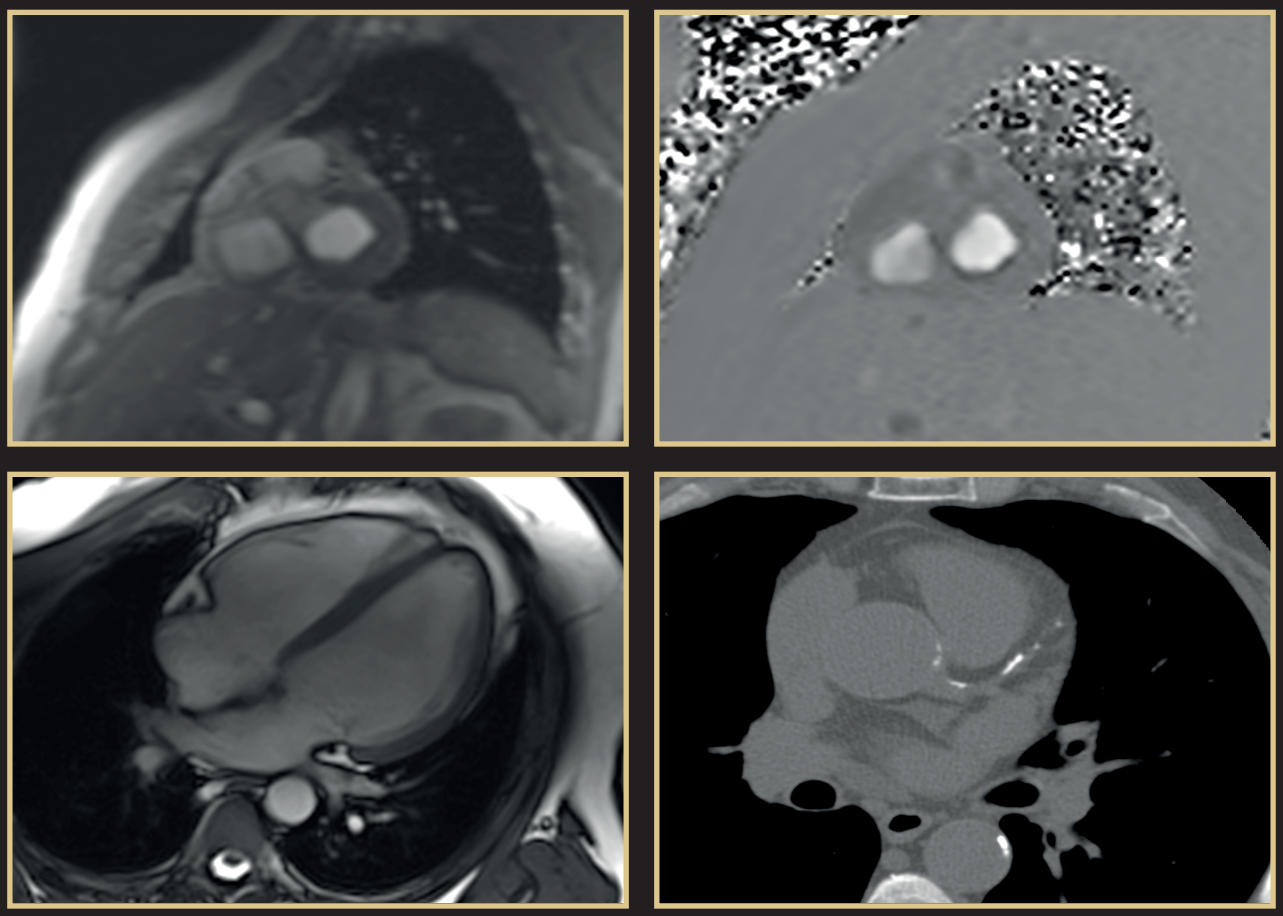

Bruno Miguel Silva Rosa da Graça

CARDIOVASCULAR MAGNETIC RESONANCE AND COMPUTED TOMOGRAPHY IMAGING FOR THE ASSESSMENT OF CARDIOVASCULAR COMPLICATIONS OF TYPE 2 DIABETES MELLITUS

Tese de Doutoramento em Ciências da Saúde, no Ramo de Medicina, na especialidade de Medicina Interna (Radiologia e Imagiologia), orientada por Prof. Doutor Filipe Caseiro Alves e Prof. Doutor Miguel Castelo Branco, co-orientada por Prof. Doutora Maria João Vidigal Ferreira e apresentada à Faculdade de Medicina da Universidade de Coimbra.

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Bruno Miguel Silva Rosa da Graça

Cardiovascular magnetic resonance and computed tomography imaging for the assessment of cardiovascular complications of type 2 diabetes mellitus

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Supervisors: Filipe Caseiro-Alves, M.D., Ph.D.
Miguel Castelo-Branco, M.D., Ph.D.
Co-supervisor: Maria João Ferreira, M.D., Ph.D.

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A Faculdade de Medicina não aceita qualquer responsabilidade em relação à doutrina e à forma desta dissertação (Regimento da Faculdade de Medicina de Coimbra, 1931, art. 108, § único).

A todos os Meus

Ao Professor Doutor Filipe Caseiro Alves

Ao Professor Doutor Miguel Castelo Branco

À Professora Doutora Maria João Ferreira

Aos meus Mestres

Aos meus Amigos

Index

Part I: Literature review

Chapter I

Classification, epidemiology and mechanisms of cardiovascular disease in diabetes mellitus

Chapter II

Non-invasive imaging of cardiovascular disease in diabetes mellitus

Chapter III

Cardiac imaging in asymptomatic diabetes mellitus

Part II: Personal contribution

Chapter IV

Clinical study: Cardiovascular magnetic resonance imaging assessment of diastolic dysfunction in a population without heart disease: a gender-based study

Chapter V

Clinical study: Left atrial dysfunction in type 2 diabetes mellitus: insights from cardiac MRI

Chapter VI

Clinical study: Left ventricular diastolic function in type 2 diabetes mellitus and the association with coronary calcium score: a cardiovascular MRI study

Chapter VII

Clinical study: Disease duration as a major determinant of increased coronary artery calcium score in asymptomatic patients with type 2 diabetes

Chapter VIII

Summary and conclusions

Preâmbulo

Desde o início da minha formação pós-graduada como Médico Radiologista que procurei integrar o trabalho que o Serviço de Imagiologia dos HUC desenvolvia na área da imagiologia cardíaca.

Testemunhei o início da aplicação da tomografia computorizada para estudo das artérias coronárias no Serviço e tenho acompanhado o alargamento da ressonância magnética e tomografia computorizada cardíacas a novas situações clínicas.

Um destes campos activos de pesquisa é a diabetes mellitus. Pela natureza tipicamente assintomática da diabetes mellitus tipo 2, as suas manifestações e complicações, nomeadamente no foro cardiovascular, são reconhecidas tardiamente. Neste âmbito, o estudo de novas técnicas de imagem não-invasiva, poderá permitir conhecer melhor o espectro de alterações cardíacas condicionadas pela diabetes mellitus tipo 2.

Acabou por ser com enorme motivação que recebi o desafio para tentar aprofundar o conhecimento na área da imagiologia cardíaca e a sua aplicação no estudo de novos marcadores de doença cardiovascular em pacientes com diabetes mellitus tipo 2.

O trabalho que culmina na presente Tese só foi possível com o recurso a múltiplas ajudas e o concurso de muitas colaborações.

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Neste âmbito, pela logística que acarreta, o trabalho mais afectado foi o da tomografia computorizada. O meu agradecimento á Drª Luísa Teixeira pela disponibilidade demonstrada.

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Part I

Literature review

Chapter I

Classification, epidemiology and mechanisms of cardiovascular disease in diabetes mellitus

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. The causes of diabetes are multiple. Both genetic and environmental factors play roles in its etiology. The supply of insulin may be reduced by a decrease in pancreatic β cell mass and/or functional disturbances of β cells. In other cases there are both insulin resistance and relative deficiency of insulin.

If the metabolic abnormality is mild, patients may be asymptomatic, while in the presence of overt hyperglycemia, characteristic symptoms such as thirst, polydipsia, polyuria and weight loss often arise. In severe cases, ketoacidosis or hyperglycemic–hyperosmolar states may occur and lead to disturbances of consciousness, coma and even death unless treated appropriately.

The duration of this metabolic disorder leads to diabetes-specific complications, chiefly involving small vessels (retinopathy, nephropathy and neuropathy), resulting in serious outcomes such as visual disturbance, renal failure, and gangrene. Diabetes accelerates and exacerbates the occurrence of arteriosclerosis, increasing the risks for coronary disease, cerebrovascular disease and peripheral artery disease (1-3).

An increasingly obese and aged population heralds a global epidemic of diabetes mellitus, which poses a major risk to individual and public health.

1. Classification

The classification of diabetes mellitus comprises four categories: type 1, type 2, other types, and gestational diabetes.

Type 1 diabetes mellitus covers those cases where disease is due to pancreatic islet beta-cell destruction. This can be idiopathic or more commonly is due to autoimmune processes. It does not include cases where beta-cell destruction is due to a specific disease such as cystic fibrosis. Type 2 diabetes covers those cases that result from defects in insulin secretion and is often combined with a degree of insulin resistance. This category covers a large range of mechanisms from defective insulin secretion with no insulin resistance to largely insulin resistance with a degree of defective secretion. The deficiency in insulin secretion is usually relative rather than absolute (1-3).

Other specific types of diabetes include other etiopathogenic mechanisms,

e.g., genetic defects in b-cell function, genetic defects in insulin action, diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical-induced (such as in the treatment of HIV/AIDS or after organ transplantation). Gestational diabetes mellitus refers to a state of glucose intolerance occurring or detected for the first time during pregnancy (1-3).

2. Diagnosis

The diagnosis of diabetes mellitus is established when a patient presents with classic symptoms of hyperglycemia (thirst, polyuria, weight loss, blurry vision) and has a random blood glucose value of 200 mg/dL (11.1 mmol/L) or higher, and confirmed on another occasion. In an asymptomatic individual the diagnosis can be established with any of the following criteria: fasting plasma glucose values ≥126 mg/dL (7.0 mmol/L), two-hour post oral glucose test values of ≥200 mg/dL (11.1 mmol/L), and hemoglobin A1c values ≥6.5%. An abnormal result should be confirmed by repeat measurement with the same test (1-3).

Prediabetes

It is recognized an intermediate group of individuals whose glucose levels, although not meeting criteria for diabetes, are nevertheless too high to be considered normal and have a relatively high risk for the future development of diabetes. The onset of type 2 diabetes is gradual, with most individuals progressing through a state of prediabetes, which is defined as one or more of the following: fasting plasma glucose 100 to 125 mg/dL (5.6 to 6.9 mmol/L), two-hour post oral glucose test values of 140 to 199 mg/dL (7.8 to 11.0 mmol/L) or hemoglobin A1c 5.7% to 6.4% (1-3). Although individuals can spend years in a prediabetes stage, an expert American Diabetes Association (ADA) panel estimated that up to 70% of individuals with prediabetes will eventually progress to type 2 diabetes (2).

3. Epidemiology

The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity.

Diabetes mellitus is a major public health problem in the western world. The International Diabetes Federation estimates that in 2025 there will be 380 million diabetics, which corresponds to 7.1% of the population

between 20 and 79 years. In Europe it is considered that affects about 60 million people: 10.3% of men and 9.6% of women over 25 (3). The number of diabetics has been growing also in Portugal. The last Report of the National Observatory of Diabetes, pointed to a prevalence of 12.3% in the population between 20 and 79 years and an incidence of 511.4 new cases per 100 000 inhabitants (4).

Type 2 diabetes mellitus is the commonest type of diabetes accounting for between 80 and 95% of cases of diabetes.

4. Diabetes and the heart

Type 2 diabetes, the most common form of the disease, may remain undetected for many years and physicians often face this disease at an advanced stage, when vascular complications have already occurred in most of patients.

Cardiovascular disease is the leading complication of type 2 diabetes and approximately one half of patients with type 2 diabetes will die of a cardiovascular cause (5). Indeed, the National Cholesterol Education Program report from the United States and guidelines from the European Society of Cardiology consider type 2 diabetes to be a cardiovascular equivalent (6, 7), thereby establishing diabetics a high risk population. This classification was based in part upon the observation that patients with type 2 diabetes without a prior myocardial infarction were at the same risk for myocardial infarction and coronary mortality as patients without diabetes who had a prior myocardial infarction (8).

Apart from an increased incidence, coronary artery disease is also more extensive and accelerated among diabetic patients (9). Diabetic individuals have a higher incidence of two-and three-vessel disease and a greater number of diseased vessel segments than do their nondiabetic counterparts (10). Multivessel coronary heart disease is also common in asymptomatic patients with type 2 diabetes (11).

When coronary artery disease occurs in diabetics, the course of disease is particularly aggressive and associated with worse outcomes than in non-diabetics (12). Diabetes is associated with an increased risk of myocardial infarction. In diabetics; following a myocardial infarction, the risk of a subsequent myocardial infarction and development of heart failure are higher than in non-diabetics (13).

Furthermore, the presence of coronary atherosclerosis without significant luminal narrowing may not be entirely benign (14, 15). Indeed, previous

studies have demonstrated that myocardial infarction and unstable angina are frequently caused by coronary lesions deemed to be nonsignificant before the event (15). Plaque composition may be crucial to define coronary risk. In a histologic study of atherectomy specimens from patients with and without diabetes, coronary tissue from diabetics contained a greater amount of lipid-rich atheroma, more macrophage infiltration, both of which are associated with a greater risk for plaque rupture, and a higher incidence of thrombosis (16).

In addition to the increase in cardiovascular events, patients with type 2 diabetes also have a high rate of asymptomatic coronary disease as determined by the presence of coronary artery calcification and by inducible silent ischemia on stress imaging (1, 6, 17).

It has been well recognized that patients with type 2 DM have impaired coronary flow reserve reflecting coronary microvascular function even in the absence of epicardial coronary atherosclerosis (18, 19). Coronary microvascular dysfunction may be the underlying mechanism in patients with symptoms and signs of myocardial ischemia without angiographically detectable coronary artery disease, and has independent prognostic information in diabetic patients (19).

4.1 Diabetic cardiomyopathy

Chronic heart failure is a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill with or to eject blood. Systolic heart failure arises from a compromise in the contractility of the heart and is defined as a left ventricular ejection fraction of < 45%. Diastolic dysfunction interferes with the heart's ability to relax and fill with blood.

The Framingham study firmly established the epidemiologic link between diabetes and heart failure (20). Coronary heart disease and arterial hypertension are the main mechanisms responsible for heart failure. However, heart failure in diabetes also occurs in the absence of underlying coronary artery disease and hypertension, an entity known as diabetic cardiomyopathy, that is defined based on exclusion of other potential causes.

Diabetic cardiomyopathy development affects myocardial remodeling and eventually leads to cardiac diastolic and systolic dysfunction.

Diastolic dysfunction is a major characteristic in diabetic cardiomyopathy. It has been reported that there is a significant impairment in diastolic function in individual with diabetes even without coronary artery disease

and overt heart failure (21-23). Studies on type 2 diabetic patients with normal blood pressure and glucose levels found a prevalence over 40% of diastolic dysfunction, compared to healthy controls (22, 23). Recently, studies with tissue Doppler strain analysis, could also detect systolic abnormalities in diabetic patients with normal left ventricular ejection fraction (24-26).

4.2 Diabetic cardiac autonomic neuropathy

Diabetic cardiac autonomic neuropathy encompasses damage to the autonomic nerve fibers that innervate the heart and blood vessels, resulting in abnormalities in heart rate control and vascular dynamics. Cardiac autonomic neuropathy is particularly associated with an increased risk of silent myocardial infarction and sudden cardiac death. Autonomic nervous system dysfunction is believed to be the primary underlying mechanism for impaired recognition of ischemia in diabetic patients. Supporting this hypothesis is the observation that the uptake of metaiodobenzylguanidine (MIBG), a norepinephrine analog, is reduced in diabetic patients with silent ischemia (27-29).

The prevalence of cardiac autonomic neuropathy in type 2 diabetic patients is estimated to be around 20-30% of patients.

Diabetic cardiac autonomic neuropathy has a wide range of manifestations: resting tachycardia, orthostatic hypotension, exercise intolerance, intraoperative cardiovascular liability, and silent myocardial infarction. The diagnosis of this entity is based on the evaluation of heart rate response to deep breathing, standing and Valsalva maneuver, and blood pressure response to standing. However, these tests are indirect assessments of the autonomic nervous system and are less sensitive than direct assessments by cardiac radionuclide imaging with SPECT or PET (27-29).

5. Mechanisms of cardiovascular disease in diabetes mellitus

The etiology of cardiovascular disease in diabetes is complex and multifactorial, mainly due to prolonged exposure to hyperglycemia clustering with other risk factors such as arterial hypertension, dyslipidemia, obesity, as well as genetic susceptibility.

The development of cardiovascular disease in people with insulin resistance is a progressive process, characterized by early endothelial dysfunction and vascular inflammation leading to monocyte recruitment, foam cell formation and subsequent development of fatty streaks. Over many years, this leads to atherosclerotic plaques, which, in the presence of enhanced inflammatory content, become unstable and rupture to promote occlusive thrombus formation.

5.1 Endothelial dysfunction, oxidative stress and vascular inflammation

Vascular endothelial cells play a major role in maintaining cardiovascular homeostasis. In addition to provide a physical barrier between the vessel wall and lumen, the endothelium secretes a number of mediators that regulate platelet aggregation, coagulation, fibrinolysis and vascular tone.

Hyperglycemia, elevated free fatty acids, and insulin resistance which occur in states of diabetes mellitus, act in concert to target endothelial cells, resulting in oxidative stress and endothelial dysfunction (30-32).

Endothelial cells secrete several mediators that can alternatively mediate either vasoconstriction, such as endothelin and thromboxane A2, or vasodilation such as nitric oxide and prostacyclin.

Nitric oxide plays an important role in the regulation of vascular tone, inhibition of platelet aggregation, and suppression of vascular smooth muscle cell proliferation (31).

When the endothelium is dysfunctional, the vasoconstrictor effects are unopposed and arterial tone is increased. In addition, pathological states are associated with increased endothelial production of endothelin-1 and other endothelium-derived vasoconstrictors that may further promote vasospasm and increase arterial stiffness.

Reduction or loss of endothelial nitric oxide availability results in monocyte and vascular smooth muscle cell migration into the vascular intima layer and the formation of macrophage foam cells, the initial stages

of atherosclerosis (30-32).

Increased nutritional fatty acid intake and increased lipolysis in diabetes will lead to increased free fatty acid delivery to nonadipose tissues, such as muscle, liver, pancreas and also the heart (32, 33).

The metabolic effects of type 2 diabetes mellitus on the myocardium have been previously described in experimental models, and include increased myocardial nonesterified fatty acid utilization, an increased glucose flux and subsequently triglyceride accumulation, and subsequent increased formation of reactive oxygen species. These processes, commonly referred to as "gluco-lipotoxicity," contribute to decreased adenosine triphosphate synthesis, mitochondrial dysfunction, and finally to apoptosis of myocardial cells

5.2 Macrophage dysfunction

Extracellular deposition of lipids is followed by an inflammatory infiltrate of monocytes and T lymphocytes to form fatty streaks. Monocytes develop in to macrophages and scavenge modified lipids to form foam cells. The inflammatory infiltrate and foam cells secrete inflammatory mediators and produce reactive oxidative species resulting in the migration and proliferation of vascular smooth muscle cells (32-35).

5.3 Atherogenic dyslipidemia

Atherogenic dyslipidemia comprises a triad of increased blood concentrations of small, dense low-density lipoprotein particles, decreased high-density lipoprotein particles, and increased triglycerides. A typical feature of obesity, the metabolic syndrome, insulin resistance, and type 2 diabetes mellitus, atherogenic dyslipidemia has emerged as an important risk factor for myocardial infarction and cardiovascular disease.

In type 2 diabetes, increased free fatty acid release to the liver, due to lipolysis, lead to enhanced hepatic dense low-density lipoprotein particles production.

The lipid profile of these subjects is characterized by high triglycerides, low high-density lipoprotein cholesterol, increased remnant lipoproteins, apolipoprotein B synthesis and small, dense low-density lipoprotein particles. This low-density lipoprotein particles subtype plays an important role in atherogenesis being more prone to oxidation (32-35).

5.4 Coagulation and platelet function

Among factors contributing to the prothrombotic condition, which characterize patients with diabetes mellitus, platelet hyperreactivity plays a pivotal role. Platelets of diabetes mellitus patients are characterised by dysregulation of several signalling pathways leading to intensified adhesion, activation and aggregation.

Hyperglycaemia may increase platelet reactivity by glycating platelet surface proteins (impairing membrane fluidity and therefore increasing platelet adhesion), activating protein kinase C (a mediator of platelet activation) and inducing P-selectin (a surface adhesion protein) expression.

Insulin deficiency also plays an important role in platelet dysfunction by different mechanisms: increased intracellular calcium concentration leading to enhanced platelet degranulation and aggregation; impaired response to nitrous oxide and prostacycline, which enhances platelet reactivity (32, 34-36).

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Chapter II

Non-invasive imaging of cardiovascular disease in diabetes mellitus

Introduction

The present chapter reviews diabetic cardiovascular disease and illustrates the role of multimodality cardiac imaging in its assessment.

We will discuss:

a) Diabetic heart disease, including: i. Myocardial dysfunction; ii. Metabolic heart disease; iii. Coronary artery disease; and iv. Cardiac autonomic neuropathy;

b) Cerebrovascular disease, and

c) Peripheral arterial disease.

1. Myocardial dysfunction

1.1 Diastolic dysfunction

Diabetes mellitus affects both systolic and diastolic left ventricular (LV) function. Initially, myocardial dysfunction is only very subtle, without clinical symptoms, and often occurs before diabetes mellitus is apparent (1). The first signs of myocardial dysfunction manifest in diastole (2). In patients with type 2 diabetes mellitus, a high prevalence of diastolic dysfunction of 43%–75% (3, 4) and the occurrence of heart failure in the presence of preserved systolic function have been reported (5, 6).

Diastole is the period of the cardiac cycle between closure of the aortic valve and that of the mitral valve. During this time, the left ventricle relaxes and refills, ready for the next systolic contraction. Conventionally, diastole is divided into four physiological phases: (i) isovolumetric relaxation: from closure of the aortic valve to opening of the mitral valve; (ii) early rapid filling: due transmitral pressure gradient that drives LV filling; (iii) diastasis: period of low flow in mid-diastole; (iv) late rapid filling: due to atrial contraction.

Diastolic dysfunction is characterized pathophysiologically by impaired relaxation or abnormal myocardial passive properties. Consequently, with exercise, there is an inability to augment LV end-diastolic volume despite increasing LV end-diastolic pressure. With the development of early diastolic dysfunction, the proportion of LV filling permitted to occur in early diastole is reduced. The relative importance of atrial contraction is thus increased, so-called grade I diastolic dysfunction. As

diastolic dysfunction worsens, pressure raises within the left atrium, such that immediately following mitral valve opening, blood is forced under positive pressure, rather than sucked by negative pressure, into the left ventricle. Although the proportion of LV filling occurring in early diastole returns to normal, the underlying physiology is not normal, hence the designation of pseudonormal LV filling, or grade II diastolic dysfunction. Grade III diastolic dysfunction is characterized by a further increase in filling pressure that will increase the gradient between the left atrium and the left ventricle during early diastole. When filling pressures are high, flow into the ventricle will start early and filling will terminate quickly, but with reversibility with the Valsalva maneuver. Grade IV diastolic dysfunction is characterized by irreversibility with the Valsalva maneuver.

Currently, echocardiography is the method of choice for diastolic function testing in clinical practice. One of the first simple tests that is performed when evaluating diastolic function is the assessment of mitral inflow velocities from the leaft atrium to the left ventricle with pulsed-wave Doppler ultrasonography (US) (Figure 1). Time-velocity curves are obtained from a sample volume positioned on the mitral leaflet tips, and waveform analysis results in quantification of early (E) and atrial (A) peak filling velocity, and subsequently calculation of the E/A ratio and the deceleration time of the E peak filling velocity (DT), for which cutoff criteria are defined for diastolic dysfunction classification (7).

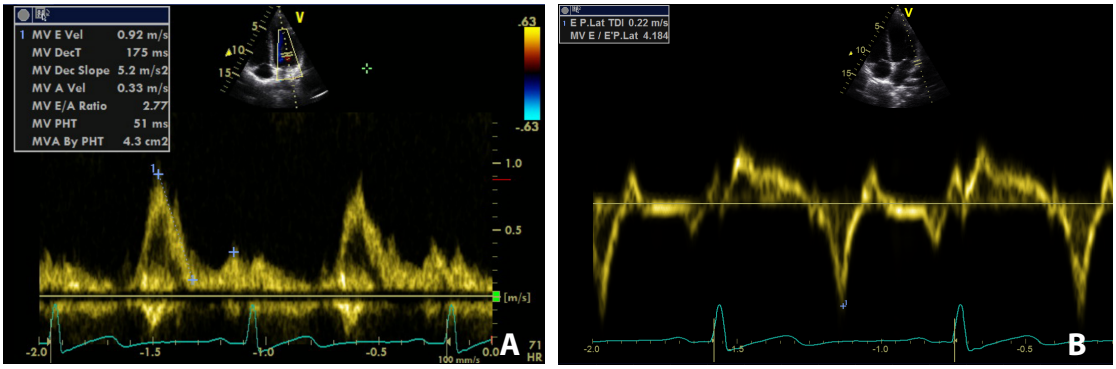

Fig 1. Estimation of left ventricular diastolic pressure by tissue Doppler imaging. Estimation of left ventricular diastolic pressure by the ratio of transmitral E (A) and mitral septal annular velovity (B), E/E'. The E/E' ratio in this subject is 4, which is within normal limits. *Courtesy of Dr Rogério Teixeira.*

In contrast to the wealth of studies in LV systolic dysfunction, there is relative inexperience in cardiac MR assessment of diastolic LV function. Nonetheless, a number of MR techniques have been used to evaluate diastolic function. Phase-contrast MR imaging with velocity encoding offers an attractive alternative for the assessment of the transmitral flow

velocity. From a time-resolved phase-contrast acquisition with velocity encoding perpendicular to the mitral plane (Figure 2), a time-velocity curve representing the transmitral in- flow is obtained. Similar to Doppler echo US, waveform analysis provides the assessment of the E/A ratio and DT.

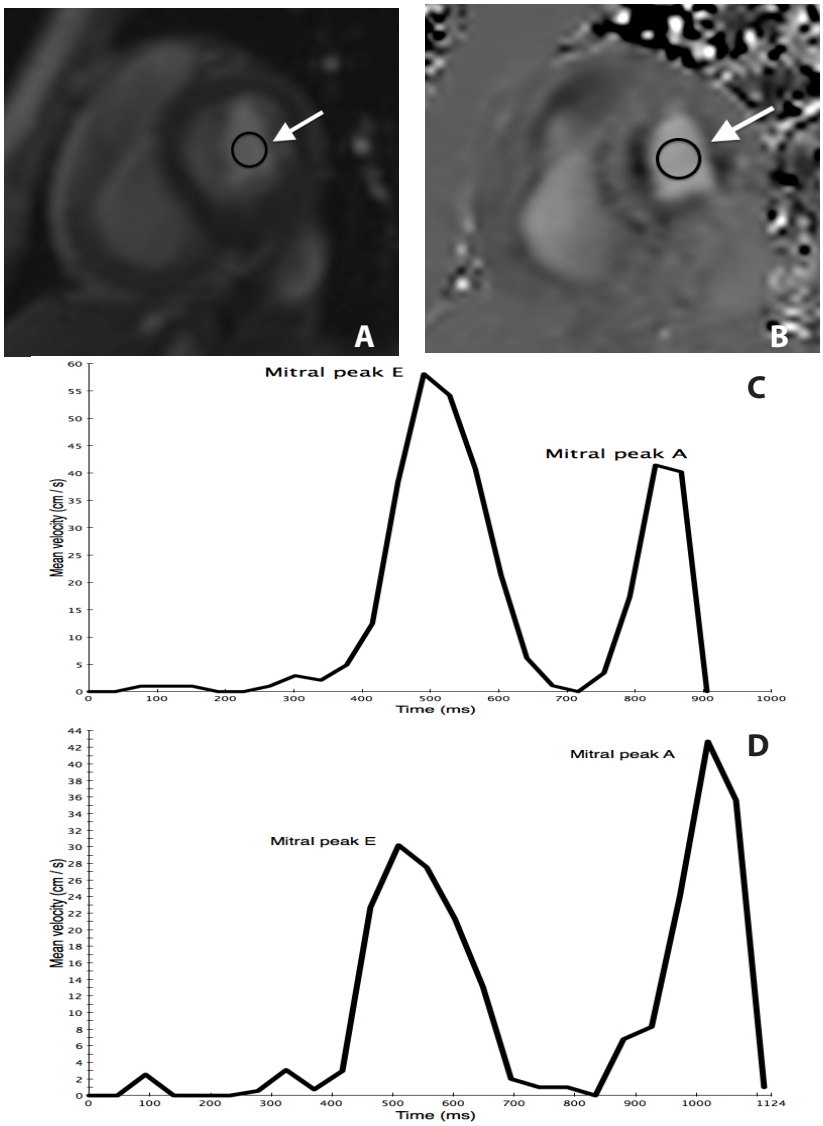

Fig 2. Measurement of transmitral flow. A and B) Phase-encoded MRI obtained with a phase-contrast sequence shows a region of interest placed at the centre of the mitral orifice. The transmitral flow curve is obtained from this region of interest. C) Normal diastolic function. The transmitral flow curve is composed of two peaks. The first one is the E wave and second one is the A wave. The E wave corresponds to rapid LV filling at early diastole; the A wave corresponds to late LV filling during end-diastole, secondary to LA contraction. D) Transmitral flow curve indicative of impaired myocardial relaxation, with a peak A superior to peak E.

With the advent of tissue Doppler imaging, quantification of longitudinal myocardial velocities has significantly simplified the interpretation of LV diastolic function. Mitral annular velocities in the longitudinal direction are obtained at the interventricular septum and lateral wall, and waveform

analysis results in early (E') and late (A') annular velocities. E' is affected by LV relaxation, preload conditions, and LV minimal pressure (7). Therefore, E' and the E/E' ratio play an important role in the estimation of LV filling pressures (Figure 1). Velocity-encoded MR imaging and tissue Doppler imaging can be used interchangeably for assessment of longitudinal myocardial velocities (8), with comparable values between MR imaging and tissue Doppler imaging for diastolic function assessment (9).

A number of novel indices of regional and global LV diastolic function were developed in recent years, including myocardial strain and strain rate imaging by echocardiography, that permits the evaluation of myocardial deformation and quantifies LV rotation, twist, and untwist (10, 11). For a better understanding of these concepts of strain and strain rate imaging, it is important to realize that: wall motion measurements (displacement and velocity) cannot differentiate between active and passive movement of a myocardial segment, whereas deformation analyses (strain and strain-rate imaging) allow discrimination between active and passive myocardial tissue movement.

Strain imaging can also be performed by MRI. Cardiovascular MR tagging remains the most widely available and validated cardiovascular MR modality for myocardial strain quantification. Cine displacement encoding with stimulated echoes (DENSE) has been proposed as a method that offers increased spatial resolution related to the pixel wise displacement encoding and more direct computation of displacement (12). Nevertheless, the value of improved acquisition MR imaging techniques such as the application of tagging and strain imaging in diastolic function testing is still under investigation.

1.2 Systolic dysfunction

Traditionally, the clinical standard in the assessment of global LV systolic function is primarily based on the quantification of LV chamber volumes to derive LV ejection fraction. Although quantification of systolic function by LV ejection fraction is easily understandable and reasonably reproducible, it is highly dependent in preload and afterload. Myocardial velocity, strain, and strain rate imaging are more sensitive systolic markers than LV ejection fraction. Similarly, quantification of systolic function by myocardial velocity strain, and strain rate imaging has been shown to be significantly correlated with global LV systolic function. Previous studies have demonstrated subclinical myocardial systolic dysfunction in diabetic patients using 2-dimensional speckle tracking echocardiography (13-15). Cine DENSE, a motion-encoding MR imaging technique for myocardial

strain assessment with high spatial resolution, also identified subclinical myocardial dysfunction in patients with DM2 (16).

2. Metabolic imaging of diabetic heart disease

Magnetic resonance spectroscopy (MRS) is a companion technique to the more familiar magnetic resonance imaging (MRI) scan.

Cardiac 1H-MRS studies use the same hardware (i.e., magnet, gradient and radiofrequency coils) as any cardiac MR imaging examination. Presently, MRS is technically difficult, requires lengthy scans, and specialist expertise, as well as additional magnetic resonance hardware and software.

Whereas MR imaging determines the spatial distribution of water (and lipid) protons across a region of interest, MRS measures the chemical content of MR-visible nuclei. The most widely studied nuclei are phosphorus (31P), hydrogen (1H) and to a lesser extent sodium (23Na). The advent of hyperpolarisation techniques has opened a new field for carbon-13 (13C)-MRS. Each of these nuclei assesses different aspects of cardiac metabolism.

MRS is particularly advantageous for assessing metabolism because the chemical properties and environment of each nucleus determine the frequency at which it appears in the MR spectrum, giving rise to peaks corresponding not only to specific metabolites but also to the constituent nuclei of each metabolite.

The proton is the nucleus with the highest MR sensitivity and natural abundance in living tissue (>99.9 %). Therefore, 1H-MRS can potentially become a powerful technique as it can detect and quantify a variety of myocardial metabolites including lipids, taurine, carnitine and total creatine.

Increased nutritional fatty acid intake and increased lipolysis in diabetes will lead to increased free fatty acid delivery to nonadipose tissues, such as muscle, liver, pancreas and also the heart. The metabolic effects of type 2 diabetes mellitus on the myocardium have been previously described in experimental models, and include increased myocardial nonesterified fatty acid utilization, an increased glucose flux and subsequently triglyceride accumulation, and subsequent increased formation of reactive oxygen species. These processes, commonly referred to as “gluco-lipotoxicity,” contribute to decreased adenosine triphosphate synthesis, mitochondrial dysfunction, and finally to apoptosis of myocardial cells (17).

1H-magnetic resonance spectroscopy (1H-MRS) has proved to be a reliable

method to noninvasively quantify cardiomyocytic triglyceride content in vivo (18). As the heart moves due to contraction and breathing, MRS is subject to motion artefacts. Therefore, ECG triggering is necessary to correct for motion of the heart throughout the cardiac cycle. Respiratory motion compensation has been performed with respirometer triggered acquisition, breath-hold scanning sequences, and by using navigator echoes (19-21). Given the location of the heart inside the body, dedicated localisation techniques are required to obtain the metabolite signals from the myocardium only. The most commonly used single-voxel techniques, based on either a double spin-echo method (PRESS–Point-RESolved Spectroscopy sequence) or stimulated echo sequence (STEAM–STimulated Echo Acquisition Mode sequence) (22).

The voxel for cardiac MRS is typically placed in the inter-ventricular septum to avoid contamination of the spectrum with signals arising from pericardial fat. A typical myocardial 1H MRS will display signals arising from water, creatine, choline, and fat. As the water signal is approximately 100-1000 times that of the fat signal, acquisition of 1H MRS both with and without water suppression is essential for reliable quantification of intramyocardial triglyceride content. Using dedicated software, signal amplitudes from the intracellular triglycerides and water are quantified and expressed as triglyceride/water ratio.

In a large human study using 1H-MRS, McGavock et al (23) showed that cardiac steatosis occurs in patients with impaired glucose tolerance even before the onset of type 2 diabetes mellitus and precedes cardiac dysfunction. Another study compared patients with type 2 diabetes mellitus with controls of the same age and body-mass index and showed that there is an increase in myocardial triglyceride content in uncomplicated type 2 diabetes mellitus and is associated with an impairment of LV diastolic function (24).

Over the last decade, 1H-MRS has become an invaluable technique to non-invasively assess cardiac lipid in clinical and pre-clinical research.

MRS may provide insights into the early cardiac metabolic changes in diabetes that may lead to heart disease and help identify potential therapeutic targets.

However, technical challenges, limited spatial and temporal resolution have hampered its widespread development, especially as a clinical diagnostic tool.

3. Coronary artery disease

Compared to individuals without diabetes, those with diabetes have a higher prevalence of coronary artery disease, a greater extent of myocardial ischemia, and are more likely to have a myocardial infarction and silent myocardial ischemia

Various non-invasive methods are available for the non-invasive assessment of CAD in diabetes and their diagnostic and prognostic value will be discussed.

The "gold standard" for detection of CAD remains invasive angiography with vessel-selective contrast injection of the coronary arteries. Both spatial (0.2 mm) and temporal (5 ms) resolution of the technique are extremely high, and the degree of luminal narrowing can be quantified precisely. This is an invasive and expensive procedure with a small but definite risk for complications. Noninvasive testing is increasingly used to assess CAD, and multiple methods are now available.

The various non-invasive imaging modalities available for investigation of CAD can be broadly divided into the categories below:

(a) Anatomical imaging, which allow direct visualisation of the coronary arteries:

- coronary calcium score (CAC)

- coronary computed tomography (CT) angiography using electron beam CT (EBCT) or multidetector CT (MDCT)

- MR angiography of the coronary arteries.

(b) Assessment of the functional significance of coronary stenosis:

- myocardial perfusion scintigraphy, which includes single photon emission CT (SPECT) and positron emission tomography (PET)

- stress echocardiography (SE)

- contrast echocardiography

- cardiac MR imaging including stress MR imaging and delayed enhancement sequences.

3.1 Anatomical imaging

There is a consensus in the literature about an increased prevalence of coronary atherosclerotic plaques in diabetic hearts, with such plaques bearing a higher propensity for rupture.

Anatomical imaging assesses atherosclerosis by direct visualization of the coronary arteries. Since the coronary arteries are small, tortuous, and move substantially during the cardiac cycle, imaging remains technically challenging. As a result, all techniques have shortcomings and limitations, but with recent and ongoing technical advances, image quality and diagnostic accuracy are continuously improving. Besides noninvasive angiography, these techniques may also allow assessment of plaque composition in the near future.

In the following topics we will discuss the role of non-invasive imaging for the direct visualization of coronary artery disease in diabetes mellitus.

3.1.1 Coronary artery calcium scoring

Since the presence of calcium is related to the presence of atherosclerosis, coronary calcifications serve as a direct marker for coronary artery disease.

Coronary artery calcium (CAC) scoring used to be obtained using EBCT, which has now been replaced by MDCT (Figure 3). CAC is a reflection of an individual's global atherosclerotic burden and is reported either in Agatston units (25) or by the volume scoring method, both of which correlate well with each other (26). The Agatston score assigns a CT factor to the coronary calcium based on the Hounsfield unit in all the coronary arteries and compiles a total score based on age and gender.

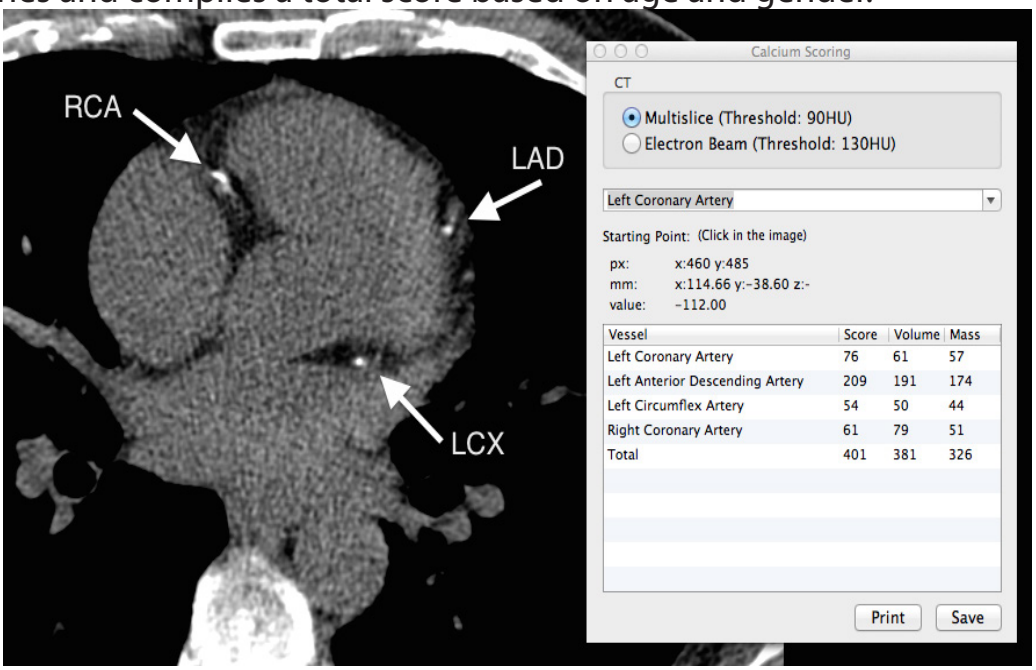

Fig 3. Measurement of coronary artery calcium scoring by MDCT. Arrrows are pointing to the main coronary arteries (RCA right coronary artery; LAD left anterior descending artery; LCX left circunflex artery). The table on the left illustrates the results obtained.

CAC has been shown to be independently associated with cardiovascular morbidity and mortality (27). Furthermore, CAC has been consistently additive to Framingham risk score (27-31) and results in improved reclassification (32-34). This powerful body of literature culminated in the incorporation of CAC screening in the ACCF/AHA Guidelines for Assessment of Cardiovascular Risk in Asymptomatic Adults (35). In general, a calcium score of 0 is considered to rule out CAD, whereas calcium scores >400 represent extensive calcifications and as a consequence extensive CAD. Studies exploring the presence and extent of calcium between patients with and without diabetes mellitus showed considerably higher calcium scores in patients with diabetes mellitus as compared to their non-diabetic counterparts (36, 37). Moreover, asymptomatic patients with diabetes mellitus presented with similar extent of CAC as compared to symptomatic patients without diabetes mellitus (38). Accordingly, coronary calcium may be used to identify CAD non-invasively in patients with diabetes mellitus.

Absence of CAC has been noted in 15%–38% of patients (39, 40). Patients with 0 CAC represent a very important cohort as their cardiovascular disease (CVD) event rate was found to be similar to patients without diabetes (40, 41), challenging the notion that diabetes is a CVD equivalent (42). Based on this extensive evidence, the ACCF/AHA recommended the use of CAC to risk stratify adults >40 years of age with diabetes (35).

In the general population coronary calcium score has been demonstrated as a strong predictor for coronary events (26-28, 43). Data on prognostic stratification with calcium scoring in patients with diabetes mellitus are presently less available. In a study by Raggi et al (41), 10,377 patients (903 with diabetes) were followed for a period of 5.0 ± 3.5 years after CAC imaging. Mortality increased with increasing baseline CAC levels for both diabetic and non-diabetic individuals. However, despite similar CAC scores, there was a greater increase in mortality in diabetic than non-diabetic patients for every increase in CAC score

3.1.2 Coronary CT angiography

It is important to realize that although calcium strongly correlates with total atherosclerotic burden, it is not directly related to the degree of stenosis. Also, the technique is not site-specific and high grade lesions may be observed at sites with limited calcium, whereas extensive calcium can occur in the absence of significant stenosis. Accordingly, the presence of obstructive CAD cannot be diagnosed with this technique, and the strength in calcium scoring mainly lies in risk stratification based on the

evaluation of total atherosclerotic burden.

More detailed information on the coronary anatomy can be derived from noninvasive contrast-enhanced coronary CT angiography, which permits direct visualization of the coronary arteries (Figure 4).

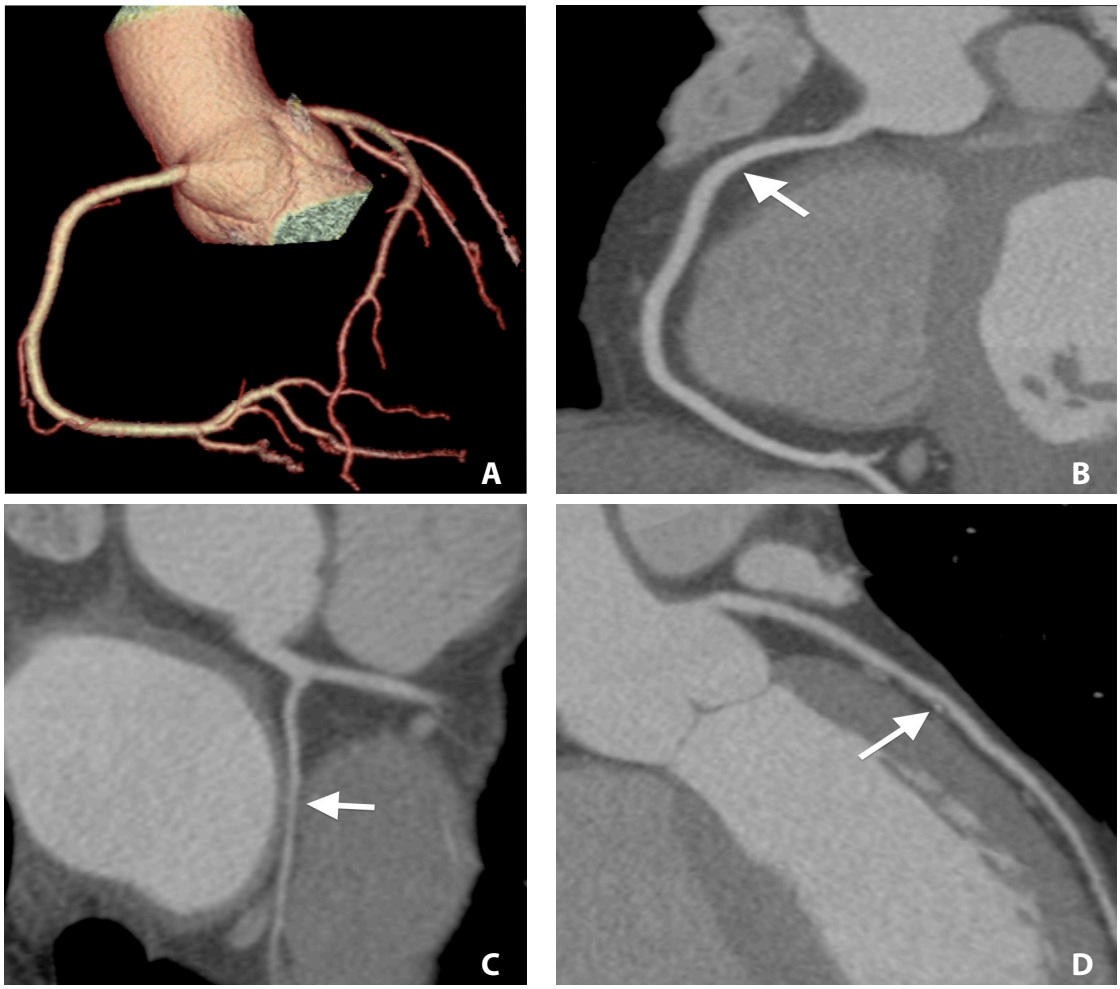

Fig 4. Coronary CT angiography scan. A) Volume rendering of the coronary tree. B) Curvilinear reformat of a normal right coronary artery. C) Curvilinear reformat of a normal left circunflex artery. D) Curvilinear reformat of the left anterior descending artery showing a small, non-significant mixed plaque in the mid left anterior descending artery.

The accuracy of coronary CT angiography to detect significant (>50%) stenosis as compared to conventional coronary angiography has been evaluated in numerous studies.

In the general population, the sensitivity and specificity to detect CAD are 91 and 96%, respectively (44). At present only two studies dedicated to patients with diabetes mellitus are available, showing similar values of accuracy for the detection of coronary stenosis as demonstrated in the

general population (45, 46). The advantage of this technique is the high negative-predictive value that approaches 100%

An interesting feature of MDCT is the fact that not only is the coronary lumen visualised but also the vessel wall, allowing the possibility to non-invasively detect non-calcified plaques (Figure 5).

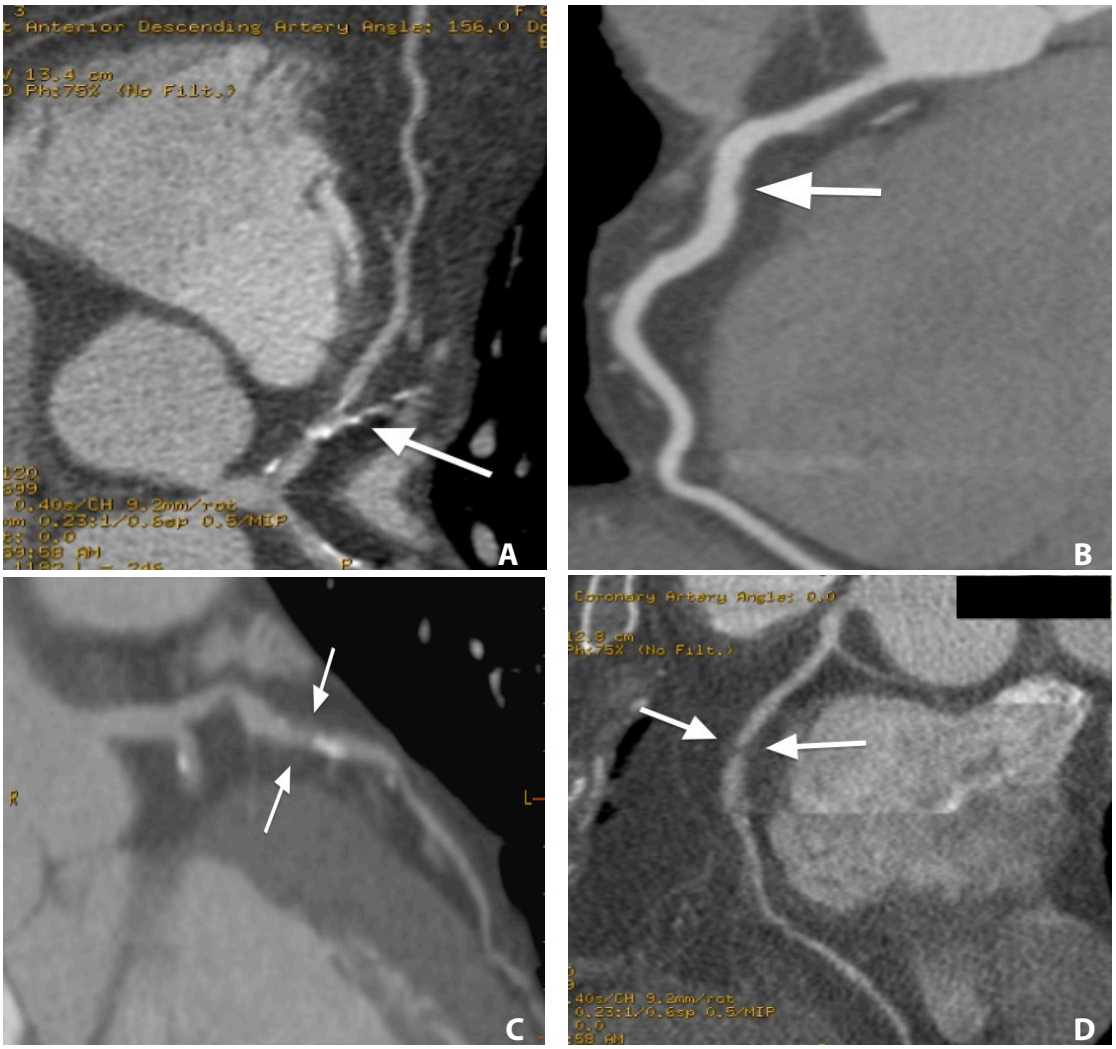

Fig 5. Coronary CT angiography scan, curvilinear reformats. A) Calcified significant plaques (arrow) in proximal left anterior descending and first diagonal arteries. B) Non-signicant small non-calcified plaque in proximal right coronary artery (arrow). C) Mixed significat plaque (arrows) in the left anterior descending artery. D) Significant non-calcified plaque (arrows) in the right coronary artery.

There is a good correlation between plaque volume estimation on intravascular ultrasound and on CT (47). Plaque volume estimation has the potential for monitoring response to lipid-lowering therapy (48). However, it is affected by several variables including the image quality

and interobserver variability (47, 49, 50).

To a limited extent, information on plaque composition can also be derived. Low attenuation plaques (i.e. non-calcified plaques), plaques with spotty calcification (mixed plaques) and those associated with constrictive remodelling are more likely to result in an acute coronary syndrome (47-50).

Using coronary CT angiography, it has been demonstrated that diabetic patients have more extensive, diffuse coronary atherosclerosis compared with matched nondiabetic patients (45, 46).

The CONFIRM Registry, a large international multicenter study, strengthened the prognostic value of MDCT coronary angiography in the general population (51). Preliminary results suggest that CT angiography has incremental prognostic information over baseline clinical variables also in diabetic patients (52, 53). In both patients with and without diabetes mellitus with absence of disease, the event rate was 0% over a median follow-up of 20 months. The event rate increased in patients without diabetes mellitus but with obstructive CAD and was highest in patients with diabetes mellitus and obstructive CAD (53).

3.1.3 Coronary MR angiography

MR imaging has attempted to provide noninvasive images of the coronary arteries. While an initial report of 39 patients suggested a sensitivity and specificity of 90 and 92%, respectively (54), additional reports were less optimistic. Recent developments, including free breathing, navigator techniques, and three-dimensional acquisition techniques, permit superior visualization of the coronary arteries. In the general population, the sensitivity and specificity for the detection of CAD are 72 and 86%, respectively (28 studies, 903 patients) (55). However, up to 30% of all segments had to be excluded due to uninterpretability.

Currently, despite recent developments such as free breathing and navigator techniques, the performance of MDCT coronary angiography is considered to be better than that of coronary MR angiography. Dedicated studies in patients with diabetes have not been published.

3.2 Assessment of the functional significance of coronary stenosis

The basis of functional imaging is the detection of CAD by assessing the hemodynamic consequences (i.e., ischemia) of CAD rather than direct visualization of the coronary arteries.

A sequence of events occurs during induction of ischemia, referred to as "the ischemic cascade" (56). Myocardial oxygen consumption and oxygen delivery determine the myocardial tissue oxygenation. Development of ischemia is the end result of a sequence of events resulting from an imbalance between myocardial oxygen consumption and myocardial oxygen delivery called the ischemic cascade. It refers to the temporal sequence of pathophysiological events that occurs within seconds of occlusion of a coronary artery. Early (within seconds) in the ischemic cascade, perfusion abnormalities occur, and systolic wall motion abnormalities follow within 10–20 s. Electrocardiogram (ECG) changes and angina occur only at the end of the cascade.

Accordingly, exercise ECG is predictably not the most sensitive technique, and its diagnostic accuracy has been demonstrated to be low in patients with diabetes (57). Conversely, abnormalities in perfusion and systolic wall motion are early markers of ischemia.

The ischemic changes become irreversible after 30min, when myocardial necrosis sets in. It starts in the subendocardium and moves towards the epicardium as a wave front phenomenon.

For ischemia assessment, imaging needs to be performed during stress and at rest. Comparison of the stress and rest images reveals whether stress-inducible perfusion or systolic wall motion abnormalities are present, indicating ischemia. The stress can be performed using bicycle or treadmill exercise or (in patients unable to exercise) pharmacological agents. Pharmacological stressors include dobutamine (a β1–specific agonist), which increases heart rate, contractility, and arterial blood pressure, resulting in increased myocardial oxygen demand; adenosine (a direct vasodilator); or dipyridamole, which act indirectly by inhibiting cellular uptake and breakdown of adenosine.

Adenosine has a shorter half-life than dipyridamole; in case of significant side effects, termination of adenosine infusion alone may suffice whereas with dipyridamole, reversal of drug action with aminophylline is often required. Dobutamine perfusion imaging is potentially indicated in patients with contraindications to dipyridamole and adenosine (obstructive pulmonary disease, heart block).

The presence of myocardial perfusion defects during stress is due to heterogeneous flow distribution because of 2 potential mechanisms: obstructive epicardial coronary artery disease due to atherosclerosis and subsequently reduced flow during stress (58); or insufficient vasomotor response in the coronary microvasculature due to endothelial dysfunction resulting in relative hypoperfusion during stress (59).

Functional imaging performed using gated SPECT, (contrast) stress echocardiography and MR imaging allow integrated assessment of perfusion and function at rest and after stress.

It is important to realize that the predictive value of these functional tests is not only defined by their sensitivity and specificity but also by the prevalence of disease in the investigated population. The incremental value of non-invasive stress testing is largest when the pre-test likelihood is intermediate because the test result is likely to have the largest effect on the post-test probability of CAD and hence on clinical decision making. Some authors have proposed the use of clinical scoring in diabetes mellitus to select patients with intermediate risk for further testing (60).

3.2.1 Myocardial perfusion imaging/nuclear imaging

Stress perfusion imaging with thallium-201, technetium-99m sestamibi or technetium-99m tetrafosmin detects heterogeneous flow distribution due to decreased coronary flow reserve during exercise or pharmacological vasodilatation.

Current state of the art is ECG gated SPECT. Images are taken following exposure to stress and at rest, allowing the identification of fixed and reversible defects (Figure 7). The dimensions of the left ventricle and ejection fraction can also be determined.

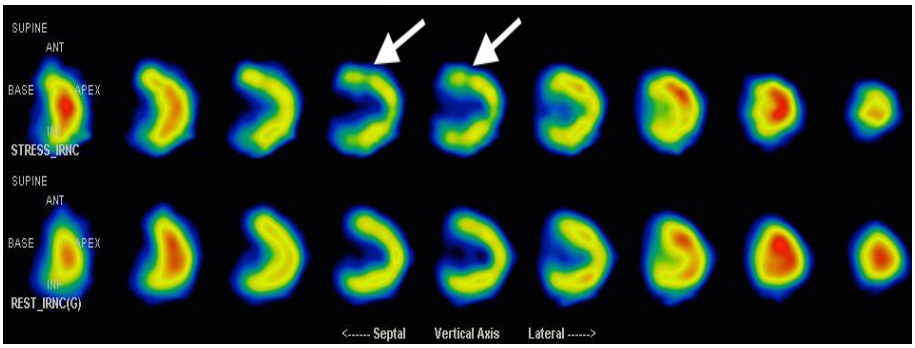

Fig 7. Myocardial perfusion using gated SPECT at rest and after adenosine stress. Resting images are depicted in the lower row. Stress images are depicted in the upper row. Reversible perfusion defects were observed in the anteroseptal regions (arrows). *Courtesy of Dr Maria João Cunha.*

Stress testing combined with nuclear imaging has a sensitivity of 86% and a specificity of 74% to detect significative coronary artery stenosis in the general population (based on pooled analysis of 79 studies, 8964 patients), and has a similar diagnostic accuracy among diabetics (61).

The prevalence of myocardial perfusion defects as assessed by SPECT myocardial perfusion imaging in asymptomatic diabetic patients ranges from 20% to 40% in several prospective studies (73, 74), and the prognostic value of SPECT myocardial perfusion imaging in diabetic patients has been confirmed in several previous studies (62-66).

A 5-year follow-up of the DIAD study (67) found that SPECT myocardial perfusion imaging provides good risk stratification, with a 6-fold higher risk of cardiac death/nonfatal myocardial infarction in patients with moderate to large defects versus no or small defects (2.4% vs. 0.4%, $P<0.001$). However, it does not appear that this information leads to improved clinical care because there was no significant decrease in cardiac death/nonfatal myocardial infarction in asymptomatic diabetic individuals screened for ischemia versus routine clinical care (67). One possible explanation for a lack of screening benefit is the lower risk of this population given that patients with known CAD were excluded. It is also possible that increased use of evidence-based medical therapy in both the screened and unscreened groups minimized the differences between them.

Despite the prognostic benefit of SPECT myocardial perfusion imaging, the rate of cardiac events is unacceptably high in diabetic patients with normal myocardial perfusion (68), up to 14% in diabetic men. This high rate is likely secondary to both false-negative studies for significant CAD by SPECT myocardial perfusion imaging and to the increased prevalence of mild stenosis with a higher risk of plaque rupture.

SPECT perfusion studies in some patients with three-vessel or left main CAD can appear normal with uniform tracer uptake due to balanced ischemia. In this situation, flow reserve is reduced in all three coronary supply regions yielding no focal defects. Similarly, some patients with diffuse endothelial and/or microvascular dysfunction causing symptoms can show homogenous tracer distribution on post-stress scans. This may be why the annual cardiac death or nonfatal myocardial infarction rate in rather high in diabetic patients with normal SPECT perfusion studies.

Positron Emission Tomography myocardial perfusion imaging can overcome these limitations of SPECT perfusion imaging.

PET consists of perfusion imaging with a perfusion tracer (rubidium-82, nitrogen-13 ammonia or oxygen-15 water) and functional metabolic

imaging with 18F- fluorodeoxyglucose (FDG).

Mismatch between flow and metabolism, i.e. reduced flow with normal or increased FDG uptake, suggests reversible ischemia. Matched reduction in blood flow and metabolism suggests an infarct.

PET myocardial perfusion imaging has been applied in research to examine coronary microvascular reactivity, in which absolute blood flow and myocardial flow reserve can be accurately measured using tracer kinetic models and dynamic imaging. This is, in part, due to the high temporal resolution and attenuation correction inherent in PET technology (69).

At present no specific PET imaging studies have addressed the diagnostic and prognostic value of cardiac PET imaging stress testing in a diabetic population.

Another active field of research is the detection of vulnerable coronary plaques with PET-CT. Studies have already shown the usefulness of FDG PET-CT as a marker of vascular inflammation and macrophage burden (70, 71). A recently published study using PET-CT with the tracer 18F-sodium fluoride could identify ruptured and high-risk atherosclerotic plaques in patients with symptomatic coronary artery disease (72).

Future studies are needed to demonstrate the diagnostic accuracy of these techniques and investigate their risk/benefit ratio.

3.2.2 Stress echocardiography

Echocardiographic evaluation typically focuses on functional changes due to coronary artery narrowing or occlusion - specifically systolic wall thickening and endocardial motion. Both physical exercise and pharmacological stress can be used. Resting wall motion abnormalities mainly represent infarcted myocardium. Stress-induced new or worsening regional or global wall motion abnormality is a reliable predictor of ischemia.

The accuracy of stress echocardiography is dependent on the degree of stenosis, the amount of myocardium at risk and the degree of induced wall motion abnormality (73). False-negative results are more likely with submaximal exercise (in the case of exercise-induced stress), single-vessel disease and moderate stenosis (50– 70%).

Stress echocardiography has a mean sensitivity of 86% and a specificity of 81% in the general population (74).

There are limited data that specifically address the utility of stress echocardiography in patients with diabetes mellitus. Hennessy et al

(75) evaluated 52 patients with diabetes mellitus referred for cardiac assessment using dobutamine stress echocardiography (DSE). Sensitivity, specificity, and positive and negative predictive values of DSE for significant CAD detection were 82%, 54%, 84%, and 50%, respectively. Although the study was limited by the small size of the cohort, it demonstrated similar diagnostic accuracy for DSE in a diabetic population.

Another small study compared several forms of non-invasive stress testing in 56 asymptomatic diabetic patients, demonstrating a positive predictive value of 60% for exercise ECG, 69% for DSE, and 75% for myocardial perfusion imaging (76).

A negative stress echocardiography study confers a 0.5–0.8% risk of cardiac death or non-fatal myocardial infarction (77). An abnormal stress echocardiography study is associated with an increased risk of adverse cardiac events. The risk is increased with resting left ventricular dysfunction, extensive ischemia and extensive wall motion abnormality (78).

The prognostic value of stress echocardiography among diabetic patients has been evaluated in several larger studies. Either pharmacological or exercise echocardiography was applied in diabetic patients with cardiac symptoms. The prevalence of abnormal studies ranged from 40-60% (79). In 2012, van der Sijde et al (80) studied the prognostic value of DSE in 396 diabetic patients during a mean time of 13 years. DSE provided incremental value over clinical characteristics and stress test parameters for prediction of mortality and cardiac events. Survival analysis showed that DSE provided optimal risk stratification up to 7 years after initial testing; after that period, the risk of adverse outcome increased comparably in both normal and abnormal DSE patients.

3.2.3 Myocardial contrast echocardiography

With recent developments in echocardiographic equipment and microbubble contrast agents, real time perfusion imaging of the myocardium is now feasible. The rate of replenishment within the myocardium is dependent on the presence of intact microvasculature and myocardial blood flow rate, and the intensity at which the contrast effect plateaus is dependent on myocardial blood volume.

This technique is promising because a perfusion abnormality would be expected to occur before a regional wall motion abnormality in the ischemic cascade. However, at present its use is limited to a few centers only and further standardization of the methodology is required before the technique is established (81).

3.2.4 Magnetic resonance perfusion imaging and dobutamine stress imaging

Magnetic resonance imaging combines superb image quality and the advantage of conducting myocardial perfusion and wall motion measurements at rest and under stress conditions during a single session examination. For perfusion imaging with MRI, adenosine is the first choice agent. For the detection of wall motion abnormalities both adenosine and dobutamine can be administered as a stressor. MRI offers the assessment of first pass myocardial perfusion with high spatial resolution of 2-3 mm, which even permits differentiation of transmural and sub-endocardial perfusion defects (Figure 8).

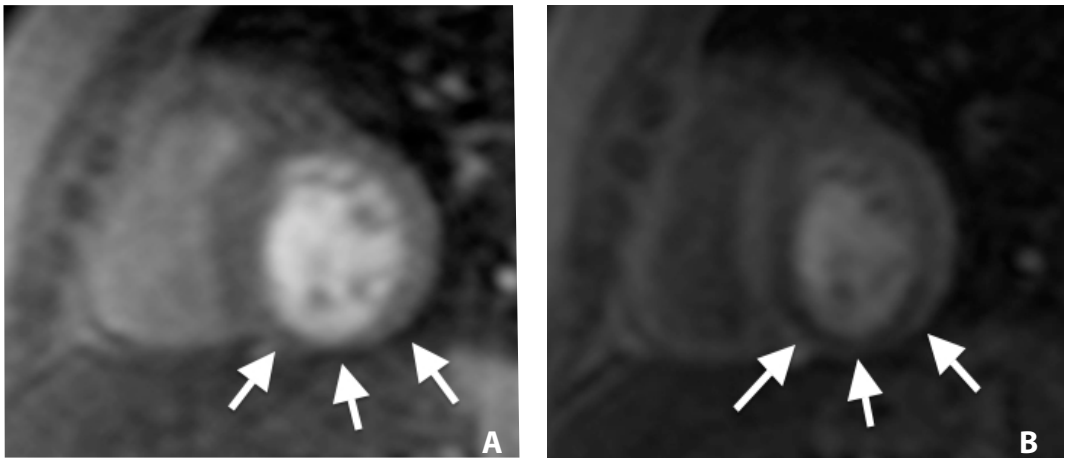

Fig 8. Myocardial perfusion imaging by MRI during the first pass of an intravenously injected contrast bolus. Rest image (A) shows a small subendocardial defect in the infero-septal wall. In the stress image (B) there is a large subendocardial perfusion defect in the inferior, septal and infero-lateral wall (arrows), confirming the presence of acute ischemia.

Many publications have shown that MR myocardial perfusion imaging has similar or superior diagnostic accuracy to the more established nuclear techniques for myocardial perfusion imaging (82-84). Studies that combine MR myocardial perfusion and delayed enhancement imaging have demonstrated significant improvements in the overall diagnostic performance of this technique for the detection of CAD (82-85). The recent Clinical Evaluation of Magnetic Resonance Imaging in Coronary Heart Disease (CE-MARC) (86) trial employed a multiparametric MRI protocol (consisted of rest and adenosine stress perfusion, cine imaging, late gadolinium enhancement, and MR coronary angiography) and demonstrated superior sensitivity and negative predictive value vs

myocardial perfusion scintigraphy (86% and 90% vs 66% and 79%, respectively) for the diagnosis of coronary artery disease.

A recent meta-analysis confirmed a high sensitivity of 89% and a moderate specificity of 80% for the diagnosis of significant CAD in a population with a high prevalence of CAD of 57% (87). The value of stress MR imaging in low-prevalence populations is not clear. False positive tests can be attributed to the presence of artefacts due to susceptibility (called dark rim artefacts), poor gating and motion artefacts (82-85, 88).

An abnormal adenosine stress MR imaging was associated with an increased risk of a cardiac event, and an abnormal dobutamine stress perfusion was associated with a high risk of a cardiac event over a follow up period of 2.3 years (89).

Furthermore, a negative MR myocardial perfusion study seems to be associated with an excellent long-term prognosis. Several studies have emphasised the high negative predictive value of MR myocardial perfusion imaging for event-free survival (89-91).

In addition to first-pass perfusion stress imaging, MRI can be used to detect and quantify abnormalities of myocardial wall motion following the administration of dobutamine, similar to stress echocardiography.

MRI has the inherent advantage over echocardiography of higher spatial resolution, good tissue contrast and higher reproducibility (92).

The object of this study is to induce reversible myocardial ischemia by the action of positive inotropic and chronotropic agents.

Using high, incremental doses of dobutamine, it is possible to diagnose coronary artery disease by the depiction of new stress-induced wall-motion abnormalities that were not present in the rest scans.

The same principle is used in the evaluation of myocardial viability, but with a low-dose dobutamine stress test. Myocardium that is viable but functionally abnormal shows a brief improvement in contractility in response to positive inotropic pharmacologic stimulus.

High–dose stress dobutamine MRI as demonstrated good sensitivities (83–96%) and specificities (80–100%) for detection of significant CAD (93).

At present no specific MR imaging studies have addressed the diagnostic and prognostic value of cardiac MR imaging stress testing in a diabetic population.

3.2.5 Delayed enhancement MRI

Myocardial fibrosis is a frequent occurrence in patients with diabetes mellitus and one of the major factors predisposing to the development of heart failure. Cardiac fibrosis is the consequence of extracellular cardiac matrix remodeling. Previous studies have also demonstrated histological evidence of increased diffuse microscopic fibrosis in the myocardium of diabetic patients (94, 95).

Currently, MR imaging with delayed contrast enhancement is the gold standard for noninvasive visualization of myocardial fibrosis (96). This technique is based on an inversion recovery pulse sequence and delayed imaging of the heart at approximately 10-20 minutes after administration of gadolinium-based contrast agents. Due to the chemical charge and molecular size of these gadolinium-based contrast agents, they rapidly diffuse from the intravascular to extracellular space, but do not enter the intracellular space. Consequently, contrast accumulates within infarcted or scarred myocardial tissues. By selecting an appropriate inversion time to "null" normal myocardium, scar tissue will appear as bright hyperenhanced regions (Figure 6) (97).

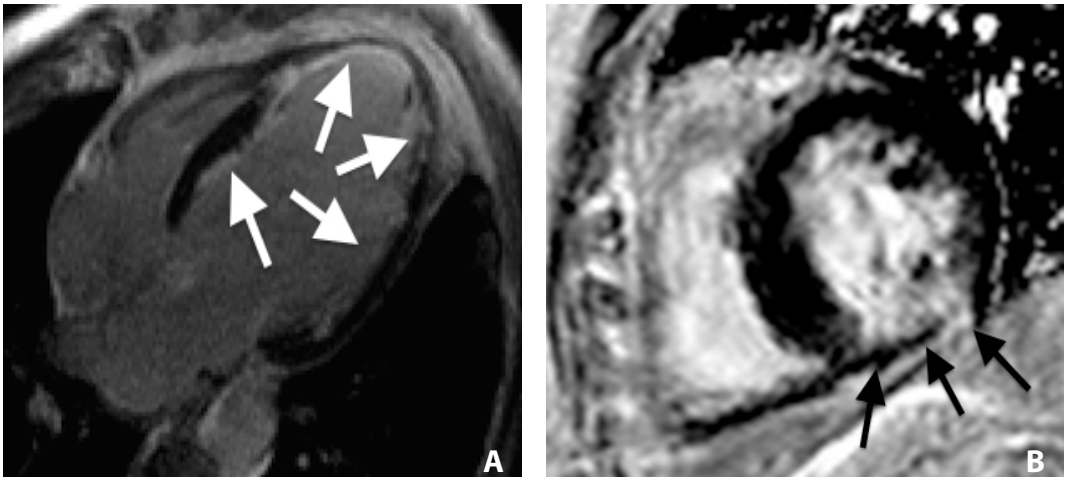

Fig 6. Delayed-enhancement MRI study. A) Four-chamber image showing difuse subendocardial enhancement in the left ventricular myocardium (arrows), from coronary artery disease. B) Short-axis image showing transmural enhancement in the left ventricular inferior wall (arrows), from myocardial infarction.

Different from replacement fibrosis, where regional collagen deposits appear in areas of myocyte injury, delayed enhancement has a limited sensitivity for interstitial diffuse fibrosis (97, 98). Therefore, to image diffuse interstitial fibrosis within the myocardium other techniques might be more suitable.

While echocardiogram backscatter techniques may be applied for that purpose (99), myocardial tissue characterization is definitely an area where MR imaging plays a large role.

In MR imaging, gadolinium-based contrast agents accumulate and have increased washout times within these myocardial fibrous tissues because of the absence of viable myocytes and an increased volume of distribution. By directly quantifying T1 values for each voxel in the myocardium, a parametric map can be generated representing the T1 relaxation times of any region of the heart.

Contrast-enhanced myocardial T1 mapping may noninvasively quantify diffuse interstitial myocardial fibrosis in diabetic patients. A shorter global contrast-enhanced myocardial T1 time (suggestive of a higher burden of interstitial myocardial fibrosis) may be present in diabetic patients and relate to myocardial dysfunction (100).

Future studies are needed to evaluate the prognostic relevance of myocardial fibrosis detection by late gadolinium enhancement or T1 mapping in diabetes mellitus.

4. Cardiac autonomic neuropathy

In clinical practice cardiac autonomic neuropathy (CAN) was diagnosed based on a battery of autonomic tests that mostly depend on the heart rate variability and its response to various stimuli that affect the autonomic nervous system.

There is evidence to suggest that scintigraphic assessment is more sensitive in detecting cardiac autonomic neuropathy that indirect autonomic reflex testing, because MIBG uptake is reduced in patients with normal autonomic tests (101, 102).

SPECT and PET imaging are available for the assessment of cardiac sympathetic adrenergic innervation and activation. Essentially, adrenergic nerve imaging is based on 2 principles: synthesis of false neurotransmitters (catecholamine analogs) or the labeling of true adrenergic neurotransmitters. Both techniques allow evaluation of abnormalities in cardiac sympathetic innervation by visualizing the uptake and storage of radiolabeled neurotransmitters transported into the presynaptic nerve terminals.

Abnormalities in sympathetic innervation can be assessed using 123-iodine meta-iodobenzylguanidine (123I MIBG), a norepinephrine analog that is taken up and accumulated in the presynaptic nerve terminals. Currently, 123I MIBG represents the most commonly used tracer in clinical cardiology to evaluate cardiac sympathetic innervation patterns. Planar and tomographic SPECT images are acquired 10-20 minutes (early) or 3-4 hours (late) after MIBG administration. From the planar images, semiquantitative measurements, such as heart-to-mediastinum ratio and cardiac washout rate, are used to evaluate global sympathetic innervation. SPECT images are used to assess regional abnormalities in sympathetic innervation.

Diabetic patients with CAN show a decreased myocardial uptake of this tracer, which has been independently correlated with the occurrence of cardiovascular events on long-term follow-up (102). In patients with diabetes mellitus, CAN was associated with a threefold increase in the progression of heart failure over a 2-year period (103).

Nevertheless, no standardized methodology or normative values exist, and available data on reproducibility are limited. Scintigraphic studies are appropriate to explore the effects of sympathetic dysfunction on cardiac metabolism and function and are useful in assessing cardiac sympathetic function in research studies (101-104).

5. Diabetes and cerebrovascular disease

Cerebrovascular disease is a leading cause of morbidity and mortality in diabetes. Compared with non-diabetic patients, diabetic patients have at least twice the risk for stroke, earlier onset of symptoms, and worse functional outcomes (105, 106). Approximately 20% of diabetic patients will die from stroke, making it one of the leading causes of death in this population. Diabetic patients more often develop ischemic strokes and have an increased proportion of lacunar strokes that may be clinically silent. Allied to this, diabetes increases the risk of stroke-related dementia.

The pathogenesis of cerebrovascular disease in diabetes may include: accelerated atherosclerosis, autonomic neuropathy, atrial fibrillation and microvascular disease in deep penetrating branches of the cerebral arteries (105, 106).

The relation between stroke and carotid atherosclerosis is established.

The majority of cerebral ischemic events are caused by atherosclerotic diseases, with most changes affecting the carotid bifurcation. This can occur via embolic transformation of an atherosclerotic lesion or secondary to ischemia caused by flow-limiting carotid artery stenosis (107).

It has been reported that extracranial carotid atherosclerosis with the resulting atherothromboembolism may account for up to 20% of ischemic strokes (108). In patients with asymptomatic carotid artery stenosis > 60%, the risk of stroke is 1–2% per year (109). This makes the degree of carotid artery stenosis on of the most important factor in determining the risk of stroke (107).

Besides luminal stenosis, plaque composition and morphology are key determinants of the likelihood that a plaque will cause cardiovascular events. Diabetic patients seem to be at exceptionally high risk for the development of vulnerable plaques (110).

High-frequency ultrasound transducers produce the high spatial resolution required to measure intima-media thickness (IMT) in vessel walls, the region that will show thickening with atherosclerotic plaque (Figure 9).

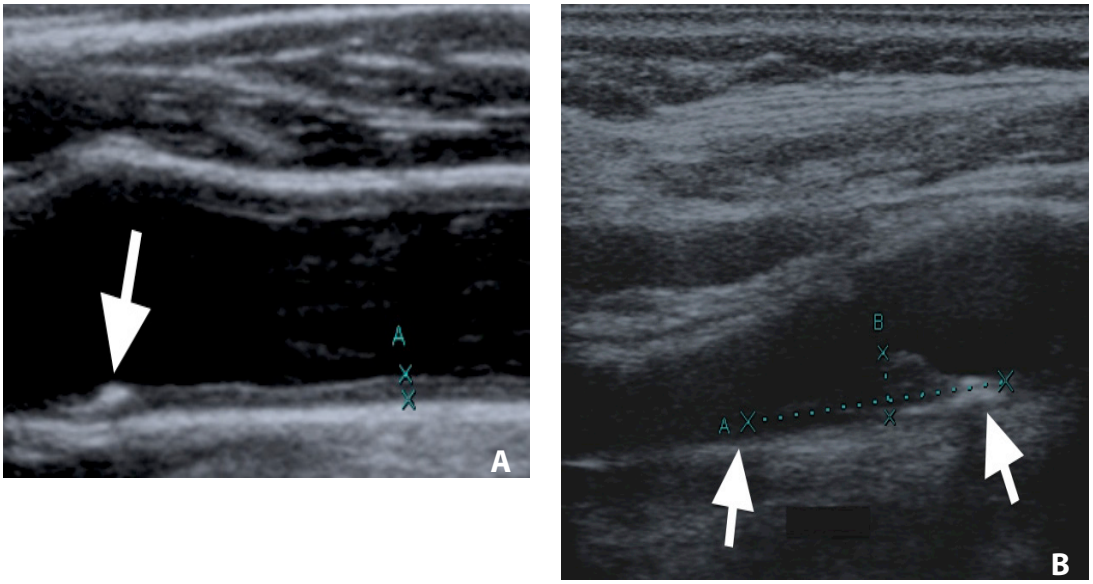

Fig 9. Carotid ultrasound studies of 2 asymptomatic subjects with type 2 diabetes mellitus. A) Carotid intima-media thickness measured at the far wall of the left common carotid artery (between calipers) showed difuse increase of the IMT (1-1.2 mm). Non-significant calcified plaque in the left carotid bulb (arrow). B) Mixed non-significant plaque in the right carotid bulb and origin of the internal carotid artery (arrows).

IMT measurements show good reproducibility (111). IMT is increased in type 2 diabetic patients with cardiovascular disease (111-113) and is an independent predictor of coronary events (113). However, the magnitude of its predictive value when added to other risk factors is questionable.

MR imaging, which has been used for evaluation of the carotid vessels, makes it possible not only to quantify the size of the atherosclerotic plaque but also to assess intraplaque hemorrhage and the integrity of the fibrous cap (114).

It is technically possible to combine magnetic resonance angiography (quantification of degree of stenosis and its spatial distribution) with high-resolution MRI imaging (characterization of the arterial wall and the composition of plaque).

Nevertheless, detailed characterization of plaque, including the identification of high-risk features, remains difficult at present. Although much is expected from current developments, evidently more data are needed before plaque characterization with MR imaging may be used clinically for the identification and management of patients at risk (114, 115). No specific studies have been performed in diabetes.

6. Diabetes and peripheral artery disease

Diabetic patients are at high risk for peripheral arterial disease.

The complex pathophysiology of diabetic foot lesions results from a number of factors present in the diabetic population that leads to foot pathology in a synergistic fashion. These factors include peripheral neuropathy, which leads to structural and sensory changes within the foot; microvascular changes, nonocclusive changes in the microcirculation leading to the impairment of normal cellular exchange; infection, often aggressive and polymicrobial and atherosclerosis of the peripheral arteries (116).

In diabetes mellitus, peripheral artery disease begins earlier, progresses more rapidly and is more commonly asymptomatic. Regarding distribution of the disease, distal arterial involvement of the tibial and peroneal arteries is the predominant pattern (117).

Patients with peripheral artery disease may be asymptomatic or may develop symptoms of intermittent claudication or symptoms of critical limb ischaemia, characterized by pain in the peripheries at rest, ischemic ulceration or gangrene. In diabetic patients, the combination of lower extremity neuropathy and ischemia secondary to atherosclerosis can lead to pressure necrosis, ulceration, and microbial infection. The clinical features are aggravated because of the decreased ability to fight infection by delaying or preventing delivery of oxygen, nutrients, components of a proper immune response, and antibiotics to the injured and infected area (116-118).

Noninvasive testing can be performed by peripheral Doppler ultrasonography, color duplex scanning, ankle-brachial pressure index measurement, plethysmography, transcutaneous oximetry, computed tomography and magnetic resonance angiography (116-118).

The results of revascularization procedures for proximal lesions are similar to those in non-diabetic patients, but results in distal bypasses are poor in the long term. Amputation rates after revascularization are much higher in diabetic than in non-diabetic patients (116-118).

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Chapter III

Cardiac imaging in asymptomatic diabetes mellitus

Introduction

In the present chapter we will discuss the issue of screening cardiovascular disease in asymptomatic diabetic patients. We will first address possible role of imaging in assessing CAD in asymptomatic diabetes mellitus. Then, we will discuss the issue of myocardial functional evaluation in subclinical cardiovascular diabetes mellitus.

1. Coronary artery disease in asymptomatic diabetes mellitus

The issue of screening for coronary artery disease (CAD) in asymptomatic diabetic patients has been raised and debated intensively.

Nevertheless, there are no strong data to support a benefit of screening asymptomatic patients with type 2 diabetes for silent myocardial ischemia.

In the Detection of Ischemia in Asymptomatic Diabetics (DIAD) study (1), 1123 asymptomatic diabetic patients were randomized to be screened with adenosine-stress radionuclide myocardial perfusion imaging or no screening. The primary endpoint was the occurrence of death or nonfatal myocardial infarction at follow-up. All patients received aggressive preventative therapies. The study failed to show any benefit in terms of the primary endpoint or for any secondary endpoints in the actively screened group as compared to conventional management with a cumulative composite of death or myocardial infarction in the range of 3% in both groups after a median follow-up of 5 years. Authors conclude by emphasizing that this is not a negative study, rather it conveys the positive message that diabetic patients have a fairly good outcome with state-of-art medications when managed in a conventional manner.

To be effective, screening of asymptomatic patients must provide prognostic information that allows risk stratification, which then triggers more effective risk reduction.

Clinically, the simplest screening involves measurement of circulating markers (e.g. low-density lipoprotein, glycosylated hemoglobin, etc.) or readily assessable clinical measures (blood pressure, neuropathy) as is done with the Framingham, UK Prospective Diabetes Study, and other risk engines. However, current risk engines do not provide consistently reliable information (2-4).

So, clinicians have relied on more sophisticated techniques such as coronary

artery calcium scoring (CAC) and myocardial perfusion scintigraphy imaging to detect coronary atherosclerosis and silent ischemia.

The most recent guidelines for the assessment of risk in asymptomatic adults endorse the use of CAC to risk stratify adults >40 years of age with diabetes (5). In a large study that included more than 10,000 individuals (903 with DM), diabetic patients had a higher CAC score and higher all-cause mortality over an average follow-up of 5 years than non-diabetic patients (3.5% vs 2.0%) (6). Increasing CAC scores in this study were predictive of mortality in both diabetic and non-diabetic patients, and this relationship was stronger in diabetic patients. Perhaps more importantly, diabetic patients with a zero CAC score had a similar favorable prognosis to non-diabetic patients (6).

Use of CAC scoring for risk stratification in asymptomatic diabetic patients is not currently endorsed by the American Diabetes Association recommendations (7). However, if a strategy of testing asymptomatic diabetic individuals for the presence of silent ischemia is needed, pre-selection of individuals based on calcium scores with the intent of performing subsequent functional imaging in the presence of a substantial atherosclerotic burden should be considered.

In diabetic patients without history of CAD, Anand et al (8), using a combined approach of CAC imaging and myocardial perfusion scintigraphy, reported abnormal myocardial perfusion scintigraphy findings in 18% of patients with an CAC between 10 and 100, in 48% of patients with an CAC between 100 and 400 and in 71% of patients with CAC >1000, whereas no patients with absent or minimal CAC (score 0 to 10) showed abnormal myocardial perfusion scintigraphy. In addition, although only 20 cardiovascular events occurred in this population during a follow-up interval of 2.2 years, no event was observed in patients with absent or minimal coronary calcification.

Thus, it needs to be recognized that while in intermediate risk subjects, a two-step risk stratification strategy of an initial coronary calcium scan, followed by selective myocardial perfusion scintigraphy imaging in subjects with high CAC scores sounds attractive and would be worth testing, there is no definitive evidence to support any clinical benefit of this approach. In contrast, based on the study by Anand et al (8), it seems conceivable that in asymptomatic type 2 diabetics without known CAD, a CAC score followed by selective ischemia evaluation for subjects with significant CAC (>10) should be considered to identify candidates for invasive CAD assessment (9). Thus, myocardial perfusion scintigraphy would be potentially useful for those with evidence of coronary atherosclerosis through a positive CAC scan or, as first line test, in those with abnormal resting ECG, in

agreement with recommendations of the American Diabetes Association (7). Asymptomatic diabetics with evidence of moderate to severe inducible ischemia at myocardial perfusion scintigraphy should then undergo invasive coronary angiography as they may benefit from revascularization.

Noninvasive coronary CT angiography and MRI have not been extensively studied in the asymptomatic type 2 diabetes mellitus population.

Using coronary CT angiography in asymptomatic patients remains controversial, primarily because of the higher radiation dose, added cost, and use of nephrotoxic contrast, but it has the potential to identify useful data beyond what is derived from CAC.

Recent advances in cardiac CT imaging technology allow for further reduction of the radiation dose from coronary CT angiography (10); available new dose-reducing techniques include prospective triggering (11-13), adaptive statistical iterative reconstruction (14), and high-pitch spiral acquisition (15).

Magnetic resonance imaging is acquiring a growing body of evidence for screening asymptomatic cardiovascular disease in diabetes mellitus. In a notable study by Kwong et al (16) MRI showed myocardial scar (delayed gadolinium enhancement) in 28% of diabetic patients who had no evidence of myocardial infarction by clinical history, medical records or by electrocardiogram. In this study, the presence of delayed gadolinium enhancement was a strong independent predictor of future major adverse cardiovascular events and death (hazard ratio of 4.13). It is important to identify patients with silent myocardial infarctions because their risk of future cardiovascular events is comparable to that of patients with clinically recognizable myocardial infarctions.

The recent Clinical Evaluation of Magnetic Resonance Imaging in Coronary Heart Disease (CE-MARC) (17) trial employed a multiparametric MRI protocol and demonstrated superior sensitivity and negative predictive value vs myocardial perfusion scintigraphy (86% and 90% vs 66% and 79%, respectively) for the diagnosis of coronary artery disease. The lack of widespread availability difficult the more widely use of MRI for the investigation of coronary heart disease.

2. Myocardial dysfunction in asymptomatic diabetes mellitus

Diabetes mellitus is an independent risk factor for the development of heart failure (HF) (18, 19). The increased prevalence of CAD and hypertension in patients with diabetes mellitus contribute to the increased incidence of HF (20, 21). Furthermore, diabetic cardiomyopathy has been proposed as a primary myocardial disease in diabetic patients without significant epicardial CAD, hypertension, or valvular heart disease. This entity lead to gradual decline in left ventricular (LV) function with impairment in LV relaxation first and then followed by systolic dysfunction that may progress over time to congestive HF (22, 23). Therefore, before presenting with overt HF symptoms, diabetic patients may have long-standing subclinical myocardial dysfunction. Thus, early detection of diabetic heart disease may permit early treatment and prevention of heart failure progression (24).

The optimum process for identifying patients with diabetic myocardial dysfunction is unclear. Unfortunately, the measurement of brain natriuretic peptide does not seem to be a solution to screening. This marker of overt LV dysfunction (25) is released in response to increased transmural wall stress and appears to have limited efficacy for the detection of subclinical heart disease (26). Specifically, brain natriuretic peptide does not appear to be a worthwhile marker of diabetic myocardial disease (27, 28).

The most frequent finding in an asymptomatic patient with diabetic heart disease is diastolic dysfunction with normal left ventricular ejection fraction (22, 23, 28). Often, imaging is used to demonstrate LV diastolic and systolic dysfunction in diabetic heart disease.

Echocardiography and MRI are the 2 modalities that offer the most in this regard. They each have strengths and limitations that at present make them complementary. Echocardiography's high temporal resolution makes it the modality of choice for assessing diastolic function, whereas MRI's high spatial resolution permits precise evaluation of systolic function. Although MRI suffers from limited availability, and contraindications such as implantable metallic devices, its ability to obtain accurate information in patients with poor echocardiographic windows makes it an important alternative in the diagnosis and characterization of heart failure. Advances in MRI sequences increasingly allow characterization of diastole.

Factors causing LV filling pressure to rise will also cause left atrial (LA) pressure overload and LA dilatation (29, 30). Thus, LA volume will both reflect the severity and duration of LV diastolic dysfunction (29, 31, 32).

In agreement with this, LA size has proven to be a powerful predictor of outcome in the general population (33) and in several disease entities, including myocardial infarction, severe aortic valve stenosis and heart failure (31, 34, 35).

A recent prospective observational follow-up study of diabetes mellitus patients without overt cardiovascular disease at inclusion demonstrated that a dilated LA was a predictor of death and major cardiovascular events, even after adjustment for age and hypertension, independently of myocardial ischemia (36). Therefore, LA volume could emerge as a simple and important tool for risk stratification in type 2 diabetes mellitus patients.

Gupta et al have used MRI, the gold standard method in cardiac imaging, in 1802 subjects from the general population enrolled in the Dallas Heart Study with a mean age of 40 years (37). They correctly evaluated not only the prognostic value of LA volume and function, but also the interaction of each one with LV parameters and with two specific cardiac biomarkers, cardiac troponin T and N-terminal pro brain natriuretic peptide.

New imaging echo-based methods, in particular the most recent speckle tracking methods, are quicker, cheaper, and easier than MRI; however, their use in large outcome studies is hindered by the risk of low quality images, noise, and the lack of agreed common standards for this technique. Thus, to date, the study of LA function by 'classic' volumetric methods is the simplest, clinically applicable, and most representative of the interaction between dimensions and function (38).

LA dimensions and function are the consequence of what happens in other cardiac chambers, the left ventricle in particular. They are good markers of generalized cardiac dysfunction, potentially allowing for early detection and for an estimate of its duration. Accordingly, the study of Gupta et al shows that the contribution of LA variables (LA ejection fraction in particular) provided a significant but small increment in discrimination of all-cause mortality (37). Whether LA dimensions and function can also be therapeutic targets and not only markers of risk is a largely unexplored area and deserves future research.

The prognostic implications of these changes in LV and LA function and recommendations on systematic imaging surveillance of asymptomatic subjects with diabetes mellitus need further prospective evaluation.

Highlights

Asymptomatic diabetic adults with subclinical cardiovascular disease represent a group at high risk for cardiovascular morbidity and mortality. While risk factor scoring systems such as the Framingham risk score are useful in the general population, they may not be as predictive in diabetics as compared with the general population. CAC scoring, however, is consistently additive and helps positively reclassify patients and is therefore recommended in this population. Myocardial perfusion imaging remains controversial but may have a role in patients with higher CAC scores. Although scintigraphy has been the most widely used technique to study myocardial perfusion, it is accepted that MRI, being a more accurate method without radiation may play a greater role in the near future.

Myocardial dysfunction in diabetes mellitus is characterized predominantly by diastolic dysfunction consisting of relaxation abnormalities that are prevalent and have prognostic importance. Echocardiography and MRI could provide important data for risk stratification by studying subclinical left ventricular and left atrial dysfunction.

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Part II

Personal contribution

Introduction

1. Background

Diabetes mellitus is responsible for diverse cardiovascular complications such as increased atherosclerosis in large arteries (carotids, aorta, and femoral arteries) and increased coronary atherosclerosis. Coronary microvascular disease may contribute to cardiac pathology as well. Diabetes mellitus also can affect cardiac structure and function in the absence of changes in blood pressure and coronary artery disease, a condition called diabetic cardiomyopathy.

Due to the often masked symptoms of type 2 diabetes mellitus, the disease may only be diagnosed several years after onset when complications have already occurred. Often, the prognosis of patients with type 2 diabetes mellitus depends on the presence of cardiovascular disease.

Research on cardiovascular complications of diabetes mellitus will allow the development of appropriate markers and diagnostic techniques to identify individuals at risk, stage the disease, prognosticate, and evaluate therapy relevant to the cardiovascular complications of diabetes mellitus.

The criteria for the selection of those asymptomatic patients with type 2 diabetes that should undergo cardiac screening and the therapeutic consequences of screening remain controversial. Non-invasive techniques as markers of atherosclerosis and myocardial ischemia may aid risk stratification and the implementation of tailored therapy for the patient with type 2 diabetes mellitus.

A number of noninvasive tests are now available to detect coronary atherosclerotic disease and myocardial dysfunction.

Regarding the evaluation of coronary atherosclerotic disease, CT can non-invasively detect the presence and extent of calcified plaque in the coronary arteries (coronary artery calcium score – CAC).

Observational studies revealed that diabetic patients have significantly higher CAC than nondiabetic patients (1-4). However, in asymptomatic type 2 diabetic patients, few studies have investigated the association of CAC with other traditional risk factors of coronary artery disease.

Regarding the evaluation of myocardial dysfunction, cardiac magnetic resonance has been widely accepted as the “gold standard” for the assessment of cardiac structure and function.

Nevertheless, the use of cardiac MRI for the assessment of left ventricular

diastolic function and left atrial function is still a novelty.

So far, none MRI-based studies of left ventricular diastolic function have been carried out in healthy adult individuals.

The evaluation of left ventricular diastolic function is important in type 2 diabetes mellitus, since it represents one of the earliest preclinical manifestations of left ventricular dysfunction in this condition (5-10).

Additionally, although is known that the suggested mechanisms conducting to left ventricular dysfunction in type 2 diabetes mellitus include systemic phenomena that can also impact the function of the left atrium, there are limited published data on left atrial involvement in this condition.

Coronary artery disease is commonly cited as a mechanism underlying diastolic dysfunction (11). But there are limited and controversial published data on the relationship of CAC to left ventricular diastolic function (12-14).

2. Objectives

The purpose of the present thesis is to study the role of novel cardiac imaging techniques in assessing cardiovascular disease in patients with type 2 diabetes mellitus.

Primary objectives:

1. To study the extent of CT-derived anatomic and MR-derived functional measures of cardiovascular disease in an asymptomatic diabetic population. To evaluate the relation between these CT-derived and MR-derived measures.

Secondary objectives:

2. To evaluate the prevalence of diastolic dysfunction in a normal population and to determine gender-specific differences for left heart volumes and function, using cardiac MRI

3. To assess left ventricular diastolic function in asymptomatic type 2 diabetes mellitus with cardiac MRI, and to evaluate whether these parameters of LV diastolic function are related to coronary atherosclerosis.

4. To assess left atrial function in asymptomatic type 2 diabetes mellitus with cardiac MRI.

5. To evaluate the prevalence and severity of CAC scores in asymptomatic diabetes, and explore its association with other conventional risk factors

of atherosclerosis

Hypotheses

A considerable number of asymptomatic patients with type 2 diabetes mellitus have significant cardiovascular disease, as detected by CT and MR.

Left ventricular diastolic dysfunction is a marker of asymptomatic type 2 diabetes mellitus, but may be present in a significative percentage of apparently normal subjects. The presence of diastolic dysfunction is related to coronary artery calcium score.

Asymptomatic type 2 diabetic subjects will have reduced left atrial function, compared with normoglycemic control subjects.

Asymptomatic subjects with type 2 diabetes mellitus would show a wide range of CAC scores.

3. Material and methods

Study population

Between December 2011 and December 2013, we prospectively enrolled 73 subjects (33 women and 40 men; mean age 60.6 +/- 7.5 years) with DM2 enrolled based on referral to the outpatient clinical department of diabetology of our institution and 82 normoglycemic controls (43 women and 39 men; mean age 50.3 +/- 8.2 years) recruited from the community.

Subjects were included if they met the following inclusion criteria: age between 45-75 years, no symptoms or history of overt heart disease (cardiomyopathy, coronary artery disease or valvular heart disease), no signs or symptoms of cerebrovascular disease, no abnormal findings on routine clinical and physical examination and a normal ECG.

Exclusion criteria were: LV ejection fraction (LVEF) < 55%, regional LV wall motion abnormalities and valvular heart diseases assessed by CMR, severe renal failure, claustrophobia, CMR images unsuitable for quantification, type 1 DM, severely uncontrolled DM, defined as glycated hemoglobin > 12%, and uncontrolled blood pressure at rest (defined as systolic blood pressure > 180 mm Hg and/or diastolic blood pressure > 100 mm Hg). Subjects with contraindications to MRI were not enrolled.

The study was approved by our institutional ethics committee. Each subject gave written informed consent.

Every eligible subject (controls and diabetic patients) underwent carotid

ultrasound study and cardiac MRI. Computed tomography for coronary artery calcium scoring was performed only in subjects with type 2 diabetes mellitus.

Carotid Ultrasound study

Scanning of the extra-cranial common carotid artery, the carotid bulb, and the internal carotid artery in the neck was performed by an experienced observer bilaterally in the longitudinal and transversal planes, using a GE Healthcare logic 9 with a 9 linear (8 MHz) probe. Carotid intima-media thickness (CIMT) was measured in the common carotid artery as the distance between 2 parallel echogenic lines corresponding to the blood-intima and media-adventitia interface on the posterior wall of the artery. Determinations of carotid intima-media thickness were performed at the site of the thickest point and values below 0.8 mm were considered as normal. Localized elevated lesions with maximum thickness of more than 1 mm, having a point of inflection on the surface of the intima-media complex, were defined as "plaques". Stenosis was defined as > 50% occlusion.

Coronary artery calcium score, data acquisition and analysis

Coronary artery calcium (CAC) scoring was performed only in the DM2 group, using a 64-slice CT scanner (LightSpeed VCT XT, GE Healthcare, Milwaukee, USA). For this purpose, a non-enhanced low-dose ECG-gated scan was performed with prospective triggering at 75% of the R–R interval. All examinations included the entire coronary tree and were performed with the patient breath-holding in inspiration. Scan parameters were as follows: field of view of 25 cm, slice thickness of 2.5 mm, gantry rotation time 0.35 s, tube current 100–400 mA, and tube voltage 120 kV.

CAC score was determined by an experienced observer using dedicated software (SmartScore, version 4.0, GE Healthcare, Milwaukee, USA). Total CAC score was calculated for each patient using the Agatston method.

Cardiovascular magnetic resonance protocol

CMR imaging was performed by using a 3.0-T unit (Magnetom Trio; Siemens, Erlangen, Germany). Cardiac cine images were acquired by using steady-state free-precession sequences with retrospective electrocardiographic gating. Participants were imaged in the supine position and performed a breath-hold at end expiration for each image acquisition to eliminate respiratory motion artifacts. After scout images were obtained, cine imaging was performed in four-chamber, three-chamber, and two-chamber long- and short-axis views with the use of

the following parameters: 8-mm-thick sections with a 2-mm gap between sections, repetition time 59.04 ms, echo time1.45, number of segments 18; 50°flip angle, 256×156-mm matrix, 2.1 ×1.6-mm pixel size, acquired temporal resolution 25-40 ms; and number of reconstructed cardiac phases, 25.

A breath-held, retrospectively vector-ECG gated, two-dimensional flow-sensitive phase-contrast gradient-echo sequence was used for velocity encoded (VENC) MRI flow measurements perpendicular to the orifice of the mitral valve. VENC-MRI slices were positioned in early diastole at the tip of the mitral valve leaflets. Typical imaging parameters of VENC-MRI were as follows: slice thickness 6 mm, repetition time 35.6,ms, echo time 2.33 ms, number of segments 3, acquired temporal resolution 24-56ms, 20° flip angle, 25 calculated phases and pixel spacing 4.5×3.1 mm. Encoding velocity was set to 130 cm/s.

MR image analysis

Quantitative image data analysis was performed by using dedicated software (Segment, version 1.8, Medviso, Lund, Sweden). All functional evaluations were performed within 25 minutes per patient.

Tracing of endocardial and epicardial contours

All measurements were undertaken semi-automatically. End-diastolic and end-systolic frames were identified according to the ventricular blood pool area. In all LV short-axis slices across all temporal phases (200-250 images) endocardial and epicardial contours were semi-automatically drawn and manually corrected. This segmentation took on the order of 3–5 minutes. At the base of the left ventricle, the aortic outflow tract below the valve was included in volume measurements. The free papillary muscles were included for LV mass assessment, and excluded for left ventricular volume assessment. In the basal region of the heart where the left atrium was seen, only the portion of the slice that could be identified as the left ventricle was included for measurement.

This analysis provided the time-varying course of the LV volume during the cardiac cycle. The peak filling rate (PFR) is the steepest tangent to the first part of the filling curve. Segment software automatically determines this parameter.

Analysis of the transmitral flow

In the flow sensitive sequence, a round region of interest was placed at the center of the mitral valve orifice on a diastolic image and propagated to other phases to obtain the transmitral curve. From the analysis of the

transmitral flow curve, the following measurements were performed: early phase of ventricular filling (E) and atrial phase of ventricular filling (A) mean peak velocities (in centimeters per second) and peak E velocity–to–peak A velocity (E/A) ratio.

Left atrial measurements

Left atrial volume measurements were performed in the four- and two-chamber orientations by the biplane area-length methods. In the analysis we excluded the atrial appendage and the pulmonary veins. The parameters of LA size and function included in our analyses were:

- LA minimum volume (LAmin): LA end-diastolic volume at the first frame after mitral valve closure.

- LA maximum volume (LAmax): LA end-systolic volume right before mitral valve opening.

LA reservoir function

- Total LA stroke volume (LASV) = LAmax – LAmin.

- Total LA ejection fraction (LAEF) = total LASV/LAmax

LA contractile function

- Active LASV: directly obtained by VENC-MRI from transmitral flow curves, using a MATLAB script.

- LA volume pre-atrial contraction (LApreA) = LAmin + Active LASV

- Active LAEF = active LASV/LApreA

LA conduit function

- Passive LASV = LA max - LApreA

- Passive LAEF = passive LASV/LAmax

Statistical analysis

All data were systematically and prospectively entered in a database. The extensive database formed the basis for the studies presented in this thesis.

At the time of writing this thesis, patients are still being enrolled.

All continuous variables were tested for normal distribution. All normally distributed data are expressed as means ± standard deviations. Categorical variables are expressed as counts and percentages. Between-group differences of the average were compared using the unpaired

Student's t-test for parametric data and the Mann-Whitney U test for non-parametric data. Between-group differences in numbers and percentages were compared using the Chi-squared test (Fisher's exact test).

Pearson's correlation test was used for sets of normal variables. Independent associations were studied by using a linear regression with multivariable adjustments for potentially confounding factors.

P-values of less than 0.05 were considered statistically significant. All computations were performed using software (SPSS, version 20.0; SPSS, Chicago, IL, USA).

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Chapter IV

Cardiovascular magnetic resonance imaging assessment of diastolic dysfunction in a population without heart disease: a gender-based study

Bruno Graça, Maria João Ferreira, Paulo Donato,
Miguel Castelo Branco, Filipe Caseiro Alves

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1. Abstract

Objectives

Asymptomatic left ventricular (LV) diastolic dysfunction is increasingly recognised as an important diagnosis. Our goal was to study the prevalence and gender differences in subclinical LV diastolic dysfunction, using cardiovascular magnetic resonance imaging (CMR) at 3 T.

Methods

We prospectively studied 48 volunteers (19 male and 29 female, mean age 49±7 years) with no evidence of cardiovascular disease. We used CMR to measure left atrium (LA) and LV volumes, LV peak filling rate and transmitral flow.

Results

The overall prevalence of LV diastolic dysfunction in our cohort varied between 20% (based on evaluation of LV filing profiles) and 24% (based on the evaluation of the transmitral flow). The prevalence of diastolic dysfunction was higher in men than in women, independently of the criteria used (*P* between 0.004 and 0.022).

Indexed LV end-diastolic volume, indexed LV stroke volume, indexed LV mass, indexed LA minimum volume and indexed LA maximum volume were significantly greater in men than in women ($P<0.05$). All the subjects had LV ejection fractions within the normal range.

Conclusions

It is clinically feasible to study diastolic flow and LV filling with CMR.

CMR detected diastolic dysfunction in asymptomatic men and women.

Key Points

CMR imaging offers new possibilities in assessing left ventricular diastolic function.

The prevalence of diastolic dysfunction is higher in men than in women.

The prevalence of some diastolic dysfunction in a normal population is 24%.

2. Introduction

Diastolic heart failure (HF) is a progressive disorder characterized by impaired left ventricular (LV) relaxation, increased LV stiffness, increased interstitial deposition of collagen, and modified extracellular matrix proteins. Diastolic HF, also referred to as HF with normal ejection fraction, currently accounts for 40-50% of all HF cases and has a prognosis which is as ominous as that of systolic HF. Clinical consensus places special emphasis on the detection of subclinical LV systolic and diastolic dysfunction and the timely identification of risk factors for heart failure (1). Fundamental structural and functional properties of the left ventricle (LV) and of the left atrium (LA) including parameters of diastolic function are often assessed in the clinical setting using two-dimensional and tissue Doppler echocardiography. Over the last decade, cardiovascular magnetic resonance (CMR) imaging has been widely accepted as the "gold standard" for the assessment of cardiac structure and function because of its high spatial and temporal resolution, excellent image quality and lack of geometric assumptions (2). CMR offers a variety of alternative approaches for evaluating diastolic function (3).

So far, few MRI-based studies of left ventricular diastolic dysfunction have been carried out. Several studies have defined CMR normal ranges of LV volumes and systolic function (4-11) but none has assessed the prevalence of left ventricular diastolic dysfunction in healthy adult individuals.

The aim of the study was, therefore, to evaluate the prevalence of diastolic dysfunction in a normal population and to determine gender-specific differences for left heart volumes and function, using CMR at 3 T.

3. Materials and methods

Study population

Forty-eight healthy volunteers (19 male and 29 female, mean age 49±7 years, mean height 165±8 cm, mean weight 68±11 kg, with normal left ventricular ejection fraction, no history of cardiac disease, hypertension or other cardiac risk factors and a normal baseline electrocardiogram (ECG) were recruited. Exclusion criteria included a personal history of any condition that might be associated with systemic inflammation (such as systemic inflammatory arthritis or chronic chest disease) and signs of valvular disease in cine CMR imaging. Volunteers with contraindications to CMR were not enrolled. The study was approved by our institutional

ethics committee. Each subject gave written informed consent.

Cardiovascular magnetic resonance protocol

CMR imaging was performed by using a 3.0-T unit (Magnetom Trio; Siemens, Erlangen, Germany). Cardiac cine images were acquired by using steady-state free-precession sequences with retrospective electrocardiographic gating. Participants were imaged in the supine position and performed a breath-hold at end expiration for each image acquisition to eliminate respiratory motion artefacts. After scout images were obtained, cine imaging was performed in four-chamber, three-chamber, and two-chamber long- and short-axis views with the use of the following parameters: 8-mm-thick sections with a 2-mm gap between sections, repetition time 59.04 ms, echo time1.45, number of segments 18; 50°flip angle, 256×156-mm matrix, 2.1 ×1.6-mm pixel size, acquired temporal resolution 25-40 ms; and number of reconstructed cardiac phases, 25.

A breath-held, retrospectively vector-ECG gated, two-dimensional flow-sensitive phase-contrast gradient-echo sequence was used for velocity-encoded (VENC) MRI flow measurements perpendicular to the orifice of the mitral valve. VENC-MRI slices were positioned in early diastole at the tip of the mitral valve leaflets. Typical imaging parameters of VENC-MRI were as follows: slice thickness 6 mm, repetition time 35.6 ms, echo time 2.33 ms, number of segments 3, acquired temporal resolution 24-56 ms, 20° flip angle, 25 calculated phases and pixel spacing 4.5×3.1 mm. Encoding velocity was set to 130 cm/s (12).

MR image analysis

Quantitative image data analysis was performed by using dedicated software (Segment, Medviso, Lund, Sweden) (13). All functional evaluation was performed within 25 min per patient.

Tracing of endocardial and epicardial contours

All measurements were undertaken semi-automatically. End-diastolic and end-systolic frames were identified according to the ventricular blood pool area. In all LV short-axis slices across all temporal phases (200-250 images) endocardial and epicardial contours were semi-automatically drawn and manually corrected (Fig. 1).

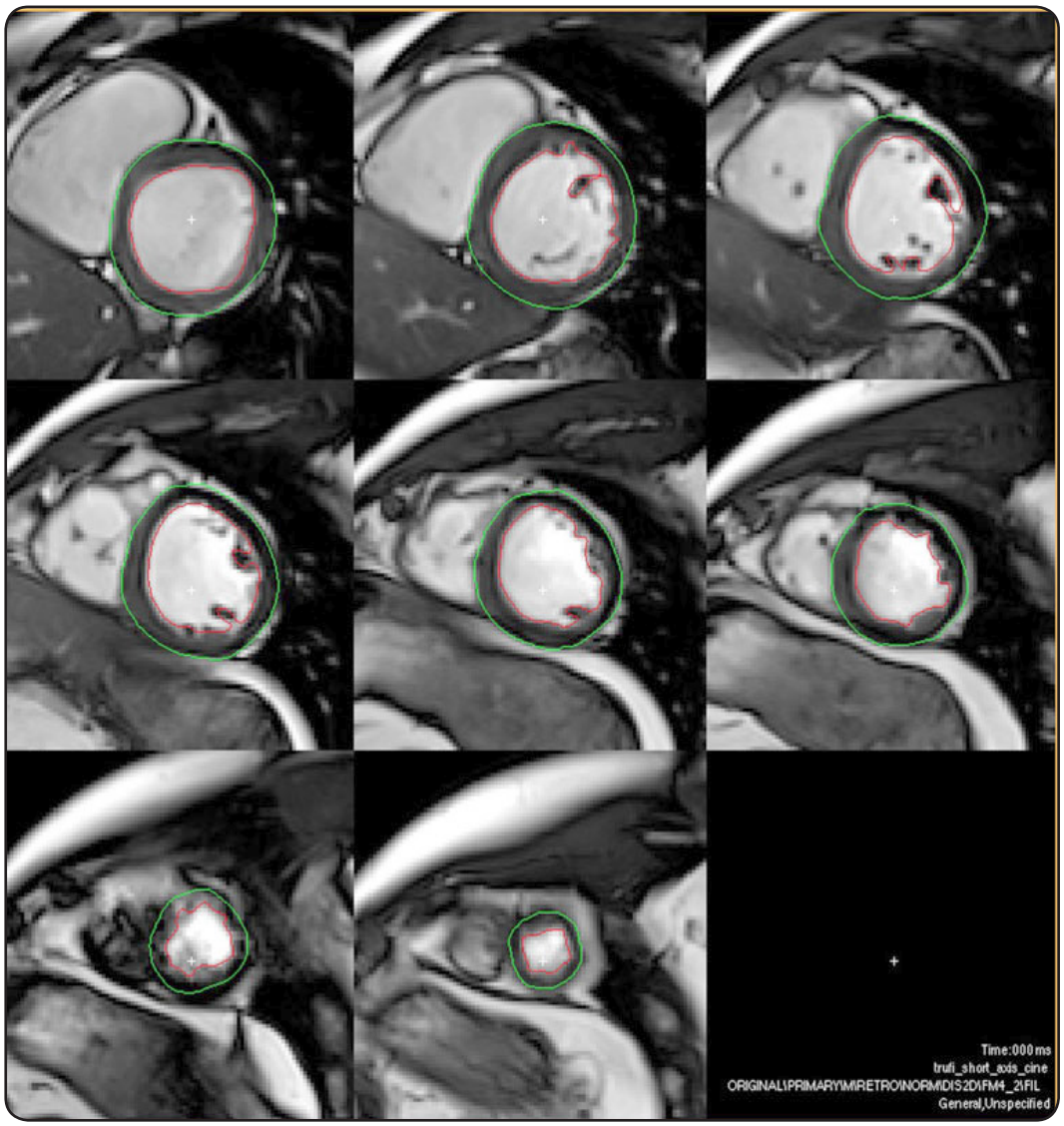

Fig. 1 Semi-automatic left ventricular (LV) segmentation performed on short-axis views obtained with cine sequences for volumetric assessment of global LV filling.

This segmentation took on the order of 3–5 min. At the base of the LV, the aortic outflow tract below the valve was included in volume measurements. The free papillary muscles were included for LV mass assessment, and excluded for left ventricular volume assessment. In the basal region of the heart where the LA was seen, only the portion of the slice that could be identified as the LV was included for measurement.

This analysis provided the time-varying course of the LV volume during the cardiac cycle. The peak filling rate (PFR) is the steepest tangent to the first part of the filling curve (Fig. 2). Segment software automatically determines this parameter.

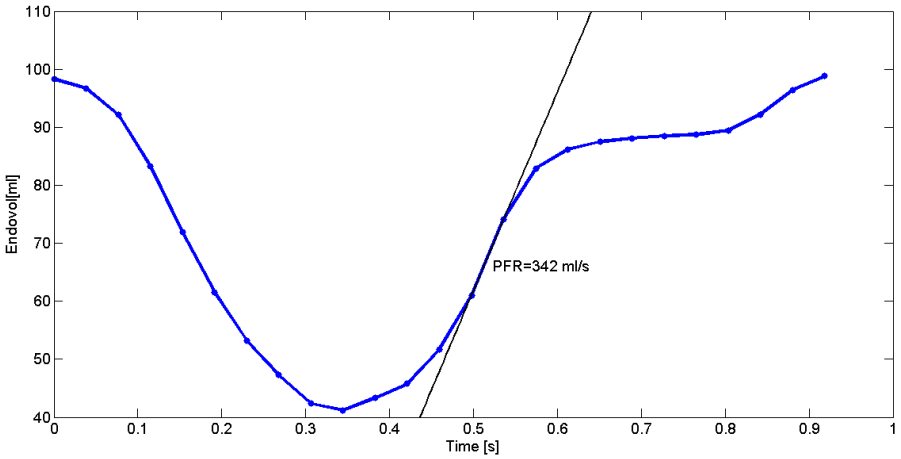

Fig. 2 Left ventricular (LV) filling volume versus time curve and its first derivative, the peak filling rate curve. The LV volume versus time curve and its first derivative (LV dV/dt) are obtained after the endocardial delineation of all LV short-axis slices across all temporal phases. The peak filling rate is the steepest tangent to the first part of the filling curve and represents the most rapid ventricular filling.

LA volume measurements were performed in the four- and two-chamber orientations by the biplane area-length methods. The following parameters of LA size and function were included in our analyses:

- LA minimum volume (LAVmin): LA end-diastolic volume at the first frame after mitral valve closure

- LA maximum volume (LAVmax): LA end-systolic volume right before mitral valve opening

- LA total emptying volume (LAEV): LAVmax – LAVmin

- LA total emptying fraction (LAEF): 100 × (LAVmax –LAVmin)/LAVmax

- LA conduit volume (LACV): LV stroke volume – LAEV

Analysis of the transmitral flow (TMF)

In the flow sensitive sequence, a round region of interest with a minimum of 1 cm2 was placed at the centre of the mitral valve orifice (Fig. 3) and propagated to other phases to obtain the TMF curve (Fig. 4).

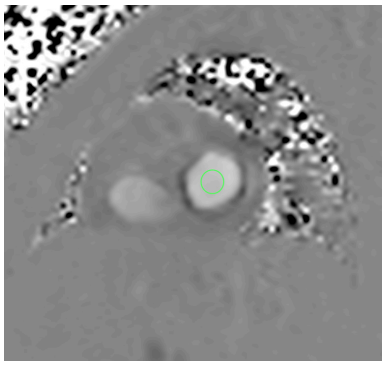

Fig. 3 Measurement of transmitral flow. Phase-encoded MRI obtained with a phase-contrast sequence shows a region of interest placed at the centre of the mitral orifice. The transmitral flow curve is obtained from this region of interest.

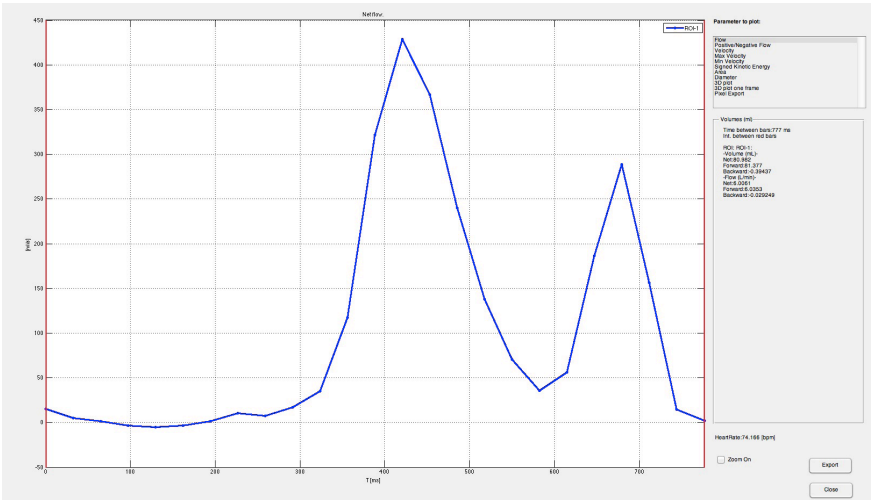

Fig. 4 Cardiac MR findings of the transmitral flow. The transmitral flow curve is composed of two peaks. The first one is the E wave and second one is the A wave. The E wave corresponds to rapid LV filling at early diastole; the A wave corresponds to late LV filling during end-diastole, secondary to LA contraction.

From the analysis of the TMF curve, the following measurements were performed: E and A mean peak velocities (in centimetres per second), E/A ratio, and mitral deceleration time (MDT) (measured from the E peak to the baseline).

Statistical analysis

All data were subjected to Shapiro-Wilk tests to establish normal distribution of the data. All normally distributed data are expressed as means ± standard deviations. Differences between means of women and men were assessed using Student's t-test for independent samples. The chi-squared test (Fischer's exact test) was used to determine a relation between the parameters diastolic dysfunction and gender. *P* values of less than 0.05 were considered statistically significant. All computations were performed using software (SPSS, version 20.0; SPSS, Chicago, IL, USA).

4. Results

This study included 48 (19 male and 29 female) subjects. Table 1 displays the baseline characteristics for the current study population.

Table 1 Baseline characteristics of the study population according to gender

	Female (n=29)	Male (n=19)	*P*
Age (years)	47±6	52±8	0.018
Height (cm)	160.4±5	172.9±5.3	< 0.001
Weight (kg)	62.9±8.7	76.6±9.8	< 0.001
BSA (m2)	1.67±0.13	1.92±0.14	< 0.001
BMI (kg/m2)	24.4±3.2	25.6±2.4	0.175

BSA body surface area, BMI body mass index

Gender differences in left ventricular measures

Table 2 lists the values obtained for LV mass, end diastolic volume (EDV), end systolic volume (ESV), stroke volume (SV) and ejection fraction (EF), according to gender.

Table 2 Left ventricular (LV) measurements according to gender

	Female (n=29)	Male (n=19)	*P*
LV EDV (mL)	103.0±17.0	136.3±24.4	< 0.001
LV EDV / BSA (mL/m2)	61.9±10.8	71.0±10.9	0.007
LV ESV (ml)	35.9±11.7	46.4±12.5	0.006
LV ESV / BSA (mL/m2)	21.6±6.6	24.1±6.1	0.178
LV SV (ml)	67±10.4	90±16.4	< 0.001
LV SV / BSA (mL/m2)	40.4±7.1	47.0±7.9	0.005
LV EF (%)	66±5.6	66.6±5.7	0.693
LV mass (g)	107.8±14.7	134.7±18.6	< 0.001
LV mass / BSA (g/m2)	65.1±7.7	70.4±8.9	0.038

LV left ventricle, EDV end diastolic volume, BSA body surface area, ESV end systolic volume, SV stroke volume, EF ejection fraction

Indexed LV end-diastolic and stroke volumes were significantly greater in men than in women (*P*=0.007 and *P*=0.005, respectively). The indexed LV myocardial masses at end diastole were significantly greater in males than in females (*P*=0.038).

All the subjects had LV EFs within the normal range (male, range 55–85%; female, range 56–85%) published for healthy individuals at MRI and no significant gender differences were noted in this parameter.

Gender differences in left auricular measures

Table 3 lists the values obtained for LA volumes and reservoir function, according to gender.

Table 3 Left atrium (LA) measurements according to gender

	Female (n=29)	Male (n=19)	*P*
LAVmin (mL)	26.3±8.2	36.9±11.7	0.002
LAVmin/BSA (mL/m2)	15.7±4.8	19.2±6.2	0.046
LAVmax (ml)	62.7±16.4	84.0±21.1	0.001
LAVmax / BSA (mL/m2)	37.5±9.3	43.8±10.1	0.032
LAEV (ml)	36.4±11.6	47.2±14.4	0.010
LAEV / BSA (mL/m2)	21.8±6.6	24.5±6.8	0.176
LACV (ml)	30.9±13.2	42.2±16.0	0.016
LACV / BSA (mL/m2)	18.7±8.4	21.8±8.8	0.235
LAEF (%)	57.4±9.3	56.4±8.9	0.698

LAVmin Left atrium minimum volume, LAVmax LA maximum volume, LAEV LA total emptying volume, LACV LA conduit volume, LAEF LA total emptying fraction, BSA body surface area

Indexed LA minimum and maximum volumes were significantly greater in

men than in women (*P*=0.046; *P*=0.032, respectively). Although absolute values of LA total emptying and conduit volumes were significantly greater in men than in women (*P*=0.01; *P*=0.016, respectively), the respective indexed volumes were comparable (*P*=0.176; *P*=0.235). Values of LAEF were similar in both genders (*P*=0.698)

Gender differences in left ventricular diastolic measures

Table 4 lists the values obtained for LV parameters of diastolic function, according to gender. Two subjects were excluded from the study because of intense motion artefacts during the flow-sensitive acquisition.

Table 4 LV diastolic function measurements according to gender

	Female (n=27)	Male (n=19)	*P*
LV PFR (mL/s)	360.9±84.0	429.6±139.7	0.043
LV PFR/EDV (mL/s)	3.58±0.8	3.22±1.2	0.246
LV PFR/SV (mL/s)	5.51±1.4	4.9±1.8	0.208
Mitral peak E velocity (cm/s)	51.8±8.3	47.7±10.2	0.164
Mitral peak A velocity (cm/s)	39.5±8.2	41.6±9.7	0.446
Mitral E/A ratio	1.35±0.4	1.19±0.3	0.147
Mitral deceleration time (ms)	164±31	181±48	0.176

LV left ventricle, PFR peak filling rate, EDV end diastolic volume, SV stroke volume

Absolute values of PFR was significantly greater in men than in women (*P*=0.043).

The indexed (to EDV and SV) values of peak filling rate in women and men subjects were comparable (*P*=0.246; *P*=0.208). The analysis of transmitral parameters showed no gender differences. Mitral peak E (*P*=0.164) and peak A (*P*=0.446) velocities were comparable in women and men. Values of the E/A ratio (*P*=0.147) and MDT (*P*=0.176) were also similar in both genders.

Prevalence of diastolic dysfunction

A PFR normalised to EDV of less than 2.5 EDV/s was considered abnormal (14-16). This analysis was repeated using a PFR normalised to the stroke volume (SV), with a PFR less than 4SV/s considered abnormal (17-18).

The criteria for diastolic dysfunction from the analysis of the TMF curve were established according to published data (12). Normal diastolic function was defined as an E/A between 1 and 2 and MDT between 150 and 220 ms. In grade I dysfunction, E/A decreases below 1. In grade II, E/A moves back into the normal range of 1–2, MDT is also normal, but there is

LA dilatation (LAVmax / BSA (mL/m2) greater than 52 in women or greater than 53 in men). In grades III and IV, E/A increase to a value above 2 and MDT drops below 150 ms. One male patient of 70 years of age with a mitral E/A ratio of 0.74 and MDT of 162 ms was established as a normal subject. We maintained the diagnosis of diastolic dysfunction in a 63-year-old man with a mitral E/A ratio of 0.68 and MDT of 234 ms (19).

Table 5 lists the prevalence of LV diastolic dysfunction, according to gender and the criteria used.

Table 5 Prevalence of LV diastolic dysfunction diagnoses according to gender

Criteria	All (n=46)	Female (n=27)	Male (n=19)	*P*
LV PFR/EDV (mL/s) < 2.5	9 (20 %)	2 (7 %)	7 (37 %)	0.022
LV PFR/SV (mL/s) < 4	9 (20 %)	2 (7 %)	7 (37 %)	0.022
Transmitral flow analysis	11 (24 %)	2 (7 %)	9 (47 %)	0.004
Mitral E/A ratio < 1	6 (13 %)	2 (7 %)	4 (21 %)	0.213
Mitral E/A ratio 1–2 and LAVmax / BSA (mL/m2) > 52 in women or > 53 in men	5 (11 %)	0 (0 %)	5 (26 %)	0.008

LV left ventricle, PFR peak filling rate, EDV end diastolic volume, SV stroke volume, LAVmax left atrium maximum volume, BSA body surface area

When patients were classified as having normal and abnormal PFR on the basis of a threshold of 2.5 EDV/s, 9 (7 male and 2 female, *P*= 0.022) patients were found to have diastolic dysfunction. When the PFR was normalised to the SV and analyses were repeated using a PFR less than 4 SV/s as abnormal, the number of patients with diastolic dysfunction was also 9 (7 male and 2 female, *P*= 0.022).

When the patients were classified as having diastolic dysfunction on the basis of TMF analysis and LA size, 11 (9 male and 2 female, *P*=0.004) patients were found to have grade I or grade II diastolic dysfunction. None of the subjects showed grade III diastolic dysfunction.

5. Discussion

Gender differences in left ventricular measures

Gender-specific differences include all absolute functional and morphological values except for EF. Normalisation to BSA eliminated

differences in LV ESV. Several studies (5–11) have described overall values of EDV, ESV, SV, LV mass and EF, which are consistent with findings from other imaging techniques and are broadly consistent with the findings in this study. Regarding the use of 3 T equipment in our study, published data suggest that field strength does not have an influence on the quantification of cardiac volume or mass, and normal values for cardiac volumes and mass established at 1.5 T can be applied to images obtained at 3 T (20).

Gender differences in left atrial measures

We observed that nearly all non-indexed LA volumes were significantly higher in men, except for the left atrial emptying fraction. While these differences disappeared in most normalised parameters, indexed LA minimum and maximum volumes remained higher in men. Hudsmith et al (6, 21) also reported higher absolute LA volumes in male patients with similar ejection fraction in patients of both genders. Regarding indexed LA volumes, our findings are broadly consistent with Maceira et al (4). The effect of age may explain the greater indexed LA maximum volumes observed in men. Gender differences in the indexed LA minimum volume may be due to the decreased ejection force of the atrial pump in men, in a Frank Starling-like mechanism (22). This could be further clarified by the analysis of LA active pumping volume and index, which we did not perform.

Gender differences in left ventricular diastolic measures

Although absolute LV PFR values were significantly higher in men, the parameter indexed for EDV or SV was higher in women, but not significantly so.

Regarding the evaluation of the TMF, there were no gender differences.

One of the largest cohort studies with mitral flow-derived indices published (23) also showed no gender difference in E/A ratio. Previous studies have demonstrated a good correlation between cardiac MRI and echocardiography Doppler for measurement of flow velocities (2, 24, 25). Nevertheless there are no significant data from cardiac MR studies in a general population.

Prevalence of diastolic dysfunction

The proportion of patients with an abnormal diastolic function is variable in the population depending on the criteria used. The prevalence reported here (between 20% and 24%) is consistent with larger population studies (26-29).

The gold standard for assessing diastolic function remains the pressure-volume relationship, but this requires an invasive approach. Conventionally, echocardiographic Doppler measurements of MV flow conditions are used to assess diastolic function. CMR is a valid alternative for those patients who do not have adequate echocardiographic image quality to reliably obtain these parameters (1).

Flow analysis with CMR allows the quantitative assessment of blood velocity, with the advantage that the tomographic plane of interest can be positioned optimally in a larger area than Doppler echocardiography. CMR provides velocities (metres per second) as well as volume flow (millilitres per second) (30) and is less operator and angle dependent (31). Lower transmitral E/A ratios reflect impaired myocardial relaxation, characterised by decreased early, but enhanced atrial filling of the LV. In subjects with transmitral E/A ratio between 1 and 2, to establish a diagnosis of grade II diastolic dysfunction, we used the criterion of increased LA maximum volume, as it often reflects the cumulative effects of filling pressures over time (1).

Left ventricular filling profiles have been used to assess diastolic function by other imaging investigations, such as radionuclide cineangiography and SPECT. Similar applications for CMR imaging have been impractical because manual planimetry of all LV images across all temporal phases would typically require tracing of more than 200 images per patient. With the improvements in post-processing tools it is now possible to semi-automatically segment all phases and quickly provide the time-varying course of the LV volume during the cardiac cycle. The ventricular relaxation abnormalities can be diagnosed by a low PFR. This evaluation is based on conventional short-axis sequences, without the necessity to perform a dedicated acquisition. The cut-off used to define the abnormal PFR has been applied to nuclear medicine tests. The use of indexed values of PFR to EDV and SV minimises the dependency of PFR from EDV and heart rate. We demonstrated that the left ventricular PFR can be easily obtained as an addition to the assessment of LV systolic function with semi-automatic contour detection and it may become a valuable asset to the evaluation of LV diastolic function.

Gender differences in the prevalence of diastolic dysfunction

The effects of age and hormonal protection may explain the lower prevalence of diastolic dysfunction in women.

The incidence of diastolic dysfunction increases with age (26) and seems to be affected by the postmenopausal state. Hormone replacement

therapy may improve LV diastolic function as one of the mechanisms of its cardioprotective effects (32).

Although we did not find any gender differences regarding body mass index (BMI), the increased weight and body surface area (BSA) of men compared with women may also be related to the higher prevalence of diastolic dysfunction in this gender.

We recognise the limitations of our study. The sample size of the study is modest and our findings need to be validated in a larger population.

The normal controls did not undergo a stress test to rule out latent ischaemia. However, none of the controls had significant cardiac risk factors.

We considered that the analysis of cine and TMF images was sufficient to exclude left-sided valvular disease. Regarding the exclusion of infiltrative myocardial disease, we did not study myocardial delayed enhancement nor did we perform endomyocardial biopsy. Nevertheless, none of our subjects had increased LV myocardial end-diastolic thickness, pericardial thickening, pericardial effusion or pleural effusion.

Cardiac catheterisation was not performed to evaluate LV diastolic function.

The use of another imaging technique such as echocardiography Doppler to study TMF and to assess LA size and function was considered beyond the scope of the present study.

Although good correlation between echo Doppler studies and MRI has been reported, the cut-off values used in echocardiography cannot simply be translated to these MRI-assessed indices based on the time–flow rate curves.

Normalising early mitral velocity (E) for the influence of myocardial relaxation by combining E with early diastolic mitral septal tissue velocity (Ea) may be performed by MR tissue phase contrast imaging, and this is an important criterion that has been established (19). On the other hand there are still conflicting data regarding the relation between E/Ea ratio and LV filling pressure (33, 34).

It is demonstrated that an LA volume index greater than 34 mL/m2 is an independent predictor of death, heart failure, atrial fibrillation, and ischaemic stroke. However, we recognise that dilated left atria may be seen in patients with bradycardia and four-chamber enlargement, anaemia and other high-output states, atrial flutter or fibrillation, and significant mitral valve disease, in the absence of diastolic dysfunction. We used a cut-off that was clearly higher (LAVmax / BSA (mL/m2) greater than 52 in women

or greater than 53 in men subjects) and we think that this eliminated most if not all of these potential confounding conditions.

This study highlights certain key points for the routine use of CMR to study diastolic function. Firstly, it is clinically feasible to study left heart volumes and function, including diastolic flow and LV filling data in a single CMR examination. Secondly, the prevalence of diastolic dysfunction in a normal adult population has clinical importance in view of the high risk in patients with impaired LV diastolic function.

In conclusion, our observations provide promising initial results for the routine use of CMR to study the prevalence and gender differences in subclinical LV diastolic dysfunction.

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Chapter V

Left atrial dysfunction in type 2 diabetes mellitus: insights from cardiac MRI

Bruno Graça, Maria João Ferreira,
Paulo Donato, Leonor Gomes,
Miguel Castelo Branco, Filipe Caseiro Alves

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1. Abstract

Objectives

The left atrium (LA) modulates left ventricular filling through reservoir, conduit and booster pump functions. Only limited data exist on LA involvement in type 2 diabetes mellitus (DM2).

This study sought to assess LA function in asymptomatic DM2 with cardiac MRI. We hypothesized that cardiac MRI can detect LA dysfunction in asymptomatic DM2.

Methods

Forty-five patients with asymptomatic DM2 and 24 normoglycemic controls were studied. MRI cine scan was performed to measure LA maximal and minimal volumes. A flow-sensitive phase-contrast gradient-echo sequence was used for flow measurements perpendicular to the orifice of the mitral valve, to quantify active LA stroke volume. LA total, passive and active emptying volumes, and fractions were calculated.

Results

LA reservoir function, namely LA total ejection fraction, was significantly greater in controls compared to patients with DM2 (62.2±5.2 vs 57.0±7.6%, P=0.004). LA passive ejection fraction was also greater in the controls (26.2±9.5 vs 16.1±11.0%, P<0.001). Regarding parameters of LA booster pump function, LA active ejection fraction were not significantly different between groups. DM2 demonstrated to be an independent determinant of LA function.

Conclusions

Cardiac MRI enables the detection of LA dysfunction in asymptomatic DM2, characterized by a reduction in LA reservoir and conduit functions.

2. Introduction

Patients with type 2 diabetes (DM2) have an increased risk of developing cardiovascular disease, resulting in significant cardiac morbidity and mortality (1). In DM2 morphological changes suggestive of heart disease may appear before symptoms arise and the prevalence of subclinical left ventricular (LV) dysfunction is increased among these patients (2).

Several mechanisms may underlie LV dysfunction in DM2, including atherosclerosis, microinfarctions, mitochondrial dysfunction, lipotoxicity and accumulation of advanced glycation end products, leading to myocyte hypertrophy, perivascular fibrosis and increased quantities of matrix collagen (3). All suggested mechanisms conducting to LV dysfunction in DM2 include systemic phenomena that can also impact the function of the left atrium (LA).

Left atrial function has been conventionally divided into three phases across the different phases of the cardiac cycle. First, during ventricular systole and isovolumetric relaxation, the LA acts as a reservoir and stores pulmonary venous return. Then, during ventricular diastole, LA emptying consists of two distinct components in subjects with sinus rhythm: the early component is related to passive blood flow from the LA, the pulmonary veins and the LA appendage into the LV; and the late component is related to active LA contraction and is referred to as the LA booster function for LV filling (4). Through these varying mechanical functions, the LA modulates LV filling and plays a key role in maintaining an optimal cardiac performance.

LA volume and function are robust markers of cardiovascular risk and adverse cardiac outcome across a broad range of cardiovascular pathologies (5-7). A recent prospective observational follow-up study of DM2 patients without overt cardiovascular disease demonstrated that a dilated LA was a predictor of death and major cardiovascular events (8). Regarding LA function, its prognostic importance in diabetes has not been evaluated.

Fundamental structural and functional properties of the left atrium are often assessed in the clinical setting using two-dimensional echocardiography.

Cardiac MRI offers several inherent advantages for the assessment of LA size and function due to its high spatial resolution, superior endocardial border definition, and capacity for unrestricted multiplanar imaging, independent of acoustic windows that may limit echocardiography (9). Basic measurements of LA size and function can be achieved with cardiac MRI by using the images routinely taken for LV function assessment.

There are limited published data on LA involvement in DM2 (10, 11), and as far as we are aware, there is no study about the role of MRI in the evaluation of LA function in DM2. Accordingly, the purpose of this study was to compare cardiac MRI–derived parameters of LA function between well-controlled uncomplicated DM2 and normoglycemic control subjects. Moreover, obesity and arterial hypertension are common in the clinical setting of DM2 and could also induce LA dysfunction.

So, we also aim to evaluate how DM2, arterial hypertension and body-mass index influences LA function.

We hypothesized that asymptomatic DM2 patients will have reduced LA function compared with normoglycemic control subjects.

3. Materials and methods

Study population

Forty-five patients with DM2 were prospectively recruited from the Endocrinology Department of our institution. Inclusion criteria included age between 45-75 years, no symptoms or history of overt heart disease, no signs or symptoms of cerebrovascular disease, no abnormal findings on routine clinical and physical examination and a normal rest ECG. Patients who had LVEF <55%, regional LV wall motion abnormalities and valvular heart disease, contraindications to MRI, glomerular filtration rate <30 mL/min, glycated hemoglobin > 12%, systolic blood pressure > 160 mm Hg and/or diastolic blood pressure > 100 mm Hg, underlying cardiomyopathy, previous myocardial infarction, coronary revascularization, or previous cardiac surgery were excluded. Twenty-four healthy volunteers recruited from the local population served as control and had no history of heart disease, diabetes mellitus, or high cholesterol. They had a normal physical examination and ECG. All subjects gave informed consent to participate in the study, which was approved by our institutional ethics committee.

Cardiac MRI protocol

All images were acquired with electrocardiographic gating, breath-holding, and the patient in a supine position. Subjects were imaged on a 3T MR system (Tim Trio, Siemens, Erlangen, Germany). The basic protocol consisted of cine steady-state free-precession imaging (TR, 3.4 ms; TE, 1.2 ms; in-plane spatial resolution, 1.6 × 2.1 mm) for LV function and mass. Cine imaging was obtained in 8 to 12 matching short-axis (8 mm thick with 2mm gap) and 3 standard long-axis planes (2-, 3- and 4-chamber views). For the calculation of LV mass and function, the endocardial and epicardial

borders of the LV myocardium were manually traced on successive short-axis cine images at end-diastole and systole.

A breath-held, retrospectively vector-ECG gated, two-dimensional flow-sensitive phase-contrast gradient-echo sequence was used for velocity-encoded (VENC) MRI flow measurements perpendicular to the orifice of the mitral valve. VENC-MRI slices were positioned in early diastole at the tip of the mitral valve leaflets. Typical imaging parameters of VENC-MRI were as follows: slice thickness 6 mm, repetition time 4.5 ms, echo time 2.33 ms, number of segments 3, acquired temporal resolution 36 ms, 20° flip angle, 25 calculated phases and pixel spacing 4.5×3.1 mm. Encoding velocity was set to 130 cm/s (12, 13). Flow analysis was performed on the through-plane VENC-MRI phase-contrast and magnitude images acquired across the mitral valve. To determine total blood flow and volume, region of interest curves on the VENC-MRI phase-contrast images were drawn in the diastole at the mitral valve orifice and propagated to all phases to obtain the transmitral flow (TMF) curve (14) (Figure 1). From the analysis of the TMF curve, the following measurements were performed: E and A mean peak velocities (in centimetres per second), E/A ratio, and mitral deceleration time (MDT) (measured from the E peak to the baseline).

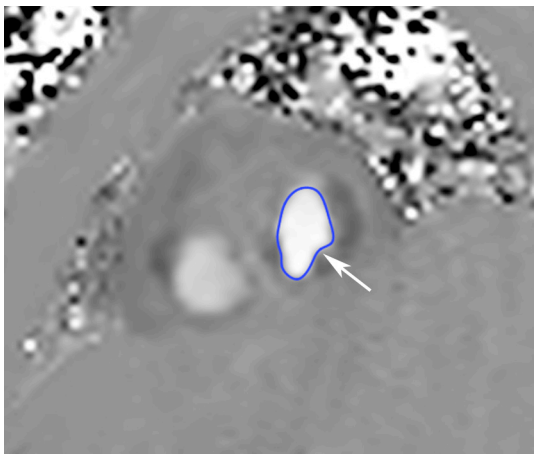

Fig. 1 Measurement of transmitral flow. Phase-encoded MRI image obtained with a phase-contrast sequence show a region of interest placed at the mitral orifice (arrow). The transmitral flow curve was obtained from this region of interest.

Left atrial measurements

Left atrial volume measurements were performed in the four- and two-chamber orientations by the biplane area-length methods. In the analysis we excluded the atrial appendage and the pulmonary veins. The parameters of LA size and function included in our analyses were:

- LA minimum volume (LAmin): LA end-diastolic volume at the first frame after mitral valve closure (Figure 2A and 2B).

- LA maximum volume (LAmax): LA end-systolic volume right before mitral valve opening (Figure 2C and 2D).

LA reservoir function

- Total LA stroke volume (LASV) = LAmax – LAmin.

- Total LA ejection fraction (LAEF) = total LASV/LAmax

LA contractile function

- Active LASV: directly obtained by VENC-MRI from transmitral flow curves (Figure 3), using a MATLAB script.

- LA volume pre-atrial contraction (LApreA) = LAmin + Active LASV

- Active LAEF = active LASV/LApreA

LA conduit function

- Passive LASV = LA max - LApreA

- Passive LAEF = passive LASV/LAmax

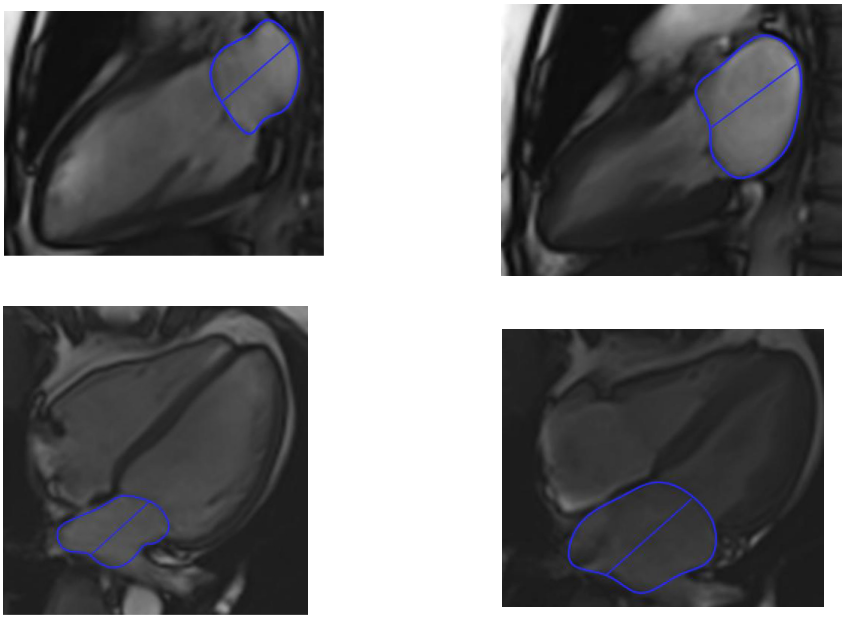

Fig. 2 Measurement of left atrial minimal and maximal volumes. Cine-MRI slices were acquired in the two-chamber (upper row) and four chamber (lower row) long-axis orientation. Minimal (A and B) and maximal left atrial (C and D) volumes were calculated using the biplane area-length method. The atrial appendage and the pulmonary veins were excluded from the measurements.

MR image analysis

Quantitative image data analysis was performed by using dedicated software (Segment, Medviso, Lund, Sweden) (15). All functional evaluations were performed within 25 minutes per patient.

Reproducibility

In 5 randomly selected studies from each group, 2 readers independently measured the LAmax, LAmin and active LASV. One observer remeasured the same 10 studies at a separate time to determine intraobserver agreement from the baseline studies.

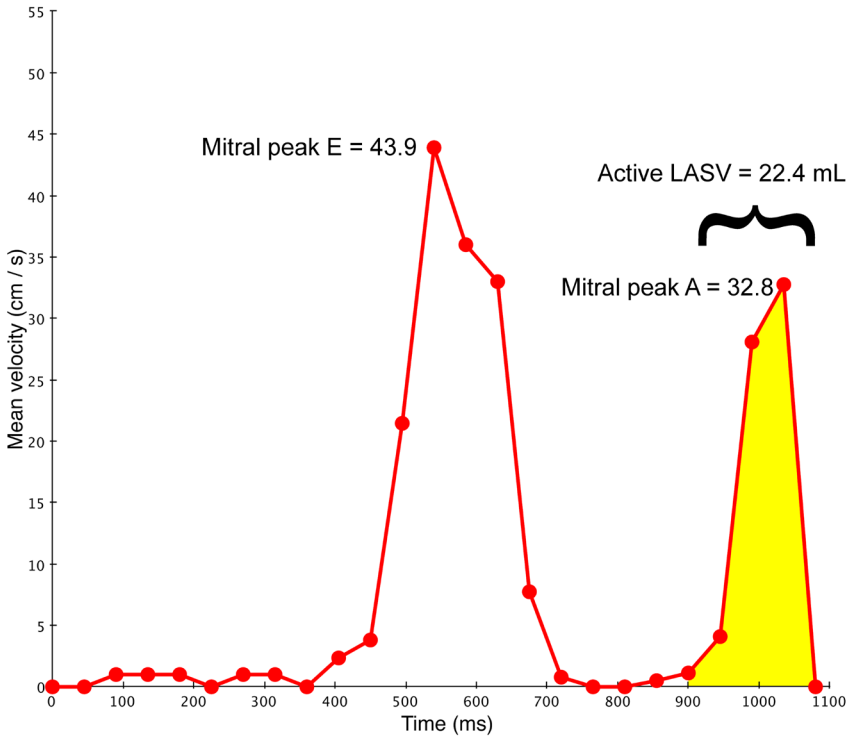

Fig. 3 MRI findings of transmitral flow. The transmitral flow curve is composed of two peaks. The first one is the E wave and second one is the A wave. The E wave corresponds to rapid LV filling at early diastole; the A wave corresponds to late LV filling during end-diastole, secondary to LA contraction. Active left atrial stroke volume was directly obtained from the analysis of the area under the A wave (filled in yellow).

Statistical analysis

All continuous variables were tested for normal distribution. All normally distributed data are expressed as means ± standard deviations. Categorical variables are expressed as counts and percentages. Differences between means of the DM2 group and controls were examined by the unpaired t-test. Between-group differences in numbers and percentages were compared using the Chi-squared test.

Univariate and multiple analyses with a forward selection procedure were performed. The goal of these analyses was to determine which factors were responsible for the difference in LA phasic function (total LAEF and passive LAEF) between groups. We employed a 2-step strategy for the selection of variables. The first step was that a variable had to be significantly different between groups. If so, in univariate analysis there had to be an association between this variable and the dependent variable with $P<0.1$. The variables

fulfilling these criteria were then entered in a multivariable regression analysis, and those with $P<0.05$ were considered independently related to the dependent variable. Intraobserver and interobserver variability were assessed by using a Bland-Altman analysis. All computations were performed with software (SPSS, version 20.0; SPSS, Chicago, IL).

4. Results

Demographic data and LV parameters of the DM2 group vs. controls are presented in Table 1. Body mass index was significantly increased in the DM2 group (P<0.001). Our population of DM2 had a higher (53%) prevalence of systemic arterial hypertension (HT), although not significantly greater than the control group.

All left ventricular volumes and masses were in the normal range, with no significantly difference between groups.

Subjects with DM2 had significantly decreased diastolic functional parameters with greater mitral peak A velocity and lower mitral E/A ratio (P=0.010; P=0.002, respectively).

Table 1 Baseline characteristics of the study population and left ventricular measurements

	DM2 group (n=45)	Normoglycemic group (n=24)	*P value*
Age, y	58.9±6.9	55.8±6.8	0.081
Male, n (%)	24/45 (53)	14/24 (58)	0.801
Body surface area, m2	1.87±0.21	1.83±0.18	0.374
Body mass index, kg/m2	29.5±4.4	25.6±3.1	< 0.001
Hypertension, n (%)*	24/45 (53)	8/24 (33)	0.011
LV EDV / BSA, mL/m2	77.4±14.4	79.8±13.9	0.507
LV EF, %	61.6±7.2	64.5±5.6	0.119
LV mass / BSA, g/m2	64.6±13.1	62.4±13.4	0.529
Mitral peak E velocity, cm/s	42.3±11.9	46.4±11.6	0.168
Mitral peak A velocity, cm/s	50.3±10.7	43.9±8.9	0.010
Mitral E/A ratio	0.86±0.27	1.08±0.27	0.002

BP, blood pressure; LV, left ventricle; EDV, end-diastolic volume; BSA, body surface area; EF, ejection fraction.

*Blood pressure > 140/90 mmHg or treatment with anti-hypertensive medication.

Intraobserver and interobserver variability of left atrial measurements

The intraobserver limits of agreement were −2.1 mL to 0.6 mL for LAmin, 0.5 mL to 3.9 mL for LAmax and −2.1 mL to 1.2 mL for active LASV. The interobserver limits of agreement were 0.02 mL to 4.2 mL for LAmin, −3.0 mL to 2.6 mL for LAmax and −0.9 mL to 1.2 mL for active LASV. The corresponding intraclass correlation coefficient values were 0.99, 0.99, and 0.95 for the intraobserver analysis and 0.97, 0.98, and 0.98 for the interobserver analysis for LAmin, LAmax and active LASV, respectively.

Left atrial measures

Indexed LA minimum and maximum volumes were not significantly different between groups (Table 2).

Table 2 Left atrial measurements

	DM2 group (n=45)	Normoglycemic group (n=24)	*P* value
LAmin / BSA, mL/m2	16.7±6.9	13.9±3.9	0.096
LAmax / BSA, mL/m2	37.7±11.0	36.0±7.6	0.488
Total LASV / BSA, mL/m2	21.0±5.8	22.1±4.4	0.431
Active LASV / BSA, mL/m2	13.8±3.6	13.2±3.9	0.522
Passive LASV / BSA, mL/m2	7.2±5.6	9.0±5.5	0.243
Total LAEF, %	56.9±8.2	62.2±9.3	0.034
Passive LAEF, %	27.6±18.2	45.3±15.5	< 0.001
Active LAEF, %	46.7±11.8	49.0±12.9	0.513

BSA, body surface area; LAmin, left atrial minimum volume; LAmax, left atrial maximum volume; LASV, left atrial stroke volume; LAEF, left atrial ejection fraction.

Parameters of LA reservoir function, namely total LAEF value, was significantly greater in the control group (*P*=0.034).

Parameters of LA conduit function, namely LA passive ejection fraction value was also significantly greater in the control group (*P*<0.001).

Regarding parameters of LA booster pump function, including indexed LA active stroke volume and active LAEF values were not significantly different between groups.

Factors that influence LA function

Table 3 outlines the univariate Pearson correlations for total and passive LAEF.

Table 3 Univariate Pearson correlation coefficients analysis of total LAEF and passive LAEF

	Total LAEF		Passive LAEF	
	Correlation Coefficient	*P* value	Correlation Coefficient	*P* value
Age	0.20	0.876	-0.30	0.017
Body mass index	-0.22	0.083	-0.32	0.012
LV EDV / BSA, mL/m2	0.084	0.522	0.004	0.977
LV mass / BSA, g/m2	-0.172	0.184	-0.228	0.077
Mitral peak A velocity	0.15	0.190	-0.29	0.024
Mitral E/A ratio	0.07	0.583	0.48	<0.001

LAEF, left atrial ejection fraction.

There was no significant correlation between total LAEF and age, body-mass index, LV ejection fraction, indexed LV mass, indexed end-diastolic LV volume, mitral peak A velocity and mitral E/A ratio.

There was a significative, low to moderate negative correlation between passive LAEF and age, body mass index and mitral peak A velocity. There was a significative, moderate positive correlation between passive LAEF and mitral E/A ratio. There was no significant correlation between passive LAEF and LV ejection fraction, indexed end-diastolic LV volume and indexed LV mass.

To identify the independent determinants of total and passive LAEF univariate predictors with $P<0.10$ were all entered into a multiple linear regression model as covariates (Table 4).

Table 4 Independent determinants of total LAEF and passive LAEF

	β	***P* value**
Total LAEF		
Diabetes mellitus	-0.15	0.017
Arterial hypertension	-0.01	0.918
Body mass index	1.10	<0.001
Passive LAEF		
Diabetes mellitus	-0.18	0.182
Hypertension	0.04	0.67
Body mass index	-0.03	0.927
Mitral peak A velocity	0.22	0.328
Mitral E/A ratio	0.89	<0.001

LAEF, left atrial ejection fraction.

On multivariable analysis, total LAEF was independently influenced and reduced in DM2 and with increased BMI. Passive LAEF was related to E/A ratio.

5. Discussion

In this study, MRI-derived parameters of LA function in patients with DM2 were investigated. Differences in LA function were observed between DM2 patients and control subjects. LA phasic function is changed in asymptomatic DM2, with an associated decrease in reservoir (total LAEF) and conduit (passive LAEF) functions. Conversely, there was no change in LA booster function.

We have demonstrated that DM2 and body-mass index are independent determinants of LA reservoir function on multivariate analysis. Mitral E/A ratio is an independent determinant of LA passive function.

Left atrial reservoir function

During LV systole and isovolumic relaxation, the LA functions as a reservoir, receiving blood from the pulmonary veins and storing energy in the form of pressure. This atrial function is mainly modulated by LV contraction, through the descent of the LV base during systole and by LA properties (i.e., relaxation and chamber stiffness) (16).

Our group of DM2 patients demonstrated lower total LA ejection fractions compared to normoglycemic controls. This could by explained by a decrease in LA compliance in the DM2 group (17) in the context of diabetic cardiomyopathy. An impairment of LA compliance in DM2 has been recently demonstrated by Kadappu et al (11), by showing that echocardiographic parameters of global and segmental strains of the LA were significantly reduced in subjects with DM2.

Left atrial conduit function

During early LV diastole, the pressure in the left atrium falls, and flow in the pulmonary veins increases. During this period, the left atrium acts as a passive conduit (conduit function of the left atrium) and blood is transferred into the LV through the LA via a small pressure gradient and flows passively from the pulmonary veins into the LV. Traditionally the LA passive stroke volume is not easily measured, because, while the mitral valve is open, some blood flows directly from the pulmonary veins and LA appendage (16). In our study, we obtained the LA active atrial emptying volume directly by measurement of transmitral total flow across

all the area of the mitral valve, and added this volume to LA minimum volume to establish the LA volume before LA contraction. The LA passive stroke volume was obtained by subtracting this LA volume before LA contraction to LA maximum volume. This method is not confounded by passive diastolic blood flow from the pulmonary veins and LA appendage (18). Passive LA ejection fraction is the proportion of the LA passive stroke volume to LA maximum volume.

In our study DM2 patients demonstrated lower LA passive ejection fraction compared to normal controls. The explanation for this finding could reside in the LV. Left atrial passive stroke volume is effectively drawn into the left ventricle via LV suction, and it may be more appropriately viewed as a property of LV diastolic function rather than intrinsic LA function. This conduit function is modulated especially by LV diastolic properties (LV relaxation and early diastolic pressures) (19).

DM2-related changes in LV diastolic properties are well recognized with a decrease in early diastolic filling, directly influenced by abnormal LV relaxation, and an increased passive stiffness due to remodeling (20). In fact, the DM2 group of our study showed higher mitral peak A velocity and lower mitral E/A ratio compared to normal controls. Also, mitral E/A ratio was an independent determinant of LA conduit function. Higher mitral peak A velocity and lower mitral E/A ratio are markers of impaired LV relaxation (21), suggesting that there may be a link between LV diastolic properties and LA function.

Left booster pump function

In the presence of a sinus rhythm, LV filling is completed by atrial contraction. The LA is a contractile chamber that actively empties immediately before the onset of LV systole and establishes final LV end-diastolic volume. LA booster pump function is mostly dependent on intrinsic atrial contractility and becomes increasingly important to the preservation of cardiovascular performance in the presence of reduced LV compliance (16).

In our study, the parameters of LA booster pump function (active LA stroke volume and active LA ejection fraction) were similar in both groups.

Muranaka et al (10) showed also a reduction in atrial phasic function in diabetes mellitus, as measured by strain rate parameters. Their results point also to an impairment of LA reservoir and conduit functions in patients with DM2 (10). Asbun et al (17) demonstrated a relation between diabetic cardiomyopathy and a reduction in LA compliance. A recent study from van-Schinkel et al (22) also demonstrated an association of type 1 diabetes mellitus, aortic stiffness (determined by MRI-assessed pulse wave velocity)

and decrease in LA compliance (measured with echocardiographic speckle tracking strain analysis). Kadappu et al (11) evaluated LA function by strain and strain rate derived from 2D speckle tracking in patients with DM2. Patients with DM2 had altered phasic LA function with impaired LA reservoir, conduit and contractile functions (11).

The findings of our study contribute to the available knowledge, favoring the hypothesis that there is a relationship between DM2, obesity and left atrial dysfunction.

The originality of our study is the use of cardiac MRI–derived parameters of LA function.

While MRI represents the current gold standard for assessment of LA size and function (23), there are limited data available on this topic.

Traditionally, LA size and function has been studied with performing 2-dimensional echocardiography. However, cyclic changes of LA volume may not be observed directly by 2-dimensional echocardiography because the shape of the LA changes during the heart cycle, and the pattern is influenced by the loading conditions (24). Therefore, 2-dimensional echocardiography presents only a snapshot view of the LA function.

In contrast, MRI data acquisition is distributed to several cardiac cycles in segmented ECG-gated sequences. Thus, cardiac MRI has been proved to be an effective alternative for accurately assessing the LA volume and phasic function (25).

There is growing evidence that LA size and function serves as an important diagnostic and prognostic factor in a variety of conditions, including DM2 (8). Therefore, comprehensive evaluation of LA function might be an important clinical factor to stratify the risk of preclinical cardiovascular disease and could be integrated into a routine cardiac MRI protocol for the evaluation of high-risk subjects, including patients with DM2.

There are a few limitations to our study. This study was a case-control study with a relatively small number of subjects and our findings need to be validated in a larger population.

Our findings are limited by an inability to eliminate causal relationships with important clinical factors, such as medications used, serum concentrations of glucose, and glycosilated haemoglobin, dyslipidaemia, retinopathy, microabuminuria and smoking status.

The possibility of influence of myocardial ischemia or fibrosis on LV or LA function cannot be completely excluded. Although stress imaging is recommended for symptomatic type 2 diabetics, there is still no consensus on the best approach for screening asymptomatic diabetic

subjects without known coronary artery disease (26). In the absence of sufficient clinical indication, it was not deemed ethical to subject these asymptomatic subjects to gadolinium myocardial perfusion and delayed enhancement.

For the analysis of the LA volumes, a true volumetric approach such as the use of a contiguous short-axis stack, is preferred. However, the biplane area-length method is a validated good compromise between accuracy and speed, as it does not require extra image acquisition apart from that routinely taken for LV function assessment (27).

This study highlights certain key points for the routine use of cardiac MRI to study LA function in diabetic patients.

First, evaluation of LA function is feasible with cardiac MRI. Second, body-mass index and diabetes independently influence LA function. Third, cardiac MRI shows a decrease in LA reservoir and conduit functions in DM2 and may allow the diagnosis of subclinical LA dysfunction in this high-risk population.

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Chapter VI

Left ventricular diastolic function in type 2 diabetes mellitus and the association with coronary calcium score: a cardiovascular MRI study

Bruno Graça, Paulo Donato, Maria João Ferreira,
Miguel Castelo Branco, Filipe Caseiro Alves

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1. Abstract

Objectives

The purpose of the current study was to compare cardiovascular magnetic resonance imaging (CMR)-derived parameters of left ventricular (LV) diastolic function between uncomplicated type 2 diabetes mellitus (DM2) and normoglycemic controls, and to evaluate whether these parameters of LV diastolic function are related to coronary atherosclerosis.

Methods

We prospectively studied 41 subjects with DM2 and 21 normoglycemic controls (30 women and 32 men, mean age 57.2 ± 7.1 years) with no evidence of overt cardiovascular disease. We used CMR to measure LV volumes, LV peak filling rate (PFR) and transmitral flow; and CT to determine coronary artery calcium scores.

Results

Absolute values of the peak filling rate (PFR) were significantly lower in DM2 patients than in control subjects (mean ± SD, 293.2 ± 51.7 vs 375.7 ± 102.8 mL/s, respectively; $P < 0.001$). Mitral peak E velocities (mean ± SD, 42.8 ± 10.7 vs 48.8 ± 10.4 cm/s; $P = 0.040$) and peak E velocity–to–peak A velocity ratios (0.88 ± 0.3 vs 1.1 ± 0.3; $P = 0.002$) were also lower in DM2 patients compared with control subjects. DM2 patients with coronary artery calcification showed a lower PFR normalized to stroke volume (SV) (mean ± SD, 4.4 ± 1.0 vs 5.3 ± 1.4, respectively; $P = 0.038$) and lower mitral peak E velocities (40.1 ± 11.3 vs 48.0 ± 7.3 cm/s; $P = 0.024$) than DM2 patients without coronary calcification. PFR normalized to SV was independently associated with the presence of coronary artery calcification ($\beta = -1.5$, $P = 0.005$).

Conclusions

Type 2 diabetes mellitus decreases CMR-derived parameters of left ventricular diastolic function. Patients with type 2 diabetes mellitus and coronary atherosclerosis show a more impaired left ventricular diastolic function compared with patients without coronary atherosclerosis.

2. Introduction

Cardiovascular disease is the most frequent cause of death and disability in type 2 diabetes mellitus (DM2).

Type 2 diabetes mellitus is responsible for diverse cardiovascular complications such as increased coronary atherosclerosis and left ventricular (LV) dysfunction. Left ventricular diastolic dysfunction is highly prevalent in patients with DM2 (1, 2), representing the earliest preclinical manifestation of LV dysfunction in this condition (3-6).

Over the last decade, cardiovascular magnetic resonance (CMR) imaging has been widely accepted as the "gold standard" for the assessment of cardiac structure and function because of its high spatial and temporal resolution, excellent image quality and lack of geometric assumptions (7). CMR offers a variety of alternative approaches for evaluating diastolic function (8, 9).

The presence of coronary calcium (CAC) is indicative of coronary atherosclerosis (10), which can be detected non-invasively by multidetector computed tomography (CT).

Coronary artery disease is commonly cited as a mechanism underlying diastolic dysfunction (11). Many of the same factors that contribute to atherosclerosis may also produce LV diastolic dysfunction by either direct mechanisms (e.g., hypertension and age-related vascular stiffening) or secondarily via coronary artery disease progression and resulting changes in myocardial compliance (11).

There are limited and controversial published data on the relationship of CAC to LV diastolic function. Edvardsen et al (12) demonstrated that coronary atherosclerosis is associated with depressed regional left ventricular systolic and diastolic wall strain, measured by MRI tagging. Colletti et al (13) demonstrated that CAC scores greater than 100 predicts an increased likelihood of clinically unsuspected left ventricular regional wall motion abnormalities, which are associated with lower LV ejection fractions and peak filling rates, as assessed with cardiac MRI. Eleid et al (14) study pointed to different conclusions. Their results in asymptomatic patients with normal LV ejection fraction and negative cardiac stress test showed that CAC does not correlate with LV diastolic function as defined by established Doppler echocardiographic criteria.

Although all of these studies included patients with DM2, none specifically studied this population. Accordingly, the purpose of the current study was to compare CMR-derived parameters of diastolic function between well-controlled uncomplicated DM2 and normoglycemic controls, and to

evaluate whether these CMR-derived parameters of LV diastolic function are related to coronary atherosclerosis

Cardiovascular magnetic resonance imaging was used to measure parameters of LV diastolic function, while the presence of coronary atherosclerosis was assessed by means of CAC scoring.

We hypothesize that in asymptomatic diabetic patients, diastolic function as measured by mitral flow velocities and peak filling rate (PFR) will be reduced with the presence of coronary atherosclerosis. We also hypothesize that asymptomatic diabetic patients will have reduced diastolic function compared to normoglycemic controls.

3. Materials and methods

Study population

We prospectively enrolled 41 subjects (21 women and 20 men) with DM2 enrolled based on referral to the outpatient clinical department of diabetology of our institution and 21 age and gender-matched normoglycemic controls (9 women and 12 men) recruited from the community.

Subjects were included if they met the following inclusion criteria: age between 45-75 years, no symptoms or history of overt heart disease (cardiomyopathy, coronary artery disease or valvular heart disease), no signs or symptoms of cerebrovascular disease, no abnormal findings on routine clinical and physical examination and a normal ECG.

Exclusion criteria were: LV ejection fraction (LVEF) < 55%, regional LV wall motion abnormalities and valvular heart diseases assessed by CMR, severe renal failure, claustrophobia, CMR images unsuitable for quantification, type 1 DM, severely uncontrolled DM, defined as glycated hemoglobin > 12%, and uncontrolled blood pressure at rest (defined as systolic blood pressure > 180 mm Hg and/or diastolic blood pressure > 100 mm Hg). Subjects with contraindications to CMR were not enrolled.

The study was approved by our institutional ethics committee. Each subject gave written informed consent.

Coronary artery calcium score, data acquisition and analysis

Coronary artery calcium (CAC) scoring was performed only in the DM2 group, using a 64-slice CT scanner (LightSpeed VCT XT, GE Healthcare, Milwaukee, USA). For this purpose, a non-enhanced low-dose ECG-gated scan was performed with prospective triggering at 75% of the R–R interval.

All examinations included the entire coronary tree and were performed with the patient breath-holding in inspiration. Scan parameters were as follows: field of view of 25 cm, slice thickness of 2.5 mm, gantry rotation time 0.35 s, tube current 100–400 mA, and tube voltage 120 kV.

CAC score was determined by an experienced observer using dedicated software (SmartScore, version 4.0, GE Healthcare, Milwaukee, USA). Total CAC score was calculated for each patient using the Agatston method (15). For the final analysis patients were categorized in two groups: Group 1, CAC score > 0; Group 2, CAC score = 0.

Cardiovascular magnetic resonance protocol

CMR imaging was performed by using a 3.0-T unit (Magnetom Trio; Siemens, Erlangen, Germany). Cardiac cine images were acquired by using steady-state free-precession sequences with retrospective electrocardiographic gating (16). Participants were imaged in the supine position and performed a breath-hold at end expiration for each image acquisition to eliminate respiratory motion artifacts. After scout images were obtained, cine imaging was performed in four-chamber, three-chamber, and two-chamber long- and short-axis views with the use of the following parameters: 8-mm-thick sections with a 2-mm gap between sections, repetition time 59.04 ms, echo time1.45, number of segments 18; 50°flip angle, 256×156-mm matrix, 2.1 ×1.6-mm pixel size, acquired temporal resolution 25-40 ms; and number of reconstructed cardiac phases, 25.

A breath-held, retrospectively vector-ECG gated, two-dimensional flow-sensitive phase-contrast gradient-echo sequence was used for velocity encoded (VENC) MRI flow measurements perpendicular to the orifice of the mitral valve. VENC-MRI slices were positioned in early diastole at the tip of the mitral valve leaflets. Typical imaging parameters of VENC-MRI were as follows: slice thickness 6 mm, repetition time 35.6,ms, echo time 2.33 ms, number of segments 3, acquired temporal resolution 24-56ms, 20° flip angle, 25 calculated phases and pixel spacing 4.5×3.1 mm. Encoding velocity was set to 130 cm/s (9).

MR image analysis

Quantitative image data analysis was performed by using dedicated software (Segment, version 1.8, Medviso, Lund, Sweden) (17). All functional evaluations were performed within 25 minutes per patient.

Tracing of endocardial and epicardial contours

All measurements were undertaken semi-automatically. End-diastolic and

end-systolic frames were identified according to the ventricular blood pool area. In all LV short-axis slices across all temporal phases (200-250 images) endocardial and epicardial contours were semi-automatically drawn and manually corrected (Fig. 1). This segmentation took on the order of 3–5 minutes. At the base of the left ventricle, the aortic outflow tract below the valve was included in volume measurements. The free papillary muscles were included for LV mass assessment, and excluded for left ventricular volume assessment. In the basal region of the heart where the left atrium was seen, only the portion of the slice that could be identified as the left ventricle was included for measurement.

This analysis provided the time-varying course of the LV volume during the cardiac cycle. The peak filling rate (PFR) is the steepest tangent to the first part of the filling curve (Fig. 2). Segment software automatically determines this parameter.

Analysis of the transmitral flow

In the flow sensitive sequence, a round region of interest with a minimum size of 1 cm2 was placed at the center of the mitral valve orifice on a diastolic image and propagated to other phases to obtain the transmitral curve (9). From the analysis of the transmitral flow curve, the following measurements were performed: early phase of ventricular filling (E) and atrial phase of ventricular filling (A) mean peak velocities (in centimeters per second) and peak E velocity–to–peak A velocity (E/A) ratio.

Statistical analysis

All continuous variables were tested for normal distribution. All normally distributed data are expressed as means ± standard deviations. Categorical variables are expressed as counts and percentages. Between-group differences of the average were compared using the unpaired Student's t-test for parametric data and the Mann-Whitney U test for non-parametric data. Between-group differences in numbers and percentages were compared using the Chi-squared test (Fisher's exact test).

The distribution of CAC score was skewed, and therefore, medians and ranges were reported.

Logarithmic transformation of CAC scores— that is, log (total CAC score + 1)—was used for parametric evaluation.

Pearson's correlation test was used for sets of normal variables. Independent associations between coronary artery calcification and LV diastolic values were studied by using a linear regression with multivariable adjustments for potentially confounding factors (eg, age, gender, history of hypertension, body mass index, duration of diabetes, LV ejection fraction and LV mass

index).

P-values of less than 0.05 were considered statistically significant. All computations were performed using software (SPSS, version 20.0; SPSS, Chicago, IL, USA).

4. Results

Forty-one DM2 patients and 21 age- and sex-matched normoglycemic control sub- jects were studied. Demographic, clinical, and imaging data of the patients and control subjects are presented in Table 1. The mean age was 57.2 ± 7.1 years (age range, 45–72 years) and 32 (52%) patients were male.

As expected, patients with DM2 had higher BMI (P<0.001) than controls. Our population of DM2 patients had a higher (P=0.001) prevalence of systemic arterial hypertension (HT), compared with controls.

Left ventricular function

Patients with DM2 had normal conventional parameters of systolic function including LVEF, and a normal LV mass index, compared with controls.

The absolute values of PFR were significantly higher in controls (Fig. 1), compared with DM2 patients (Fig. 2) (P<0.001).

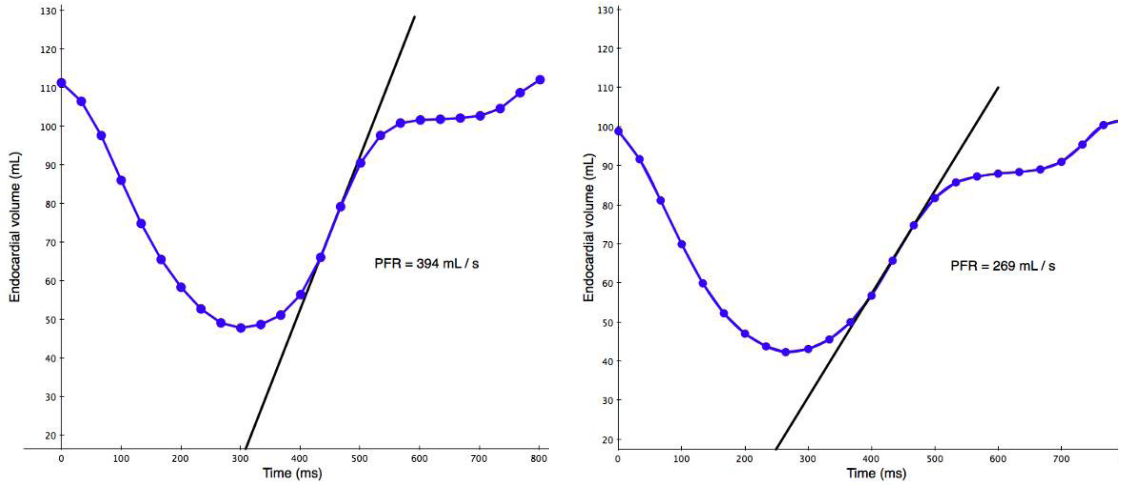

Fig. 1–A) 48-year-old normoglycemic man. Left ventricular filling volume versus time curve and its first derivative, the peak filling rate curve. The LV volume versus time curve and its first derivative (LV dV/dt) are obtained after the endocardial delineation of all LV short-axis slices across all temporal phases. The peak filling rate is the steepest tangent to the first part of the filling curve and represents the most rapid ventricular filling. The value obtained was 394 mL/s. B) 45-year-old man with type 2 diabetes mellitus. Left ventricular filling volume versus time curve and the peak filling rate obtained (269 mL/s).

Table 1 Characteristics of the study population

Characteristics	DM2 group (n=41)	Controls (n=21)	*P*
Age (years), mean ± SD	58.2±7.2	54.9±6.5	0.078
Male gender, n (%)	20 (49)	12 (57)	0.533
BSA (m2), mean ± SD	1.85±0.21	1.80±0.16	0.359
BMI (kg/ m2), mean ± SD	29.3±4.6	25.0±1.7	< 0.001
Diabetes duration (y), mean ± SD	13.3±8.9	-	
Hypertension n(%)a	30 (73)	6 (29)	0.001
LV EDV (mL), mean ± SD	96.3±20.8	118.7±25.1	0.001
LV EDV / BSA (mL/m2), mean ± SD	52.2±10.9	65.6±11.4	< 0.001
LV SV (mL), mean ± SD	63.7±14.5	79.9±14.4	< 0.001
LV EF (%), mean ± SD	66.7±8.0	67.9±5.3	0.485
LV mass / BSA (g/m2), mean ± SD	68.2±13.6	65.6±10.0	0.474
LV PFR (mL/s), mean ± SD	293.2±51.7	375.7±102.8	< 0.001
LV PFR/EDV (mL/s), mean ± SD	3.1±0.7	3.2±0.8	0.616
LV PFR/SV (mL/s), mean ± SD	4.7±1.1	4.7±1.0	0.979
Mitral peak E velocity (cm/s), mean ± SD	42.8±10.7	48.8±10.4	0.040
Mitral peak A velocity (cm/s), mean ± SD	49.8±11.2	45.0±8.6	0.069
Mitral E/A ratio, mean ± SD	0.88±0.3	1.1±0.3	0.002

Note—Dash (—) indicates not applicable. DM2 = type 2 diabetes mellitus, BSA = body surface area, BMI = body mass index, LV = left ventricular, EDV = end-diastolic volume, SV = stroke volume, LVEF = left ventricular ejection fraction, PFR = peak filling rate, E/A ratio = peak E velocity–to–peak A velocity ratio. Blood pressure > 140/90 mm Hg or treatment with antihypertensive medication.

The values of PFR indexed to end-diastolic volume (EDV) and stroke volume (SV) in DM2 patients and control subjects were similar ($P = 0.616$, $P = 0.979$, respectively).

An analysis of the transmitral parameters showed lower mitral peak E velocities ($P = 0.040$) and lower E/A ratios ($P = 0.002$) in DM2 patients compared with control subjects. Mitral peak A velocities were higher in the DM2 group, although not significantly so ($P = 0.069$).

To further address the relationship between hypertension and diastolic dysfunction in the patient population, we performed a subgroup analysis of the parameters of diastolic dysfunction in the DM2 group according to the presence or absence of hypertension. The results obtained are presented in Table 2.

In this subgroup comparison, all parameters of LV diastolic function were similar in both groups.

Table 2 Left ventricular diastolic function parameters in the DM2 group according to the presence of systemic arterial hypertension

Parameters	DM2 only group (n=11), Mean±SD	DM2 and HT group (n=30), Mean±SD	*P*
LV PFR (mL/s)	293.0±52.6	292.1±52.7	0.964
LV PFR/EDV (mL/s)	3.0±0.8	3.2±0.7	0.356
LV PFR/SV (mL/s)	4.5±1.1	4.8±1.2	0.454
Mitral peak E velocity (cm/s)	43.0±9.4	42.8±11.4	0.948
Mitral peak A velocity (cm/s)	50.4±6.5	49.5±12.6	0.774
Mitral E/A ratio	0.85±0.19	0.89±0.27	0.610

Note—PFR = peak filling rate, EDV = end-diastolic volume, SV = stroke volume, E/A ratio = peak E velocity–to–peak A velocity ratio.

Coronary atherosclerosis

In the DM2 group, average CAC score was 344 ±754 (range 0 – 4697). CAC was was less than 10 in 18 (44%) DM2 patients. Nine of 42 (21%) DM2 patients had CAC scores greater than 10.

Parameters of diastolic function and calcium score

Table 3 shows baseline and left ventricular systolic and diastolic parameters of DM2 subjects according to the presence or absence of coronary artery calcification.

Table 3 Characteristics of patients with DM2 according to the presence or absence of coronary calcification

	Calcium score > 0 (n=27)	Calcium score = 0 (n=14)	*P*
Age (y), mean ± SD	60.2±6.7	54.6±6.8	0.018
Male gender, n (%)	15 (56)	5 (36)	0.228
BSA (m2), mean ± SD	1.86±0.17	1.84±0.27	0.763
BMI (kg/m2), mean ± SD	29.1±4.5	30.0±4.6	0.550
Diabetes duration (y), mean ± SD	14.9±9.0	9.4±8.4	0.060
Hypertension, n(%)a	19 (70)	11 (79)	0.574
LV EDV (mL), mean ± SD	97.5±22.7	93.9±17.1	0.567
LV EDV / BSA (mL/m2), mean ± SD	52.4±10.8	51.8±11.4	0.863
LV SV (mL), mean ± SD	65.9±15.6	59.4±11.5	0.139
LV EF (%), mean ± SD	68.3±8.9	63.7±6.6	0.065
LV mass / BSA (g/m2), mean ± SD	70.9±14.3	63.1±10.6	0.055
LV PFR (mL/s), mean ± SD	286.6±54.2	306.4±45.3	0.220
LV PFR/EDV (mL/s), mean ± SD	3.0±0.7	3.4±0.8	0.142
LV PFR/SV (mL/s), mean ± SD	4.4±1.0	5.3±1.4	0.038
Mitral peak E velocity (cm/s), mean ± SD	40.1±11.3	48.0±7.3	0.024
Mitral peak A velocity (cm/s), mean ± SD	49.8±13.2	49.8±6.2	0.997
Mitral E/A ratio, mean ± SD	0.83±0.3	0.96±0.2	0.086

BSA, body surface area; BMI, body-mass index; LV, left ventricle; EDV, end diastolic volume; SV, stroke volume; EF, ejection fraction; PFR, peak filling rate.

[a]Blood pressure > 140/90 mmHg or treatment with anti-hypertensive medication.

DM2 patients with coronary calcification were older (*P*=0.018) and had longer duration of diabetes mellitus, although not significantly so (*P*=0.060). Comparing DM2 subjects according to the presence or absence of coronary calcification, we found no differences in terms of gender, BSA, BMI or the presence of hypertension. The analysis of LV systolic function and LV mass showed also no statistically significant difference between DM2 with or without coronary calcification.

Regarding parameters of diastolic function, DM2 subjects with coronary calcification showed lower PFR/SV (Figs. 3A and 3B) (*P*=0.038) and lower mitral peak E velocities (*P*=0.024), compared to DM2 subjects without coronary calcification (Figs. 4A and 4B).

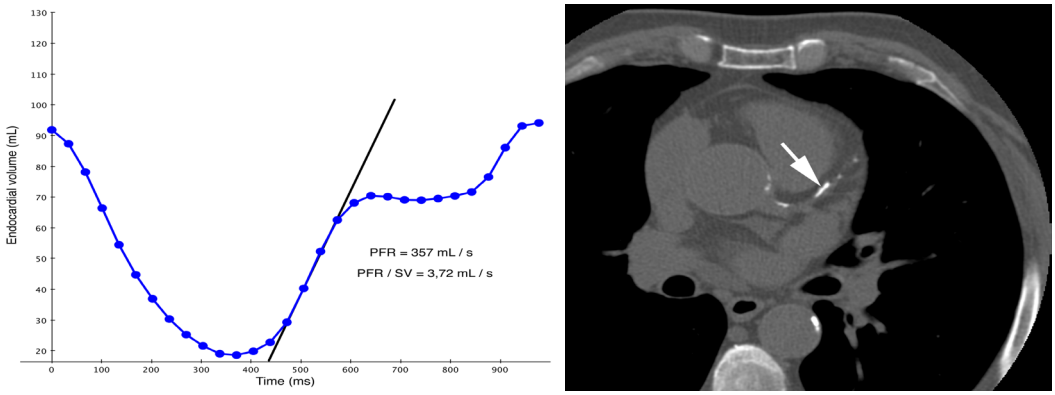

Fig. 3-67-year-old man with type 2 diabetes mellitus and coronary artery calcification.

A, Left ventricular filling volume versus time curve and peak filling rate (PFR) obtained (357 mL/s). PFR is steepest tangent (line) to first part of filling curve and represents most rapid ventricular filling. PFR indexed to stroke volume (PFR/SV) was 3.72 mL/s.

B, CT image used for coronary artery calcium scoring shows coronary artery calcifications (arrow). Agatston calcium score calculated by CT was 410.

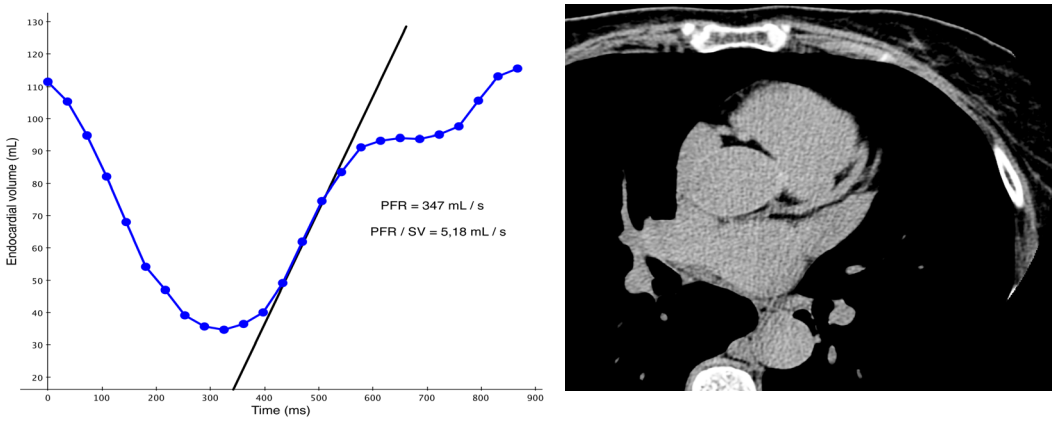

Fig. 4—55-year-old woman with type 2 diabetes mellitus and no coronary artery calcification.

A, Left ventricular filling volume versus time curve and peak filling rate (PFR) obtained (347 mL/s). PFR is steepest tangent (line) to first part of filling curve and represents most rapid ventricular filling. PFR indexed to stroke volume (PFR/SV) was 5.18 mL/s.

B, CT image used for coronary artery calcium scoring shows no coronary artery calcifications. Agatston calcium score calculated by CT was 0.

Univariable and Multivariable analysis

In the DM2 group, an increasing CAC score was negatively correlated with PFR/SV (r=-0.68, *P*<0.001; Fig. 5).

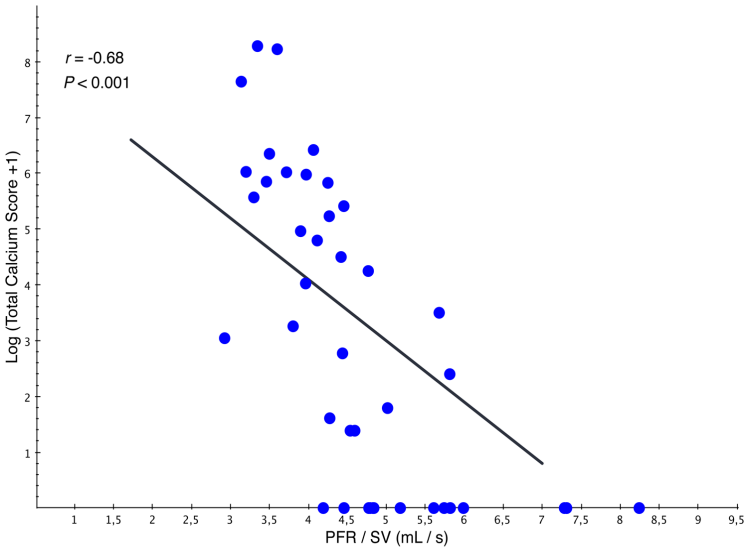

Fig. 5 – Relationship of PFR / SV with CAC

By multivariable analysis, PFR/SV was independently associated with the presence of coronary calcification (β = –1.5, $P = 0.005$) after adjustment for age, sex, BMI, presence of hypertension, diabetes duration, LVEF, and LV mass index.

5. Discussion

In this study, coronary artery calcium score and parameters of diastolic function in patients with type 2 diabetes mellitus were investigated. The major observations were as follows: A) Differences in diastolic function were observed between DM2 patients and control subjects, in particular, in LV PFR values, mitral peak E velocities and E/A ratios; B) Parameters of diastolic function were different between DM2 patients with or without coronary calcification, in particular LV PFR/SV values and mitral peak E velocities.

Differences in left ventricular diastolic measures

Left ventricular filling profiles have been used to assess diastolic function by other imaging investigations, such as radionuclide cineangiography and SPECT. Similar applications for CMR imaging have been impractical because manual planimetry of all LV images across all temporal phases would typically require tracing of > 200 images per patient. With the improvements in post-processing tools it is now possible to semi-automatically segment all phases and quickly provide the time-varying course of the LV volume during the cardiac cycle. The ventricular relaxation

abnormalities can be diagnosed by a low peak filling rate. This evaluation is based on conventional short-axis sequences, without the necessity to perform a dedicated acquisition. The use of indexed values of PFR to EDV and SV minimizes the dependency of PFR from EDV and heart rate. We demonstrated that the left ventricular peak filling rate can be easily obtained as an addition to the assessment of LV systolic function with semi-automatic contour detection and it may become a valuable asset to the evaluation of LV diastolic function.

In the current study, parameters of LV diastolic function were impaired in DM2 patients compared with normoglycemic controls. Mitral peak E velocity, E/A ratio and LV peak filling rate were lower in DM2 patients, indicating impaired myocardial relaxation and/or increased myocardial stiffness, which are the hallmarks of diastolic dysfunction (18). DM2-related changes in LV diastolic properties are well recognized with a decrease in early diastolic filling, directly influenced by abnormal LV relaxation, mainly due to a derangement in energy supply, and an increased passive stiffness due to remodeling (19).

We acknowledge that coexisting arterial hypertension augments the impairment of LV diastolic function in diabetic patients, and may partially explain the changes reported.

Interestingly, Di Bonito et al observed diastolic dysfunction in 16 normotensive patients with type 2 diabetes without microvascular complications and a short disease duration (even less than 1 year) (20). Likewise, previous studies showed that more than 40% to 75% of normotensive diabetic patients under excellent glycemic control had diastolic dysfunction (1, 2, 21). In our study parameters of diastolic function were not different in the patients with DM2 and HT, compared to patients with DM2 without HT.

Diastolic function and coronary atherosclerosis

Our study in DM2 patients shows an association between diastolic function assessed by CMR and coronary calcification assessed by CT.

In our cohort of DM2 patients, lower PFR/SV and mitral peak E values were significantly associated with coronary calcification. Furthermore, the presence of coronary calcification was independently associated with PFR/SV, increasing the strength of this association. Our results are consistent with the findings of other studies. Colletti et al (13) studied 386 subjects, including 39 patients with diabetes mellitus, and also found a relation of a CAC > 100 with decreased MRI-derived parameters of LV diastolic filling. Scholte et al (22) have reported similar conclusions. Their results point to an association of coronary atherosclerosis with subclinical

left ventricular dysfunction evaluated in type 2 diabetic patients, using echocardiographic derived indices of diastolic (transmitral and pulmonary vein flow recording) and systolic function (global longitudinal strain) (22).

Mechanisms underlying the relationship of subclinical atherosclerosis and diastolic function are probably related to vascular function.

Poulsen et al (23) suggested that abnormal LV filling in DM2 patients is closely associated with abnormal myocardial perfusion on myocardial perfusion scintigraphy. Macrovascular coronary disease could also contribute to this subclinical myocardial damage (22). In coronary macrovasculature, formation of atheroma may lead to luminal obliteration, recurrent thrombosis, distal embolization, and clinically silent micro-infarctions (11).

The findings of the present study contribute to the available knowledge, favoring the hypothesis that there is a link between subclinical LV diastolic dysfunction and asymptomatic atherosclerosis.

Our DM2 patients with coronary artery calcification had greater LV mass than the DM2 patients without coronary calcification, but the difference did not quite achieve acceptable levels of statistical significance (P=0.055). LV mass represents an end point of cumulative LV insult and although not a diagnostic criteria for LV diastolic dysfunction, increased LV mass is associated with diastolic dysfunction, from abnormal relaxation to coexistent restrictive physiology due to increased myocardial stiffness (24). Systemic arterial hypertension and diabetes mellitus are known causes LV hypertrophy. Notwithstanding the high prevalence of arterial hypertension in our population, none of our subjects had an LV mass above the normal range (LV mass indexed to BSA > 86g/m2 for males and >72g/m2 for females) (25, 26).

The originality of our study is the use of CMR-derived parameters of diastolic function and the relatively high number of DM2 patients with low CAC. We had a relatively large percentage of DM2 patients with CAC equal to zero, something that we were not expecting in a population of DM2 patients between 45 and 72 years of age.

A basic limitation of this study must be acknowledged. This was a cross-sectional study with a relatively small number of subjects; therefore, studies using larger patient cohorts are needed to clarify the association between LV diastolic function and coronary atherosclerosis.

Our findings are limited by an inability to eliminate causal relationships with important clinical factors, as medication used, serum concentrations of glucose, and HbA1c, dyslipidemia, retinopathy, microabuminuria and smoking status. Also, the high prevalence of hypertension in our population

makes it difficult to interpret the findings and clarify the responsibility of diabetes in the results obtained.

The possibility of influence of myocardial ischemia on LV diastolic function cannot be completely excluded. The evaluation of coronary stenosis and plaque characterization was not performed in our study, since CT angiography is not recommended in asymptomatic type 2 diabetic patients. Since myocardial perfusion, endothelial function, coronary flow reserve and arterial stiffness were not evaluated in the present study, a more comprehensive understanding of the pathophysiological process should be the focus of future research.

We considered that the analysis of cine and transmitral flow images was sufficient to exclude left-sided valvular disease as a potential pitfall of abnormal transmitral flow. Regarding the exclusion of infiltrative myocardial disease, we did not study myocardial delayed enhancement nor did we performed endomyocardial biopsy. Nevertheless, none of our subjects had increased LV myocardial end-diastolic thickness, pericardial thickening, pericardial effusion or pleural effusion.

Recent findings (27) shows improvement in transmitral flow assessment by applying three-dimensional three- directional velocity-encoding, over the conventional approach of two-dimensional one- directional through-plane velocity-encoded MRI with a fixed acquisition plane used in our study.

Normalizing early mitral velocity (E) for the influence of myocardial relaxation by combining E with early diastolic mitral septal tissue velocity (Ea) may be performed by MR tissue phase contrast imaging, and this is an important parameter of diastolic function that has been established (28). On the other hand there are still conflicting data regarding the relation of E/Ea ratio and LV filling pressure (29, 30).

This study highlights certain key points for the routine use of CMR to study diastolic function in diabetic patients and the relation of diastolic function with coronary atherosclerosis. Firstly, CMR shows a decrease in diastolic function in DM2 and may allow the diagnosis of subclinical diastolic dysfunction in this high-risk population. Secondly, diastolic function is possibly related to the presence of coronary atherosclerosis.

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Chapter VII

Disease duration as a major determinant of increased coronary artery calcium score in asymptomatic patients with type 2 diabetes

Bruno Graça, Maria João Ferreira,
Paulo Donato, Leonor Gomes,
Miguel Castelo Branco, Filipe Caseiro Alves

Submitted

1. Abstract

Background

Coronary artery calcium (CAC) measurement reflects coronary atherosclerosis and predicts coronary events beyond conventional risk factors. We sought to evaluate the prevalence and severity of CAC scores in asymptomatic diabetes, and explore its association with other conventional risk factors of atherosclerosis.

Methods

We prospectively studied 66 asymptomatic subjects with type 2 diabetes mellitus aged 40-75 years (mean age 60,2 years) who underwent coronary calcium CT scanning to assess CAC score. Clinical, demographic and laboratorial data were collected. Carotid atherosclerosis was studied by carotid ultrasonography. Statistical analysis was performed.

Results

In our population of asymptomatic subjects with type 2 diabetes mellitus, coronary calcified plaque was found in 70% of subjects. In 41% of subjects, the CAC score was <10; in 27% of subjects, the CAC score was > 400. There was a statistically positive correlation of CAC score with age (rho = 0.257; $P = 0.037$), duration of diabetes (rho = 0.327; $P = 0.008$) and carotid atherosclerosis (rho = 0.420; $P < 0.001$). In the multivariate analysis, male gender ($P < 0.001$) and diabetes duration ($P = 0.002$) demonstrated to be independent predictors of the CAC score.

Conclusion

In asymptomatic patients with type 2 diabetes mellitus, disease duration is an independent determinant of CAC score and could explain the heterogeneity of CAC scores observed in this population. These findings emphasize the critical importance of aggressive cardiovascular disease risk reduction in patients with type 2 diabetes mellitus early in the course of the disease.

2. Introduction

Coronary artery disease (CAD) is a major cause of death and impairment of quality of life (1) in patients with type 2 diabetes mellitus. However, type 2 diabetic patients may have long-standing subclinical coronary artery disease (2). Thus, early detection of CAD may improve prognosis (3). Cardiovascular risk assessment based on conventional risk factors proved to be consistently unreliable in this high risk population, since they not directly reflect structural and functional changes in arteries associated with CAD (4, 5).

In asymptomatic type 2 diabetic patients, few studies have investigated the association of coronary calcium with other traditional risk factors of CAD or with other markers of peripheral atherosclerosis, such as ultrasonographically assessed carotid atherosclerosis (6).

Previously published reports have demonstrated, in diabetes mellitus, greater coronary artery calcium (CAC) score (7-10). The present study supplements the existing reports of the association between CAC score on CT and asymptomatic diabetes, and explores its association with other risk factors of CAD and with carotid atherosclerosis.

Our hypothesis was that asymptomatic subjects with type 2 diabetes mellitus would show a wide range of CAC scores, and we sought to investigate what factors could influence the CAC score in this population.

3. Materials and methods

3.1. Study population

Sixty-six patients (31 women and 35 men) with type 2 diabetes mellitus were prospectively recruited based on referral to the outpatient clinical department of diabetology of our institution. Detailed information regarding symptoms, physical examination, medical history and risk factors were collected prospectively at the time of presentation. Patients were included if they met the following inclusion criteria: age between 40-75 years, no symptoms or history of overt heart disease (cardiomyopathy, coronary artery disease or valvular heart disease) and therapy for DM2 including oral hypoglycaemic agent or insulin.

Hypertension was defined as a blood pressure >140/90 mm Hg or treatment with antihypertensive medication. Hypercholesterolemia was

defined as a total cholesterol level > 190 mg/dL or use of lipid-lowering medication. There was no history of smoking habits in our population.

Exclusion criteria were: severely uncontrolled diabetes mellitus (glycated hemoglobin > 12%), uncontrolled blood pressure at rest (defined as systolic blood pressure > 180 mm Hg and/or diastolic blood pressure > 100 mm Hg) and the presence of ventricular and supraventricular arrhythmias.

The study was approved by our institutional ethics committee. Each subject gave written informed consent.

3.2. Coronary artery calcium score, data acquisition and analysis

Agatston score was performed using a 64-slice CT scanner (LightSpeed VCT XT, GE Healthcare, Milwaukee, USA). For this purpose, a non-enhanced low-dose ECG-gated scan was performed with prospective triggering at 75% of the R–R interval. All scans included the entire coronary tree and were done with breath held in inspiration. Scan parameters were as follows: field of view of 25 cm, slice thickness and increment of 2.5 mm, gantry rotation time 0.35 s, tube current 100–400 mA, and tube voltage 120 kV.

CAC score was determined by an experienced observer using dedicated software ('SmartScore', GE Healthcare, Milwaukee, USA). Total CAC score was calculated for each patient using the Agatston method (11).

3.3. Carotid Ultrasound study

Scanning of the extra-cranial common carotid artery, the carotid bulb, and the internal carotid artery in the neck was performed by an experienced observer bilaterally in the longitudinal and transversal planes, using a GE Healthcare logic 9 with a 9 linear (8 MHz) probe. Carotid intima-media thickness (CIMT) was measured in the common carotid artery as the distance between 2 parallel echogenic lines corresponding to the blood-intima and media-adventitia interface on the posterior wall of the artery. Determinations of carotid intima-media thickness were performed at the site of the thickest point and values below 0.8 mm were considered as normal. Localized elevated lesions with maximum thickness of more than 1 mm, having a point of inflection on the surface of the intima-media complex, were defined as "plaques". Stenosis was defined as > 50% occlusion, according to established consensus (12). Patients were

categorized in three groups: Group 1, no atherosclerosis (normal carotid ultrasound study); Group 2, mild atherosclerosis (presence of increased CIMT); Group 3, moderate to severe atherosclerosis (presence of carotid plaque or stenosis).

3.4. Statistical analysis

All continuous variables were tested for normal distribution. All normally distributed data are expressed as means ± standard deviations. Categorical variables are expressed as counts and percentages. The distribution of Agatston scores was skewed, and therefore, medians and ranges were reported.

Logarithmic transformation of Agatston scores, log (total calcium score +1), was used for parametric evaluation.

Bivariable correlations of CAC scores were performed using the Pearson or Spearman method as appropriate.

Cross-tabulated statistics were provided in the categorical comparison between the Agatston groups, and the level of significance was determined using the Chi-Square test. Multiple linear regression analysis was used for prediction of LnAgatston score. *P*-values of less than 0.05 were considered statistically significant. All computations were performed using software (SPSS, version 20.0; SPSS, Chicago, IL, USA).

4. Results

Baseline characteristics of the study population are presented in Table 1.

Table 1 Baseline characteristics of the study population

	DM2 subjects (n=66)
Age (years)	60.2±7.4
Male gender, n (%)	35 (53)
Height (cm)	162.3±9.9
Weight (kg)	78.7±12
Body-mass index (kg/m2)	30.1±5.3
Diabetes duration, years	13.6±8.9
Hypertension*, n (%)	46 (70)
Hypercholesterolemia†, n (%)	30 (46)
Glycosylated hemoglobin (%)	9.2±2.2
Agatston score	534.6±1021.5
LnAgatston	3.65±2.98

LnCAC, logarithmically transformed CAC score; BMI, body mass index
Data are averages ± SDs or numbers of patients (percentages).
* Blood pressure > 140/90 mm Hg or treatment with antihypertensive medication.
† Total cholesterol level > 190 mg/dL or use of cholesterol-lowering medication.

Mean CAC score was 535 ±1021 (range 0 – 4697) in the total population. In 20 patients (30%) CAC score was absent; in 27 patients (41%) CAC score was <10; in 36 patients (55%) CAC score was < 100; in 18 patients (27%) CAC score was > 400.

Regarding extra-cranial carotid atherosclerosis, 14% of the subjects (n=9) had a normal carotid ultrasound study. In 22 patients (33%) carotid ultrasound found increased CIMT; in 33 patients (50%) were reported non significative plaques and in 2 patients (3%) were diagnosed with carotid stenosis.

3.1. Determinants of coronary calcium score

Logarithmic transformed CAC score showed a statistically positive correlation with age, duration of diabetes (Fig. 1) and carotid atherosclerosis. The results are presented in Table 2.

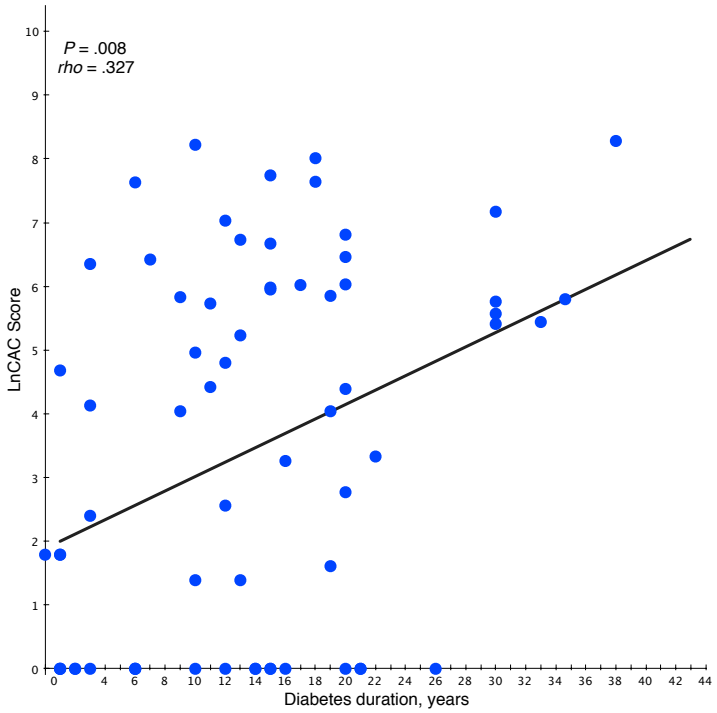

Fig. 1 – Relationship of Agatston score with duration of diabetes

Table 2 Correlation between LnAgatston scores and other parameters.

	rho	*P* value
Age (years)	0.257	0.037
Height (cm)	0.203	0.201
Weight (kg)	0.133	0.288
BMI (kg/m2)	-0.147	0.240
Diabetes duration, years	0.327	0.008
Glycosylated hemoglobin (%)	-0.211	0.091
Carotid atherosclerosis	0.420	<0.001

LnCAC, logarithmically transformed CAC score; BMI, body mass index

To evaluate the relation of gender, hypertension and hypercholesterolemia with CAC scores we performed a Chi-squared analysis. The results are presented in table 3. Male gender was significantly associated with an Agatston score > 10.

Table 3 Relation of Agatston scores with gender, hypertension and hypercholesterolemia

	CAC score < 10 (n=27)	CAC score >=10 (n=39)	*P* Value
Male gender, n (%)	9 (33)	26 (67)	0.008
Hypertension*, n (%)	20 (74)	26 (67)	0.520
Hypercholesterolemia†, n (%)	13 (48)	17 (44)	0.715

Data are numbers of patients (percentages).
* Blood pressure > 140/90 mm Hg or treatment with antihypertensive medication.
† Total cholesterol level > 190 mg/dL or use of cholesterol-lowering medication.

In order to determine the factors affecting the LnCAC score, a model was created with age, male gender, duration of diabetes and carotid atherosclerosis (Table 4). Regression analysis predicted male gender and diabetes duration as statistically significant factors affecting the LnCAC score (Table 4).

Table 4 Predictors of CAC score identified by multiple linear regression analysis

	β	*P* value	95% confidence interval
Age, years	-0.003	0.948	-0.097 -0 .090
Male gender	2.706	<0.001	1.466 - 3.946
Diabetes duration, years	0.117	0.002	0.046 - .189
Carotid atherosclerosis	0.755	0.109	-0.173 - 1.683

5. Discussion

IIn this study, coronary artery calcium score and carotid atherosclerosis in asymptomatic patients with type 2 diabetes mellitus were investigated. The major observations were as follows:

First, CT could detect coronary calcified plaques in 70% of asymptomatic patients with type 2 diabetes. In addition, in 27% of patients a markedly elevated CAC score (>400) was observed. Furthermore, we had a relatively large percentage (41%) of type 2 diabetes mellitus patients with CAC score < 10, something that we were not expecting in a population of type 2 diabetes mellitus patients between 45 and 72 years of age.

After correction for traditional risk factors, male gender and diabetes duration were found to be independent predictors of increasing coronary calcium scores.

In our population, carotid atherosclerosis was not an independent predictor of coronary calcium scores.

When compared to other traditional risk scoring systems, calcium scores have been shown to be significantly superior in predicting silent myocardial ischemia and short-term outcomes, compared to United Kingdom Prospective Diabetes Study Risk Score, UKPDS and Framingham risk score (13). In an 8-year follow up study it was shown that those who had higher CAC score (>400) had significantly higher prevalence of cardiac events compared to those with lower scores (14).

However, on the other side, knowing that diabetics constitute a higher risk group, studies showed that having a low calcium score could be helpful to re-stratify them into a lower risk category with low cardiac events and excellent survival rates (15). This was also demonstrated by Anand et al that prospectively measured CAC score in asymptomatic type 2 diabetic subjects without prior cardiovascular disease. No cardiac events or perfusion abnormalities occurred in subjects with CAC score ≤10 up until 2 years of follow-up (13). In a 5-year follow up study for 903 asymptomatic diabetics, patients suffering from diabetes with no coronary artery calcium demonstrated a survival similar to that of individuals without diabetes and no detectable calcium (3).

Using coronary CT angiography in asymptomatic patients remains controversial, primarily because of the higher radiation dose, added cost, and use of nephrotoxic contrast, but it has the potential to identify useful data beyond what is derived from CAC score.

As detailed in the 2010 Guideline for Appropriate Use Criteria for Cardiac Computed Tomography, coronary CT angiography is not recommended for cardiovascular risk assessment in asymptomatic adults (16).

The relation of male gender with coronary atherosclerosis demonstrated in our study is in line with previous publications. In the general population, as with the prevalence of clinical coronary disease, CAC scores are higher in men compared to women (17). One of the largest studies that examined the age and gender distribution of CAC score in asymptomatic diabetic subjects found that at any given age, men exhibited significantly greater calcified plaque burden, as compared with women (10).

Moreover, the influence of age at onset and duration on the impact of diabetes on cardiovascular disease risk is not fully established.

Previous studies (18-20) suggested that cardiovascular disease risk in patients with diabetes escalates significantly with disease duration. A recent study evaluating the prevalence and severity of calcified plaque in coronary arteries in patients aged <40 years included 142 diabetics and

demonstrated that a high percentage of young patients with diabetes have detectable coronary atherosclerosis (21).

Nevertheless, the impact of diabetes duration on coronary artery calcification is not yet established.

In this asymptomatic population of type 2 diabetes mellitus subjects, a significant relation was observed between disease duration and the presence of atherosclerosis determined by the CAC score. Furthermore, after correction for traditional risk factors, diabetes duration was found to be an independent predictor of the CAC score.

A longer exposure to chronic hyperglycemia may be the explanation. Other factors, such as worsening beta-cell function and thus insulin insufficiency, could also play a role (22). Beta-cell function is not easy to measure in the clinical practice. Because chronic hyperglycemia and insulin insufficiency generally coexist in persons with longer diabetes duration, it is difficult to determine their independent roles. Irrespective of mechanisms, the clinical implication is that this emphasizes the need to be aggressive with cardiovascular disease risk reduction in patients with type 2 diabetes early in the course of the disease.

The relationship between carotid CIMT and coronary artery disease on CT has been previously studied in different populations. A recent study demonstrated that carotid plaque and increased CIMT are associated with coronary artery disease on coronary CT angiography in a mostly nondiabetic white cohort (3). Previous studies in the general population using electron beam CT also found an association between carotid artery disease and CAC score (24, 25).

Fewer data are available concerning the relation of carotid atherosclerosis to coronary artery disease in asymptomatic patients with diabetes. Djaberi et al (6) studied 150 asymptomatic diabetic patients and found a significant relation between CIMT and the presence of atherosclerosis determined by CAC score and coronary CT angiography. A recent study evaluated 241 asymptomatic type 2 diabetic patients and concluded that there is an association of CIMT with coronary artery stenosis assessed by coronary CT angiography (26).

No study assessing the relation between carotid and coronary atherosclerosis incorporated other features of carotid atherosclerosis, as carotid plaques or stenosis.

Although we found a significant positive correlation between the severity of carotid atherosclerosis and CAC scores, this relation is not independent of gender or duration of diabetes.

Several limitations must be acknowledged. The present analysis was

restricted to an evaluation of the relation between carotid atherosclerosis and Agatston score in diabetic patients, and assessment did not include a nondiabetic control group. As coronary CT angiography is accompanied by radiation exposure, it is not feasible to perform a similar assessment in asymptomatic subjects.

6. Conclusions

In conclusion, we have demonstrated that, among asymptomatic patients with type 2 diabetes mellitus, disease duration is an independent determinant of CAC score and could explain the heterogeneity of CAC scores observed in this population. In the light of current trends of rising prevalence of type 2 diabetes combined with a decrease in the age at onset (27), these findings emphasize the critical importance of aggressive cardiovascular disease risk reduction in patients with type 2 diabetes mellitus early in the course of the disease.

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Chapter VIII

Summary and conclusions

Summary and conclusions

Diabetes mellitus is responsible for diverse cardiovascular complications such as increased atherosclerosis in large arteries (carotids, aorta, and femoral arteries) and increased coronary atherosclerosis. Coronary microvascular disease may contribute to cardiac pathology as well. Diabetes mellitus also can affect cardiac structure and function in the absence of changes in blood pressure and coronary artery disease, a condition called diabetic cardiomyopathy.

Due to the often masked symptoms of type 2 diabetes mellitus, the disease may only be diagnosed several years after onset when complications have already occurred. Often, the prognosis of patients with type 2 diabetes mellitus depends on the presence of cardiovascular disease.

Research on cardiovascular complications of diabetes mellitus will allow the development of appropriate markers and diagnostic techniques to identify individuals at risk, stage the disease, prognosticate, and evaluate therapy relevant to the cardiovascular complications of diabetes mellitus.

The criteria for the selection of those asymptomatic patients with type 2 diabetes that should undergo cardiac screening and the therapeutic consequences of screening remain controversial. Non-invasive techniques as markers of atherosclerosis and myocardial ischemia may aid risk stratification and the implementation of tailored therapy for the patient with type 2 diabetes mellitus.

A number of noninvasive tests are now available to detect coronary atherosclerotic disease, myocardial dysfunction and myocardial ischemia.

The potential of cardiovascular imaging for the assessment of cardiovascular complications of type 2 diabetic patients is an active field of research.

The purpose of the present research is to study the role of novel cardiac imaging techniques in assessing cardiovascular disease in patients with type 2 diabetes mellitus.

Chapter 1 provides an overview of the definition, classification, diagnosis and prevalence of diabetes mellitus. Moreover, are described the cardiovascular manifestations of diabetes mellitus and discussed the pathophysiological mechanisms responsible for diabetic cardiovascular disease.

Chapter 2 provides an overview of the background of different noninvasive imaging modalities that are used for the evaluation of cardiovascular disease in diabetes mellitus.

In **Chapter 3** we discussed the issue of screening cardiovascular disease in asymptomatic diabetic patients.

In **Chapter 4**, we studied the prevalence and gender differences in subclinical LV diastolic dysfunction, using MR imaging. We concluded that the prevalence of some diastolic dysfunction in apparently normal subjects is up to 25%, and this diastolic dysfunction is higher in men than in women.

In **Chapter 5**, we prospectively assessed for the presence of left atrial function in asymptomatic patients with type 2 diabetes mellitus, using MR imaging. We concluded that left atrial function is altered in patients with type 2 diabetes mellitus, characterized by a reduction in reservoir and conduit functions, without a compromise of booster pump function. We have also demonstrated that type 2 diabetes mellitus was an independent determinant of left atrial reservoir function. These findings point out that left atrial dysfunction might be a component of the heart phenotype in type 2 diabetes mellitus.

In **Chapter 6**, we compared MR-derived parameters of left ventricular diastolic function between asymptomatic diabetic subjects and normoglycemic controls. We also evaluated whether these parameters of LV diastolic function were related to coronary atherosclerosis. We concluded that type 2 diabetes mellitus decreases MR-derived parameters of left ventricular diastolic function, and this effect is related to coronary atherosclerosis.

The purpose of the study in **Chapter 7** was to evaluate the prevalence of coronary and carotid atherosclerosis in asymptomatic patients with type 2 diabetes mellitus. The major observations were as follows: First, CT could detect coronary calcified plaques in 70% of asymptomatic patients with type 2 diabetes. In addition, in 27% of patients a markedly elevated Agatston score (>400) was observed. Furthermore, we had a relatively large percentage (41%) of type 2 diabetes mellitus patients with Agatston score < 10. After correction for traditional risk factors, male gender and diabetes duration were found to be independent predictors of increasing coronary calcium score. Carotid atherosclerosis was not an independent predictor of coronary calcium scores.

Conclusions and future perspectives

In this thesis, we evaluated several different cardiac imaging techniques for the assessment of complications in asymptomatic patients with type 2 diabetes mellitus.

Left ventricular diastolic dysfunction seems to be a biomarker of asymptomatic type 2 diabetes mellitus, but may be present in a significative percentage of apparently normal subjects.

Our study was one of the pioneers in using novel MRI imaging parameters to identify left atrial dysfunction in type 2 diabetes mellitus.

We demonstrated that asymptomatic subjects with type 2 diabetes mellitus show a wide range of coronary calcium scores, and that disease duration is an independent predictor of increasing coronary calcifications.

We also demonstrated that the presence of coronary calcification is related to left ventricular diastolic dysfunction.

The relationship of these cardiac imaging biomarkers with other biological and genetical markers of target-organ damage will be addressed in future studies.

The prognostic value of all these cardiac imaging biomarkers for risk stratification of asymptomatic patients with type 2 diabetes mellitus will be determined in future follow-up studies.

The challenge is to identify those subjects at increased risk for cardiovascular morbidity and mortality, adequate treatment and improve the outcome of these patients.

Resumo e conclusões

A diabetes mellitus é responsável por diversas complicações cardiovasculares, incluindo aterosclerose em artérias de grande calibre (carótidas, aorta e artérias femorais) e nas artérias coronárias. A doença microvascular coronária é um dos outros mecanismos de doença coronária na diabetes mellitus. Por fim, a diabetes mellitus pode também afectar a estrutura e a função cardíaca na ausência de alterações na pressão arterial e de doença arterial coronária, uma condição denominada miocardiomiopatia diabética .

Pela natureza tipicamente assintomática da diabetes mellitus tipo 2, o diagnóstico pode ser efectuado muito tardiamente, quando já ocorreram complicações graves. Frequentemente, o prognóstico dos pacientes com diabetes mellitus tipo 2 depende da presença e do grau de doença cardiovascular.

A investigação no campo das complicações cardiovasculares de diabetes mellitus poderá permitir o desenvolvimento de marcadores e técnicas de diagnóstico, no sentido de melhor identificar os pacientes de risco, estadiar a doença, estabelecer o prognóstico e avaliar a terapêutica instituída.

Não estão estabelecido os critérios de selecção de pacientes assintomáticos com diabetes mellitus tipo 2 que devem efectuar rastreio de doença cardiovascular, bem como as potenciais consequências terapêuticas desse rastreio.

Uma série de técnicas não-invasivas estão agora disponíveis para detectar doença coronária aterosclerótica, disfunção e isquémia miocárdica.

O potencial da imagiologia cardíaca na avaliação das complicações cardiovasculares em pacientes com diabetes tipo 2 é um campo activo de pesquisa.

O objectivo da presente investigação é estudar o papel de novos métodos de imagiologia cardíaca na avaliação de doença cardiovascular em pacientes com diabetes tipo 2.

No **capítulo 1**, foi efectuada uma revisão da definição, classificação, diagnóstico e epidemiologia da diabetes mellitus. Além disso, são descritas as manifestações cardiovasculares da diabetes mellitus e discutidos os mecanismos fisiopatológicos responsáveis pela doença cardiovascular na diabetes.

O **capítulo 2** fornece uma visão geral das diferentes modalidades não-invasivas de imagem que são utilizadas para a avaliação da doença

cardiovascular em pacientes com diabetes mellitus.

O **capítulo 3** discute a temática do rastreio da doença cardiovascular em diabéticos assintomáticos.

O objectivo do trabalho apresentado no **capítulo 4** foi avaliar a prevalência e diferenças de género na disfunção diastólica do ventrículo esquerdo em controlos assintomáticos, através de estudo de RM cardíaca. Concluiu-se que a prevalência de disfunção diastólica em indivíduos aparentemente normais é de até 25%, e esta disfunção diastólica é maior em homens do que em mulheres.

No **capítulo 5**, estudámos a função da aurícula esquerda em diabéticos assintomáticos, através de RM. Concluiu-se que a função da aurícula esquerda está alterada em pacientes com diabetes mellitus tipo 2, caracterizado por uma redução nas funções de reservatório e conduto, sem um compromisso da contracção activa. Também demonstramos que a diabetes mellitus tipo 2 é um determinante independente da função de reservatório da aurícula esquerda. Estes resultados indicam que a disfunção da aurícula esquerda pode ser um componente do fenótipo cardíaco em pacientes com diabetes mellitus tipo 2 .

No **capítulo 6** comparámos parâmetros de disfunção diastólica entre diabéticos assintomáticos e controlos, utilizando a RM cardíaca. Além disso, avaliámos a relação entre marcadores de disfunção diastólica do ventrículo esquerdo e a arteriosclerose coronária. Concluiu-se que a diabetes mellitus tipo 2 associa-se a redução da função diastólica do ventrículo esquerdo, e que este efeito está relacionado com a aterosclerose coronária.

O objectivo do estudo descrito no **capítulo 7** foi avaliar a prevalência de aterosclerose coronária e carotídea em pacientes assintomáticos com diabetes mellitus tipo 2. As principais observações foram as seguintes: a TC detectou placas calcificadas coronárias em 70% dos pacientes assintomáticos com diabetes tipo 2. Além disso, em 27 % dos pacientes foi observada um score de cálcio marcadamente elevado (> 400). Por fim, uma percentagem relativamente grande (41%) dos pacientes com diabetes mellitus tipo 2 tinha um score de cálcio <10. Após a correcção dos fatores de risco tradicionais, o género masculino e duração da diabetes demonstraram ser predictores independentes do aumento do score de cálcio. Não se verificou relação entre a arteriosclerose coronária e o score de cálcio nas artérias coronárias.

Conclusões e perspectivas futuras

Na presente tese, foram avaliadas diferentes técnicas de imagem para o estudo de complicações cardiovasculares em pacientes assintomáticos com diabetes mellitus tipo 2.

A disfunção diastólica do ventrículo esquerdo parece ser um biomarcador de diabetes mellitus tipo 2 assintomática, mas pode também estar presente num número significativo de indivíduos aparentemente normais.

O nosso estudo foi um dos pioneiros no uso de novos parâmetros de imagem de ressonância magnética para identificar disfunção da aurícula esquerda na diabetes mellitus tipo 2.

Demonstramos que indivíduos assintomáticos com diabetes mellitus tipo 2 apresentam uma grande variabilidade de scores de cálcio coronário, e que a duração da doença é um predictor independente do aumento do grau de calcificação coronária.

Demonstramos ainda que a presença de aterosclerose coronária está relacionada com a disfunção diastólica do ventrículo esquerdo.

A relação desses biomarcadores de imagem cardíaca com outros marcadores biológicos e genéticos de lesão de órgão-alvo será abordada em estudos futuros.

O valor prognóstico de todos esses biomarcadores de imagem cardíaca para estratificação do risco em pacientes assintomáticos com diabetes mellitus tipo 2 será determinada em estudos futuros.

O desafio será identificar os indivíduos com maior risco de morbilidade e mortalidade cardiovascular, adequar o tratamento e melhorar o prognóstico destes pacientes .

List of publications

1. Cardiovascular magnetic resonance imaging assessment of diastolic dysfunction in a population without heart disease: a gender-based study

Bruno Graça, Maria João Ferreira, Paulo Donato, Miguel Castelo Branco, Filipe Caseiro Alves

European Radiology, January 2014

2. Left atrial dysfunction in type 2 diabetes mellitus: insights from cardiac MRI

Bruno Graça, Maria João Ferreira, Paulo Donato, Leonor Gomes, Miguel Castelo Branco, Filipe Caseiro Alves

European Radiology, July 2014

3. Left ventricular diastolic function in type 2 diabetes mellitus and the association with coronary calcium score: a cardiovascular magnetic resonance study

Bruno Graça, Paulo Donato, Maria João Ferreira, Miguel Castelo-Branco, Filipe Caseiro-Alves

American Journal of Roentgenology, June 2014

4. Relationship of cardiovascular risk factors with coronary artery calcium in asymptomatic patients with type 2 diabetes

Bruno Graça, Paulo Donato, Maria João Ferreira, Leonor Gomes, Miguel Castelo Branco, Filipe Caseiro Alves

Submitted