regard to the microcirculatory variables by LDF and expressed as perfusion units at randomisation (I-group 6.2±2.8 and O-group 6.0±3.1). At the end of the study, there were no significant differences between the two treatment groups regarding skin microcirculation at rest (I-group 6.3±3.1 and O-group 5.9±2.7; Δp=1.00). The perfusion units during hyperaemia did not differ between the two groups, as outlined in Figure 18.

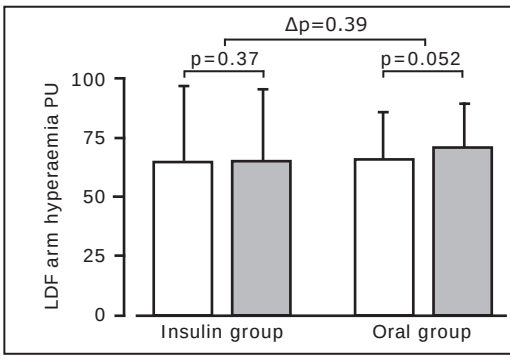


Figure 18. Laser Doppler fluxmetry, presented as perfusion units by treatment group before (open bars) and after (filled bars) glucose normalization.

Values are presented as mean and 95% CI. Δ*P* refers to the in between group comparison before and after intensified glucose control.

General Discussion

Ever since the Framingham Study [37, 112] pinpointed the increased risk and severe prognosis of heart failure in people with diabetes almost forty years ago, efforts have been made to understand the pathophysiology, establish methods for early detection and find preventive treatment opportunities. As outlined in the introduction, compromised diastolic function is an early sign of myocardial involvement in the diabetic heart. It appears that this may occur independently of other reasons for heart failure, such as hypertension and coronary artery disease, and that with time it may progress and contribute to clinically manifest heart failure. This thesis focuses on two issues of relevance – the early detection of diastolic myocardial involvement and the opportunity to interact with this kind of dysfunction in patients with diabetes using meticulous glycaemic control.

Diagnostic aspects

Diagnosing diastolic disturbances is problematic as a result of the continuing lack of a generally accepted definition [20, 113]. In spite of this, it is important to find safe, simple methods for screening a diabetic population for the presence of these disturbances, together with methods that can be used to follow the impact of various therapeutic interventions intended to prevent or at least retard further deterioration or eventually cure the disease. Echocardiography is currently the most commonly applied method for the evaluation of myocardial function, a method that is recommended by international guidelines as the first in line among diagnostic tools [6]. Left ventricular diastolic dysfunction is then usually evaluated by means of mitral and pulmonary vein Doppler echocardiography.

The results are, however, not easy to interpret, as they are influenced by a multitude of variables, most importantly left atrial and ventricular pressure and compliance, the dynamic change in the mitral annulus area, heart rate and age [109, 114-117]. TDI, which estimates myocardial tissue velocities, thereby offering a relatively load-independent measurement of left ventricular relaxation, is a complementary method [67, 68].

In Study I, TDI was compared with conventional echocardiography and with the atrio-ventricular plane-displacement technique. The protocol was designed and conducted at a time when TDI was a fairly new method and its value for diagnosing diastolic dysfunction had still not been firmly established. The patients that were included did not specifically have diabetes, as the main purpose was to study the method in patients with heart failure with preserved vs. reduced systolic function compared with controls. Study I showed that patients with diastolic heart failure could not be differentiated from controls by M-mode and regular two-dimensional echocardiography. The use of TDI did, however, add important information relating to regional systolic and diastolic velocities and time intervals during the cardiac cycle. Moreover, TDI revealed that patients with diastolic myocardial dysfunction often have signs of mild systolic involvement as well, an early finding that was subsequently confirmed by others [88, 118]. It therefore appears that TDI is at least as good as conventional MPWD for the detection of diastolic myocardial disturbances. In some patients with heart failure and an ejection fraction of > 45%, there were evident changes in TDI parameters during the systolic and diastolic phase. The TDI technique therefore appeared to be a useful

diagnostic tool in patients with mild systolic or diastolic left ventricular function compared with the traditional MPWD technique, as this group of patients might be more difficult to identify, especially because of the pseudonormalisation phenomenon that occurs in MPWD. TDI did, however, not offer any major advantage when it came to the diagnostic capacity of MPWD, while the atrio-ventricular plane-displacement method was inferior to these two techniques. It must be acknowledged that the use of an isolated MPWD variable or Doppler pattern is insufficient when assessing myocardial diastolic function [68]. The main explanation is the pseudonormalisation phenomenon seen with MPWD and the fact that several of the variables obtained by this method, not least the E/A ratio, are age dependent [108]. In spite of less load dependence, a similar limitation was found with TDI, as the predictive value only improved when several variables were combined. Our findings are in accordance with currently accepted algorithms for the diagnosis of diastolic dysfunction [6, 20, 89], all of which include different methods and criteria added together to make a semi-quantitative assessment in categories of diastolic dysfunction or normal function. The present ESC algorithm goes even further, requiring elevated diastolic filling pressures as a criterion for diastolic impairment. Increased filling pressure is one of the two cornerstones of the haemodynamics of heart failure. Another important limitation with Doppler-based techniques is the angle dependence of this method [72, 119].

In Study II, diastolic function was classified by a combination of diastolic variables derived by Doppler echocardiography and TDI according to previously established criteria [27, 89]. The patient population consisted of patients with type 2 diabetes mellitus without any previously known cardiovascular events. The assumption was that the addition of a measurement of the maximum LA volume could improve the diagnostic correctness when diagnosing left ventricular diastolic dysfunction by mitral and pulmonary vein Doppler echocardiography and myocardial tissue velocities [120, 121]. The basis for this hypothesis is that the left atrium has multiple functions, serving both as a reservoir and as a conduit for the passage of blood from the pulmonary veins to the left ventricle and that it also performs a contractile function by optimising left ventricular filling. The size of the LA varies [122, 123]and the LA volume has been considered useful when describing LA function [124]. It is therefore of great interest to evaluate the capability of new methods to analyse in greater detail the impact of LA function when mapping early signs of diastolic impairment.

Study II was based on the assumption that Velocity Vector Imaging, a new angle-independent technique, could serve as a complement to traditional methods when screening a diabetic population for early signs of myocardial diastolic dysfunction. This technique was therefore compared with the already established techniques. It was found that patients with uncomplicated type 2 diabetes mellitus and early “traditional” signs of diastolic dysfunction had a lower systolic LA strain as revealed by VVI. LA strain also appeared to be related to left ventricular function, decreasing linearly with the increasing severity of left ventricular diastolic dysfunction. Accordingly, VVI was feasible for the quantification of regional LA function and LA volume with acceptable measurement variability. Unfortunately, despite these advantages, VVI did not turn out to be a useful, single tool for the detection of mild to moderate diastolic dysfunction in a diabetic population. When validating the method, an overlap between VVI and traditional methods was revealed when separating normal from pathological diastolic function. In conclusion, left atrial VVI may be useful for the detailed

analysis of left ventricular diastolic function, but, due to its low positive predictive value, it has limited value as a screening tool. Therefore we did not add the VVI technique to the conventional Doppler echocardiography and TDI techniques to study the impact of glycaemic control on diastolic myocardial dysfunction in patients with type 2 diabetes.

Therapeutic aspects

In addition to impaired myocardial diastolic function, there are other early expressions of diabetes-related cardiovascular involvement; they include endothelial dysfunction causing a reduction in the myocardial blood flow reserve. Previous observations suggested that hyperglycaemia-related early myocardial and microcirculatory disturbances are dynamic and that they may be reversed by improved metabolic control [59, 125]. Studies of this possibility were, however, difficult to accomplish due to a lack of suitable methodology until the ultrasound-based techniques became more generally available for the quantitative assessment of myocardial function [126] and perfusion [100].

The hypothesis behind Studies III-IV was based on the outcome of an observational study performed by von Bibra et al.[59] showing beneficial effects on myocardial diastolic dysfunction and perfusion following improved glycaemic control. The patients in the observational study had fairly long-standing diabetes and, although efforts were made to eliminate those with obvious cardiovascular complications, such as hypertensive or coronary artery disease, the possibility could not be ruled out that these conditions may have been part of the myocardial involvement. Another shortcoming was that, although this study favoured the assumption that insulin-based glycaemic control was superior to oral drugs, the distribution in terms of treatment modality was not based on chance. These considerations were taken into account when planning the DADD protocol. In this prospective trial, patients with type 2 diabetes were randomly allocated to insulin or oral glucose-lowering therapy, with the primary intention of verifying previous observations of myocardial function but also to expand them by incorporating a sub-study of peripheral microcirculation. Patients were intentionally selected as being free from previous cardiovascular events and signs of congestive heart failure or coronary artery disease. Hypertension was permitted if it was well controlled and only if there were no echocardiographic signs of left ventricular hypertrophy. The reason for these strict inclusion criteria was the desire to avoid confounding factors and to isolate the impact of intense glucose control on early signs of myocardial dysfunction. Moreover, DADD was designed as a prospective, open, blinded evaluation (PROBE) study to avoid information on group allocation, insulin versus oral therapy, influencing the interpretation of the results. The lack of a placebo-treated control group could be regarded as a limitation. It was, however, felt that it would be unethical to leave patients with an established, albeit fairly short, history of diabetes without glucose-lowering treatment.

Since the prevalence of myocardial diastolic dysfunction has often been referred to as fairly high [28, 127, 128] it may seem surprising that as many as 121 patients had to be investigated to find 39, corresponding to 32% of the screened population, that fulfilled the inclusion criteria. Although the screening process did not represent a truly population-based, epidemiological investigation, the yield was somewhat low. Available information on the actual prevalence varies considerably. In fact, Cosson et al. [129] reported a non-existent prevalence following the exclusion of patients with any micro- or macrovascular complication, while Boyer et al. [28] reported a prevalence of 75% in a study of 61 consecutive, normotensive patients with type

2 diabetes. This huge difference could be at least partially explained by patient selection, varying diagnostic methods and different definitions. The present criteria were fairly strict as it was thought to be very important to select patients that had unequivocal signs of diastolic dysfunction that could not be related to concomitant diseases or complications of the diabetic state apart from hyperglycaemia. In spite of this, some of the present patients had well-controlled hypertension. It is therefore possible that this may have contributed to diastolic dysfunction to some extent although not as the full explanation since well controlled hypertension seems mainly to induce mild dysfunction [130].
The targeted normalisation of glucose control was accomplished relatively quickly and was maintained during the four-month study period. A large percentage of the patients, 77%, had an HbA1c level below 5.5% by the end of the study, without any difference between insulin- and oral-based treatments. Glycaemic normalisation did not, however, as had been hypothesised, improve diastolic function in either of the two groups of patients. In an attempt to improve the opportunity to detect a relationship between the improved glycaemic control and diastolic function, regression plots between the actual improvement in HbA_{1c} and the change in E/E´ were constructed, but there was still no correlation.

The disappointing discrepancy between the previous findings and the outcome of the present study calls for further consideration. One obvious explanation is that observational data, although important as sources for the creation of hypotheses, are not necessarily true when tested in carefully designed prospective clinical trials. The study that was the basis for DADD recruited a partially heterogeneous set of patients referred to the department of endocrinology due to perceived difficulties in obtaining satisfactory glycaemic control. Apart from this, there were no detailed clinical or echocardiographic inclusion criteria. It is also possible that DADD recruited patients with very modest diastolic dysfunction compared with the observational study in which the patients had more prominent dysfunction. It may in fact be more difficult or impossible to further improve diastolic function at this early stage of the disease. The present data do not permit a more detailed analysis to elucidate these assumptions. Time of follow-up is another variable that should be considered. It could be argued that four months of follow-up is too short to improve the existing myocardial structural abnormalities. Lack of time would not, however, explain the discrepancy between DADD and the observational study in which the follow-up time was only three weeks.

As expected, stress increased myocardial blood flow, which may be regarded as a positive control of the opportunity to detect changes by means of the contrast-enhanced echocardiographic technique chosen for this part of the study. Following the normalisation of glucose control, there was a small increase in MBVI during stress when all the patients were combined. A change is this direction was, however, only noted among insulin-treated patients and the corresponding difference between the two groups was statistically insignificant. The observed changes may therefore be coincidental rather than an expression of a true opportunity to improve myocardial blood flow reserve by means of meticulous glycaemic control, once more negating the hypothesis. The present findings are supported by a report by Bengel et al.[131]. In their study, nateglinide and placebo were compared in patients with type 2 diabetes over a period of four months. There was no improvement in myocardial blood flow despite a normalisation of glycaemic control following the administration of nateglinide.

Study IV, a sub-study of DADD, raised the question of whether glycaemic normalisation might counteract macro- and microvascular function in the patients recruited for the DADD study. The rationale for this study was previously reported observations of disturbed endothelial function and thereby vascular reactivity in patients with an early stage of type 2 diabetes [132-134]. In accordance with the lack of an effect on myocardial blood flow, there was no improvement in the flow-mediated vasodilatation of the brachial artery or the skin microcirculation. This result does, however, contrast with observations made by Vehkavaara et al.[135], reporting an improvement in endothelial function when insulin was added to ongoing treatment with metformin and subsequently stating that this effect was long lasting [136]. A comparison of these two studies reveals that there are important differences both in the patient material and in the study design. Vehkavaara et al. measured total blood flow, mainly reflecting resistance vessel function, by means of venous occlusion plethysmography following intra-arterial infusions of acetylcholine and sodium nitroprusside, while Study IV used a non-invasive method reflecting conduit artery function. The same methodology has been used at our laboratory, demonstrating that aggressive lipid-lowering treatment improves endothelial function in patients with type 2 diabetes with coronary artery disease [137]. In this study, there was a significant improvement in endothelial function studied using flow-mediated vasodilatation but not using venous occlusion plethysmography. There may therefore be differences in response depending on vessel segments. It is interesting to note that the baseline flow-mediated vasodilatation level in Study IV was similar to that in the lipid-lowering study and was thereby compromised and reasonably possible to improve by means of pharmacological tools, if at all possible. The trend towards a reduction in brachial artery endothelium-dependent vasodilatation in insulin-treated patients in Study IV should not be ignored, but, in the absence of a significant difference between the two treatment groups, it has to be considered with great caution. It is particularly interesting, in the light of recent reports of potentially negative consequences of insulin treatment compared with oral glucose-lowering drugs [133, 138]. It could at least serve as a hypothesis-generating observation warranting further studies with tight insulin-based glycaemic control to elucidate the effects on endothelial function. In this context, data reported by Avogaro et al, [139]are of interest. These authors were unable to detect any differences between patients with uncomplicated type 2 diabetes and controls in terms of endothelium-dependent vasodilatation determined by intra-brachial acetylcholine. They concluded that their findings argued against the hypothesis that the impaired generation of nitric oxide and blood flow regulation played a role as a cause of insulin resistance in these patients. This is supported by the finding that acetylcholine-induced, endothelium-dependent forearm vasodilatation was not significantly impaired in a group of clinically healthy subjects with insulin resistance [140].

Summarising the overall negative outcome of the DADD study, one important issue for discussion is whether the current patients, like those in the report by Avogaro et al.[139], were too “healthy” to be influenced. A common denominator in studies with a positive outcome is that glycaemic control was considerably worse, with a fairly high HbA1c level and fasting plasma glucose levels at study start of about 11 mmol/l. In these investigations, the reported decrease following intensified glycaemic control was 7-8 mmol/l [59, 135, 136], a level at which the DADD patients in fact started at baseline. It is therefore possible to speculate that the proportionate or, in other words, important glucose control for myocardial and endothelial dysfunction was more apparent in the positive studies. The DADD population started with relatively reasonable glycaemic control and, despite glucose normalisation, may have been more difficult to influence than more hyperglycaemic patients.

Reflections for the future

For the future, clearer definitions of diastolic dysfunction than those presently available, which are fairly complex and difficult to apply, would be welcome. To be useful, they have to be universally accepted and uniformly applied in epidemiological and treatment-oriented studies. There is clear-cut need for further, well-conducted epidemiological studies of the true prevalence and long-term impact of early signs of impaired myocardial diastolic function, according to generally accepted diagnostic criteria, in patients with type 2 diabetes free from concomitant disease. Those detected with a condition of this kind should be followed over substantial periods of time to obtain a better understanding of the rate of progress and whether diastolic dysfunction relates to future systolic impairment or whether other factors such as progressive coronary artery disease or hypertension-induced hypertrophy are more important for the pathogenesis.

Finally, it should be underlined that it is still too early to abandon the hypothesis of a favourable relationship between glycaemic control and myocardial diastolic dysfunction. The potential lack of efficacy of available glucose-lowering therapies has not been addressed in the present context. It would be valuable to study new agents, such as incretins, in future trials. In the light of the present results, these and other drugs have to be explored in prospective, randomised clinical trials recruiting patients at a more advanced stage of the disease than those selected for the DADD study. The problem then is the obvious risk of bias caused by hypertensive and coronary artery disease and other complications of diabetes. Accordingly, these protocols must include a detailed examination of the patients with this in mind.

CONCLUSIONS

DTI is a useful method for diagnosing diastolic myocardial dysfunction, with an accuracy that is similar to that of conventional echocardiography with mitral pulse wave Doppler flow.

Left atrial deformation measured as regional and overall systolic strain is impaired in patients with uncomplicated type 2 diabetes mellitus and mild to moderate left ventricular diastolic dysfunction. Strain measurements appear to be of value in distinguishing patients with normal diastolic function from those with abnormal diastolic function. Although the discriminatory power of VVI as a single measurement to detect early left ventricular diastolic dysfunction in patients with type 2 diabetes mellitus is too limited, it nonetheless offers new information on regional LA function and LA volumes and may replace or add to some of the traditional Doppler echocardiography measurements.

The hypothesis that improved glycaemic control, in particular insulin-based control, would reverse early signs of myocardial diastolic and endothelial dysfunction in patients with type 2 diabetes was not proven. It remains to be established whether it is possible to influence more pronounced dysfunction, particularly in patients with less well-controlled and long-standing diabetes, in the setting of controlled clinical trials. This is an important issue in view of the obvious risk of deterioration towards overt heart failure, which has a very serious prognosis in these patients.

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