The Study of Myocardial Metabolism and Its Role in The Pathophysiology of Early Diabetic Cardiomyopathy

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Declaration and statements

Declaration

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature.

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Statement 1

This thesis is the result of my own investigation, except where otherwise stated. Where correction services have been used, the extent and nature of the correction is clearly marked in a footnote(s). Other sources are acknowledged by footnote giving explicit references. A bibliography is appended.

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Statement 2

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Statement of contribution to research

The studies were conceived and designed jointly by me and Professor Frenneaux.

Execution

I performed all of the recruitment and organisation of the appointments involved in the study. I performed metabolic exercise tests, echocardiogram, cardiac MRI and cardiac MRS studies in the University of Birmingham. In conjunction with Dr Dave Hauton, I performed the ketones and HOMA-IR measurements. Metabolic exercise tests were reported by Rebekah Weaver. Perhexiline assays and LFT were performed by Dr Alan Hutchins at Llandough Hospital, Cardiff.

Analysis

All data were collated and analysed by myself. Inter-observer reproducibility data were analysed by Dr Thanh Phan and Dr K Abozguia.

Publication

This thesis for the Doctoral Degree PhD in cardiovascular medicine is based on the following publication listed below:

1. **Dr G Nallur Shivu**….. Prof M Frenneaux. Relationship Between Coronary Microvascular Dysfunction and Cardiac Energetics Impairment in Type 1 Diabetes Mellitus. **Circulation** 2010 Mar 16;121(10):1209-15

2. **Dr G Nallur Shivu**….. Prof M Frenneaux. Increased left ventricular torsion in uncomplicated type 1 diabetes: the role of coronary microvascular function. **Diabetes Care** 2009 Sep;32(9):1710-2 (doi:10.2337/dc09-0408)

3. **Dr G Nallur Shivu……..** Prof M Frenneaux. ^{31}P Magnetic Resonance Spectroscopy to Measure In Vivo Cardiac Energetics in Normal Myocardium and Hypertrophic Cardiomyopathy: Experiences at 3 Tesla. **European Journal of Radiology**: 2010;73:255-259.

4. **Dr G Nallur Shivu*,** Dr K Abozguia**…..** Prof M Frenneaux. The heart metabolism : Pathophysiological aspects in ischaemia and heart failure **Current Pharmaceutical Designs:** 2009; 15(8): 827-35 **(*Joint first author)**

5. Dr K Abozguia, **Dr G Nallur Shivu…..** Prof M Frenneaux Potential of metabolic agents as adjunct therapies in heart failure– **Future cardiology**- 2007 Sep; 3(5):525-35.

6. **Dr G Nallur Shivu*,** Dr T Phan ….. Prof M Frenneaux. Multi-Centre Experience on the use of Perhexiline in Chronic Heart Failure and Refractory Angina: Old Drug, New Hope. **European Journal of Heart Failure** 2009 Sep; 11(9):881-6. **(*Joint first author)**

Abstracts

Moderated poster presentations:

1. Mechanisms responsible for cardiac energetic impairment in type 1 diabetes: **Welsh Cardiovascular Society 2010**

2. Left ventricular untwisting patterns and its relation to diastolic relaxation in patients with uncomplicated type 1 diabetes: **European Society of Cardiology 2009.**

3. Increased left ventricular twist as an early manifestation of diabetic cardiomyopathy in patients with uncomplicated type 1 diabetes: **British Cardiovascular Society 2009.**

4. Mechanisms responsible for cardiac energetic impairment in type 1 diabetes: **British Cardiovascular Society 2009.**

5. LV torsion and strain patterns in uncomplicated type 1 diabetes: **Welsh Cardiovascular Society 2009**

Other:

1. The Relationship of Left Ventricular Untwisting to Diastology in Type 1 Diabetes: **AHA Scientific Sessions 2009.**

2. The Role of Coronary Microvascular Function and Rotational Deformation Delay in the Development of Increased Left Ventricular Torsion in Uncomplicated Type 1 Diabetes: **AHA Scientific Sessions 2009.**

3. Increased left ventricular torsion in uncomplicated type 1 diabetes: the role of coronary microvascular function: **ESC 2009**

4. Pathophysiology of cardiac energetic impairment in type 1 diabetes: **ESC 2009**

5. Impaired cardiac energetics is independent of coronary microvascular function in patients with type 1 diabetes: **ESC Heart Failure Congress 2009**

6. Increased left ventricular torsion in patients with uncomplicated type 1 diabetes: **ESC Heart Failure Congress 2009**

7. Impaired cardiac energetics in type 1 diabetic patients in the absence of coronary artery disease or heart failure: **ESC Congress 2008**

8. Divergent response of perhexiline, a metabolic modulator on insulin sensitivity in patients with and without diabetes **: BCS/BSCR 2008**

9. Effects of perhexiline on ketone body and triglyceride synthesis in patients with refractory angina or heart failure : **BCS/BSCR 2008**

10. ^{31}P Nuclear Magnetic Resonance Spectroscopy of the myocardium at 3 Tesla : **13th World Congress on Heart Disease 2007**

Abstract:

The human myocardium is a metabolic omnivore and utilises fatty acids, glucose, ketones, amino acids and lactate to produce energy. Altered metabolism results in cardiac muscle dysfunction and can play a potentially significant role in development of heart failure. Metabolic modulators like Perhexiline are potentially significant new treatments in the management of heart failure and coronary artery disease.

Diabetes is a metabolic disorder that results in altered high energy phosphate kinetics in the myocardium. We demonstrate that microvascular disease plays little role in the development of impaired cardiac energetics in young patients with uncomplicated type 1 diabetes. We have shown an increase in left ventricular torsion in these patients with normal ejection fraction. Coronary microvascular disease and rotational deformation delay play a significant role in the development of increased torsion in these individuals which counteracts the early diastolic dysfunction. Furthermore the left atrial contribution to left ventricular filling is increased in these individuals.

We demonstrate that Perhexiline has a differential action on insulin sensitivity in subjects with and without diabetes. It also increased plasma ketones and triglycerides in these patients. Finally we demonstrate that Perhexiline can be safely used and provides good relief of symptoms when used clinically in subjects with refractory angina and heart failure.

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List of abbreviations used:

2D: 2 dimensional
2,3-DPG: 2,3- Diphosphoglycerate
ADP: Adenosine di-phosphate
ALP: Alkaline phosphatase
AMP: Adenosine mono-phosphate
AMPK: AMP-activated protein kinase
ARB: Angiotensin II receptor blocker
AST: Aspartate aminotransferase
ATP: Adenosine tri-phosphate
BMI: Body mass index
CABG: Coronary artery bypass grafting
CAD: Coronary artery disease
CAN: Cardiac autonomic neuropathy
CCS: Canadian cardiovascular society
CHF: Chronic heart failure
CK: Creatine kinase
CPT: Carnitine palmitoyl transferase
CVD: Cardiovascular disease
DecT: Deceleration time
ECG: Electrocardiogram
ESV: End systolic volume
EDTA: Ethylene diamine tetra acetic acid
EDV: End diastolic volume
EF: Ejection fraction
FFA: Free fatty acids
HC: Healthy controls
HCM: Hypertrophic cardiomyopathy
ISIS: Image selected invivo spectroscopy
IVRT: Isovolumetric relaxation time
LA: Left atrium
LFT: Liver function tests
LV: Left ventricular
LVH: Left ventricular hypertrophy
MPRI: Myocardial perfusion reserve index
MRI : Magnetic Resonance Imaging
MRS: Magnetic resonance spectroscopy
NMR: Nuclear magnetic resonance
NOE: Nuclear overhauser enhancement
NYHA: New York Heart Association
PCI: Percutaneous coronary intervention
PCr: Phosphocreatine

PDC: Pyruvate dehydrogenase complex
PET: Positron Emission Tomography
PFR: Peak filling rate
PPAR α: Peroxisome proliferators-activated receptor alpha
RER: Respiratory exchange ratio
ROS: Reactive oxygen species
SNR: Signal to noise ratio
STE: Speckle tracking echocardiography
SV: Stroke volume
T1DM: Type 1 diabetes mellitus
T2DM: Type 2 diabetes mellitus
TD: Trigger delay
TDI: Tissue Doppler imaging
TR: Repetition time
UCP: Uncoupling proteins
VO2 max: Maximal oxygen consumption at peak exercise

CHAPTER ONE

INTRODUCTION

Publications

1. **Dr G Nallur Shivu*,** Dr K Abozguia**…..** Prof M Frenneaux. The heart metabolism : Pathophysiological aspects in ischaemia and heart failure **Current Pharmaceutical Designs:** 2009; 15(8): 827-35 **(*Joint first author)**

2. Dr K Abozguia, **Dr G Nallur Shivu…..** Prof M Frenneaux Potential of metabolic agents as adjunct therapies in heart failure– **Future cardiology**- 2007 Sep; 3(5):525-35.

Burden of ischaemic heart disease and heart failure

The global burden of coronary artery disease (CAD) is ever increasing. Around 18 million people die worldwide every year from cardiovascular disease (CVD) [97], of which 4.3 million are from Europe, accounting for nearly half of all deaths in Europe [10]. It is the main cause of disease burden in the western world. Diabetes is an important risk factor for the development of CVD and the global burden of diabetes is increasing [59;266;282]. Approximately 80% of deaths in patients with diabetes are secondary to cardiovascular complications [64;83]. The economic costs are alarming: CVD cost the European Union around 192 billion Euro in 2006 and this figure is increasing every year [10]. The prevalence of heart failure has dramatically increased throughout the western world. The incidence and prevalence of heart failure increases with increasing age [19;73] and ageing of the population accounts in part for the increasing prevalence of heart failure. Increased survival rates in patients suffering acute myocardial infarction [24] (due to reperfusion therapies) has also been proposed to contribute. It has been estimated that there are currently 6.5 million chronic heart failure (CHF) patients in Europe and 5 million in the USA [260]. CHF has a poor prognosis with nearly 50% of the patients dying in the first four years of diagnosis and nearly half of those with severe heart failure dying within 1 year [155;249;256]. These dismal figures are in spite of the dramatic improvements in the treatment of CHF over the past 20 years or so. These have focused mainly on the blockade of maladaptive neuro-hormonal activation[66;69]. Recently, there has been increasing interest in metabolic agents (which alter the fuel usage of the heart) as treatment for both CAD and CHF [5;6]. Understanding myocardial metabolism and its various adaptive and maladaptive features in conditions like CAD and CHF is key to further developments in this field. Studying changes in myocardial metabolism in diabetes can provide further insights into the patho-physiology of development of CHF in diabetes. Heart

failure occurs more frequently in diabetes [109] and is commonly due to epicardial CAD and/or hypertension [101;126;250]. However in some patients left ventricular (LV) dysfunction occurs in the absence of significant epicardial CAD or hypertension [214]. This indicates that diabetes may have a direct effect on the heart, which can contribute to the development of LV dysfunction. This condition is sometimes termed 'diabetic cardiomyopathy'. Hence further insights into myocardial metabolism and its alteration may play a significant role in improving outcomes in patients with CAD and CHF.

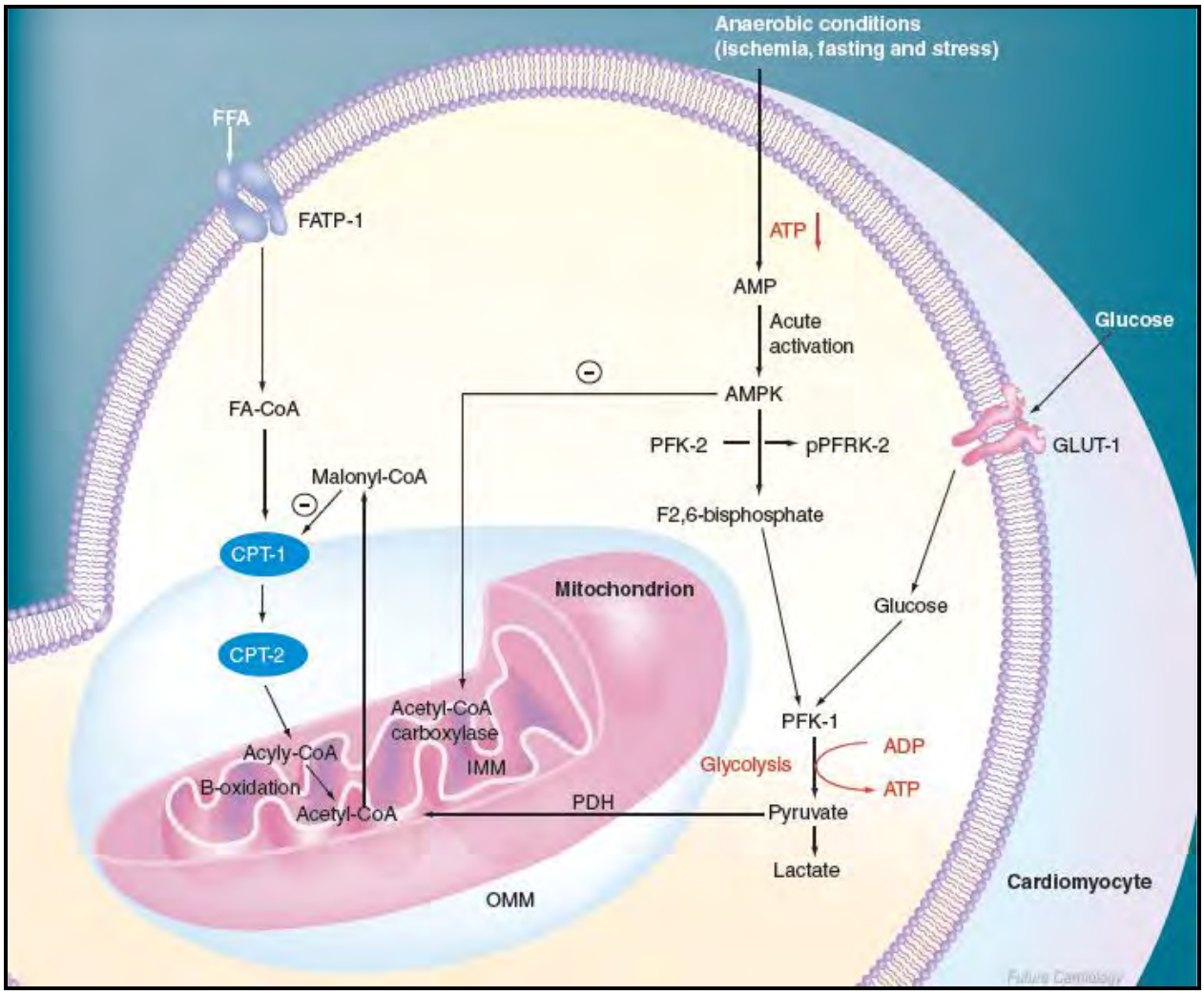
Myocardial metabolism in normal hearts

The human heart is a relentless pump that never stops working during life. It beats for approximately 100,000 times a day and requires a vast amount of energy to fulfil its function. Therefore it would not be a surprise that impaired cardiac energetics may play a key role in the pathogenesis and progression of diabetes to CHF. Cardiac energetic impairment has been shown to be an adverse prognostic feature in patients with CHF [176]. The myocardium is a metabolic omnivore and is able to metabolise glucose, free fatty acids (FFAs), amino acids, ketones and lactate for energy production. In normal circumstances 60-90% of the acetyl Co-A which enters the Krebs cycle comes from beta-oxidation of FFAs and 10-40% from oxidation of pyruvate derived from glycolysis [242;243] [Fig (1.1)]. Glucose metabolism consists of two important components, glycolysis and carbohydrate oxidation. Glycolysis which occurs in the cytosol involves conversion of glucose to pyruvate, while carbohydrate oxidation involves the subsequent mitochondrial oxidation of pyruvate. Pyruvate derived from glucose and lactate enters the Krebs cycle via the pyruvate dehydrogenase complex (PDC) that catalyses the conversion of pyruvate to acetyl Co-A [53;147]. Glycolysis only contributes approximately 5% of the total adenosine tri-phosphate (ATP) generated by the heart during

aerobic conditions. During ischaemia, glycolysis is increased. However, this may result in accumulation of protons and lactate if carbohydrate oxidation does not increase in parallel. Ischaemia inhibits the PDC and results in anaerobic glycolysis[189;201]. Therefore, accelerated glycolysis during ischaemia can have detrimental consequences. Unlike glucose metabolism, fatty acid metabolism produces all of the ATP that it generates in the mitochondria via beta oxidation. Fatty acid oxidation consumes more oxygen to produce the same amount of ATP as compared with glucose oxidation. Also increased fatty acid metabolism results in a concomitant decrease in glucose oxidation due to Co-A mediated inhibition of PDC. This can lead to an uncoupling of glycolysis from carbohydrate oxidation with a resultant increase in proton and lactate production [183].

The myocardium normally works at 15-20% of its maximal oxidative capacity at rest [65;244]. It is able to adapt its substrate utilisation according to its needs. At low to moderate intensity exercise there is a net increase in glucose and lactate utilisation with no change in FFA utilisation [72]. At high intensity exercise, glucose uptake falls compared to lower intensity exercise [113]. There is a concomitant 10 fold increase in lactate uptake which serves as the main substrate during high intensity exercise.

Figure 1.1



Myocardial metabolism.

CPT-carnitine-palmitoyl-transferase; IMM-inner mitochondrial membrane; OMM-outer mitochondrial membrane; PDH-pyruvate dehydrogenase; GLUT-1- Glucose transporter-1; FATP-1- fatty acid transporter protein-1; PFK- phosphofructokinase ; AMPK- AMP-activated protein kinase. Reproduced from Abozguia et al Future Cardiology. (2007) 3(5), 525-535(11) with permission of Future Medicine Ltd [6]

Substrate Utilisation and Myocardial Oxygen Utilisation

Simple stoichiometry suggests that utilisation of fatty acids should cost approximately 10-12% more oxygen per unit of ATP generated than glucose. In practice, the increased oxygen requirement when plasma FFAs are increased compared with dominant glucose utilisation appears to be substantially greater (up to 40 %) [98;120;162], implying the presence of an 'oxygen wasting' phenomenon with increased FFA usage. In the isolated perfused rat heart, increasing the palmitate and octanoate in the perfusate disproportionately increased oxygen utilisation and this was only partially abolished by administration of an inhibitor of fatty acid beta oxidation, suggesting that a component of the oxygen 'wasting effect' of increased FFAs is not related to mitochondrial long chain fatty acid oxidation [100]. It is possible that it may be due to the up-regulation of uncoupling protein expression in the mitochondria via FFA activation of peroxisome proliferators-activated receptor alpha (PPAR $\propto$) [23]. Normally the electron transport chain generates a net proton gradient across the inner mitochondrial membrane and this drives the phosphorylation of adenosine di-phosphate (ADP) to ATP. However, when mitochondria are 'uncoupled' there is proton leakage and the electrochemical gradient is dissipated as heat [226]. Emerging evidence suggests that the primary role of uncoupling proteins (UCP) is the export of lipid peroxides and long chain fatty acids out from the mitochondrion which helps prevent oxidative damage to mitochondrial DNA [74]. This occurs when fatty acid delivery exceeds oxidative capacity and when there is an accumulation of lipid peroxides. PPAR α activation by fatty acids increases the expression of UCP [23] and furthermore, the activity of UCP is increased by lipid peroxides [217]. Heart failure is associated with elevated free fatty acid concentrations [139] which might increase uncoupling. Further, Murray et al showed that mitochondrial uncoupling protein expression in human cardiac muscle correlated positively with plasma FFA concentration in patients undergoing elective

coronary artery bypass surgery [166]. This relationship was noted in patients with heart failure as well. Furthermore, high levels of FFA may trigger intracellular futile metabolic cycles such as intra-myocardial lipolysis and re-esterification [21;120;161].

Metabolic changes in ischaemic heart disease

All metabolic adaptive mechanisms during ischaemia, whether physiological or pharmacological, aim at improving the oxygen efficiency of the myocardium by shifting substrate use towards glucose metabolism. This shift can be beneficial as it can result in up to 30-40% reduction in oxygen usage as compared to FFA [98;120;162]. As the myocardium works at only 15-20% of oxidative capacity under normal conditions, even during subtotal ischaemia, the myocardium continues to derive a large proportion of its energy from oxidative metabolism. During more marked ischaemia, there is a shift towards greater use of glucose as a substrate for energy production [118]. Despite this shift, the predominant source of ATP in the ischaemic heart continues to be FFA oxidation [134]. During myocardial ischaemia, FFA oxidation is not only more oxygen demanding, it also suppresses glucose oxidation by directly inhibiting PDC [110;134;143]. The consequence of this is the accumulation of lactate and hydrogen ions in ischaemic cells and associated wastage of ATP in restoring intracellular ionic homeostasis [144]. Phosphocreatine (PCr) also begins to deplete at this point because it is used to generate ATP. This not only generates inefficiency but may also have detrimental effects on myocardial contractile function. Low intracellular pH and increased intracellular lactate are associated with reduced contractile function of myocardial segments [89]. Diastolic function and rhythm stability may be adversely affected by the accumulation of metabolic intermediates generated by the beta oxidation of fatty acids during ischaemia [114;165]. Ischaemia also results in opening of the late inward sodium current leading to intracellular sodium

increase and secondary increases in calcium, in turn causing a marked slowing of LV active relaxation [18].

Myocardial stunning and hibernation

Myocardial stunning is a phenomenon which results from brief ischaemic injury followed by reperfusion [273]. It is seen in many clinical circumstances including spontaneous episodes of ischaemia following percutaneous coronary intervention (PCI), thrombolysis and /or coronary artery bypass surgery. The pathogenesis of stunning is via production of oxygen free radicals, alterations in calcium homeostasis and oxidative metabolism and possible changes in muscle protein structure [156;289]. Gerber et al demonstrated normal oxygen consumption in the dysfunctional myocardium following reperfusion by PCI [71]. This indicated a marked reduction in the regional mechanical efficiency (work done divided by oxygen consumption). McNulty et al demonstrated increased glucose uptake with decreased glycogen synthesis in post-ischaemic myocardial stunning models of rats 24hrs after ischaemic insult [156]. This suggests an important role of enhanced glycolysis in the recovery from myocardial stunning [156]. Based on this, glucose-insulin-potassium (GIK) infusion therapy which drives glucose uptake was noted to be beneficial in one study of diabetic patients with acute myocardial infarction [63]. Although insulin drives glucose uptake and glycolysis, the inhibition of PDC that occurs in ischaemia results in uncoupling of glycolysis and glucose oxidation [144]. This leads to accumulation of protons and lactate which can further enhance cell damage. Another recent study of diabetic patients with acute myocardial infarction did not show any benefit of GIK followed by intensive glucose control [158].

Myocardial stunning is an acute phenomenon and potentially reversible. However when the myocardium receives repeated insults of ischaemic injury these result in a phenomenon called myocardial hibernation. The hibernating myocardium is still viable but not contracting, and timely revascularisation will restore contractile function. Hibernating myocardium exhibits reduced oxidative metabolism and FFA utilization [229;258]. Ischaemia-induced increased glucose utilization is also supported by studies of preconditioning which demonstrated markedly increased glucose uptake [102]. Following revascularisation improvement of contractile function is associated with an increase in FFA metabolism. It has been suggested that recovery of myocardial function may be related to the improvement in FFA metabolism [235]. However, increases in FFA metabolism may be a consequence of improved oxygen availability due to revascularisation rather than the cause of myocardial contractile restoration. This is supported by the fact that metabolic modulating agents that putatively shift metabolism from FFA towards glucose also result in improvement in systolic function in patients with chronic ischaemic LV dysfunction [132;210;276].

For patients experiencing myocardial ischaemia, any agent that shifts substrate utilisation towards glucose might be anticipated to offer considerable benefit. A shift towards increased utilisation of glucose by the myocardium can be induced by any intervention that suppresses FFA uptake and/or oxidation. Currently available metabolic agents achieve increased glucose utilisation by inhibiting FFA uptake into the mitochondria, or by inhibiting beta oxidation (Table 1.1). However, it is important to note that modulating FFA metabolism may not be without risk. Inherited disorders of fatty acid metabolism, particularly involving Acyl-CoA dehydrogenase, have been associated with dilated cardiomyopathy and arrhythmogenesis in infants, possibly secondary to accumulation of free-fatty acid metabolites [25;151].

Table 1.1

Classification of metabolic agents*
• **FFA uptake inhibitors**
Perhexiline
Oxfenicine
Etmoxir
? Beta-Blockers
? Amiodarone
• **FFA β-Oxidation inhibitors**
Trimetazidine
? Ranolazine
• **Others**
D-Ribose
Proprionyl-L-Carnitine
Glucagon-like Peptide-1

* Mechanism of action not confirmed in vivo

Metabolic changes in Heart Failure

In animal models of left ventricular hypertrophy (LVH) and heart failure a relative switch towards increased glucose uptake and reduced fatty acid oxidation has been demonstrated. In the canine rapid pacing model of heart failure, there was a relatively normal substrate utilisation in the early stages of heart failure. A shift towards relatively greater glucose uptake was however noted in the end stages of heart failure [203]. This shift in metabolism was attributed to reduced expression and activity of retinoid X receptor Alpha (RXR-alpha), a receptor known to stimulate expression of Medium Chain acyl CoA Dehydrogenase (MCAD) and enzymes involved in FFA oxidation. Studies of canine microembolisation-induced heart failure indicate that substrate change to glucose is a late phenomenon [38]. Various other complex mechanisms may be responsible for the shift to this foetal phenotype. Increased adenosine mono-phosphate (AMP) and AMP kinase activity has been reported in hypertrophied rat hearts undergoing transition to heart failure [263]. Increased AMP kinase activity increases glucose uptake via GLUT-1 transporters and increased glycolysis via phosphorylation of 6-phosphofructo-2-kinase. Chronic activation of AMP kinase also decreases FFA utilisation via decreased expression of carnitine palmitoyl transferase-1 (CPT-1) and MCAD enzymes [148;263].

There remains a controversy as to whether the shift towards increased glucose uptake in animal models of LVH and heart failure is adaptive or maladaptive. Human genetic disorders of fatty acid beta oxidation may be associated with the development of heart failure [25;151]. Furthermore, in animal models of both LVH and heart failure, whilst glucose uptake and glycolysis are increased, glucose oxidation is reduced because of reduced activity of the PDC. Therefore pyruvate conversion to acetyl coenzyme A is reduced and anaplerotic reactions fail

to compensate [241]. Unlike the above animal experimental models that undergo transition from LVH to heart failure and in which there is a shift to a 'fetal' pattern, the available evidence suggests that human heart failure may not be associated with a shift towards predominant glucose utilisation; indeed the converse may be true. Using Positron Emission Tomography (PET) technique, Taylor et al demonstrated increased myocardial FFA uptake and reduced glucose uptake in human heart failure [259]. There are several potential explanations for this. Firstly, insulin resistance is commonly present in CHF. Indeed, Nikolaidis et al demonstrated a progressive increase in cardiac insulin resistance during disease progression in the canine rapid pacing heart failure model [180]. There was a reduced cardiac basal and insulin stimulated glucose uptake (associated with reduced insulin stimulated GLUT-4 translocation). Secondly, increased plasma FFAs are characteristically found in CHF due to sympathetic activation [186] and contribute to insulin resistance. Despite the uncertainty of whether the shift towards glucose metabolism in experimental models of heart failure is adaptive or maladaptive in nature, our observations with Perhexiline (over 2 months) [132]and those with Trimetazidine (up to 18 months) [56] in patients with CHF suggest that pharmacologically-induced inhibition of fatty acid metabolism has beneficial effects on cardiac performance, exercise capacity and symptoms. Interestingly however, acute reduction of FFA uptake/oxidation may not be beneficial in heart failure. For, in a recent study using acipimox, patients with heart failure developed acute deterioration of LV function [268]; Acipimox significantly reduces plasma concentrations of FFA and consequently FFA uptake by the myocardium. One plausible explanation for the deterioration in LV function is that the reduced FFA uptake was not associated with concomitant increase glucose oxidation due to the presence of insulin resistance in heart failure patients. In other words, depriving the heart of an important source

of fuel (FFA) may result in deterioration of LV function. It would be interesting to assess the effects of chronic acipimox use in CHF patients.

Myocardial energetics in heart failure

There is now strong evidence indicating impaired cardiac energetics playing a significant part in the pathogenesis of heart failure even in patients without CAD. Animal models have shown that there is a global loss of the total adenine nucleotide (TAN) pool [136;234]. The energetic abnormality may in turn exacerbate the loss of TAN in that increased AMP activates cytosolic 5'AMP –specific 5'nucleotidase, the primary enzyme responsible for the conversion of AMP to adenosine in muscle cells. In a study by Shen and colleagues, a canine rapid pacing model was used to investigate the changes of cardiac energetics during the evolution of heart failure. They showed a progressive monotonic decay in total adenine nucleotides and creatine from the onset of pacing. Interestingly, the onset of the decline in energetic status preceded objective evidence of cardiac contractile dysfunction [234]. The cardiac creatine content is determined primarily by the activity of the creatine transporter which is responsible for creatine uptake into the myocytes against a concentration gradient [177]. Two thirds of the creatine in the heart is phosphorylated via the creatine kinase (CK) reaction to form PCr. PCr is a vital energy buffer molecule that provides precious phosphoryl groups to ADP to generate ATP rapidly during periods of physiological stress e.g. exercise. This reaction is also mediated via CK and can generate ATP 10 times faster than that occurs via oxidative phosphorylation. Interestingly, heart failure studies in animals and humans have shown a progressive reduction in the creatine pool, as much as 60% loss, and the level of reduction is related to heart failure severity [171;262;288]. This reduction in the total creatine pool consequently causes a reduction in PCr and in PCr/ATP ratios, as measured by phosphorus (^{31}P) Nuclear Magnetic Resonance (NMR) spectroscopy [45;82]. Evidence originating from

Cardiac Magnetic Resonance Spectroscopy (MRS) studies have shown reduced myocardial energetic status in patients with systolic heart failure [81], insulin resistance [167;221] and ischaemic heart disease[284]. Similarly, patients with hypertrophic cardiomyopathy (HCM) also have impaired cardiac energetics as shown by a diminished resting PCr/ATP ratio [107]. Data from vivo ^{31}P NMR spectroscopy studies and human biopsy specimens indicates a 25-30% reduction of ATP in the failing human heart. The magnitude of the fall in PCr/ATP ratio predicts mortality [176]. The reduction in PCr in heart failure is further compounded by a decreased number of creatine transporters [177] and a reduction in CK isoenzyme expression, particularly mitochondrial CK which leads to a reduced [PCr]/[Total Creatine] [51].There is some evidence to suggest that iNOS-derived nitric oxide may also play a role in the reduction of mitochondrial CK activity [77]. Some studies have shown that short term oral creatine supplementation in CHF increases skeletal muscle function, however LV ejection fraction (EF) remains unchanged [13;75]. Competitive athletes have been known to take creatine supplements to increase their performances but this has little benefit because myocyte levels of creatine is limited by membrane transporters [103;164].

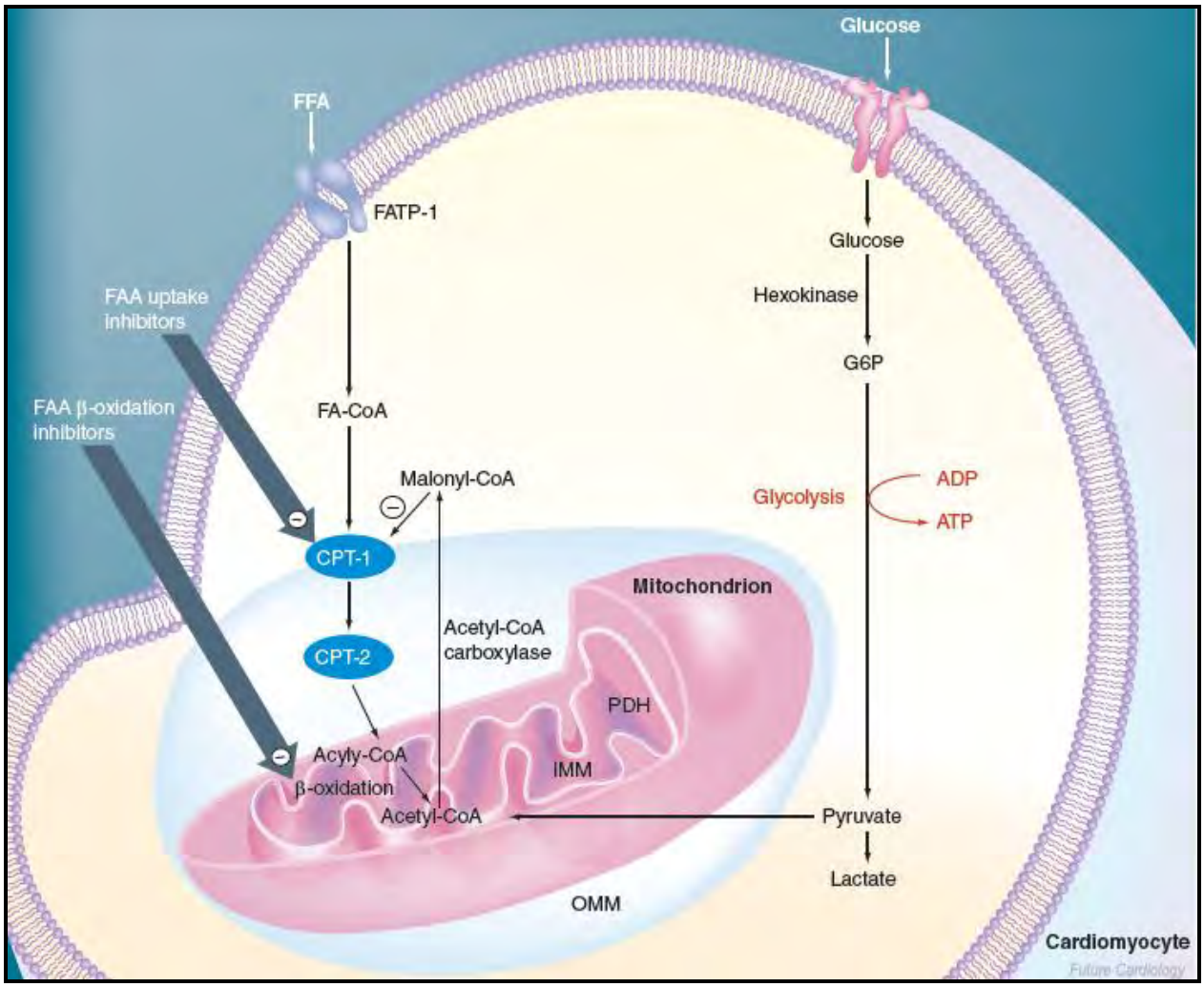
Studies with Trimetazidine, a beta-oxidation inhibitor have demonstrated improvement in cardiac energetics associated with improvements in New York Heart association (NYHA) class and LV function when given to patients with heart failure [68]. Therefore, the impaired cardiac energetics in heart failure could be secondary to increased FFA uptake and metabolism. However, the metabolic changes that occur in ischaemic heart disease i.e. shifts towards glucose metabolism may be at least partially beneficial to restore the cardiac energy reserve. In summary, in the myocardium of a failing heart even in the absence of CAD (or of myocardial hypoxia) there is now strong evidence indicating a reduction of cardiac energy

reserve. This appears to be related to the degree of heart failure but it nevertheless appears to begin early in the evolution of heart failure even before the development of overt signs and symptoms of heart failure [274].

Metabolic therapies in ischaemia and heart failure

It appears that shifting the metabolism towards glucose is beneficial in both ischaemic heart disease and heart failure. In a study in the late 1990's intracoronary pyruvate was shown to acutely increase stroke volume (SV) and reduce pulmonary capillary wedge pressure, implying acute beneficial effects of a shift away from fatty acid metabolism [87]. This has driven interest in the potential beneficial effects of modifying substrate utilisation in CHF. A number of pharmacological agents have been shown to inhibit FFA utilisation in the heart, either by inhibiting FFA uptake into the mitochondrion or by inhibiting beta oxidation (Table 1.1 and Figure 1.2). Some of these agents have been used as antianginal agents because of their 'oxygen sparing' actions. A summary of clinical trials of metabolic agents is shown in table 1.2.

Figure 1.2



Effects of metabolic agents on myocardial metabolism in cardiomyocyte mitochondria.

FFA uptake inhibitors such as perhexiline, oxfenicine, and etomoxir prevent uptake of free fatty acids via inhibition of carnitine palmitoyltransferase I, which is a key enzyme in this process in mitochondria. FFA β-oxidation inhibitors such as Trimetazidine inhibit β-oxidation of free fatty acids. These actions shift myocardial substrate use from free fatty acids to glucose, which is more efficient in terms of energy production, leading to an oxygen-sparing effect.

Abbreviations: CoA, coenzyme A; CPT, carnitine palmitoyltransferase; FA-CoA, fatty acid coenzyme A; FATP1, fatty acid transporter protein 1; FFA, free fatty acid; G6P, glucose-6-phosphate; IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane; PDH, pyruvate dehydrogenase. Reproduced from Abozguia et al Future Cardiology. (2007) 3(5), 525-535(11) with permission of Future Medicine Ltd [6]

Table 1.2

Metabolic Agents	Study (Ref)	Study design	No Patients	Patient Characteristics	Study duration	Outcome
Perhexiline	Lee *et al* 2005[132]	RC PCT* Double blind	56	EF≤ 45 Non-ischaemic and ischaemic CHF NYHA II-IV	2 months	↑ LVEF ↑ $V_{o2\ max}$ Improved quality of life (QOL)
Trimetazidine	Vitale C *et al* 2004[276]	RC PCT	47	EF< 50% Chronic stable angina NYHA I-III	6 months	↑ LVEF Better QOL
Trimetazidine	Di Napoli et al 2005[56]	Open label	61	LVEF <40% Ischaemic LV dysfunction NYHA II-IV	18 months	↑ LVEF Improved -NYHA
Trimetazidine	Fragasso G *et al* 2006[67]	Open label	55	LVEF<45% Ischaemic and non-ishchaemic CHF NYHA II-III	13 months	↑ LVEF Improved -NYHA
Etomoxir	Schmidt-Schweda *et al* 2000[222]	Open label	10	LVEF<40% Dilated Cardiomyopathy NYHAII-III	3 months	↑ LVEF Increased Stroke volume during exercise
Etomoxir	Holubarsch CJ *et al* 2007[92]	RC PCT	350	LVEF<40% Ischaemic and non-ischaemic moderate CHF NYHA II-III	6 months	Study stopped prematurely because of hepatotoxicity.
D-Ribose	Omran *et al* 2003[181]	RC PCT Cross over	15	Ischaemic Cardiomyopathy NYHA II-III	3 weeks	Improved functional capacity and diastolic function
L-Carnitine	Anand *et al* 1998[12]	RC PCT Single blind	30	LVEF<40% NYHA II-III Ischaemic and non-ischaemic cardiomyopathy	4 weeks	↑ VO2 max No change in LVEF
L-Carnitine	Eur Heart journal 1999[2]	RC PCT Double Blind	537	LVEF <40% NYHA II-III Ischaemic and non-ischaemic cardiomyopathy	6 months	No increase in exercise capacity
Glucagon-like Peptide 1	Sokos *et al* 2006[239]	Open label	12	NYHA III-IV	5 weeks	↑ LVEF Improved QOL

* RC PCT: Randomized Placebo Controlled Trial

Summary of human clinical trials of metabolic agents for treatment of CHF

FFA Uptake Inhibitors

Perhexiline

Perhexiline is a metabolic modulator which causes a shift in cardiac substrate use from fatty acids to carbohydrates. It is a reversible inhibitor of both CPT1 and CPT2 which are key enzymes involved in the transport of FFA into the mitochondria [114;115]. Perhexiline was frequently used as an antianginal agent in the 1970s and is an effective monotherapy for relieving symptoms of angina [95;277;281], improving exercise tolerance, and increasing the workload needed to induce ischaemia [44;93]. Its use however declined dramatically in the late 1970s and early 1980s following reports of hepatotoxic effects and peripheral neuropathy [28;192;208]. These effects were later demonstrated to occur in patients in whom hydroxylation was slow because they possesed a genetic variant of the cytochrome P450 enzyme, which metabolizes the drug, resulting in drug accumulation and, consequently, accumulation of phospholipids in the liver and nerves [163]. However, the risk of toxic effects is virtually eliminated by maintaining plasma concentrations at between 150 and 600 ng/ml, at this level the drug also remains efficacious [44;96]. As a result, in countries such as Australia, the use of perhexiline was re-introduced as an adjunctive therapy for refractory angina, with good results. At therapeutic concentrations, perhexiline has no effects on systemic vascular resistance and is not negatively inotropic [121;190;191]. In a double-blind, randomized, placebo-controlled trial, our group have shown short-term beneficial effects of perhexiline in patients with ischaemic or non-ischaemic heart failure [132]. Patients taking optimal conventional medical therapy were randomized to perhexiline or placebo for 8 weeks, with dummy dose adjustment in the placebo group. We noted a large increase in the combined primary endpoint of peak oxygen uptake (≈ 3 ml kg–1 min–1) and LVEF (≈ 10 percentage points), and improvement in symptoms as assessed by the Minnesota Living with Heart Failure

Questionnaire. The study design involved separate randomization of the ischaemic and non ischaemic groups, permitting separate analysis of the primary endpoint in each group. Peak oxygen uptake was significantly increased in both the ischaemic and non-ischaemic groups; therefore suggesting the mechanism of benefit is not primarily anti-ischaemic. The study was however of short duration (8 weeks) and therefore, further work is required to show whether the clinical benefits of perhexiline are prolonged and whether it reduces hospitalization and mortality.

Oxfenicine

Oxfenicine is an irreversible inhibitor of carnitine palmitoyltransferase I (CPT1). Oxfenicine has been shown to delay development of terminal heart failure in animal models, attenuate adverse haemodynamic changes, prevent wall thinning, prevent the activation of matrix metalloproteinases, and result in the transcriptional downregulation of CPT1 and of key enzymes involved in cardiac energy metabolism in animal models of heart failure [137]. However a dose-related increase in cardiac weight due to uniform myocardial fiber hypertrophy of all cardiac chambers was noted in animal studies [76]. There was an increase in liver and kidney weights as well [16]. No human studies have been reported with use of Oxfenicine in heart failure.

Etomoxir

Etomoxir, an irreversible inhibitor of CPT1, was initially introduced as a therapy for diabetes due to its hypoglycemic properties [4;202]. It has been shown to favourably modify ventricular mass, geometry and function in pressure-overloaded rats [267], and reduce the occurrence of ventricular failure in diabetic rat hearts [142]. In humans, a 3-month, open-label, uncontrolled

trial in 10 patients with NYHA class II–III heart failure, etomoxir treatment in addition to standard therapy significantly improved LVEF, cardiac output at peak exercise, and clinical status [222]. However, recently a large randomized placebo controlled study was terminated prematurely because of the development of significant hepatotoxicity in four subjects [92]. Therefore etomoxir may not be a suitable metabolic modulator for use in heart failure.

Beta Blockers

β-adrenoreceptor blockade improves LV function and prognosis in patients with ischaemic or nonischaemic cardiomyopathy [31;185]. Interestingly, Wallhaus et al. demonstrated that in patients with heart failure carvedilol caused a 57% reduction in myocardial free fatty acid uptake [278]. However, neither mean myocardial uptake of labelled glucose tracers, nor the rates of glucose use were significantly increased. This could be because of a type II error, since a marked fall in the ratio of myocardial free fatty acid to glucose use does suggest a so-called metabolic shift. This effect may be due to CPT1 inhibition. In another study, Al-Hesayen and colleagues showed that after 4 months of carvedilol therapy, myocardial lactate consumption was increased and myocardial uptake of FFA in patients with CHF was reduced [9]. This suggests that carvedilol therapy may cause a significant shift in myocardial substrate use from FFA towards carbohydrates.

FFA β-Oxidation inhibitors

Trimetazidine

Trimetazidine reduces the oxidation of FFA via inhibition of the enzyme long-chain 3-ketoacyl coenzyme A thiolase, which is crucial in the β-oxidation pathway [111]. It is an effective anti-anginal agent with no significant vasodilator properties at rest or during

dynamic exercise [257]. In a double-blind, placebo-controlled trial involving 47 patients with CAD and a reduced LV function, limited by angina but not by heart failure, trimetazidine therapy improved LV systolic and diastolic function and quality of life [276]. A number of other studies have also demonstrated benefits with Trimetazidine. Di Napoli et al demonstrated in an 18 months, open-label study a significant improvement in LV function in patients with ischaemic cardiomyopathy with reduced LVEF [56]. Patients were excluded if they had experienced acute myocardial infarction less than 3 months previously, or had acute heart failure. Many patients however were taking suboptimal conventional therapy. Rosano and colleagues also demonstrated improvements in LVEF among patients with diabetes and coronary heart disease and LV systolic dysfunction, but without frank heart failure, following 6 months of trimetazidine therapy [210]. Recently, Fragasso et al demonstrated in patients with either ischaemic or non-ischaemic cardiomyopathy, an increase in LVEF and decrease in NYHA class with the use of Trimetazidine [67]. However, in another study, which assessed the effects of trimetazidine in patients with heart failure who were diabetic, revealed no significant improvement on exercise capacity and only minor effects on LV systolic function in both resting and exercise states [261].

Ronalazine

Ranolazine has been shown to be a partial inhibitor of fatty acid β oxidation [43;152], but this has not been reliably replicated in large experimental or human studies. Ranolazine also reduces late sodium entry into ischaemic myocardial cells and, therefore, is thought to indirectly reduce calcium uptake via the sodium–calcium exchanger, so preserving ionic homeostasis, and reversing ischaemia-induced contractile dysfunction [153]. The drug has anti-anginal properties. In a study of a canine microembolization model of heart failure, intravenous

ranolazine increased myocardial work without increasing myocardial oxygen use, which implies an increase in cardiac efficiency [39]. Acute intravenous administration of ranolazine also improved LV systolic function in dogs with heart failure [215]. So far there have been no studies looking at the effects of ranolazine in humans with CHF. There have been reports that the drug slightly prolongs the QT interval on electrocardiograms (ECGs). This effect raises concerns about possible complications such as torsade de pointes, polymorphic ventricular tachycardia and sudden cardiac death [133]. However, in the MERLIN TIMI 36 study on >6000 patients, ronalazine decreased the incidence of ventricular and supraventricular arrhythmias providing support for its safety [230].

Glucagon-Like Peptide 1

Glucagon-like peptide-1 (GLP-1) is a naturally occurring incretin with both insulinotropic and insulinomimetic properties with resultant increases in myocardial glucose uptake. In a dog model of heart failure, GLP-1 improved LV function and systemic haemodynamics [179]. In a small open label study, chronic infusion of GLP-1 significantly improved LV function, functional status, and quality of life in patients with severe heart failure [239]. However, in a recent study involving patients with heart failure, infusion of GLP-1 for 48 hours had no effects on LV ejection fraction or cardiac index [80]. GLP-1 is administered subcutaneously because of breakdown in the gut and it is given as an analogue because it is broken down by DPP IV intravascularly. However, there are now DPP IV inhibitors available that inhibit the breakdown of endogenous GLP-1 which are used to treat diabetes [169;198;211].

D-Ribose

D-Ribose is a pentose sugar which enhances ATP production by entering the pentose phosphate pathway and bypassing rate limiting steps of glycolysis. Omran et al studied the effect of ribose supplementation on cardiac haemodynamics and quality of life in patients with ischaemic cardiomyopathy. This was a prospective, double blind, randomised, cross over design study which showed improved quality of life and improved diastolic function with ribose supplementation [181]. However this has not been confirmed in large scale studies.

Proprionyl-L-Carnitine

Previous studies have demonstrated carnitine deficiency in heart failure patients [204;205;255]. Carnitine is an important co-factor in intermediary metabolism of the myocardium which improves utilisation of pyruvate in Krebs cycle. Studies with carnitine supplementation have shown mixed results. A small study by Anand et al showed improvement in maximal oxygen consumption at peak exercise (VO2 max) but no change in EF [12]. However a larger study in 1999 with 537 patients showed no improvement in exercise capacity [2].

Conclusion

Myocardial metabolism is altered in ischaemia and heart failure and plays a key role in the pathogenesis and progression of these diseases. Acute and chronic ischaemia causes a shift towards increased glucose utilization which could partially compensate the oxygen deficiency. However, myocardial metabolism in heart failure appears to be complicated and depends on aetiology, duration and whether associated comorbid disorders are present. Nevertheless, medications that can augment myocardial metabolism would seem to have a potential role in the management of these heart diseases.

Diabetes and the heart

Diabetes is a metabolic disorder characterised by hyperglycaemia, insulin resistance and/or deficiency and dyslipidaemia. Its prevalence is rapidly increasing throughout the western world [117]. It is associated with hypertension, microvascular disease [1;122;173;206] and premature atherosclerosis in the large and medium sized arteries. Heart failure occurs more frequently in diabetes patients [109] and is commonly due to epicardial CAD [101;126;250] and/or hypertension. However in some patients LV dysfunction occurs in the absence of significant epicardial CAD or hypertension [214]. The term 'diabetic cardiomyopathy' has been applied to this syndrome. The Framingham study demonstrated increased incidence of heart failure in both diabetic males (2.4:1) and females (5:1) [109]. Diabetes patients also have a far greater risk of developing heart failure and have poorer prognosis following myocardial infarction than patients without diabetes[101;250]. Diabetic cardiac consequences initially involve diastolic dysfunction but ultimately this may progress to systolic dysfunction, which may be severe. Cardiac fibrosis [112;207] and myocyte hypertrophy are prominent histological features [138].

Previous rodent studies in type 1 diabetes (T1DM) (streptozotocin-induced or genetic nonobese diabetic mice) and type 2 diabetes (T2DM) (Zucker diabetic fatty rats and ob/ob or db/db mice) have demonstrated systolic and diastolic dysfunction [3;253;265]. In vivo studies have shown elevated diastolic pressure with decreased systolic pressure and altered dP/dt (change in pressure over time) [108]. Rodents are resistant to atherosclerosis and hence all the changes can be attributed directly to diabetes [11]. However it is important to note that experimental diabetes does not precisely mimic either type 1 or type 2 diabetes and hence the pathophysiology of diabetic cardiomyopathy may be different in humans.

Metabolic changes in the myocardium in diabetes

The metabolic changes in the myocardium in patients with diabetes potentially play a significant role in the development and progression of CAD and CHF. Both type 1 and type 2 diabetes result in significant changes to cardiac energy metabolism which may contribute to the development of contractile dysfunction and increases the potential for ischaemia induced injury. Diabetes results in alteration of plasma substrates. Decreased glucose utilisation (because of insulin deficiency and/or resistance) and increased hepatic glucose production results in hyperglycaemia. Enhanced lipolysis and increased production of lipoprotein lipases by the liver results in increased plasma FFA. Diabetes is associated with decreased glucose transport, glycolysis and oxidation [141]. The reduced uptake of glucose via GLUT4 receptors which is insulin-dependent decreases the availability of glucose in the myocardium [243]. However FFA entry into the myocardium is not hormone dependent and high levels of circulating FFA result in their accumulation in the myocardium. In normal hearts, 60-90% of the energy is derived from fatty acid oxidation. In diabetes this can increase to 90-100% [140]. The increased utilization of FFA to generate energy has been demonstrated in PET studies on patients with T1DM [88]. Increased FFA usage has a greater oxygen cost, in part explicable by stoichiometry but also possibly due to increased mitochondrial uncoupling [225] and wasteful cycling of FFAs through intramyocardial lipolysis and esterification [91;209]. In T1DM, a recent PET study demonstrated reduced cardiac glucose uptake and utilisation and markedly increased FFA uptake and utilisation [88]. The increased FFA uptake results in lipid accumulation in the form of triglycerides and ceramides in spite of the increased FFA oxidation [29]. Lipid accumulation can worsen the myocardial insulin resistance which in turn worsens glucose uptake [188]. Also increased FFA oxidation produces more reactive oxygen

species (ROS) which can oxidize lipids and proteins resulting in cell damage. ROS impairs mitochondrial coupling resulting in decrease in ATP production.

There is a direct relationship between altered energy metabolism and cardiac muscle dysfunction. Systolic and diastolic dysfunction has been demonstrated by echocardiography in streptozotocin-induced type 1 diabetic rats [106] and elevated LV end diastolic pressure and reduced systolic pressure has been demonstrated by invasive measurements [108]. An increase in FFA metabolism with decrease in uptake and utilisation of glucose has also been demonstrated in this animal model and directly implicated in contractile dysfunction [232;243]. Cardiac dysfunction begins with diastolic abnormalities and finally leads to systolic dysfunction. However, it has been shown that altering cardiac metabolism reverses the contractile abnormalities [40;178]. Similarly systolic and diastolic dysfunction has been detected by echocardiography in type 2 diabetic mice associated with abnormal metabolism [231].

The impact of diabetes on the heart can occur via several other mechanisms including:

Unrecognised hypertension - Patients presenting in heart failure may be viewed as normotensive but have prior, unrecognised hypertension. Furthermore, increased large artery stiffness [48;223;237] is a prominent feature in diabetes patients and in these circumstances central aortic systolic pressure may be considerably higher than brachial pressures.

Microvascular disease – This occurs frequently in both type1 and type2 diabetes and is related to duration of diabetes [122;173] and to glycaemic control [1;206]. One of the important consequences of diabetic micro-angiopathy is the development of thickened basement

membrane. The thickened basement membrane results in increased permeability via compromised tight junctions and vesicular transport[213]. Microvascular disease causes ischaemia which along with increased capillary permeability may be involved in the pathogenesis of diabetic heart failure [149]. Previous studies have demonstrated a reduced coronary flow reserve [55;119;128;130;170;195] in asymptomatic (type 1 and 2) diabetes patients as compared to healthy controls (HC), which is a measure of microvascular dysfunction in the heart (in the absence of epicardial CAD).

Energetic impairment – Asymptomatic type 2 diabetes patients have impaired cardiac energetic status as reflected in a reduced PCr/ATP ratio on MRS and also have impaired skeletal muscle energetics (as reflected by a prolonged PCr recovery half time following exercise) [221]. Whilst microvascular ischaemia may potentially be contributory, a primary disturbance of energetic status unrelated to ischaemia is also likely. The potential role of altered cardiac substrate utilisation and of uncoupling in this energetic impairment is discussed below.

Oxidant stress – Diabetes is characterised by increased oxidant stress. Multiple sources may contribute to increased production of ROS, but a substantial body of evidence suggests an important role for nicotinamide adenine dinucleotide phosphate (NADPH) oxidase derived ROS and uncoupled nitric oxide synthase (NOS) [135]. ROS (particularly NADPH derived) have been implicated as major factors in the development of LVH and cardiac fibrosis and in the transition of LVH to heart failure [90;146]. Ventricular myocytes of streptozotocin-induced diabetic rats develop a slow diastolic decay of calcium transients and require longer times for

maximum cell shortening and re-lengthening [125]. In this model, angiotensin II mediated NADPH oxidase activation is involved in cardiomyocyte dysfunction [199;285].

Cardiac autonomic neuropathy (CAN) – Autonomic dysfunction potentially plays a significant role in development and progression of cardiomyopathy. It also predisposes to life threatening arrhythmias. Previous studies by our group have demonstrated that CAN is associated with impaired myocardial blood flow using PET [197;246]. The sympathetic dysinnervation of the heart commenced in the distal LV regions near the apex and then spread towards the base in patients with advanced disease [247].

Uncoupling proteins - Mitochondrial uncoupling is a process which was first described in brown fat [212]. Long chain fatty acids and lipid peroxides [74] are exported across the mitochondrial membrane in exchange for protons via specific proteins known as UCP, resulting in dissipation of the mitochondrial electro chemical gradient. The physiological role of uncoupling has been the subject of much debate [225] but it has been proposed that its primary role is to export excess long chain fatty acids and toxic lipid peroxides. Uncoupling protein expression is upregulated by fatty acids via the PPAR α receptor [168;240]. The activity of UCP is increased by intra-mitochondrial fatty acid concentrations [228] and by ROS and lipid peroxides [74]. Since the early description of uncoupling in brown fat [84] it has been shown that UCP are also expressed in other cell types. UCP2 is ubiquitous, including expression in the heart, whereas UCP3 expression is mainly found in glycolytic rather than oxidative skeletal muscle [8;26]. Theoretically diabetes should be associated with increased uncoupling because increased cardiac and skeletal myocyte cytosolic fatty acids should upregulate UCP expression [154;218;240]. Increased intra-mitochondrial FFAs, ROS generation and lipid

peroxidation via ROS should also increase the activity of UCP [74;228]. However, unexpectedly (and requiring confirmation) one group reported that uncoupling protein expression was reduced in skeletal muscle in diabetes patients [224;227], but in streptozocin-induced diabetic mice cardiac UCP expression was upregulated [168]. Whatever the level of uncoupling protein expression, the activity of UCPs may be markedly increased in diabetes patients for the reasons noted above and this may be more important than UCP expression levels in determining uncoupling.

Structural changes in the heart associated with diabetes

Although there is increasing evidence for the presence of diabetic cardiomyopathy as a separate entity, detection of early changes in the myocardium is challenging in patients with diabetes. Various imaging modalities have been used to detect these changes.

Echocardiography: LVH is one of the commonest structural abnormalities demonstrated in diabetes. Previous studies with 2 dimensional (2D) and tissue doppler echocardiography have demonstrated abnormalities in various diastolic parameters prior to the onset of overt systolic dysfunction. The trans-mitral pulsed wave doppler demonstrates abnormalities in mitral inflow velocity and deceleration time (DecT), isovolumetric relaxation time (IVRT) and filling patterns [196]. However these measurements are load-dependent and can alter depending on left atrial (LA) pressures. Tissue doppler imaging (TDI) is a relatively load independent method of deriving mitral annular velocities. Previous studies in subjects with both T1DM and T2DM have demonstrated a reduction in long axis function indicated by reduced S and E' wave velocity in the presence of normal EF [61;275]. The E/E' is a good measure of LV end diastolic pressure and increased E/E' is associated with diastolic heart failure.

Computed Tomography: Electron beam tomography is able to measure coronary artery calcium which is one of the early markers of atherosclerosis. Coronary artery calcium scoring is highly sensitive in predicting the presence of coronary atherosclerosis[32;34]. However, it lacks specificity for predicting obstructive coronary artery disease[32;33]. Coronary artery calcium also has prognostic value and predicts future cardiovascular events [54]. Previous studies in young patients with T1DM have demonstrated significantly increased prevalence of coronary artery calcium indicating early onset of CAD in these individuals [245]. In another study invoving patients with T2DM Wolfe et al demonstrated increased coronary artery calcium scoring in patients with diabetes as compared with patients without diabetes even when matched for other risk factors [283]. Hence computed tomography can be utilised to detect patients with high risk of developing cardiovascular complications.

Magnetic Resonance Imaging (MRI) and Spectroscopy: MRI provides improved resolution in imaging the myocardium. In particular, tagged MRI allows measurement of myocardial velocities and LV torsion. In streptozotocin-induced type 1 diabetic mice, high resolution MRI demonstrated increase LV wall volume indicating LVH and also features suggestive of increased stiffness due to fibrosis [138]. A tagged MRI study in T1DM patients has shown increased LV torsion and torsion rate in the presence of tight glycaemic control [42].

Conclusions

The development of heart failure in diabetes is multi-factorial. Diabetes is a metabolic disorder associated with metabolic alterations in the myocardium. Insulin resistance and/or deficiency results in increased FFA utilisation and decreased glucose utilisation. This

potentially contributes significantly to the development of cardiomyopathy. Studying myocardial metabolism provides insights into the patho-physiology of heart failure in diabetes.

Aims and hypotheses of all the studies

MRS feasibility and reproducibility at 3T

Traditionally cardiac MRS is done at 1.5T. 3T offers increased signal to noise ratio (SNR). The increased sensitivity can be traded for either increased spatial resolution or improved quantification precision and hence might improve specificity of cardiac MRS as a diagnostic tool. The aims of this study were –

1) To develop a robust, practical and reproducible protocol for cardiac MRS studies at 3T.

2) To test the feasibility and reproducibility of performing cardiac MRS at 3T.

Perhexiline studies

Perhexiline was being used on a named patient basis at University Hospital Birmingham NHS Trust and Cardiff and Vale NHS Trust for patients with refractory angina and/or refractory CHF. Patients were on maximal tolerated medical therapy and were deemed not amenable for revascularisation. We aimed to test the feasibility of administering Perxiline in this clinical setting. We sought to study the effect of Perhexiline on glucose and fat metabolism in these patients. Previous animal studies have demonstrated a concentration-dependent differential inhibition of CPT-1 by Perhexiline in the liver or muscle [115]. This can result in variable effects on glucose and fat metabolism. Studying these effects of Perhexiline will throw more light on the CPT-1 inhibition patterns in humans. The aims of the Perhexiline studies were as follows–

1) To measure the impact of Perhexiline on insulin sensitivity as measured by the HOMA method.

2) To assess the impact of Perhexiline on plasma FFA, ketones, triglycerides and glycerol.

3) To assess the feasibility of safely administering Perhexiline in a clinical setting with adequate monitoring of drugs levels and side effects.

Hypotheses

1) By inhibiting beta-oxidation of FFA and enhancing glucose utilisation Perhexiline improves insulin sensitivity.

2) Inhibition of betaoxidation of FFA results in accumulation of FFA in the plasma.

3) Perhexiline can be safely administered with regular monitoring of drug levels.

Studies in diabetes patients

We conducted all our studies in patients with type 1 diabetes. We chose a cohort of young patients with no history of hypertension, primary hyperlipidemia, renal failure, coronary artery disease or heart failure. In order to tease out the pathophysiological effects of diabetes on the heart we divided the patients into two subgroups; newly diagnosed diabetes (diagnosis in the last 5 years) and long term diabetes (diagnosed more than 10 years ago). The aims of the studies were as follows-

1) To confirm the presence of cardiac energetic impairment in these patients.

2) To study the pathophysiological mechanisms responsible for the development of cardiac energetic impairment, in particular the role of coronary microvascular dysfunction and metabolic effects of diabetes.

3) To assess the early changes in LV torsion in diabetes and its relationship to coronary microvascular dysfunction.

4) To assess LV untwisting patterns in diabetes and its relation to diastolic function and LV filling.

5) To assess LA contribution to LV filling in the early stages of diabetic cardiomyopathy

Hypotheses

1) Cardiac energetic impairment occurs early in diabetes and is primarily due to metabolic effects of diabetes.

2) Coronary microvascular disease plays little role in the development of cardiac energetic impairment.

3) Study of changes in LV torsion will provide insights into the early changes in systolic function in diabetes.

4) Study of LV untwisting patterns and LV filling will provide insights into early changes in diastolic function in diabetes.

Power calculations

Aims 1 and 2; 10 subjects would be required in each group (newly diagnosed diabetes patients, long term diabetes patients and healthy controls) to identify a difference of 25% in PCr/ATP ratio with a power of 90% and a significance (P value) of <0.05. The estimated standard deviation was 0.4 from previous studies in patients with type 2 diabetes mellitus[221]. We aimed to recruit a higher number of subjects in the long term diabetes group (15 subjects). This was to ensure that adequate number of these patients would have coronary microvascular dysfunction and hence allow us to assess its role in pathophysiology of cardiac energetic impairment. We also aimed to recruit age and sex matched controls for both sub groups of diabetes patients. Hence a total of 26 healthy volunteers were recruited.

Aims 3 and 4; 30 subjects were required in each group (diabetes patients and healthy controls) to identify a difference of 25% in LV rotation measurements with a power of 90% and significance (P value) of <0.05. The estimated standard deviation was 3.3 from previous studies of speckle tracking in healthy controls[85] .

CHAPTER TWO

METHODS

This chapter describes the different techniques used in the project.

Storage of serum samples

The blood samples were collected in lithium heparin, EDTA and sodium fluoride blood tubes. The blood tubes were centrifuged for 15 minutes at 1500 revolutions per second at 4^{o}C. The serum was pipetted into plain tubes and stored at -80^{o}C. The samples were defrosted before analysis.

Insulin assay

Frozen serum samples were used to perform insulin assay using Mercodia insulin ELIZA kit. A 96 well micro plate coated with mouse monoclonal anti-insulin antibody was used. 25 µl of calibrator or samples was pipetted into each well. 100 µl of enzyme conjugate solution (peroxidise conjugated anti-insulin antibodies) was added to this and incubated on a plate shaker at room temperature for 1 hour. Following this the micro plate was washed with wash buffer six times to remove any excessive unbound enzyme conjugate. Then 200 µl of substrate 3,3',5,5' tetra-methyl-benzidine was added to each well and incubated at room temperature for 15 minutes. At the end of 15 minutes 50 µl of stop solution (0.5 M H_2SO_4) was added and the plate read at 450 nm on a colorimetric spectrophotometer. A calibrator curve was plotted using the absorbance of the different calibrators. The concentration of insulin in the samples was determined from the calibrator curve.

Ketone assay

Frozen serum samples were used to perform the ketone assay using the Ranbut ketone analysis kit. This kit measures D-3-hydroxybutarate based on its oxidation to acetoacetate by the enzyme 3-hydroxybutarate dehydrogenase. Concomitant to this reaction NAD^+ is reduced

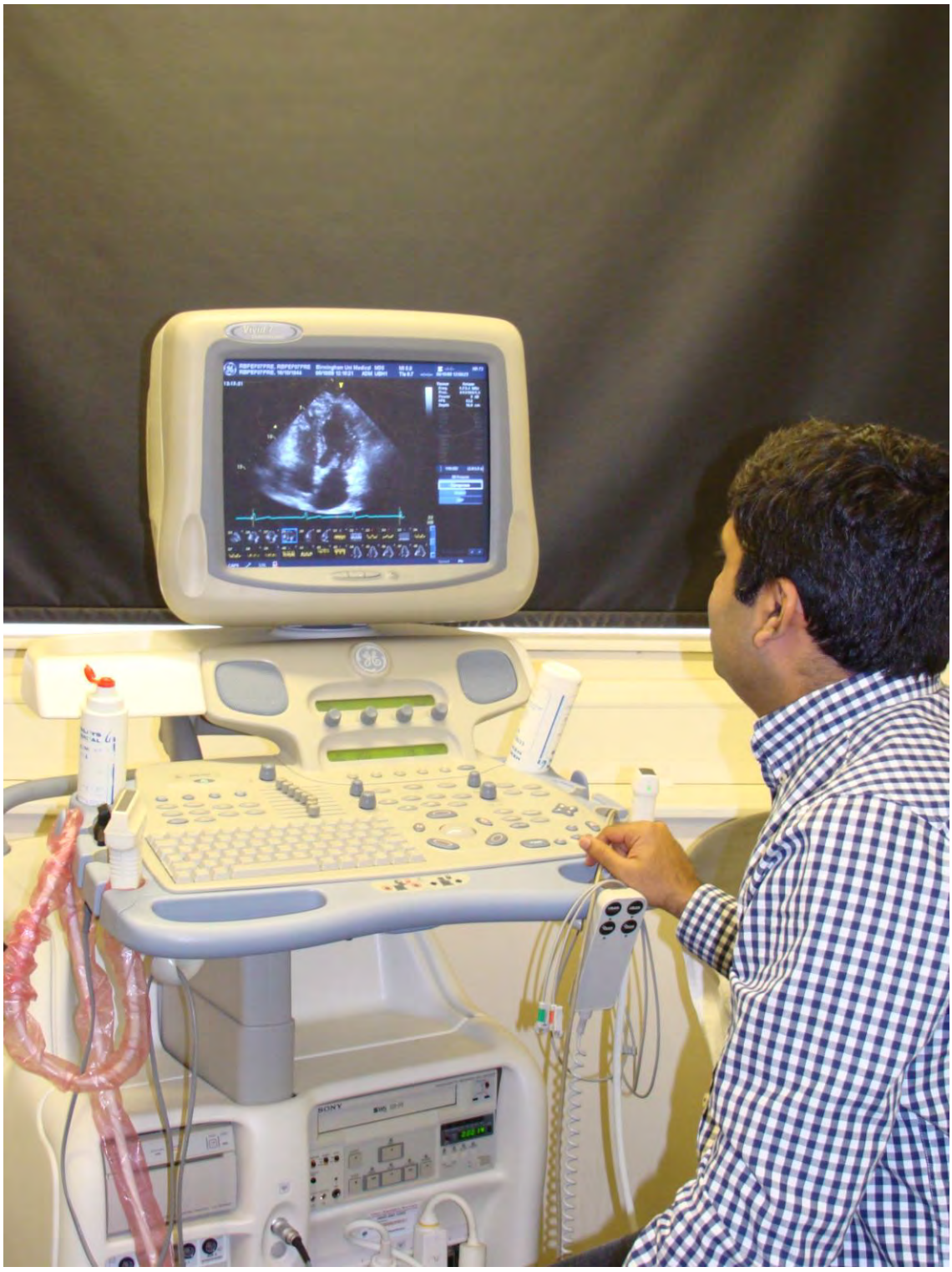
to NADH which alters the absorbance that is directly correlated with the 3-hydroxybutarate concentration. A 96 well micro plate was used and 25µl of calibrator or the serum was added to each well. 25 µl of distilled water and 1ml of reagent were added to this. The contents were mixed and then read at 340nm in a spectrometer at 37^0C following incubation for 60 sec. The 3-hydroxybutarate concentration was calculated as follows:

$$\text{3-hydroxybutarate} = \frac{\Delta A\text{sample}}{\Delta A\text{standard}} \times \text{standard concentration}$$

Echocardiography

Echocardiography was performed with participants in the left lateral decubitus position with a Vivid 7 (GE Vingmed) echocardiographic machine and a 2.5-MHz transducer (Figure 2.1). Standard echocardiographic views were obtained from parasternal (short axis grey scale images at mitral, papillary and apical levels) and apical (4 and 2 chamber views) windows. An average of three cycles was stored for each view. Trans-mitral flow profiles and tissue doppler measurements of mitral annular velocities were performed. The EF was calculated with Simpson's rule[79]. The grey scale images obtained both in the parasternal short axis and apical view were used to compute LV strain, strain rate and rotation using commercially available speckle tracking software on Echopac.

Figure 2.1



Vivid 7 echocardiographic machine

Speckle Tracking Echocardiography (STE)

Myocardial deformation was measured using a commercially available speckle tracking system in an ECHOPAC (version 4.2.0) workstation. In this system, the displacements of speckles of myocardium in each spot were analyzed and tracked from frame to frame (Figure2.2). We selected the best-quality digital two-dimensional image and the LV endocardium was traced at end-systole. The region of interest width was adjusted as required to fit the wall thickness. The software package then automatically tracked the motion through the rest of the cardiac cycle. The onset of QRS complex was taken as the beginning of systole. Adequate tracking was verified in real time. The global longitudinal and transverse strain and strain rates were derived from an average of the 12 segments from the 4 and 2 chamber views (6 segments in each view). Similarly an 18 segment model was computed from the short axis views at mitral, papillary and apical levels. The average of measurements from each of the segments was used to derive global circumferential and radial strain and strain rates. In addition, LV rotation was computed using speckle tracking. Counter-clockwise rotation was marked as a positive value and clockwise rotation as a negative value when viewed from the apex. In order to calculate LV torsion, torsion rate and untwist rates, the rotation traces of the basal and apical LV cross-sections were exported into DPlot graph software (Version 2.2.1.4, HydeSoft Computing, LLC, Vicksburg, USA). The LV twist curve was generated by calculating the difference between apical and basal rotations at each corresponding time point. LV twist rates were derived from the first derivative of the LV twist curve. Peak LV torsion was derived from LV twist divided by LV diastolic longitudinal length. Rotational deformation delay was also determined and defined as the magnitude of the time difference between time to peak basal rotation and time to peak apical rotation. In order to adjust for the

differences in heart rate between individuals the RR interval was normalised to 100% for calculation of time intervals.

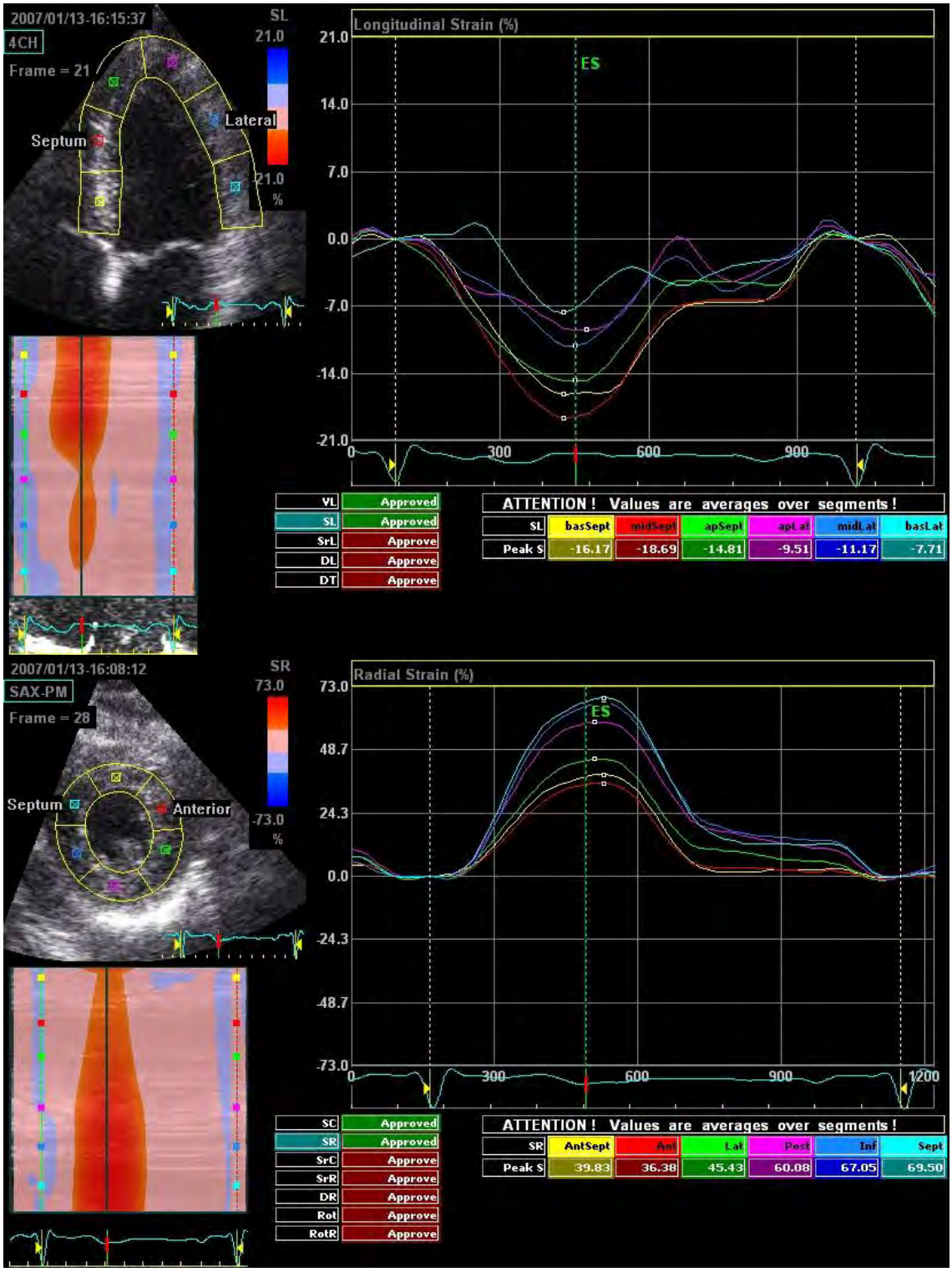


Figure 2.2: Examples of radial (bottom figure) and longitudinal (top figure) strain determined using speckle tracking echocardiography. The myocardium is divided into six equal segments and strain in each segment represented by different coloured line.

Metabolic Exercise Testing

Subjects underwent symptom-limited erect treadmill exercise testing using a standard ramp protocol with simultaneous respiratory gas analysis on a Schiller CS-200 Ergo-Spiro exercise machine. Sampling of expired gases was performed continuously, and data were expressed as 30-second means. Minute ventilation, oxygen consumption, carbon dioxide production, and respiratory exchange ratio (RER) were obtained. Peak oxygen consumption ($V0_2max$) was defined as the highest value of oxygen consumption measured during the exercise period. Blood pressure and ECG were monitored throughout. Subjects were encouraged to exercise to exhaustion with a minimal requirement of RER > 1.

^{31} P cardiac MRS

^{31}P cardiac MRS was performed using a Phillips Achieva 3T scanner (Figure 2.3) and a linearly polarized transmit and receive ^{31}P coil with a diameter of 14 cm. Localization was achieved by ISIS ^{184} volume selection. The participants were positioned supine with the coil directly over the precordium. The coil was secured in place by straps wrapped around the upper body of the subject and the coil. The participants were then positioned inside the magnet with the center of the coil at the isocenter of the magnet. Survey images were obtained to check the position of the coil (Figure 2.4). The subjects and/or the coil were repositioned if required to ensure that the distance between coil and septum and apex of the heart was minimized.

The standard phosphorus spectroscopy sequence provided by the manufacturer was used. It was based on hyperbolic secant pulses for slice selective inversion and adiabatic half passage RF pulse for non-selective excitation. In contrast to the standard procedure manual fine

adjustment of F_0 was performed if the automatic F_0 determination was not correct in order to ensure the correct voxel position. In contrast to the default iterative or FASTERMAP based shimming algorithm, which was based on the selected spectroscopy VOI, an image guided shim volume was selected that included the entire myocardium. A short axis cine scan was acquired to calculate the trigger delay (TD) for ECG triggering and check quality of shimming and F_0 determination. The TD was calculated such that the spectra were acquired in the diastolic period. The 3-D voxel of acquisition was planned to include most of the septum and apex of the heart. Care was taken to minimize blood contamination from the right ventricle as much as possible. The voxel size was kept constant at 89.54ml (44x55x37mm3) so that comparisons could be made between different subjects and scans. Initially, ^{1}H spectra were acquired from the same voxel without water suppression and repetition time (TR) of 2000 ms (total scan time of 16 sec). This helped to ensure adequate shim quality and correct F_0 determination. F_0 could be manually adjusted if necessary. Following this the ^{31}P spectrum was acquired with a TR of 10000 ms, 136 averages and 512 samples. A TR of 10000 ms was found to be optimal to adequately reduce saturation effects without increasing the scan time greatly. The spectral acquisition was ECG gated and the TD was set to acquire spectra mainly in diastole. The TD was measured by subtracting 250-300 from the total length of the cardiac cycle which allowed 250-300 msec of the cardiac cycle left for spectral acquisition (acquisition time is 170 msec). The total scan time was 23 minutes.

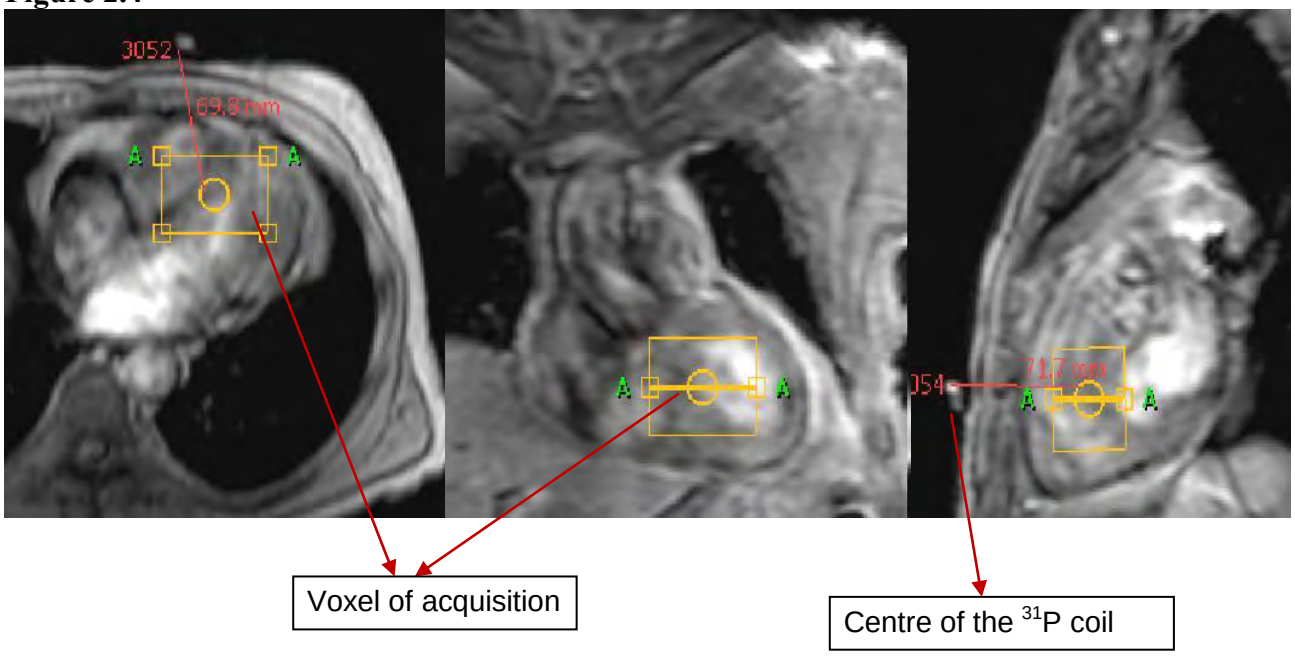
Increased chemical shift artefacts are present at 3T. In order to minimise this, slice selective inversion for ISIS encoding was based on adiabatic hyperbolic secant pulses, which achieved a pulse bandwidth between 1300 Hz (at a distance of 9 cm from the surface coil) to 2000 Hz (at a distance of 3 cm from the surface coil). This corresponds to a chemical shift

displacement of 6-10% for the investigated metabolites PCr and gamma-ATP for volumes of interest that were between 3 and 9 cm away from the coil. When subjects where scanned the distance from the coil to the ROI averaged about 7.5cm and no subjects were beyond 9.0 cm. Therefore all subjects would have a chemical shift displacement less than 10% which is acceptable.

Figure 2.3

Phillips Acheiva 3T MRI scanner which was used for magnetic resonance spectroscopy and magnetic resonance imaging (including stress MRI)

Figure 2.4



Survey images (in transverse, coronal and saggital planes) showing the position of voxel of acquisition (yellow box) and centre of the ^{31}P coil. The voxel is positioned such that it remains completely within the myocardium and as close as possible to the center of ^{31}P coil.

MRS

The spectra were analysed and quantified on jMRUI software using AMARES a time domain fitting program [271]. Post-processing was performed with 15Hz Gaussian line broadening and Fourier transformation. Phase correction was performed with the PCr peak as the reference peak. Quantification was performed with AMARES using a prior knowledge file to preselect the peaks. The concentrations of PCr, ATP and 2,3-Diphosphoglycerate (2,3-DPG) were calculated as the area under the peaks. PCr/ATP ratio was determined after correcting the ATP peak for blood contamination as described previously[46].

Cardiac MRI

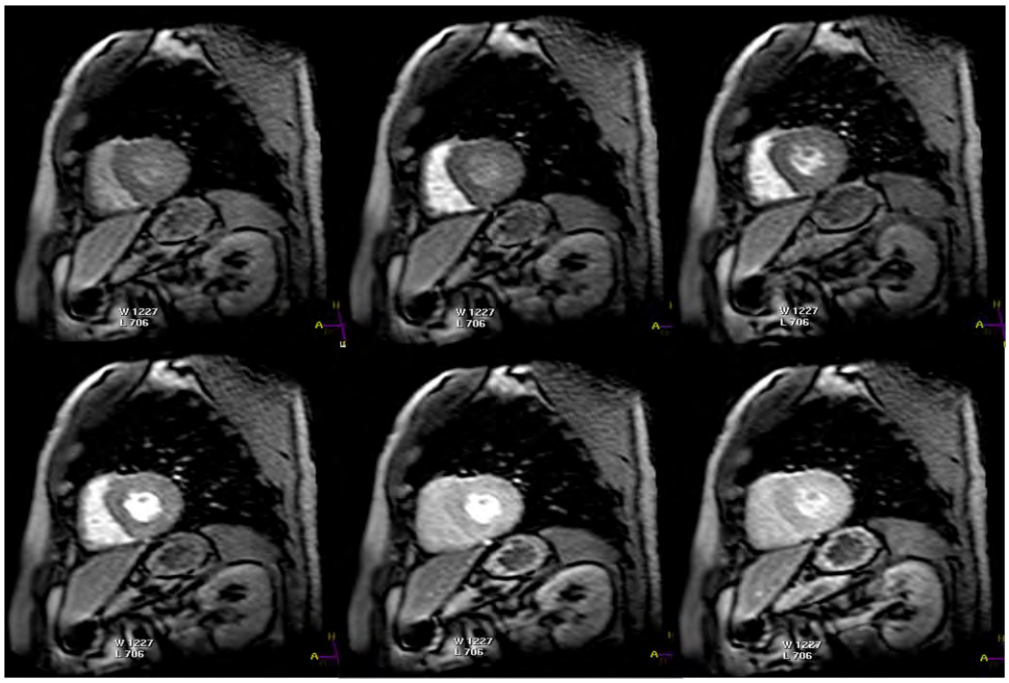
Cardiac MRI was performed on a 3 T Phillips Achieva MRI scanner (Figure 2.3). The standard protocol provided by the manufacturer was used. The subjects were positioned supine with a dedicated cardiac sense coil wrapped around the chest. The subjects were positioned in the scanner with their heart at the isocenter of the magnet. Survey images were obtained and the short axis images were planned from this. The LV was divided into slices of 8mm with a gap of 2mm. A total of 12 slices ensured complete coverage of the LV from apex to base. Short axis cine images were acquired at breath hold using sense cine sequences. LV volumes were computed from the short axis cine MRI images. The images were analyzed on the Viewforum software version 5.0. The endocardial and epicardial borders were traced at end diastole in each slice and propagated through the rest of the cardiac cycle. The software generated LV volumes at each phase of the cardiac cycle incorporating all the slices. The LV volume time curves were exported to graphical software Dplot. The first derivative of the LV volume curve was computed to represents the rate of LV volume changes. SV is defined as the difference between end-diastolic volume (EDV) and end-systolic volume (EDS). Early

and late peak filling rates (PFR) were defined as the first and second positive peaks of the first derivative curve during diastole. The early and late (atrial) components of LV filling were measured from the corresponding LV volume segments and were expressed as percentage of SV.

Stress MRI

Cardiac MRI was performed on a 3 T Phillips Achieva MRI scanner (Figure 2.3). The standard protocol provided by the German Heart Institute was used for the assessment of myocardial perfusion during adenosine mediated vasodilation (Perfusion cook book - http://www.cmr-academy.com/). The subjects were positioned supine with a dedicated cardiac sense coil wrapped around the chest. The subjects were positioned in the scanner with their heart at the isocenter of the magnet. Survey images were obtained which was followed by first pass images after Gadolinium contrast injection (0.1ml/kg body weight). For perfusion images, a single shot Turbo Field Echo SENSE pulse sequence was used with three slices per heart beat. Slice thickness was 8mm with a gap of 12mm between the slices. The field of view was 400mm with a matrix of 152x109. The TR was 3.5ms with an echo time of 1.05ms. LV was imaged in the short axis at three levels tracking the entry of contrast first into the right ventricle, and then the LV cavity followed by the illumination of the LV myocardium as the contrast passed through the coronary arterial tree (Figure 2.5). After a gap of twenty minutes which allowed the contrast to be eliminated completely from the myocardium, adenosine infusion was started at a rate of 140mcg/kg/min. At three minutes of infusion stress first pass images were obtained following injection of further Gadolinium contrast (0.1ml/kg) in a similar fashion and at the same short axis levels. ECG was continuously monitored and blood pressure was recorded every minute during the stress scan.

Figure 2.5



Short axis images of the heart depicting the passage of Gadolinium contrast through the right ventricle, then the LV cavity followed by illumination of the LV myocardium.

MPRI

Myocardial perfusion reserve index (MPRI) which is a measure of coronary microvascular function was computed from the stress MRI images. The images were analyzed on the Viewforum software version 5.0. Initially image alignment was done to reduce the motion of the heart in different cardiac cycles. Following this the endocardial and epicardial borders were traced and propagated throughout all the images. A sample volume was placed in the LV cavity as comparison for signal intensity of blood. Signal intensity curves were obtained from the software and the peak upslope of the LV myocardial illumination was computed in relation to the LV cavity illumination. A similar analysis was done on all three short axis images at rest and peak stress. The MPRI was obtained from the ratio of LV relative peak upslope at stress compared to rest.

CHAPTER THREE

^{31}P MAGNETIC RESONANCE SPECTROSCOPY OF THE HUMAN MYOCARDIUM AT 3 TESLA – TEST OF FEASIBILITY AND REPRODUCIBILITY

Publications

1. **Dr G Nallur Shivu……..** Prof M Frenneaux. ^{31}P Magnetic Resonance Spectroscopy to Measure In Vivo Cardiac Energetics in Normal Myocardium and Hypertrophic Cardiomyopathy: Experiences at 3 Tesla. **European Journal of Radiology**. 2010;73:255-259.

The following work was done in conjunction with two other PhD students, Dr Thanh Phan and Dr Khalid Abozguia. We equally contributed to this work.
Dr Roger Beadle has provided the data on further validation testing in our department on twelve healthy volunteers.

Abstract

Background: ^{31}P MRS allows measurement of *in vivo* high energy phosphate kinetics in the myocardium. Whilst traditionally ^{31}P cardiac spectroscopy is performed at 1.5 Tesla, Cardiac MRS at higher field strength can theoretically increase SNR and spectral resolution therefore improving sensitivity and specificity of the cardiac spectra. The reproducibility and feasibility of performing cardiac spectroscopy at 3 Tesla is presented here in this study in healthy volunteers and patients with HCM.

Methods: Cardiac spectroscopy was performed using a Phillips 3T Achieva scanner in 37 healthy volunteers and 26 patients with HCM to test the feasibility of the protocol. To test the reproducibility a single volunteer was scanned eight times on separate occasions. A single voxel ^{31}P MRS was performed using Image Selected Invivo Spectroscopy (ISIS) volume localisation.

Results: The mean PCr/ATP ratio of the eight measurements performed on one individual was 2.11±0.25. Bland Altman plots showed a variance of 12% in the measurement of PCr/ATP ratios. The PCr/ATP ratio was significantly reduced in HCM patients compared to controls, 1.42±0.51 and 2.11±0.57, respectively, $P<0.0001$. (All results are expressed as mean ± standard deviation)

Conclusions: Here we demonstrate that cardiac ^{31}P MRS at 3T is a reliable method of measuring in vivo high energy phosphate kinetics in the myocardium for clinical studies and diagnostics. Based on our data an impairment of cardiac energetic state in patients with HCM is indisputable.

Background

Phosphorus MRS is a non-invasive method of studying cardiac *in vivo* high energy phosphate kinetics [27]. It allows for determination of PCr, ATP, ADP and inorganic phosphate (Pi) concentrations in the myocardium. The concentrations of these substances and the ratio of PCr/ATP are measures of the cardiac energetic status. PCr is an important short-term reserve energy source that maintains a high phosphorylation potential under conditions of increased energy demand like exercise and ischaemia. The conversion of ADP to ATP by transfer of a phosphoryl group from PCr is catalysed by CK. This reaction occurs 10 times faster than ATP production via oxidative phosphorylation [22]. In patients with mild to moderate CHF, cardiac ATP flux mediated by CK is reduced by approximately 50% [280]. Animal and human studies have demonstrated that a progressive reduction of the creatine pool is directly related to the severity of heart failure [171]. This is largely due to a decrease in the number of creatine transporters at sites of energy production and utilisation [51]. In normal myocardium, two thirds of the creatine pool is phosphorylated via CK reaction to form PCr [5] and the expression and activity of this enzyme is reduced in heart failure [238;286]. Therefore in heart failure the available PCr is markedly diminished. The depletion of PCr is greater than the depletion of ATP resulting in reduced PCr/ATP ratio in heart failure as measured by MRS [45]. A reduced PCr/ATP ratio is associated with increased mortality in heart failure patients [176]. ^{31}P cardiac spectroscopy can also be used to monitor disease progress in heart failure patients. It has been used as an objective marker to show benefits of various treatments such as metabolic modulators [68].

Reduced PCr/ATP ratio is also seen in other conditions including ischaemic heart disease [279], LVH secondary to hypertension [150], valvular heart disease (mitral regurgitation and aortic

valve disease) [46;176], diabetes [221] and HCM [50;107;216]. Interestingly, patients with genotypic HCM who do not yet have hypertrophy have a similar degree of impairment of cardiac PCr/ATP ratio as do patients with marked hypertrophy, implying that the disturbance may be an early feature of the disease and is not simply due to the hypertrophy [47].

Traditionally cardiac spectroscopy in humans has been performed using 1.5 Tesla magnets. However, previous studies in animals [131] and humans demonstrated that higher field strength such as 3T [269] or 4.7T offer higher signal to noise ratio (SNR). The increased sensitivity can be traded for either increased spatial resolution or improved quantification precision and hence might improve specificity of ^{31}P cardiac MRS as a diagnostic tool. Hence it would seem desirable to perform cardiac MRS at 3T MR scanners. However, higher field strength also result in inhomogeneities of the transmit and receive B_1 field along with restrictions of the maximum achievable B_1 field strength that result in larger chemical shift displacements. Furthermore susceptibility differences between adjacent tissues have a greater effect on B_0 homogeneity [41], which results in line broadening. Hence the methods available to perform spectroscopy in 1.5 Tesla magnets cannot be reproduced in 3 Tesla magnets. We therefore conducted experiments to adapt the spectroscopy methods to suit 3 Tesla magnets. This study represents the development of a suitable method to perform cardiac spectroscopy at 3 Tesla. Data on feasibility and reliability of ^{31}P cardiac spectroscopy at 3 Tesla is presented.

Methods

Our initial aim was to develop a robust, practical and reproducible protocol for cardiac spectroscopy which could be used in all research studies. I worked along with two other PhD students on this task (Dr Khalid Abozguia and Dr Thanh Trung Phan). 9 Healthy volunteers

who provided written informed consent participated in this study. A total number of 35 scans were carried out. We performed spectroscopy using the standard pulse sequences provided by the manufacturer and initially studied the effects of various parameters like subject positioning, localisation and coil positioning, shimming, decoupling, Nuclear Overhauser Enhancement (NOE), TD and TR.

Subject positioning

The prone position potentially has several advantages such as minimising chest movements, short distance between the coil and the heart, and stability of the coil. However, most subjects cannot tolerate this position for the entire duration of the scan. We performed repeated scans in the prone and supine positions. The Cramer Rao lower bounds for prone and supine postions were 9% and 12% (PCr peak) and 13% and 22% (gamma ATP peak) respectively. Hence the spectral quality was of good quality and comparable in the two and we decided to always scan in the supine position.

Localisation and Coil position

We found the best results by positioning the centre of the 14 cm ^{31} P coil directly over the apex and septal region of the myocardium. Placing the coil in any other position would not allow the entire voxel of acquisition to lie adjacent to the coil. Survey images were taken to locate the position of the coil with respect to the apex of the heart. The coil was repositioned if necessary such that the voxel of acquisition would be at the shortest distance from the coil. We were able to acquire good cardiac ^{31} P spectra with distances up to 10 cm from the centre of the coil to the centre of voxel of acquisition. This method of coil positioning resulted in an average distance of about 7.5cm between the coil and centre of voxel of acquisition.

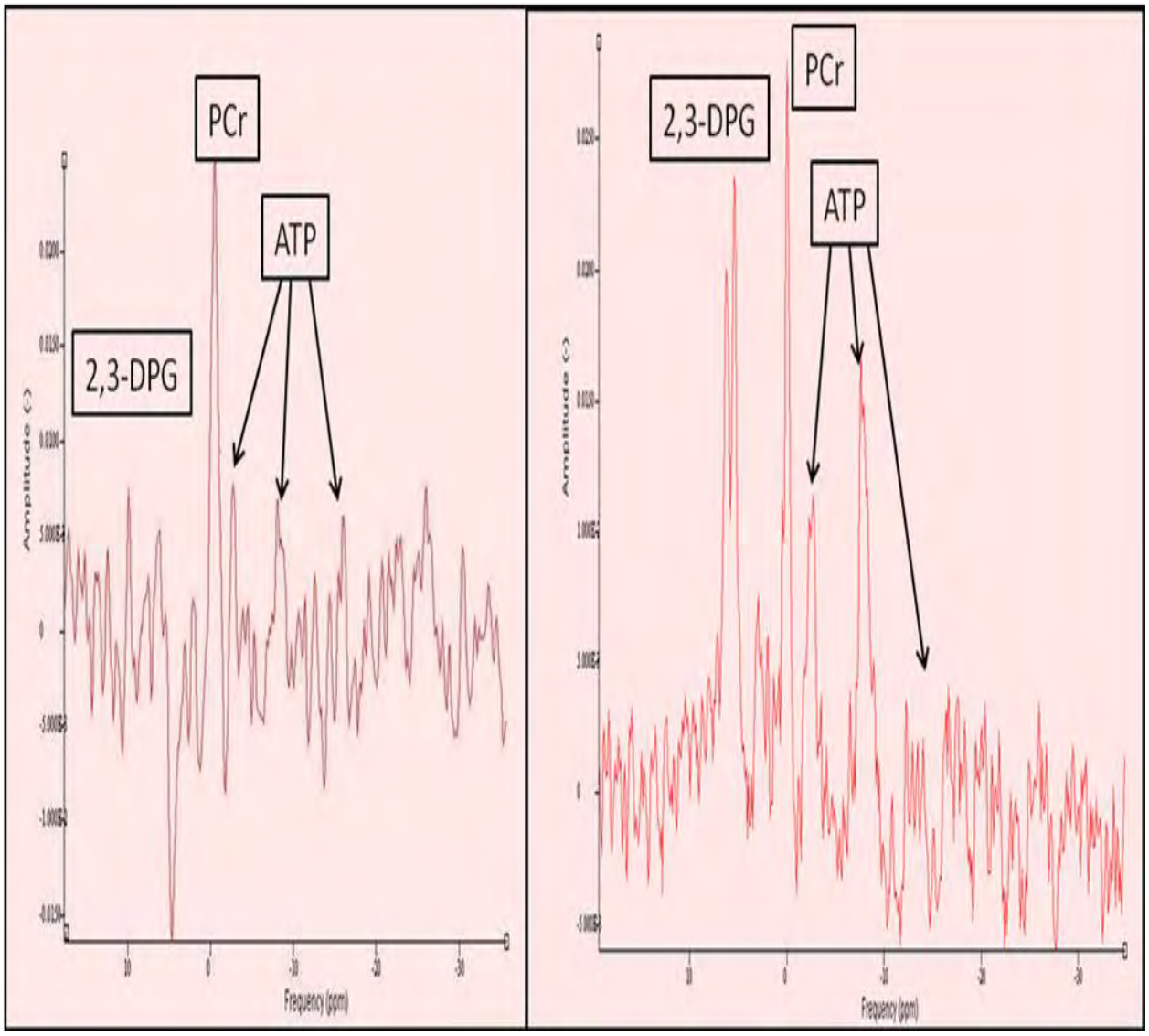
Shimming

Shimming is a process of minimising the infield inhomogenities in the region of interest. We tried various shim routines such as first and higher order shimmimg available on the scanner for spectroscopy. However none of these routine shimming methods provided spectral quality good enough for quantification. Specifically, the SNR was significantly reduced and it was not possible to accurately detect and quantify the ATP peaks (Figure 3.1). Shimming in imaging mode provided the best quality and was superior to first and second order shimming in spectroscopy mode. A short axis cine image was planned and acquired on breath hold. The short axis image provided a guide to the quality of shimming and also determined RR interval to calculate the TD. A check proton spectroscopy scan was performed to ensure good quality shim and correct F_0 determination. A line width of less than 200Hz with a single peak for water signal was required to ensure good shim quality. If the water peak was not present at zero frequency, the F_0 could be manually altered. The cine image acquisition would be repeated if the quality of shimming was found to be not optimal on proton spectroscopy.

Trigger delay

It is ideal to acquire the spectra when the heart is moving the least. This occurs during diastole and hence it is desirable to set the TD such that the spectra are acquired during diastole. The TD was measured by subtracting 250-300 from the total length of the cardiac cycle which allowed 250-300 msec of the cardiac cycle left for spectral acquisition (acquisition time is 170 msec for each spectrum).

Figure 3.1



Cardiac spectra using the routine spectroscopic shim method (figure on left) as compared to shim in imaging mode (figure on right)

PCr- Phosphocreatine; 2,3-DPG- 2,3 Diphosphoglycerate; ATP - Adenosine triphosphate.

Repetition Time

TR is the time gap between acquisition of two consecutive spectra. Short TR increases the saturation effect and reduces the signal as the phosphorus molecules are not completely relaxed before the next radio frequency pulse hits them. Long TR however increases the total acquisition time. Scans were performed at TR of 5000 msec and 10000 msec. The Cramer Rao lower bounds for TR of 5000 msec and 10000 msec were 9% and 10% (PCr peak) and 26% and 22% (gamma ATP peak) respectively. This demonstrated that the spectral quality was adequate at TR of both 5000 and 10000 msec. We chose a TR of 10000 msec to achieve a significant reduction in saturation effect without increasing the total scan time considerably (total scan time of 23 minutes).

Decoupling and NOE mode

We found no significant advantages to the quality of spectra acquisition by enabling the Decoupling mode. NOE increases the SNR but the net effect is variable on different molecules like ATP and PCr. We did not use NOE as it would interfere with quantification of the various peaks in the spectra.

Multi-rest Band

The multi rest band is used to reduce contamination from skeletal muscle and liver. Provided we positioned the voxel in a way that excluded the chest skeletal muscle and the liver, we found no significant advantages to the quality of spectra acquired with multi-rest band. Hence this was not used in our experiments.

Summary of the 31 P cardiac MRS protocol

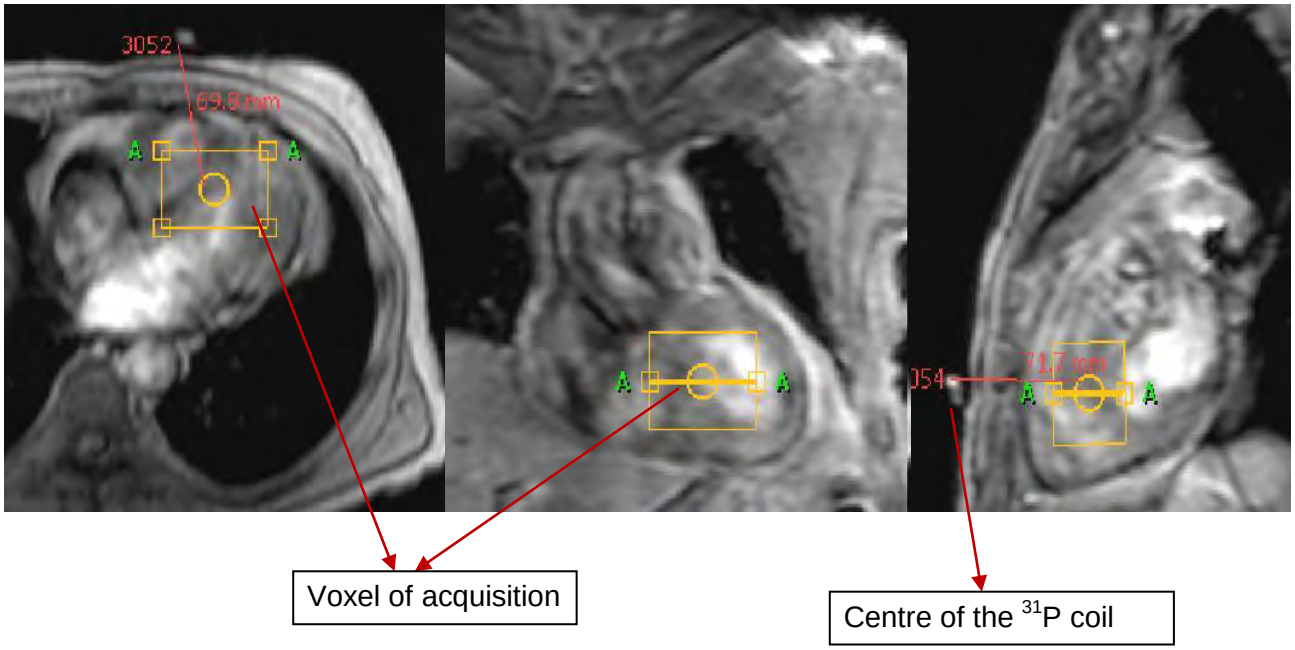
^{31}P cardiac MRS was performed using a Phillips Achieva 3T scanner and a linearly polarized transmit and receive ^{31}P coil with a diameter of 14 cm. Localization was achieved by ISIS [184] volume selection. The participants were positioned supine with the coil directly over the precordium. The coil was secured in place by straps wrapped around the upper body of the subject and the coil. The participants were then positioned inside the magnet with the centre of the coil at the isocentre of the magnet. Survey images were obtained to check the position of the coil (figure 3.2). The subjects and/or the coil were repositioned if required to ensure that the distance between coil and septum and apex of the heart was minimized.

The standard phosphorus spectroscopy sequence provided by the manufacturer was used. It was based on hyperbolic secant pulses for slice selective inversion and adiabatic half passage RF pulse for non-selective excitation. In contrast to the standard procedure, manual fine adjustment of F_0 was performed if the automatic F_0 determination was not correct in order to ensure the correct voxel position. In contrast to the default, iterative or FASTERMAP-based shimming algorithm, which was based on the selected spectroscopy VOI, an image guided shim volume was selected that included the entire myocardium. A short axis cine scan was acquired to calculate the TD for ECG triggering and check quality of shimming and F_0 determination. The TD was calculated such that the spectra were acquired in the diastolic period. The 3-D voxel of acquisition was planned to include most of the septum and apex of the heart. Care was taken to minimize blood contamination from the right ventricle as much as possible. The voxel size was kept constant at 89.54ml (44x55x37mm^3) so that comparisons could be made between different subjects and scans. Initially, ^{1}H spectra were acquired from the same voxel without water suppression at a TR of 2000 ms (total scan time of 16 sec). This

helped to ensure adequate shim quality and correct F_0 determination. F_0 could be manually adjusted if necessary. Following this the ^{31}P spectrum was acquired with a TR of 10000 ms, 136 averages and 512 samples. A TR of 10000 ms was found to be optimal to adequately reduce saturation effects without increasing the scan time greatly. The spectral acquisition was ECG-gated and the TD was set to acquire spectra mainly in diastole. The TD was measured by subtracting 250-300 from the total length of the cardiac cycle which allowed 250-300 msec of the cardiac cycle left for spectral acquisition (acquisition time is 170 msec). The total scan time was 23 minutes.

Increased chemical shift artefacts are present at 3T. In order to minimise this, slice selective inversion for ISIS encoding was based on adiabatic hyperbolic secant pulses, which achieved a pulse bandwidth between 1300 Hz (at a distance of 9 cm from the surface coil) to 2000 Hz (at a distance of 3 cm from the surface coil). This corresponds to a chemical shift displacement of 6-10% for the investigated metabolites PCr and gamma-ATP for volumes of interest that were between 3 and 9 cm away from the coil. When subjects were scanned the distance from the coil to the ROI averaged about 7.5cm and no subject was beyond 9.0 cm. Therefore all subjects would have a chemical shift displacement less than 10% which is acceptable.

Figure 3.2



Survey images (in transverse, coronal and saggital planes) showing the position of voxel of acquisition and centre of the ^{31}P coil. The voxel is positioned such that it remains completely within the myocardium and as close as possible to the center of ^{31}P coil.

Feasibility and reproducibility testing

37 Controls (22-males) and 26 HCM patients (21-males) with symptomatic non-obstructive cardiomyopathy who provided written informed consent, were included in the study. The experiment was approved by the Regional Ethics Committee at Birmingham, UK. All healthy volunteers were screened with history, echocardiography and metabolic exercise testing to rule out any structural heart diseases. All patients were recruited from cardiomyopathy clinics and had clinically proven diagnosis of non-obstructive HCM. The subject characteristics are presented in table 3.1. The mean age of the healthy volunteers was 48 ± 16 years and that of HCM patients was 55±13 (P=ns). The mean EF on echocardiography was 64±6% in controls and 64±9% (P=ns) in HCM patients. None of the healthy volunteers had any structural heart disease or ECG abnormalities. ^{31}P cardiac spectroscopy was performed eight times in one participant both - on the same and on different days - to test the reproducibility and coefficient of variation of the test. As a further test of reproducibility and validation in our department MRS was repeated twice in twelve healthy volunteers (Mean age 28±10 years).

Table 3.1: Baseline characteristics in controls as compared with HCM patients. Results expressed as mean ± standard deviation			
Parameter	**Controls (N=37)**	**HCM (N=26)**	**P Value⁎**
Age	48 ± 16	55 ± 13	ns
Male sex –no (%)	22 (59)	21 (81)	ns
EF (%)	64 ± 6	64 ± 9	ns
VO2 max	39 ± 8	24 ± 6	<0.0001
RER	1.2 ± 0.2	1.1 ± 0.1	ns
Heart rate	79.5 ± 11.7	67.5 ± 12.5	<0.01
QTc interval	421.8 ± 16.0	455.9 ± 35.0	<0.01
Systolic Blood Pressure	127.0 ± 20.4	126.1 ± 20.1	ns
Diastolic Blood Pressure	79.5 ± 9.6	75.6 ± 10.7	ns

⁎ P <0.05 considered as statistically significant.

EF- Ejection Fraction, VO2 max- Maximal oxygen consumption at peak exercise, RER – Respiratory exchange ratio, QTc- Corrected QT interval.

Analysis

The spectra were analysed and quantified on jMRUI software using AMARES a time domain fitting program [271]. Post-processing was performed with 15Hz Gaussian line broadening and Fourier transformation. Phase correction was performed with PCr peak as the reference peak. Quantification was performed with AMARES using a prior knowledge file to preselect the peaks. The concentrations of PCr, ATP and 2,3-DPG were calculated as the area under the peaks. Cramer Rao lower bounds [36] were then calculated. PCr/ATP ratio was determined after correcting the ATP peak for blood contamination as described previously [46].

Results

A typical cardiac spectrum in a healthy volunteer as compared to a patient with HCM is shown in Figure 3.3. The PCr/ATP ratio was significantly lower in HCM (1.42 ± 0.51) as compared to HC (2.11 ± 0.57, $P<0.0001$) (Figure 3.4). The mean PCr/ATP ratio for the one participant with eight measurements was 2.11 ± 0.25 (Table 3.2). Bland Altman plots were used as a test of reproducibility. The distribution of all the data points showed good reproducibility with a variance of 12% in the measurement of PCr/ATP ratios which is within limits of agreement of repeated measurements. We also measured the line width of PCr peaks in the one participant who had 8 repeated scans. The mean line width was 1.36 $\pm$ 0.07 ppm (Figure 3.5). The standard deviation of the line width was low which again confirmed the fact that the spectra were of good reproducible quality. As a further measure of the quality of spectra we calculated the Cramer Rao lower bounds [36], which was 6 ± 1% for the PCr peak and 10 ± 1% for the gamma ATP peak. Cramer Rao lower bounds were calculated for the whole group, which was 12 ± 6% for the PCr peak and 17 ± 9% for the gamma ATP peak (Table 3.4).

The MRS was repeated twice in twelve heathy volunteers to further test the variability of PCr/ATP ratio. Bland Altman plots demonstrated a variability of 0.13±0.52 (bias ± 1.96SD) with mean PCr/ATP ratio of 1.7±0.3 and 1.6±0.3. Cramer Rao lower bounds were calculated at 6 ± 3% for the PCr peak and 10 ± 3% for the gamma ATP peak. Due to an increase of susceptibility differences between heart muscle tissue, blood and air in the lungs at high field strength shim quality was slightly decreased at 3T in comparison to values reported for 1.5T cardiac spectroscopy. Hence multiplet splitting of the ATP resonances due to J-coupling is hardly visible.

Table 3.2: Table depicting the concentrations of 2,3-DPG, ATP and PCr and the calculated PCr/ATP ratios from these measurements in the subject with eight measurements.

Subject No	2,3 DPG sum	PCr	Gamma ATP	Gamma ATP (C)	PCr/Gamma ATP Ratio	PCr/Gamma ATP Ratio (C)
Subject 1A	6.72E-04	4.90E-04	3.57E-04	2.45 E-04	1.37	2.00
Subject 1B	4.27E-04	4.32E-04	3.21E-04	2.5 E-04	1.34	1.72
Subject 1C	7.32E-04	6.53E-04	4.77E-04	3.55 E-04	1.36	1.83
Subject 1D	1.00E-03	8.52E-04	5.12E-04	3.45 E-04	1.66	2.47
Subject 1E	9.39E-04	5.53E-04	3.86E-04	2.3 E-04	1.43	2.40
Subject 1F	8.29E-04	6.34E-04	4.26E-04	2.88 E-04	1.48	2.20
Subject 1G	8.66E-04	5.36E-04	3.90E-04	2.46 E-04	1.37	2.18
Subject 1H	8.32E-04	5.55E-04	4.05E-04	2.66 E-04	1.37	2.08

PCr- Phosphocreatine; 2,3-DPG- 2,3 Diphosphoglycerate; ATP- Adenosine triphosphate; (C)- Corrected for blood contamination

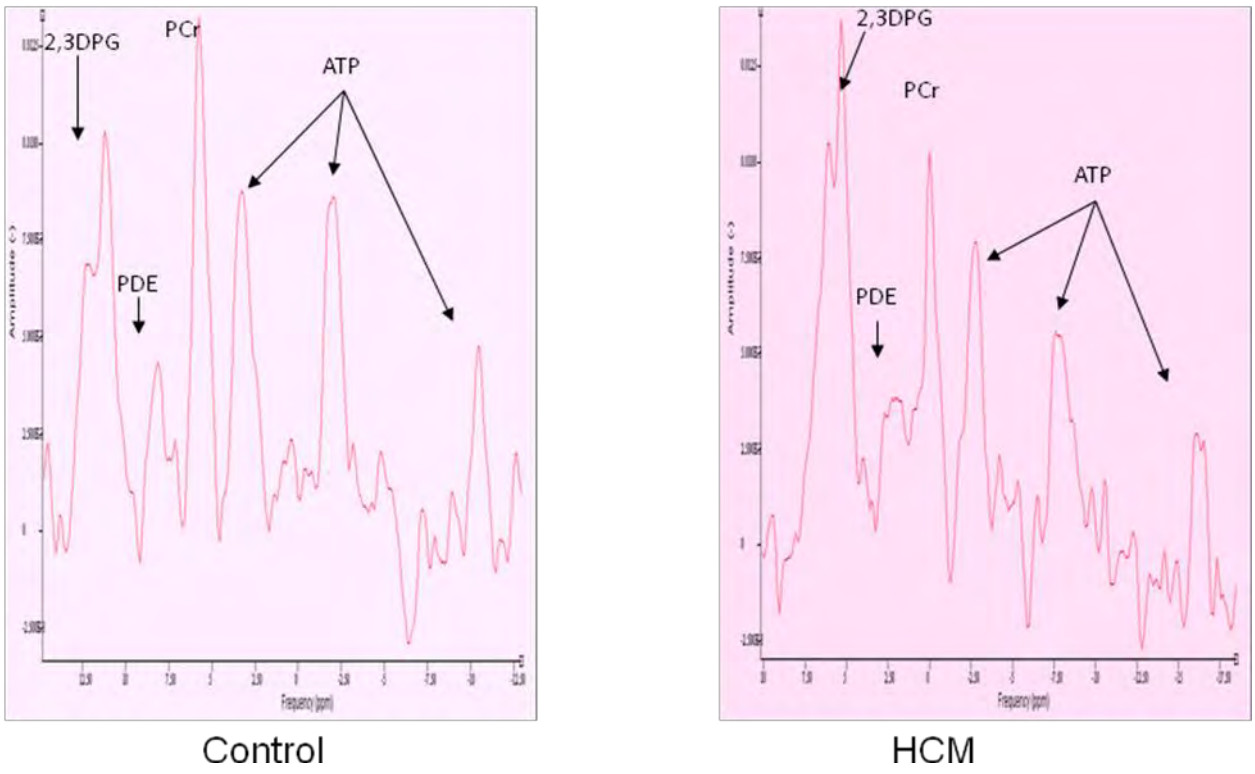
Table 3.3: Table depicting the concentrations of 2,3-DPG, ATP and PCr and the calculated PCr/ATP ratios from these measurements in controls and HCM patients. Results expressed as mean ± strandard deviation		
	Controls	**HCM**
2,3 DPG sum	9.20E-04 ± 3.40E-04	5.40E-04 ± 1.70E-04
PCr	6.80E-04 ± 4.80E-04	6.00E-04 ± 2.20E-04
Gamma ATP	4.80E-04 ± 1.80E-04	5.40E-04 ± 1.50E-04
Gamma ATP (C)	3.23E-04 ± 1.40E-04	4.20E-04 ± 1.40E-04
PCr/Gamma ATP Ratio	1.30 ± 0.30	1.1 ± 0.32
PCr/Gamma ATP Ratio (C)	2.11 ± 0.57	1.42 ± 0.51

PCr- Phosphocreatine; 2,3-DPG- 2,3 Diphosphoglycerate; ATP- Adenosine triphosphate; (C)- Corrected for blood contamination

Table 3.4: Cramer Rao Lower bounds measured to test the quality of spectra		
	PCr peak	**Gamma ATP Peak**
Healthy control (8 Measurements)	6% ± 1	10% ± 1
Whole group(Controls + HCM)	12% ± 6	17% ± 9
Controls	11% ± 5	16% ± 8
HCM	12% ± 6	17% ± 9

PCr- Phosphocreatine; ATP- Adenosine triphosphate

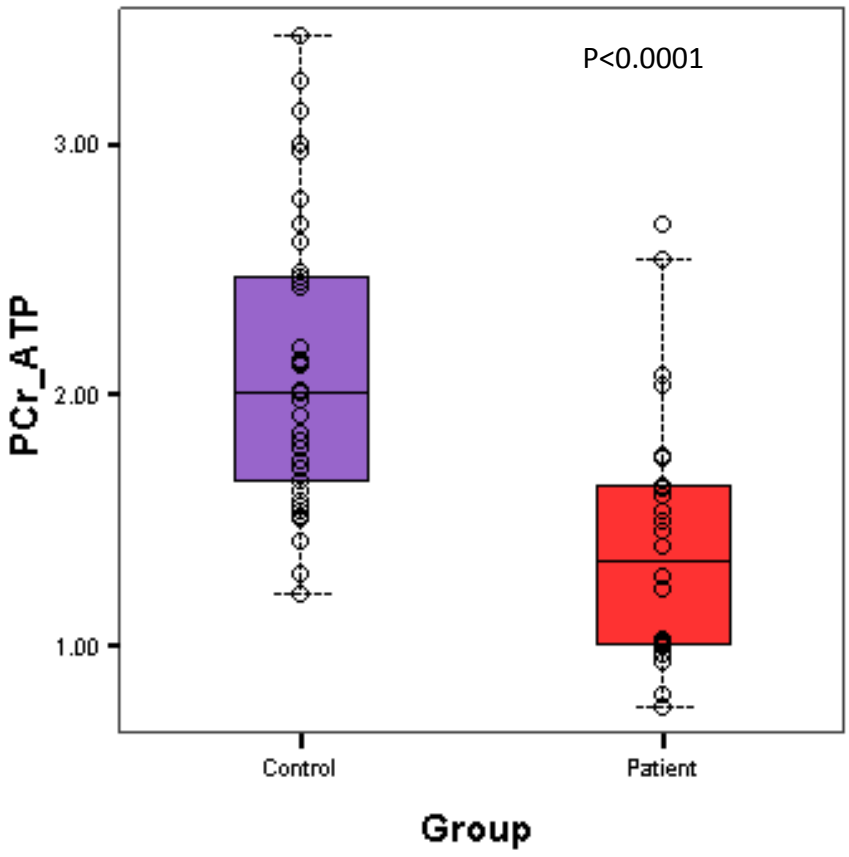
Figure 3.3



Typical cardiac spectra in a control and a patient with HCM

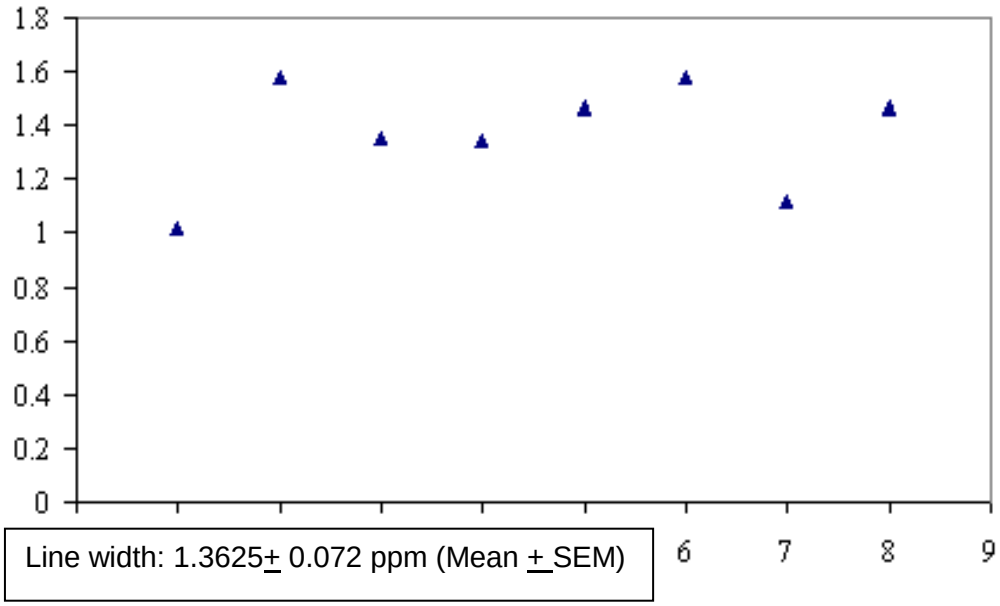
PCr- Phosphocreatine; 2,3-DPG- 2,3 Diphosphoglycerate; PDE - Phosphodiesters; ATP - Adenosine triphosphate.

Figure 3.4



Box-plots of PCr/ATP ratios in controls and HCM patients

Figure 3.5



Line width of PCr peaks from the healthy volunteer who had 8 repeated scans expressed as parts per million (ppm)

Discussion

Here we demonstrate that in-vivo cardiac ^{31}P MRS at 3T is a reliable method of measuring high energy phosphate kinetics in the myocardium. We also show reduced PCr/ATP ratio in HCM patients known to have impaired cardiac energetics as measured in 1.5T systems [47;107]. The spectra show good reproducibility indicating that ^{31}P cardiac MR spectroscopy is feasible on a clinical 3T MR system. The standard deviation for the PCr/ATP ratio for the whole group was low and comparable to previous published data on cardiac spectroscopy at 1.5 Tesla [159]. This basic method of acquiring ^{31}P spectra at 3T using pre-implemented methods such as ISIS volume localisation and iterative shimming promises to be an important diagnostic and research tool. Possible applications are the comparison of PCr/ATP ratios in conditions like heart failure, ischaemic heart disease and valvular heart disease. It might also be used to monitor disease progression and study effects of medications like metabolic modulators in heart disease.

Cardiac spectroscopy at 3T continues to face some challenges which are present at 1.5T as well, but some of them are even more pronounced at high field strength. One major drawback of cardiac spectroscopy is the effect of respiratory motion and movement of the heart itself. This can result in contamination from liver and skeletal muscle of the chest wall. Careful localization of the voxel ensures minimal contamination. We particularly paid attention to this aspect while planning our voxel of acquisition. Provided the voxel is positioned carefully, no significant advantages to the quality of spectra acquired with multiple outer volume suppression bands was observed. None of our spectra show any significant contamination with skeletal muscle or liver. It is also important to ensure that the spectral acquisition is done when the heart motion is at its minimum. This is during diastole and we set the TD such that

all of the spectral acquisition happened during diastole. One other problem is the respiratory motion. This could be partially negated by acquiring the spectra with respiratory gating as well as cardiac gating (double triggered) and volume tracking [127;219]. Further developments in these techniques should improve quality of cardiac spectra and reduce the contamination from surrounding structures.

Higher field strength of 3T offers better spatial resolution and SNR. These effects have been particularly noted in proton spectroscopy of the human brain [17]. However, increasing susceptibility differences between muscle tissue, blood and air in the lungs cause increased B_0 field inhomogeneities and hence problems with shim convergence. Various shimming techniques like iterative and FASTMAP based [78] higher order shimming were compared. Localized iterative 1st order shimming based on a volume including the entire heart offered the best and most reproducible shim quality. However, there is definitely scope for further improvement in shimming techniques. One possibility might be localized shimming based on cardiac triggered B_0-mapping as reported earlier for cardiac imaging at 3T [220]. Another problem is the shortened transverse relaxation times (T2) at 3T in comparison to 1.5T. This causes an additional increase in line width of the various peaks.

No significant advantages to the quality of spectral acquisition using proton decoupling or NOE were found. Therefore these techniques were not used. Although NOE can increase the SNR by up to 40%, this is not useful for experiments in which quantification is required. NOE in particular imparts different amounts of energy to phosphates in ATP and PCr which can result in altered PCr/ATP ratio. This is particularly important as the effect of NOE is different

for distinct molecules and depends on conditions as the pH which might be changed in the diseased myocardium.

Due to a decrease of the maximum achievable B_1 field strength at 3T together with an increase in spectral separation compared to 1.5T, the bandwidth of the excitation pulse was limited and hardly sufficient to excite the entire frequency range of interest. Using a simple surface coil this is especially a problem in large penetration depth. In addition, the excitation profile was not homogeneous. Hence different flip angles were applied to spins with different offset frequencies. This resulted in a frequency-dependent weighting of peak intensities. Hence, the intensity of the beta ATP peak, which has a large frequency offset compared to PCr, was always significantly decreased (Figure 3.3) or even lost in obese subjects, where the distance between coil and VOI is large. However the determined PCr/ γ ATP ratios were not affected, because the frequency difference between the two resonances is small and the pulse frequency offset was chosen to be between the two.

Conclusions

^{31}P MRS of the myocardium at 3T is feasible and allows reliable determination of high energy phosphate kinetics. Our reproducibility data suggests that the suggested method is robust and might be used for clinical diagnostic studies as well as for clinical experimental studies. However, to take full advantage of increased SNR and spectral separation at 3T, advances in shimming, coil and RF pulse design are necessary.

CHAPTER FOUR

THE RELATIONSHIP BETWEEN CORONARY MICROVASCULAR DYSFUNCTION AND CARDIAC ENERGETIC IMPAIRMENT IN TYPE 1 DIABETES

Publication

1. **Dr G Nallur Shivu**….. Prof M Frenneaux. Relationship Between Coronary Microvascular Dysfunction and Cardiac Energetics Impairment in Type 1 Diabetes Mellitus. **Circulation** 2010 Mar 16;121(10):1209-15

Abstract

Background: Asymptomatic subjects with diabetes have impaired cardiac energetic status that may play a significant role in development of heart failure. In this study we assessed the role of microvascular dysfunction in the development of impaired cardiac energetics in subjects with T1DM.

Methods: 25 asymptomatic subjects with T1DM (mean ± SD, age 33 ± 8 yr) and 26 age, sex and body mass index matched HC (age 32 ± 8 yr) were recruited into the study. The T1DM subjects were divided into two age matched groups: newly (<5 yr) diagnosed and longer duration (>10 yr) diabetes to assess the impact of microvascular disease. All subjects underwent echocardiogram and exercise ECG followed by MRS and stress MRI.

Results: The PCr/ γ ATP ratio was significantly reduced as compared to HC in both longer term (2.1±0.5 vs 1.5±0.4, P<0.001) and newly diagnosed subjects (2.1±0.5 vs 1.6±0.2, P<0.001). The PCr/ γ ATP ratio was similar in newly diagnosed diabetes subjects as compared to longer term (1.6±0.2 vs.1.5±0.4 P=0.44). The mean MPRI assessed by stress MRI in the longer term T1DM subjects was significantly lower than in HC (1.7±0.6 vs 2.3±0.4, P<0.05). On univariate analysis, there was no significant correlation of PCr/ γATP ratio with MPRI (r=0.21, P=0.26).

Conclusions: We demonstrate that young subjects with uncomplicated T1DM have impaired myocardial energetics irrespective of duration of diabetes and that the impaired cardiac energetics is independent of coronary microvascular function. We postulate that impairment of cardiac energetics in these subjects primarily results from metabolic dysfunction rather than microvascular impairment.

Background

Diabetes is a metabolic disorder characterized by hyperglycaemia and dyslipidaemia. The prevalence of diabetes is rapidly increasing throughout the western world [59;266;282]. Heart failure occurs more frequently in diabetes [109] and is commonly due to epicardial CAD and/or hypertension [101;126;250]. There is a markedly increased mortality associated with CAD and heart failure in patients with T1DM [264]. However in some patients, LV dysfunction occurs in the absence of significant epicardial CAD or hypertension [214]. This indicates that diabetes may have a direct effect on the heart, which can contribute to the development of LV dysfunction. The impact of diabetes on the heart could occur via various mechanisms including unrecognized hypertension, large vessel and microvascular disease, energetic impairment, autonomic neuropathy and oxidant stress. Asymptomatic subjects with T2DM have impaired cardiac energetic status as reflected in a reduced PCr/ γ ATP ratio on cardiac MRS [221]. Impairment of cardiac energetic status has the potential to play a significant role in the development of contractile dysfunction. Whilst microvascular ischaemia may potentially be contributory to energetic impairment, a primary disturbance of energetic status that is unrelated to ischaemia and due to the metabolic effects of diabetes, is also likely.

Thus, the primary aim of the study was to confirm the presence of cardiac energetic impairment in uncomplicated T1DM and to establish its relationship to coronary microvascular dysfunction. For this we recruited a cohort of young healthy T1DM subjects with no history of hypertension, primary hyperlipidemia, renal disease or coronary heart disease. In order to tease out the potential patho-physiological mechanisms in the development of cardiac energetic impairment we studied two age matched groups of T1DM subjects. The first group (newly diagnosed T1DM) were diagnosed for less than 5 years and

hence unlikely to have microvascular disease; the second group (longer term) was diagnosed for more than 10 years and were thus likely to have a certain degree of microvascular dysfunction. We used MRS to determine cardiac energetic status and used stress MRI to compute MPRI which is a measure of coronary microvascular function.

Methods

51 subjects who met the inclusion criteria and provided informed consent were recruited from the Heart of England NHS Foundation Trust and University Hospital Birmingham NHS Trust, Birmingham, UK. HC were recruited by general advertisements in the University of Birmingham and in blood banks. All the investigations were undertaken in the University of Birmingham and the project was approved by Multicenter Regional Ethics Committee in Birmingham.

Subjects

We recruited 25 subjects with T1DM (WHO definition) without a history of chest pain or breathlessness. All patients had no evidence of coronary heart disease or heart failure based on history, 12 lead ECG, a normal EF on echocardiography and metabolic exercise testing. Subjects were divided into predefined subgroups (age matched) based on duration of their diabetes as defined above: Newly diagnosed (10 subjects, age 32±10y) and longer term (15 subjects, age 33±6y).

Healthy Controls

26 age, sex and body mass index matched controls with no cardiac history or diabetes mellitus were recruited. All HC had a normal 12 lead ECG, echocardiogram and metabolic exercise test.

Subjects fasted overnight and blood samples were taken on the morning of the study. After this, a light breakfast was given and patients were allowed to take their morning dose of insulin. Following this, subjects underwent cardiac stress MRI, cardiac MRS, echocardiography and metabolic exercise testing as described in the chapter 2 (Methods). Stress MRI was done only in a subgroup of HC (8 subjects).

Analysis

MRS

The spectra were analysed and quantified on jMRUI software using AMARES a time domain fitting program [271]. Post-processing was performed with 15Hz Gaussian line broadening and Fourier transformation. Phase correction was performed with PCr peak as the reference peak. Quantification was performed with AMARES using a prior knowledge file to preselect the peaks. The concentrations of PCr, ATP and 2,3-DPG were calculated as the area under the peaks. PCr/ γATP ratio was determined after correcting the ATP peak for blood contamination as described previously [46]. We have previously published the reproducibility data in HC [236]. The MRS was repeated twice in five diabetes patients to test the variability of PCr/ γATP ratio. Bland Altman plots demonstrated a variability of 0.13±0.28 (bias ± 1.96SD) with mean PCr/ATP ratio of 1.6 and 1.5. The quality (SNR) of spectra in all subjects were further assessed by Cramer Rao lower bounds and were 8±4% for PCr peak and 11±4% for

the γATP peak in the control population and 8±2% for PCr peak and 11±3 for γATP peak in the patient population. These figures indicate that the spectra are of good quality and suggest good reproducibility of PCr/ γATP ratios in both HC and patients in our current study.

MPRI

MPRI which is a measure of coronary microvascular function was computed from the stress MRI images. The images were analyzed on the Viewforum software version 5.0. Initially image alignment was done to reduce the motion of the heart in different cardiac cycles. Following this the endocardial and epicardial borders were traced and propagated throughout all the images. A sample volume was placed in the LV cavity as comparison for signal intensity of blood. Signal intensity curves were obtained from the software and the peak upslope of the LV myocardial illumination was computed in relation to the LV cavity illumination. A similar analysis was done on all three short axis images at rest and peak stress. The MPRI was obtained from the ratio of LV relative peak upslope at stress compared to rest.

Statistics

All continuous variables are expressed as mean ± SD. Comparison between means was performed using unpaired Student T-tests. Categorical variables were compared with Pearson Chi-Square test. For comparison of variables when the diabetes patients were subdivided, one way ANOVA was used. A P value of <0.05 was considered to indicate statistical significance. Variances of data sets were determined using F-test. Pearson correlation coefficient (r) was used to describe the relationship between variables. SPSS (v15.0) was used to perform the statistical operations.

Results

The baseline characteristics and results are summarized in Table 4.1. The LVEF was 61.4±5% in HC, 60±2% in the longer term diabetes subjects and 62±6% in the newly diagnosed subjects, with no significant difference between the three groups. In the HC the E/A ratio was 1.7±0.6, the IVRT was 73±12 msec and the E/E' was 4.9±1.3. The corresponding values in longer term T1DM subjects were 1.3±0.3, 69±9 msec and 5.3±1.2 and in the newly diagnosed subjects were 1.7±0.7, 76±15 msec and 5.5±1. All of these diastolic parameters were within the normal range with no statistical difference between the subject groups.

Table 4.1

Variable	Newly diagnosed T1DM	Longer term T1DM	HC
Number	10	15	26
Age, years	32±10	33±6	32±8
Male Gender (%)	8 (80)	10 (66)	18(69)
Duration of diabetes	3.5±3	18±7	-
HbA1C, %	7.4±0.8†	8.3±1	-
BMI, Kg/m^2	23±2	26±3	25±3
Total cholesterol, mmol/L	4.3±1.2	4.4±0.7	4.9±0.9
HDL, mmol/L	1.5±0.3	1.7±0.5	1.7±0.6
Triglycerides, mmol/L	1.3±1.2	1.1±0.8	1±0.5
Free fatty acids, mmol/L	0.32±0.22	0.28±0.13	0.23±0.1
VO2 max, ml/kg/min	42.6±10.2	35.6±8.4*	44.1±7.2
PCr/γATP ratio	1.6±0.2 *	1.5±0.4*	2.1±0.5
MPRI	2.1±0.2	1.7±0.6*	2.3±0.4
Microalbuminuria, %	0	4(27)	-
Retinopathy, %	3(30) †	10(66)	-

Baseline characteristics and results in both groups of T1DM patients as compared with HC

* Significant difference as compared with HC
†Significant difference as compared to longer term diabetes subjects

BMI; Body mass index, VO2 max; Peak oxygen consumption, PCr/γATP ratio; Phosphocreatine/ gamma adenosine triphosphate, MPRI; Myocardial perfusion reserve index

MRS

Typical cardiac spectra in T1DM and HC are shown in figure 4.1. The PCr/ γATP ratio was significantly reduced as compared to HC in both longer term (2.1±0.5 vs 1.5±0.4, P<0.001) and newly diagnosed diabetes (2.1±0.5 vs 1.6±0.2, P<0.001). The PCr/ γATP ratio was similar in newly diagnosed diabetes as compared to long term diabetes subjects (1.6±0.2 vs 1.5±0.4, P=0.44). When the subject groups were divided by retinopathy status (obtained using retinal photography) the subjects with retinopathy (9-background retinopathy, 2-proliferative retinopathy and 2-maculopathy) had similar PCr/ γATP ratio as compared with those without retinopathy (1.4±0.4 vs 1.6±0.4, P=0.62) (Table 4.2).

Table 4.2

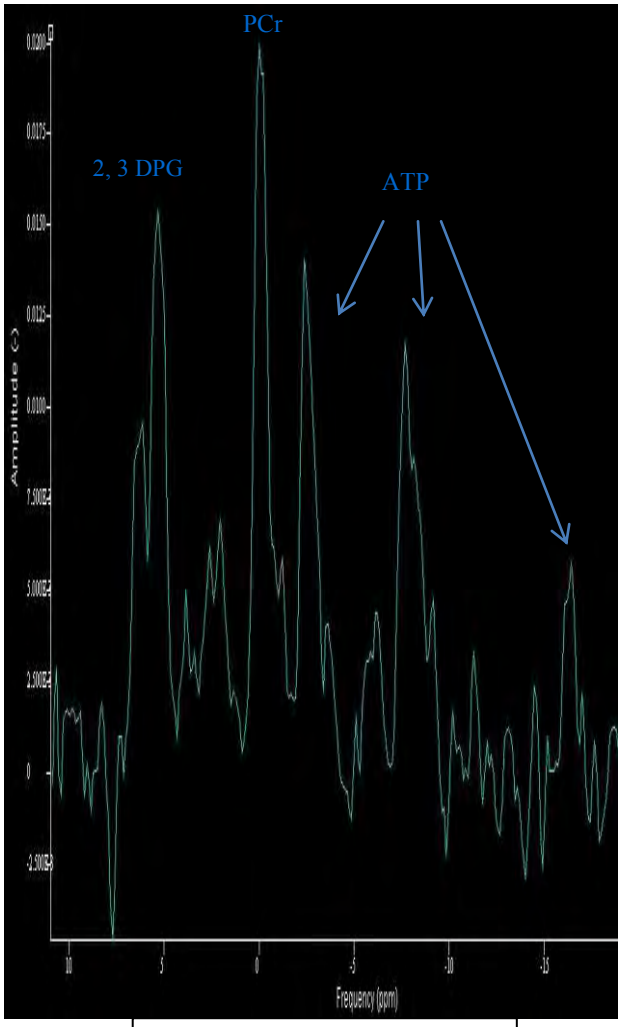
Variable	Complications absent	Complications present	HC
Retinopathy			
PCr/γATP ratio	1.6±0.4*	1.4±0.4*	2.1±0.5
MPRI			
PCr/γATP ratio	1.6±0.4*	1.4±0.2*	2.1±0.5

Cardiac energetics in subgroups of subjects with various complications (retinopathy and coronary microvascular disease).

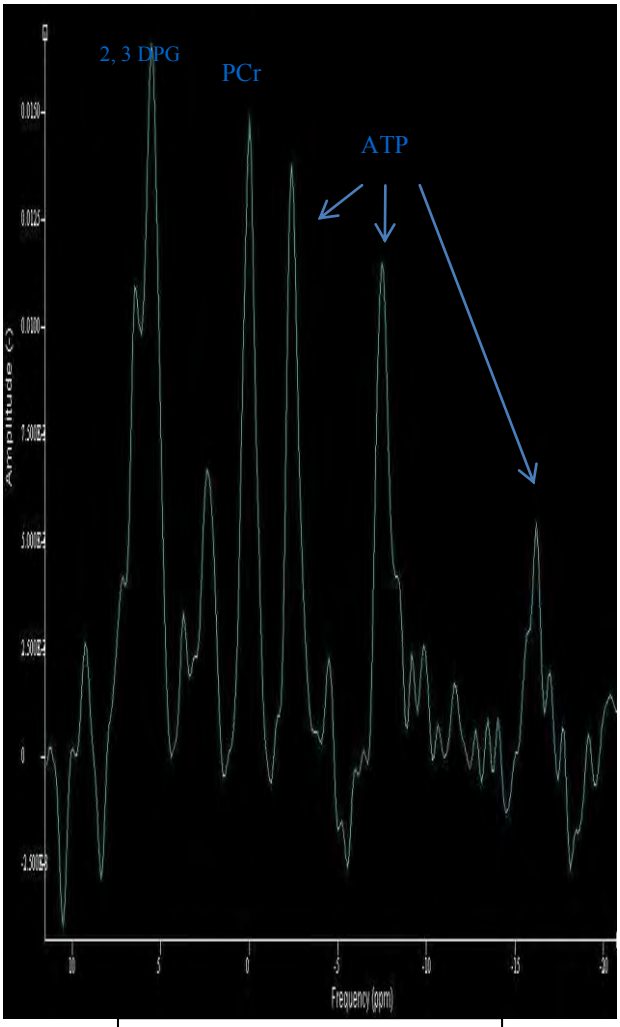
* Significant difference as compared with HC

PCr/γATP ratio; Phosphocreatine/ gamma adenosine triphosphate, MPRI; Myocardial perfusion reserve index

Figure 4.1



Typical spectra in HC



Typical spectra in T1DM

Typical spectra in T1DM and HC.

2,3 DPG: 2,3 Diphospho-glycerate, PCr: Phosphocreatine, ATP: Adenosine triphosphate

MPRI

The mean MPRI in the longer term T1DM group was significantly lower than in HC (1.7±0.6 vs 2.3±0.4, P<0.05). The MPRI in newly diagnosed T1DM was not significantly different than in longer term (1.7±0.6 vs 2.1±0.2, P=0.13) or HC. When the subject groups were divided into those with and without coronary microvascular dysfunction (MPRI of 1.5 was considered to be the lower limit of normal - 2 standard deviations below the mean for HC), the patients with and without coronary microvascular dysfunction had similar PCr/ γATP ratio (1.4±0.2 vs 1.6±0.4, P=0.64) (Table 4.2). However, those with coronary microvascular dysfunction (1.4±0.2 vs 2.1±0.5, P<0.01) and without coronary microvascular dysfunction (1.6±0.4 vs 2.1±0.5, P<0.01) had significantly lower PCr/ γATP ratio as compared to HC.

Correlation

On univariate analysis there was no significant correlation of the PCr/ γATP ratio with MPRI (r=0.21, P=0.26), the HbA1c (r=-0.27, P<0.05), FFA (r=-0.11, P=0.45) or triglycerides (r=0.09, P=0.54).

Discussion

In this study we have shown that cardiac energetics as determined by PCr/ γATP ratio is reduced in uncomplicated young T1DM subjects in the absence of ischaemic heart disease, irrespective of the duration of diabetes or presence of coronary microvascular dysfunction or other microvascular disease (retinopathy or nephropathy). As cardiac energetics did not correlate with coronary microvascular dysfunction, this suggests a primary metabolic role in the development of impaired energetics in these individuals.

Previous studies have demonstrated altered cardiac energetics in asymptomatic T1DM [160] and T2DM patients [57;221]. Our findings confirm their results. However in this study we have extended their findings by exploring the potential pathophysiology of this deficit. Subjects were characterized for the presence of macro and microvascular disease by exercise testing and stress MRI (and by history of nephropathy/retinopathy). This is important as CAD is, in itself, a cause of impaired cardiac energetic status [284] and diabetes patients in particular have a high incidence of asymptomatic CAD [175].

It has been demonstrated previously that impaired cardiac energetics play a key pathophysiological role in the development of heart failure and that the energetic abnormality usually precedes the onset of contractile dysfunction [7;234]. But, the key underlying mechanisms for this energetic impairment are not well known. Diabetes is associated with decreased glucose transport, glycolysis and oxidation [141]. The reduced uptake of glucose via GLUT4 receptors decreases the availability of glucose in the myocardium [243]. In normal hearts, 60-90% of the energy is derived from fatty acid oxidation. In diabetes this can increase to 90-100% [140]. The increased utilization of FFA to generate energy is demonstrated in PET studies [88]. Increased FFA usage has a greater oxygen cost, which is in part attributable to stoichiometry but also possibly due to increased mitochondrial uncoupling [225] and wasteful cycling of FFAs through intramyocardial lipolysis and esterification [91;209]. In type1 diabetes, a recent PET study demonstrated reduced cardiac glucose uptake and utilisation and markedly increased FFA uptake and utilisation [88]. In our study there was a nonsignificant trend towards increased plasma FFA in the diabetes subjects (0.3±0.18 vs 0.23±0.11, P=0.07). This increased FFA in the plasma coupled with increased FFA uptake could be an important factor resulting in impairment of cardiac energetics.

Increased FFA uptake results in lipid accumulation in spite of the increased FFA oxidation [29]. Lipid accumulation can worsen the myocardial insulin resistance which in turn worsens glucose uptake [188]. Also increased FFA oxidation produces more ROS which can oxidize lipids and proteins resulting in cell damage. ROS impairs mitochondrial coupling resulting in decrease in ATP production [86].

Myocardial energetic impairment might also occur secondary to ischaemia caused by microvascular disease. It is well known that development of microvascular disease is directly related to diabetes duration and glycaemic control in T1DM. Indeed the longer term T1DM subjects in our study had a lower MPRI as compared to HC. However, the study was not powered to detect significant difference in MPRI between longer term T1DM and newly diagnosed T1DM subjects. MPRI is a good indicator of coronary microvascular function. Our study demonstrates a lack of association between coronary microvascular dysfunction and energetic impairment. Moreover, cardiac energetic impairment was present even in the newly diagnosed diabetes patients (although to a slightly lesser degree) implicating metabolic impairment as the probable important mechanism. Hence microvascular disease does not appear to account for the development of cardiac energetic impairment in diabetes.

Previous studies in T2DM have demonstrated that impaired cardiac energetics may contribute to the development of diastolic dysfunction in these subjects [57]. In our study we demonstrate that impaired cardiac energetics is present even in the newly diagnosed diabetes patients. This substantiates the fact that energetic impairment precedes onset of contractile dysfunction as shown in previous animal studies [234].

Interestingly, we found that there was a significantly lower VO2 max in the longer term T1DM as compared to newly diagnosed T1DM and HC (Table 4.1). Previous studies have shown that reduced exercise capacity in T2DM is related to subclinical LV dysfunction, diabetes control and heart rate recovery [62]. In our study most of the T1DM subjects had impaired cardiac energetics. But the myocardial perfusion was impaired only in the longer term subjects. It is therefore tempting to speculate that a combination of energetic and perfusion abnormalities could result in reduced VO2 max in the longer term T1DM subjects. Skeletal muscle perfusion abnormalities could also contribute to the reduced exercise capacity, although this was not measured in the current study.

Clinical implications

Development of heart failure in diabetes is a complex process and is affected by many secondary factors like hypertension, CAD renal disease and hyperlipidemia. In this study we have demonstrated cardiac energetic impairment in uncomplicated diabetes indicating that diabetes has a direct effect on the myocardium. This impairment occurs early in the disease process and intervention at this stage to improve the myocardial energetic status may be an important method of reducing cardiovascular complications. Metabolic modulation has assumed importance recently in the management of patients with CAD and heart failure [5;6]. Since subjects with diabetes have a metabolic profile similar to these patients, metabolic agents could potentially be used to prevent heart failure in diabetes. However, large scale studies are required to substantiate this. Alternatively, intensive metabolic control early in T1DM may be able to achieve long term benefits in reducing cardiovascular complications [174].

Study Limitations

The principal limitation of the study was the small sample size of the study population. Additionally, this was a cross-sectional study which therefore gives no information on the ultimate outcome of these subclinical deficits.

CHAPTER FIVE

INCREASED LEFT VENTRICULAR TORSION IN UNCOMPLICATED TYPE 1 DIABETES: THE ROLE OF CORONARY MICROVASCULAR FUNCTION AND ROTATIONAL DEFORMATION DELAY

Publication

1. **Dr G Nallur Shivu**….. Prof M Frenneaux. Increased left ventricular torsion in uncomplicated type 1 diabetes: the role of coronary microvascular function. **Diabetes Care** 2009 Sep;32(9):1710-2 (doi:10.2337/dc09-0408)

Abstract

Background: Heart failure is a common cause of morbidity and mortality in diabetes. The early manifestations of diabetic cardiomyopathy are however not well established. LV torsion followed by untwisting during diastole is an important component that affects LV filling. We used STE to study the early changes in LV torsion in patients with uncomplicated T1DM and used stress MRI to assess its interrelationships with coronary microangiopathy.

Methods: 33 asymptomatic subjects with T1DM and 32 age-matched HC were recruited into the study. The T1DM subjects were divided into two age matched groups: newly diagnosed and longer duration diabetes to assess the impact of microvascular disease. All subjects underwent echocardiogram and metabolic exercise testing. Stress MRI was performed in 28 subjects (6 HC) to compute MPRI, a measure of coronary microvascular function. LV rotation measurements were made using a speckle tracking system.

Results: Peak LV torsion was significantly increased in the T1DM as compared to HC (1.9±0.6 vs 1.4±0.7, $P<0.01$). The mean MPRI in T1DM was 1.9±0.5 significantly lower than in HC (2.3±0.4, $P<0.05$). On multivariate regression analysis, rotational deformation delay ($r=-0.48$, $P<0.05$) and MPRI ($r=-0.44$, $P<0.05$) were independent predictors of LV torsion.

Conclusion: We demonstrate for the first time using speckle tracking that LV torsion is increased in young patients with uncomplicated T1DM. This may represent a myocardial compensatory mechanism to maintain EF during the early stages of diabetic cardiomyopathy. MPRI predicted LV torsion in T1DM subjects implicating a role for microvascular disease in the development of increased torsion in these individuals.

Background

Diabetes is a metabolic disorder associated with increased incidence of heart failure. There is a high incidence of mortality associated with CAD and heart failure in patients with T1DM [264]. However in some patients, LV dysfunction occurs in the absence of significant epicardial CAD or hypertension [214] indicating that diabetes contributes towards the development of LV dysfunction. Microvascular disease occurs frequently in subjects with both T1DM as well as T2DM and is related to duration of diabetes and to antecedent glycaemic control [1;122;173;206]. It results in both ischaemia and increased capillary permeability, both of which may be involved in the pathogenesis of diabetic heart failure [149]. Previous studies have demonstrated a reduced coronary flow reserve in asymptomatic T1DM and T2DM as compared to HC [55], which is a measure of microvascular function in the heart (in the absence of epicardial CAD). Presence of microvascular disease in the form of nephropathy or retinopathy is associated with high mortality in diabetic heart disease [264].

Although there is increasing evidence for the presence of diabetic cardiomyopathy as a separate entity, detection of early changes in the myocardium is challenging in patients with diabetes. Previous studies with 2D and tissue doppler echocardiography have demonstrated abnormalities in various diastolic parameters prior to the onset of overt systolic dysfunction. The trans-mitral pulsed wave doppler demonstrates abnormalities in mitral inflow velocity, DecT, IVRT and filling patterns [196]. However these measurements are load dependent and can alter depending on LA pressures. TDI is a relatively load independent method of deriving mitral annular velocities. Previous studies in subjects with both T1DM and T2DM have demonstrated a reduction in long axis function indicated by reduced S and E' wave velocity in the presence of normal EF [61;275]. However tissue doppler is not without limitations including its angle dependency. It relies on aligning the probe in line with the motion of the

myocardium. STE is a novel angle independent method of measuring LV strain and strain rates [58]. The added advantage is that we are now able to measure LV rotation for the first time with grey scale 2D images [85]. In order to assess early changes in the diabetic myocardium, we used speckle tracking to measure LV torsion, strain and strain rates in young patients with uncomplicated T1DM and compared it with age and sex matched controls.

In order to assess the impact of hyperglycaemia *per se* on the myocardium we chose cohorts of young subjects with T1DM and healthy non-diabetic controls without a history of hypertension, primary hyperlipidemia, peripheral vascular disease, CAD, cerebrovascular disease or renal disease. In order to assess the impact of coronary microvascular dysfunction on the development of LV dysfunction we recruited two predefined subgroups of T1DM subjects. The first group (newly diagnosed T1DM) was diagnosed for less than 5 years and hence unlikely to have microvascular disease; the second group (long term) was diagnosed more than 10 years previously and were thus likely to have a certain degree of microvascular dysfunction. These two diabetic groups were age matched. We used stress MRI to compute MPRI which is a measure of coronary microvascular function.

Methods

65 subjects who met the inclusion criteria and provided informed consent were recruited from the Heart of England NHS Foundation Trust and University Hospital Birmingham NHS Trust, Birmingham, UK. HC were recruited by general adverts in the University of Birmingham and blood banks. All the investigations were undertaken in the University of Birmingham and the project was approved by Multicenter Regional Ethics Committee in Birmingham.

Patients

We recruited 33 subjects with T1DM (WHO definition) without a history of chest pain or breathlessness. All patients had no evidence of CAD or heart failure based on history, 12 lead ECG, a normal EF on echocardiography and metabolic exercise testing. Subjects were divided into predefined subgroups (age matched) based on duration of their diabetes as defined above: Newly diagnosed (13 patients [10 male], age 31±10y) and long term (20 patients [12 male], age 34±6y). The mean HbA1c and duration of diabetes was 7.4±0.8% and 3.2±2.6y in the newly diagnosed and it was 8.3±1% ($P<0.05$) and 18.4±6.9y correspondingly in the long term diabetes patients.

Healthy Controls

32 age and sex matched controls with no cardiac history or diabetes mellitus were recruited. All HC had a normal 12 lead ECG, echocardiogram and metabolic exercise test.

The subjects underwent echocardiography, metabolic exercise testing and stress MRI as described in chapter 2 (Methods)

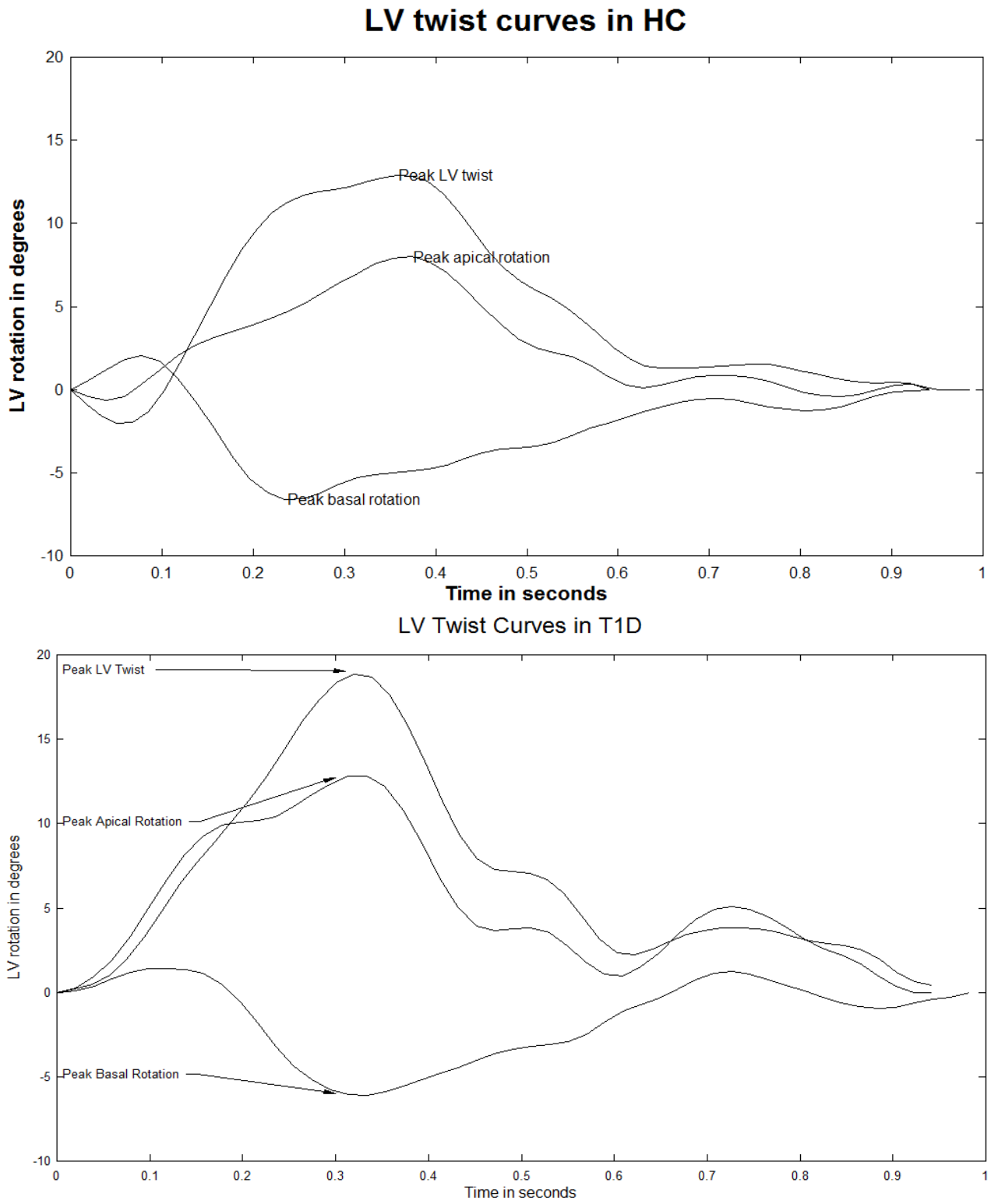
Analysis

Speckle Tracking Echocardiography

Myocardial deformation was measured using a commercially available speckle tracking system in an ECHOPAC (version 4.2.0) workstation. In this system, the displacement of speckles of myocardium in each spot were analyzed and tracked from frame to frame. We selected the best-quality digital two-dimensional image and the LV endocardium was traced at end-systole. The region of interest width was adjusted as required to fit the wall thickness.

The software package then automatically tracked the motion through the rest of the cardiac cycle. The onset of QRS complex was taken as the beginning of systole. Adequate tracking was verified in real time. The global longitudinal and transverse strain and strain rates were derived from an average of the 12 segments from the 4 and 2 chamber views (6 segments in each view). Similarly an 18 segment model was computed from the short axis views at mitral, papillary and apical levels. The average of measurements from each of the segments was used to derive global circumferential and radial strain and strain rates. In addition, LV rotation was computed using speckle tracking. Counter-clockwise rotation was marked as a positive value and clockwise rotation as a negative value when viewed from the apex. In order to calculate LV torsion, torsion rate and untwist rates, the rotation traces of the basal and apical LV cross-sections were exported into DPlot graph software (Version 2.2.1.4, HydeSoft Computing, LLC, Vicksburg, USA). The LV twist curve was generated by calculating the difference between apical and basal rotations at each corresponding time point (Figure 5.1). LV twist rates were derived from the first derivative of the LV twist curve. Peak LV torsion was derived from LV twist divided by LV diastolic longitudinal length. Rotational deformation delay was also determined and defined as the magnitude of the time difference between time to peak basal rotation and time to peak apical rotation. Of the 65 subjects in the study, 61 (94%) subjects had both adequate LV basal and apical images for speckle tracking allowing complete analysis of all LV rotational parameters.

Figure 5.1



Typical torsion curves in HC and T1DM.
HC- healthy control, T1DM – type 1 diabetes mellitus

Reproducibility of STE

Reproducibility of STE was determined within our department using a randomly selected group of 10 controls. Inter-observer measurement variability was determined by two independent observers who measured LV torsion in the 10 controls. To obtain the intra-observer variability, the first observer performed the analysis on two separate occasions 1 month apart. We performed Bland-Altman plots to assess variability of measurement. Our results showed that for LV torsion, intra-observer reproducibility was 0.24 ± 0.58 (bias ± 1.96 standard deviation of the difference (SD)) and inter-observer reproducibility was 0.15 ± 0.69 (bias ± 1.96 SD), which are acceptable.

MPRI

MPRI which is a measure of coronary microvascular function was computed from the stress MRI images. The images were analyzed on the Viewforum software version 5.0. Initially image alignment was done to reduce the motion of the heart in different cardiac cycles. Following this the endocardial and epicardial borders were traced and propagated throughout all the images. A sample volume was placed in the LV cavity as comparison for signal intensity of blood. Signal intensity curves were obtained from the software and the peak upslope of the LV myocardial illumination was computed in relation to the LV cavity illumination. A similar analysis was done on all three short axis images at rest and peak stress. The MPRI was obtained from the ratio of LV relative peak upslope at stress compared to rest.

Statistics

Continuous variables are expressed as mean ± standard deviation. Comparison between means was performed using unpaired Student T-tests. Categorical variables were compared

with Pearson Chi-Square test. For comparison of variables when the diabetes population was subdivided one way ANOVA was used. A P value of <0.05 was considered to indicate statistical significance. Variances of data sets were determined using F-test. Pearson correlation coefficient (r) was used to describe the relationship between variables. Variables of interest that were found to correlate with the dependent variable on univariate analysis were included in a stepwise linear regression analysis to identify independent predictors. SPSS (v15.0) was used to perform the statistical analysis. Bland Altman plot was used to assess data reproducibility using MedCalc (v9.2.1.0).

Results

The baseline characteristics are summarized in table 5.1. LVEF was 60.7±5% in the T1DM subjects and VO_2 max was 38.5±9.9 ml/kg/min. In the HC, the corresponding values were 61.4±5% (P=0.29 vs T1DM) and 44.1±7.2 ml/kg/min (p<0.01 vs T1DM), respectively. The mean HBA1c in the diabetic patients was 8±1%. The trans-mitral pulsed wave and tissue doppler results are summarized in table 5.2.

LV torsion

Peak LV torsion was significantly increased in the T1DM as compared to HC (1.9±0.6 vs 1.4±0.7, P<0.01). Peak LV torsion rate and peak untwisting rate were increased in T1DM but the differences did not reach statistical significance. There were significant increases in LV apical rotation, LV twist and LV twist rate in T1DM compared with HC (Table 5.3). When the patient group was subdivided based on duration of diabetes and comparisons made between the three groups (newly diagnosed vs long term vs HC) with one way ANOVA there was a statistically significant difference in LV torsion and torsion rate (P<0.05). Post hoc

Tukey tests comparing the three groups between each other revealed a significant increase in LV torsion (2±0.7 vs 1.4±0.7, $P<0.05$) and torsion rate (14.5±5.1 vs 10.9±4.8, $P<0.05$) in patients with long term diabetes as compared to HC. The LV torsion in newly diagnosed T1DM (1.7±0.4) was midway between that of long term T1DM and HC (Figure 5.2). LV torsion rates (10.4±4.3) was however very similar in newly diagnosed and HC.

Table 5.1

Variable	T1DM	HC	P value
Number	33	32	ns
Female gender (%)	11 (33)	10(31)	ns
Age,years	33±9	30±8	0.13
BMI, Kg/m^2	24±3	25±3	0.25
Resting heart rate, beats/min	82±16	77±11	0.09
Systolic blood pressure, mmHg	119±13	112±10	<0.05
Diastolic blood pressure, mmHg	75±10	70±10	0.06
VO2 max, ml/kg/min	38.5±9.9	44.1±7.2	<0.01
VO2 max, percentage predicted	98.6±16	112.2±16	<0.001
RER	1.2	1.2	0.12
Ejection fraction, %	60.7±5	61.4±5	0.29
HBA1C, %	8±1	-	-
Fasting plasma glucose, mmol/L	8.6±3.3	4.5±0.4	<0.000
Total cholesterol, mmol/L	4.4±0.9	4.9±0.9	<0.05
HDL, mmol/L	1.6±0.4	1.7±0.6	0.44

Baseline characteristics and results in T1DM patients as compared with HC. The results are expressed as mean ± standard deviation.

$P < 0.05$ was considered as statistically significant

Table 5.2

Variable	T1DM	HC	P value
Ejection fraction, %	60.7±5	61.4±5	0.29
MV E velocity, cm/sec	79.7±13	77.4±15	0.26
MV A velocity, cm/sec	58.4±15	49.1±10	<0.01
E/A ratio	1.4±0.5	1.6±0.5	0.06
Dec time, ms	252±57	248±66	0.38
IVRT, ms	72±11	72±12	0.47
TDI Peak S, cm/sec (inferior septal)	8.9±2	9.4±2	0.19
TDI Peak E', cm/sec (inferior septal)	10.6±2	12.2±2	<0.01
TDI Peak A', cm/sec (inferior septal)	8.6±2	7.8±2	0.05
E/E' (inferior septal)	7.7±1	6.4±2	<0.001
E/E' (anterior lateral)	5.3±1	4.9±1	0.06
E'/A' (inferior septal)	1.3±0.5	1.6±0.5	<0.05
E'/A' (anterior lateral)	1.8±0.6	2.1±0.8	<0.05

Mitral and tissue doppler measurements in T1DM patients as compared with HC. The results are expressed as mean ± standard deviation.

$P < 0.05$ was considered as statistically significant

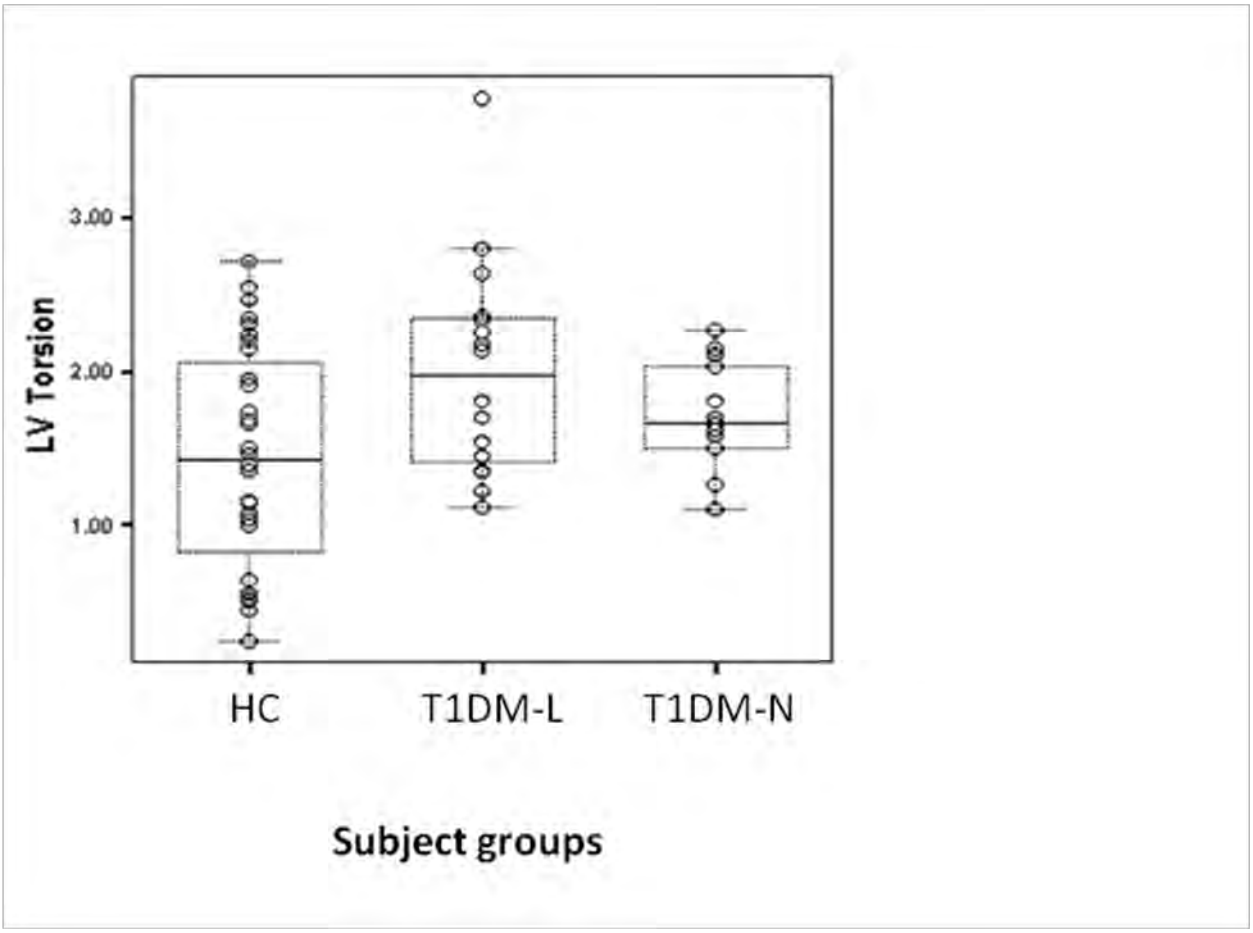
Table 5.3

Variables	T1DM	HC	P
Peak apical rotation, °	11.3±4.4	8.5±4	<0.01
peak basal rotation, °	-5.8±2.6	-4.9±2.5	0.09
Rotational deformation delay, sec	0.13±0.1	0.16±0.1	0.15
Peak LV twist, °	15.3±4.4	11.3±6	<0.01
Peak LV torsion, °/cm	1.9±0.6	1.4±0.7	<0.01
Peak twist rate S, °/sec	12.7±5.1	10.9±4.8	0.08
Peak untwist rate E, °/sec	-11.9±4.6	-11.3±4.7	0.29
Peak untwist rate A, °/sec	-6.2±3	-4.9±3.9	<0.05

LV torsion and untwist measurements in T1DM patients as compared with HC. The results are expressed as mean ± standard deviation.

$P < 0.05$ was considered as statistically significant

Figure 5.2



LV torsion measurements (expressed in degree/cm) in the HC and subgroups of patients with T1DM

HC- Healthy control, T1DM-L – Long term diabetes patient group, T1DM-N – Newly diagnosed type 1 diabetes patient group.

Rotational deformation delay

This is defined by the difference in time between the apical rotation and basal rotation achieving its peak value. The mean rotational deformation delay was greater in HC compared to T1DM (0.16± 0.1 vs 0.13±0.1, P=0.15) but the difference did not reach statistical significance.

Strain and strain rates

Global transverse and circumferential strain was significantly increased in T1DM compared to HC. Also the global circumferential and radial strain rates (S, E and A) were higher in T1DM. There was no significant difference in the global longitudinal and radial strain, although the longitudinal strain was non-significantly reduced in T1DM (table 5.4).

MPRI

The mean MPRI in T1DM was 1.9±0.5 significantly lower than in HC (2.3±0.4, P<0.05). When comparison was made between the three subgroups (new vs long term vs HC) using one way ANOVA, there was a significant difference in MPRI (P<0.05). Post hoc Tukey test revealed a significant difference in MPRI between long term T1DM and HC (1.7±0.6 vs 2.3±0.4, P<0.05). The MPRI in new T1DM tended to be higher than in long term (2.1±0.2, P=0.13), but this difference did not reach statistical significance (Figure 5.3).

Correlation and linear regression

On univariate analysis, LV torsion negatively correlated with MPRI (r=-0.40, P<0.05), VO2 max (r=-0.26, P=0.05), global circumferential strain (r=-0.35, P<0.05), circumferential strain rate S(r=-0.39, P<0.01), anterior-lateral E'/A' (r=-0.37, P<0.01) and rotational deformation

delay (r=-0.46, P<0.001). LV torsion correlated positively with fasting plasma glucose (r=0.40, P<0.01), global transverse strain (r=0.34, P<0.05) and circumferential strain rate E (r=0.37, P<0.01) and A (r=0.39, P<0.01) (table 5.5). On multivariate regression analysis only rotational deformation delay (r=-0.48, P<0.05: Figure 5.4) and MPRI (r=-0.44, P<0.05: Figure 5.5) were independent predictors of LV torsion.

Table 5.4

Variables	T1D	HC	P
Global Longitudinal Strain, %	-17.6±2.5	-18.4±2.8	0.14
Global Longitudinal Strain Rate peak S, 1/sec	-1.2±0.2	-1.2±0.2	0.22
Global Longitudinal Strain Rate peak E, 1/sec	1.5±0.3	1.5±0.3	0.14
Global Longitudinal Strain Rate peak A, 1/sec	0.8±0.2	0.8±0.3	0.46
Global Transverse Strain, %	32.6±11	26.6±10.3	<0.05
Global Radial Strain, %	30.5±8.5	28.3±12.2	0.21
Global Radial Strain Rate Peak S, 1/sec	1.6±0.3	1.3±0.4	<0.01
Global Radial Strain Rate Peak E, 1/sec	-1.7±0.5	-1.3±0.5	<0.01
Global Radial Strain Rate Peak A, 1/sec	-0.8±0.2	-0.6±0.2	<0.001
Global Circumferential Strain, %	-20.8±3	-16.8±4	<0.000
Global Circumferential Strain Rate Peak S, 1/sec	-1.7±0.3	-1.4±0.3	<0.000
Global Circumferential Strain Rate Peak E, 1/sec	2±0.5	1.6±0.4	<0.001
Global Circumferential Strain Rate Peak A, 1/sec	0.9±0.2	0.7±0.2	<0.001

Global longitudinal, radial and circumferential strain in T1DM patients as compared with HC. The results are expressed as mean ± standard deviation.

$P < 0.05$ was considered as statistically significant

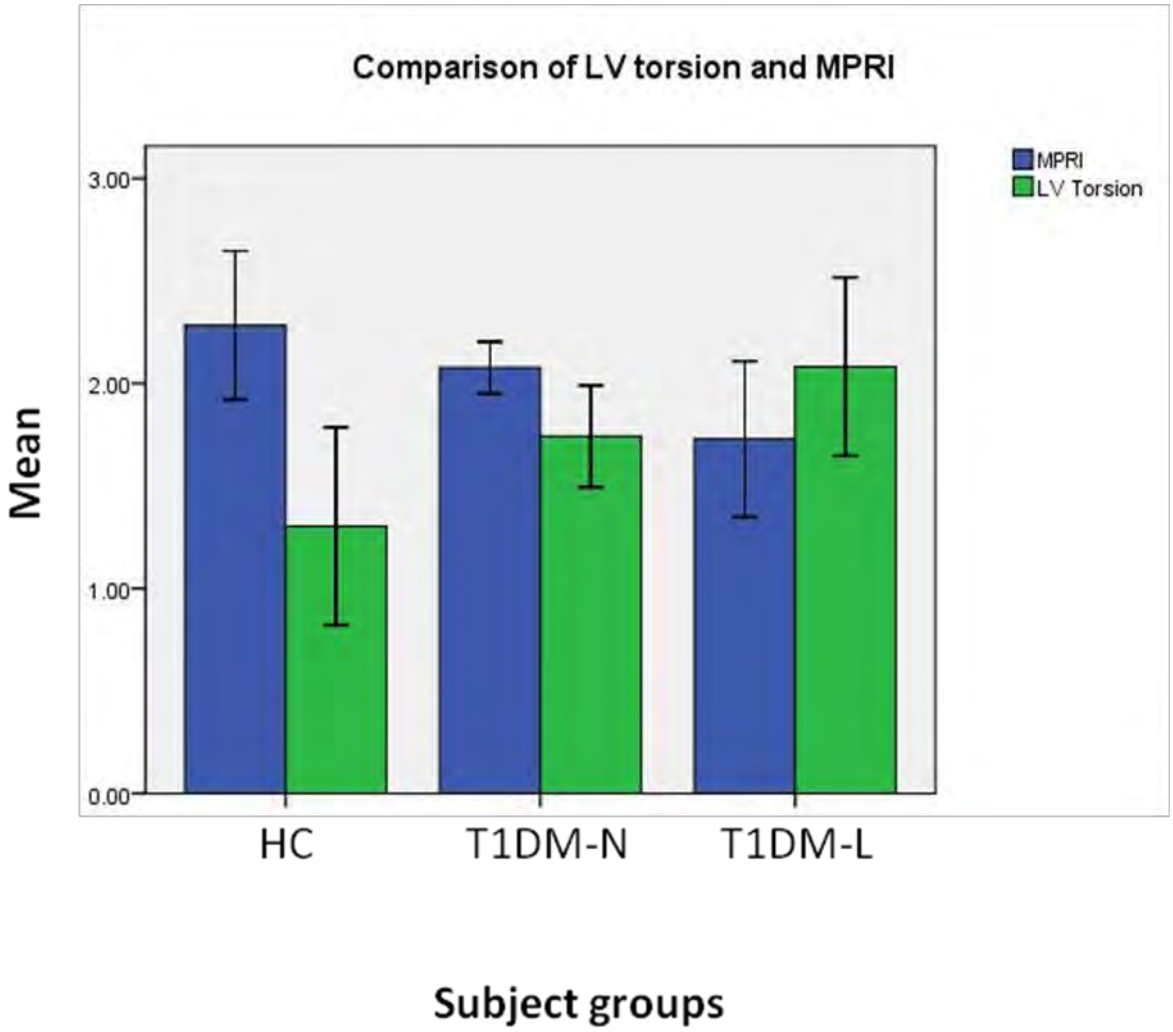
Table 5.5

Variable	R	P
MPRI	-0.40969	<0.05
Glucose	0.407503	<0.01
VO2 max	-0.25894	0.05
Rotational deformation delay	-0.45651	<0.001
Global Transverse Strain	0.337503	<0.05
Global Circumferential Strain	-0.3475	<0.05
Global Circumferential Strain Rate Peak S	-0.38668	<0.01
Global Circumferential Strain Rate Peak E	0.366952	<0.01
Global Circumferential Strain Rate Peak A	0.387069	<0.01
Anterior lateral E'/A'	-0.36961	<0.01

Correlation of variables with LV torsion in the whole group of subjects (includes HC and T1DM patients). Only the significant correlations are presented.

$P < 0.05$ was considered as statistically significant

Figure 5.3



Comparison of LV torsion (expressed in degrees) and MPRI (expressed as ratio) in HC and subgroups of T1DM

HC- Healthy control, T1DM-N – Newly diagnosed type 1 diabetes patient group, T1DM-L – Long term diabetes patient group.

Figure 5.4

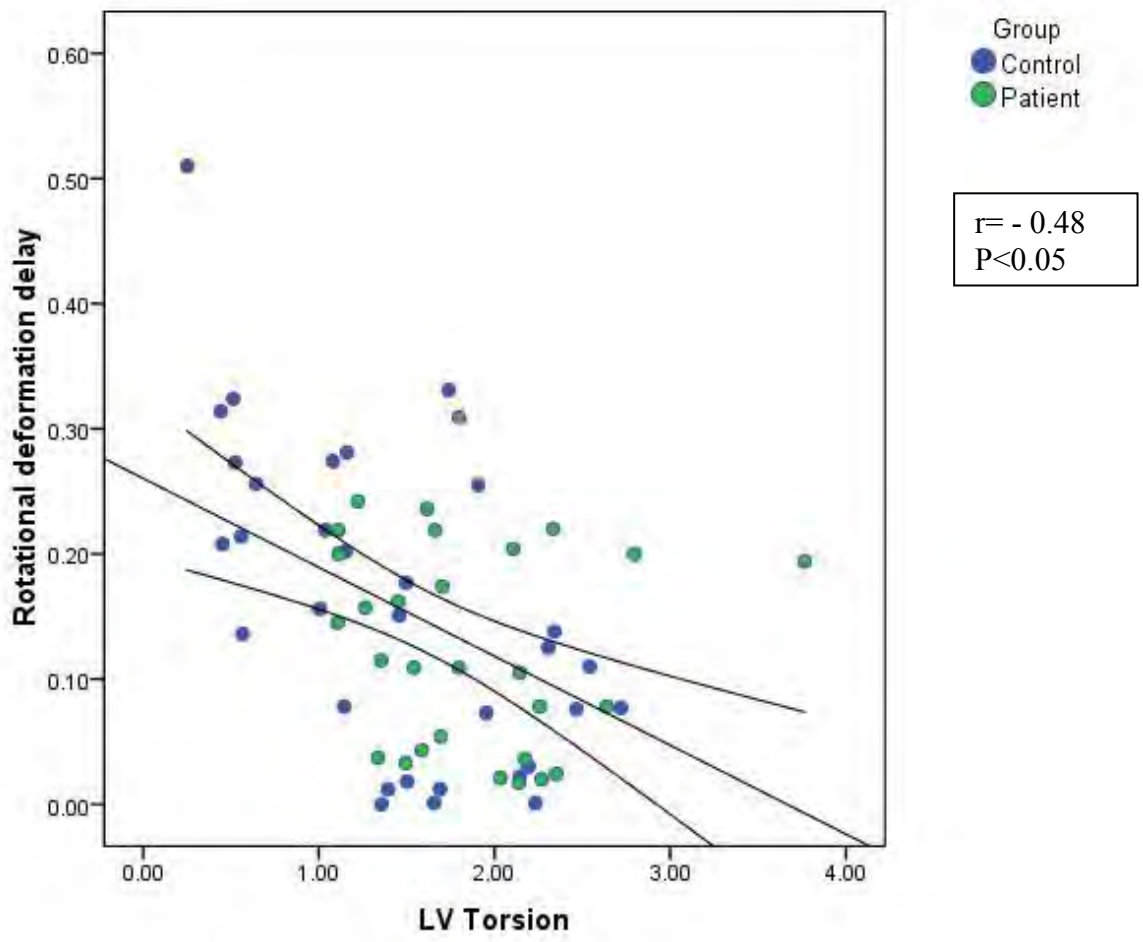


Figure depicting the significant correlation between rotational deformation delay (expressed in seconds) and LV torsion (expressed in degree/cm) in the whole subject group.

Figure 5.5

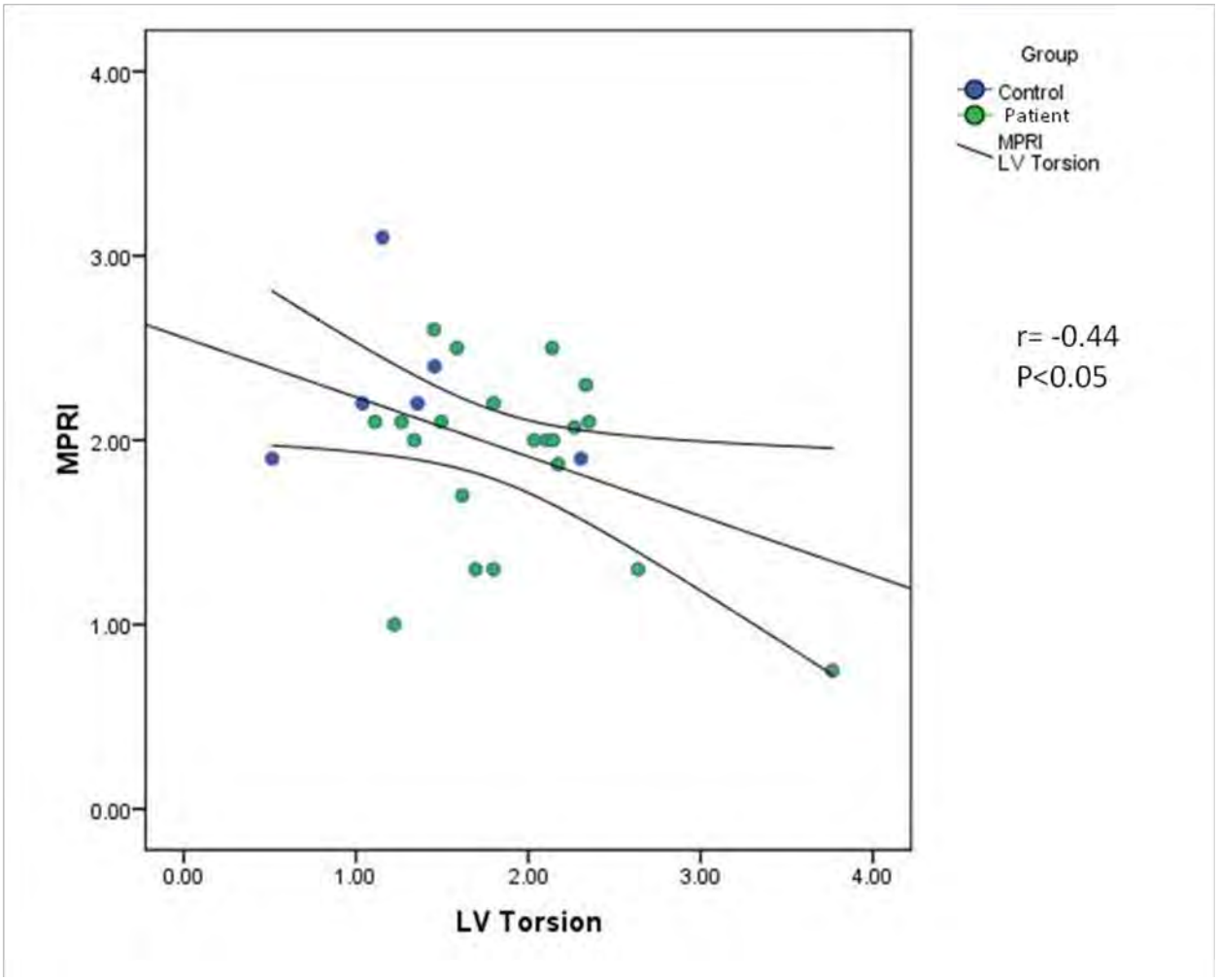


Figure depicting the significant correlation between MPRI (expressed as a ratio) and LV torsion (expressed in degree/cm) in the whole subject group.

Discussion

The principal findings in this study are: a) LV torsion is increased in young patients with uncomplicated diabetes with normal EF. b) Global transverse and circumferential strain and strain rates are increased in these patients with mild abnormalities in long axis function. c) Rotational deformation delay and coronary microvascular function may play key roles in determining LV torsion.

LV torsion is the net result of counter-clockwise rotation of the base with respect to clockwise rotation of the apex along the long axis of the LV. Normally LV torsion contributes significantly to an energy-efficient ejection during systole [14;20]. LV untwisting which follows LV twist is a key determinant of LV filling. It helps to generate the intra-ventricular pressure gradient during isovolumetric relaxation thus creating a suction effect to allow early diastolic filling to occur once the mitral valve opens [200]. In this study, we found LV torsion is increased in young T1DM. There are different plausible explanations for this. In a previous study with 53 diabetic patients with no LV hypertrophy, normal EF and no ischaemia on dobutamine echocardiography, radial contractility was increased and appeared to compensate for reduced longitudinal contractility [290]. Longitudinal (long-axis) contraction of the left ventricle is dependent on the integrity of longitudinal subendocardial myocardial fibers, whereas radial (short-axis) contraction depends on integrity of the circumferential fibers. The former is more susceptible to ischaemia and fibrosis [145;254] which may result in a relative increase in short-axis function compared with a decrease in long-axis function due to compensatory ventricular remodeling. Hence the increased torsion and circumferential strain may be a compensatory mechanism for a subclinical reduction in long axis function. Previous studies have shown increased LV torsion in aging [270], aortic stenosis [52] and HCM [287]. During

contraction the sub-endocardial fibres partially counteract the rotational motion of the sup-epicardial fibers. Contractile dysfunction of the sub-endocardial fibers due to ischaemia, fibrosis or remodeling results in a net increase in LV torsion secondary to unopposed action of the sub-epicardial fibres. Thus increased torsion may indicate sub-endocardial contractile dysfunction. Studies using tagged MRI in uncomplicated T1DM patients have previously shown increased torsion [42]. The clinical characteristics of these patients were similar to our own study population. However we have extended these findings by exploring potential mechanisms responsible for the increase in LV torsion. We noted in our study that the rotational deformation delay was higher in the controls although the difference did not reach clinical significance. This means that the apical rotation reaches its peak at a different time compared to the mitral peak. Hence the net twist would be lower as a direct consequence of rotational deformation delay. We speculate that this reduction in rotational delay at least partially explains the increase in torsion in T1DM patients. In agreement with this construct, rotational deformation delay was an independent predictor of LV torsion on multivariate regression analysis. A previous study assessing the impact of aging on LV twist concluded that reduced rotational deformation delay contributed to the increase in LV twist associated with aging [270].

In this study, we found that LV torsion was negatively correlated with MPRI and positively correlated with fasting plasma glucose. There was no significant correlation of LV torsion with HbA1c indicating that atleast in the medium term blood glucose control had no bearing on development of increased LV torsion. In fact MPRI was an independent predictor of LV torsion. In the subgroup analysis we found that the long term T1DM had the highest LV torsion followed by the newly diagnosed T1DM patients. The LV torsion in HC was the

lowest in the three subgroups. Owing to the fact that our patients had no other co-morbidities like hypertension, primary hyperlipidaemia or ischaemic heart disease, the increased torsion may be a direct consequence of the effect of hyperglycaemia on the heart which worsens with duration of diabetes. It is well known that development of microvascular disease is directly related to duration and glycaemic control in T1DM patients. Indeed the long term T1DM patients in our study had a lower MPRI as compared to HC. The study was not powered to detect significant differences in MPRI between longer term T1DM and newly diagnosed T1DM subjects. MPRI is a good indicator of coronary microvascular function. In our study MPRI was an independent predictor of LV torsion. This implies that coronary microvascular disease may play a key pathophysiological role in the development of increased torsion in these patients. Previous studies in T1DM and T2DM have suggested a role of coronary microvascular disease in the development of diabetic cardiomyopathy. Normotensive diabetic patients without large vessel CAD were found to have reduced coronary reserve flow associated with diastolic dysfunction [251;252]. Furthermore, previous studies by our group have demonstrated that CAN is associated with impaired myocardial blood flow by using PET [197;246]. The sympathetic dysinnervation of the heart commenced in the distal LV regions near the apex and then spread towards the base in patients with advanced disease [247]. Thus, dysinnervation of the apex in uncomplicated T1DM may have a role in increasing apical rotation. This will need confirmation with studies targeting this hypothesis.

Interestingly, we found that circumferential strain and strain rates were increased in the patient cohort. However, there was no significant difference in the longitudinal strain or tissue doppler mitral annular peak S, although there was a trend towards lower strain and Peak S in the diabetes patients. However, tissue doppler derived E' (inferior-septal) was significantly

lower in T1DM (10.6±2 vs 12.2±2, P<0.01). The E/E' (inferior-septal) was significantly higher in T1DM (7.7±1 vs 6.4±1, P<0.001), but still within the normal range. The tissue doppler derived E'/A' which is another marker of diastolic dysfunction was significantly reduced in T1DM (table-5.2) consistent with our previous studies [197]. There was a significant negative correlation between LV torsion and anterior lateral E'/A', implying that the increased torsion was a compensatory mechanism for the early relaxation abnormality. The increased torsion would provide the potential energy for untwisting in diastole and indeed untwisting rate was preserved in the patients' cohort in our study. A recent study looking at LV torsion in different stages of diastolic dysfunction demonstrated that LV torsion is increased in mild diastolic dysfunction but returned to normal in patients with higher grades of diastolic dysfunction [187]. Subjects in this study had diastolic dysfunction secondary to hypertension, HCM or amyloidosis. Although the reason for diastolic dysfunction in this study was different to our study population, the results are comparable in that mild diastolic dysfunction was associated with increased LV torsion. The increased LV torsion may be either a compensatory mechanism to abnormal relaxation or as a direct consequence of the abnormal relaxation. The absence of markedly reduced longitudinal function in our subjects indicates that increased torsion probably occurs before this and is one of the first stages of diabetic cardiomyopathy.

Clinical implications

Development of heart failure in diabetes is a complex mechanism and is affected by many secondary factors like hypertension, CAD, renal disease and hyperlipidemia. We have shown in our study that diabetes *per se* and microvascular disease has a major impact on the myocardium. Increased torsion may be one of the earliest features of diabetic cardiomyopathy

and it is tempting to speculate that this may herald ultimate progression to heart failure. If confirmed in large scale prospective studies it could form the basis of a screening tool for the identification of patients at risk for the development of heart failure. It could also form the basis of targeted therapy to reduce incidence of heart failure in diabetes patients.

Study Limitations

One of the main drawbacks of the study was the small sample size of the study population. Also patients were studied at one point in time and hence there is no indication as to progression to heart failure. The mean HbA1c in our study population was 8±1%. It will be important to elucidate the effect of glycaemic control on LV torsion. The subjects were not studied in a metabolically standardized environment which reflected the practical difficulties of infusing insulin and glucose during the MRI when adenosine and Gadolinium contrast also had to be infused. Our study was in a cohort of uncomplicated T1DM. However T2DM is also a major health problem with high incidence of CAD and heart failure [101;109]. Previous studies have demonstrated diastolic dysfunction as early changes in these patients [30]. There have been no studies looking at LV torsion as early manifestation of cardiomyopathy in T2DM. We expect the LV torsion to be similarly increased in uncomplicated T2DM. But T2DM is associated more often with hypertension and CAD which may have a major bearing on LV torsion.

CHAPTER SIX

LEFT ATRIAL FUNCTION AND ITS CONTRIBUTION TO LEFT VENTRICULAR FILLING IN PATIENTS WITH TYPE 1 DIABETES AND NORMAL EJECTION FRACTION

Abstract

Background: In diabetes mellitus, cardiomyopathy is characterized by the development of left ventricular diastolic and then systolic function. In this study we evaluated young patients with T1DM who had normal LVEF in order to evaluate subtle preclinical changes in cardiac function. We used STE to assess changes in LV untwisting and its relation to diastolic parameters. We used cardiac MRI to assess the LA contribution to left ventricular filling.

Methods: We recruited 33 T1DM patients and 32 age-matched HC into the study. Study participants underwent echocardiogram, cardiac MRI and metabolic exercise testing. LV rotation measurements were made using a commercially available speckle tracking system. In order to adjust for the differences in heart rate between individuals the RR interval was normalised to 100%. LV volume measurements were made using the Viewforum software from cardiac MRI images.

Results: LVEF was not different in T1DM subjects and HC (60.7±5% vs 61.4±5%, p=0.29). The early peak LV untwisting rate (E) was similar in T1DM and HC (-11.9±4.6 0/cm/sec vs -11.3±4.7 0/cm/sec, P=0.29) but the late peak LV untwisting rate (A) was significantly increased in T1DM (-6.2±3 0/cm/sec vs -4.9±3.9 0/cm/sec, P<0.05). The time to early peak untwisting rate was not different (50.9±9.6% vs 48.4±7.3%, P=0.12) but the time to late peak untwisting rate was significantly delayed in T1DM patients (80.4±12.5% vs 72.7±14.6%, P<0.05). Tissue doppler analysis demonstrated a reduction in E' (10.6±2 cm/sec vs 12.2±2, P<0.01) and E'/A' (1.3±0.5 vs 1.6±0.5, P<0.05) with an increase in E/E' (7.7±1 vs 6.4±2, P<0.001) indicating an early relaxation abnormality in T1DM subjects. Also there was increase in trans-mitral A wave velocity (58.4±15 cm/sec vs 49.1±10 cm/sec, P<0.01) and mitral annular A' (8.6±2 cm/sec vs 7.8±2 cm/sec, P<0.05). The LV filling patterns demonstrated a significantly increased LA contribution to LV filling in T1DM. On linear

regression peak late filling rate (r=0.60, P<0.000), trans mitral A wave (r=0.25, P<0.05) and A' (r=0.30, P<0.01) were predictors of LA contribution to LV filling.

Conclusion: We demonstrate for the first time using speckle tracking that LV untwisting rate E is preserved and untwisting rate A is increased and delayed in young patients with uncomplicated T1DM. This is associated with early relaxation abnormalities in the LV. The LA contribution to LV filling is increased in these patients and is directly related to increases in other indices of LA function like peak late filling rate, trans-mitral A wave and A'.

Background

Heart failure occurs more frequently in diabetes [109] and although frequently due to CAD and/or hypertension, may occur in the absence of these, when it is termed diabetic cardiomyopathy. Although there is increasing evidence for the presence of diabetic cardiomyopathy as a separate entity, detection of early changes in the myocardium is challenging in patients with diabetes. Previous studies with 2D and tissue doppler echocardiography have demonstrated abnormalities in various diastolic parameters prior to the onset of overt systolic dysfunction. Echocardiographic trans-mitral pulsed wave doppler demonstrates abnormalities in mitral inflow velocity, DecT, IVRT and filling patterns [196]. In healthy young subjects the majority of LV filling is accomplished in the early filling phase and the LA contributes the remainder. In heart failure the early phase of filling is hampered by the stiff ventricle [123;272]. In this situation the LA contributes significantly more to LV filling. Similarly there is increased reliance on the LA for LV filling in the presence of diastolic dysfunction. We demonstrated recently that increased LA filling compensates for impaired early relaxation during exercise in patients with heart failure with preserved EF [194]. LA function may be an important factor in maintaining EF in the early stages of diabetic cardiomyopathy. Loss of atrial function with the onset of atrial fibrillation may precipitate worsening heart failure in any of the above situations.

In this study we investigated various aspects of LA function in young T1DM subjects with no underlying heart failure or CAD. STE has recently been used to measure LV torsion and untwisting. LV untwisting, which follows LV torsion contributes significantly to LV filling. We studied the untwisting patterns in patients with T1DM and its relation to diastolic function and LV filling. The early untwist corresponds to the early phase of filling and late untwisting

corresponds to the LA filling phase. We used cardiac MRI to directly measure LV volumes during the cardiac cycle and hence LV filling patterns in these subjects.

Methods

65 subjects who met the inclusion criteria and provided informed consent were recruited from the Heart of England NHS Foundation Trust and University Hospital Birmingham NHS Trust, Birmingham, UK (The recruited subjects are the same as those in the previous chapter/study). HC were recruited by general adverts in the University of Birmingham and blood banks. All the investigations were undertaken in the University of Birmingham and the project was approved by Multicenter Regional Ethics Committee in Birmingham.

Patients

We recruited 33 subjects with T1DM (WHO definition) without a history of chest pain or breathlessness. All patients had no evidence of CAD or heart failure based on history, 12 lead ECG, a normal EF on echocardiography and metabolic exercise testing.

Healthy Controls

32 age and sex matched controls with no cardiac history or diabetes mellitus were recruited. All HC had a normal 12 lead ECG, echocardiogram and metabolic exercise test.

Subjects underwent echocardiography, metabolic exercise testing and cardiac MRI as described in the methods chapter (chapter 2)

Analysis

Speckle Tracking Echocardiography

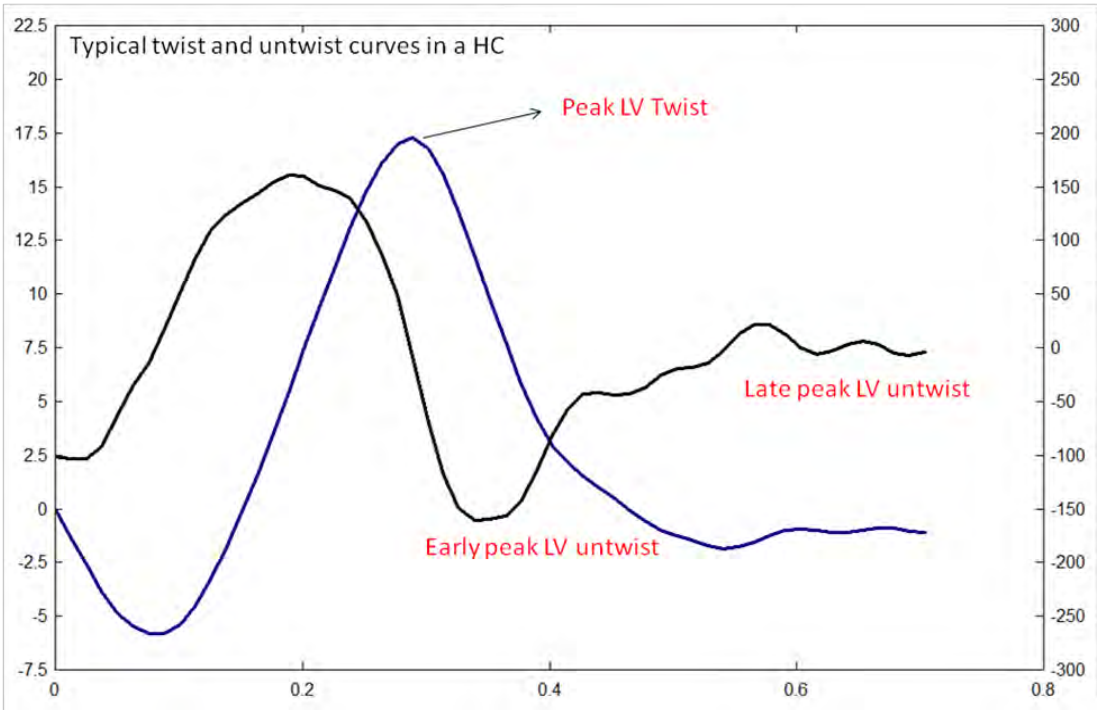
Myocardial deformation was measured using a commercially available speckle tracking system on an ECHOPAC (version 4.2.0) workstation. In this system, the displacement of speckles of myocardium in each spot were analyzed and tracked from frame to frame. We selected the best-quality digital two-dimensional image and the LV endocardium was traced at end-systole. The region of interest width was adjusted as required to fit the wall thickness. The software package then automatically tracked the motion through the rest of the cardiac cycle. The onset of the QRS complex was taken as the beginning of systole. Adequate tracking was verified in real time. Counter-clockwise rotation was marked as a positive value and clockwise rotation as a negative value when viewed from the apex. In order to calculate LV torsion, torsion rate and untwist rates, the rotation traces of the basal and apical LV cross-sections were exported into DPlot graph software (Version 2.2.1.4, HydeSoft Computing, LLC, Vicksburg, USA). The LV twist curve was generated by calculating the difference between apical and basal rotations at each corresponding time point. LV twist rates were derived from the first derivative of the LV twist curve (figure 6.1). Peak LV torsion was derived from LV twist divided by LV diastolic longitudinal length. In order to adjust for the differences in heart rate between individuals the RR interval was normalised to 100%. Of the 65 subjects in the study, 61 (94%) subjects had both adequate LV basal and apical images for speckle tracking to complete analysis of all LV rotational parameters.

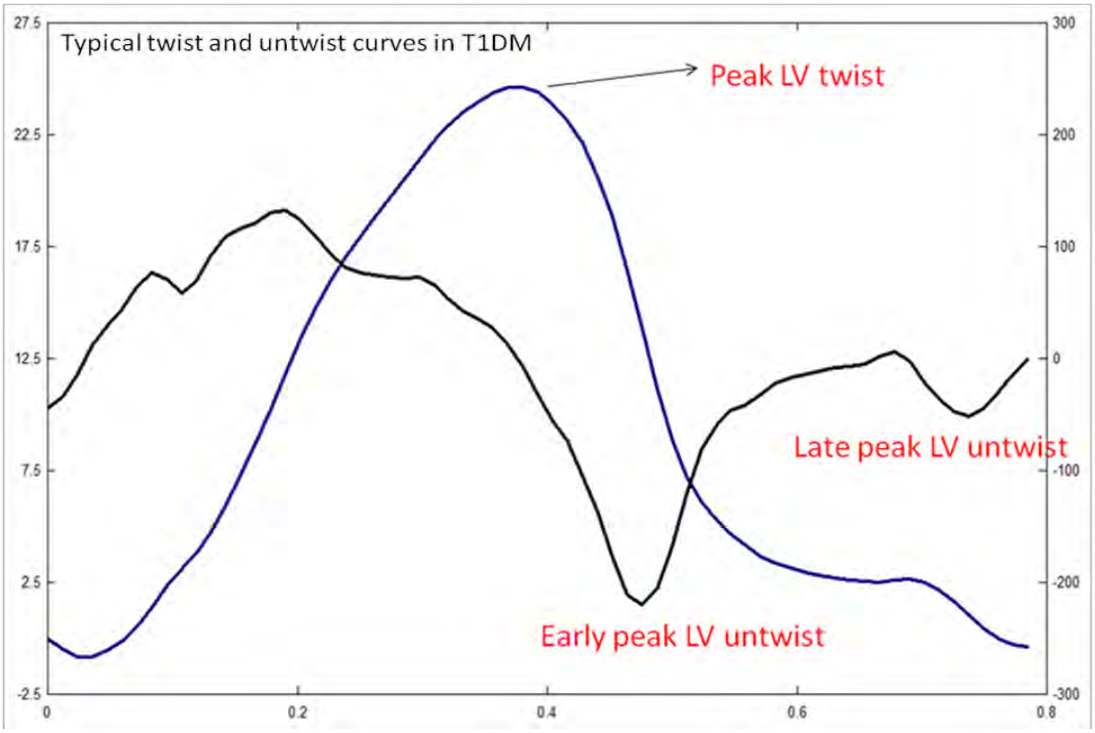
LV volumes

LV volumes were computed from the short axis cine MRI images. The images were analyzed on Viewforum software version 5.0. The endocardial and epicardial borders were traced at

end diastole in each slice and propagated through the rest of the cardiac cycle. The software generated LV volumes at each phase of the cardiac cycle incorporating all the slices. The LV volume time curves (figure 6.2) were exported to graphical software Dplot. The first derivative of the LV volume curve was computed to represents the rate of LV volume changes. SV was defined as the difference between end-diastolic volume (EDV) and end-systolic volume (ESV). Early and late PFR were defined as the first and second positive peaks of the first derivative curve during diastole. The early and late (atrial) components of LV filling were measured from the corresponding LV volume segments and were expressed as percentage of SV.

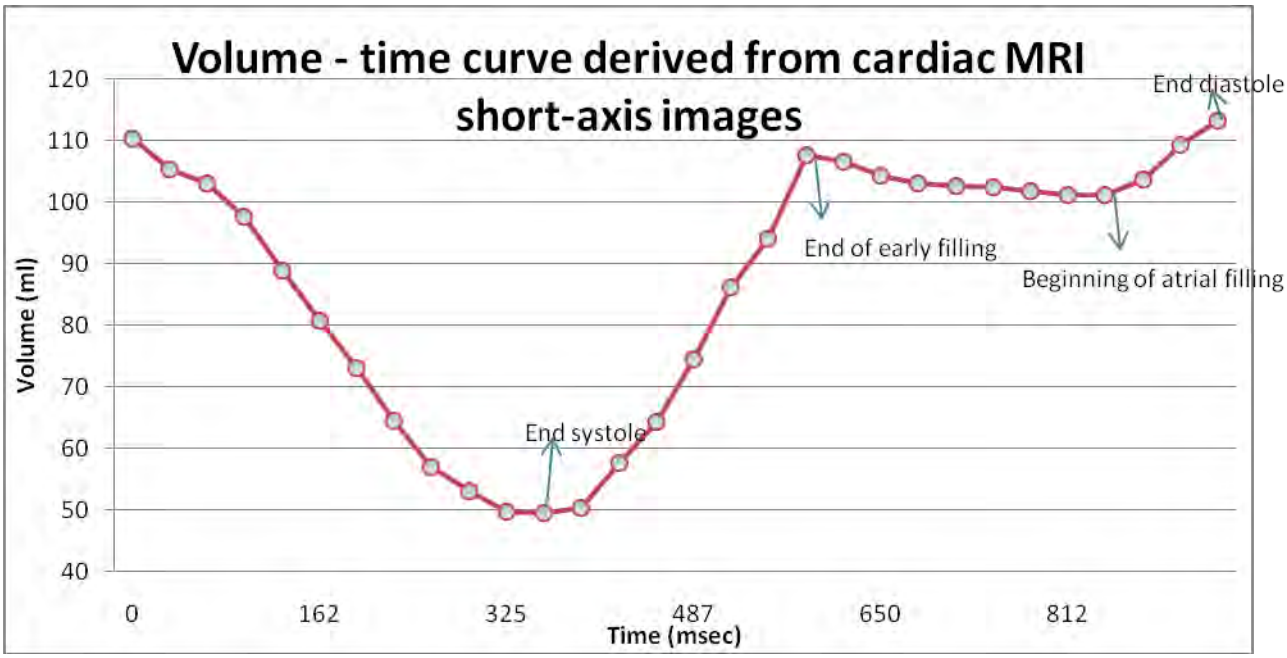
Figure 6.1

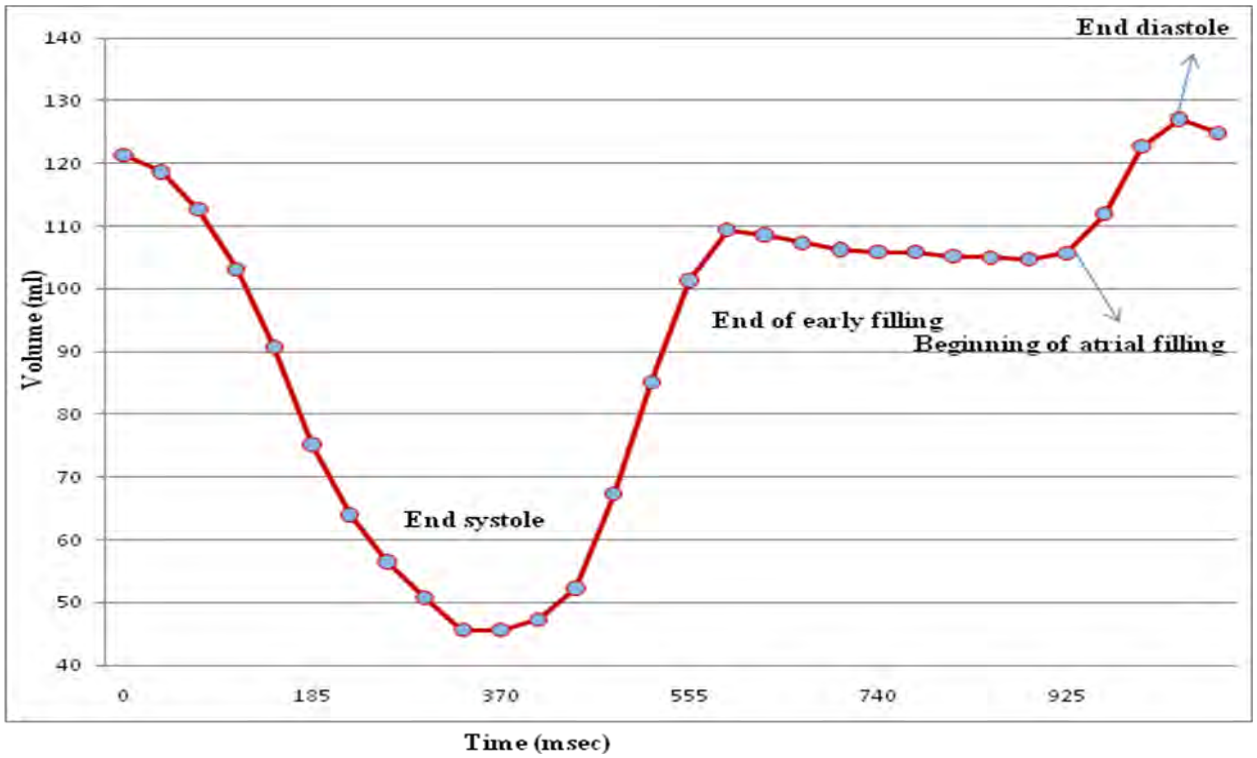




A typical twist and untwist curve in HC as compared to T1DM.
HC- healthy control, T1DM – type 1 diabetes mellitus patient.

Figure 6.2





Typical left ventricular filling curves in a healthy control (top figure) as compared with type 1 diabetes patient (bottom figure).

Statistics

Continuous variables are expressed as mean ± standard deviation. Comparison between means was performed using unpaired student T-tests. Categorical variables were compared with Pearson Chi-Square test. For comparison of variables when the diabetes population was subdivided one way ANOVA was used. A P value of <0.05 was considered to indicate statistical significance. Variances of data sets were determined using F-test. Pearson correlation coefficient (r) was used to describe the relationship between variables. Variables of interest that were found to correlate with the dependent variable on univariate analysis were included in a stepwise linear regression analysis to identify independent predictors. SPSS (v15.0) was used to perform the statistical operations.

Results

The baseline characteristics are summarized in Table 5.1. LVEF was 60.7±5% in the T1DM subjects and VO_2 max was 38.5±9.9 ml/kg/min. In the HC, the corresponding values were 61.4±5% (P=0.29 vs T1DM) and 44.1±7.2 ml/kg/min ($p<0.01$ vs T1DM), respectively. The mean HBA1c in the diabetic patients was 8±1%. The trans-mitral pulsed wave and tissue doppler results are summarized in Table 5.2. Tissue doppler analysis demonstrated a statistically significant reduction in E' and E'/A' with an increase in E/E' indicating early relaxation abnormality. There were also statistically significant increases in trans-mitral A wave velocity and mitral annular A'. The LA volume index was significantly increased in T1DM as compared to HC.

LV torsion and untwist

Peak LV torsion was significantly increased in the T1DM as compared to HC (1.9±0.6 vs 1.4±0.7, P<0.01). We noted significant increases in LV apical rotation, LV twist and LV twist rate in T1DM compared with HC (Table 6.1). The early peak LV untwisting rate was similar in T1DM and HC (-11.9±4.6 0/cm/sec vs -11.3±4.7 0/cm/sec, P=0.29). The late peak LV untwisting rate was significantly increased in T1DM (-6.2±3 0/cm/sec vs -4.9±3.9 0/cm/sec, P<0.05). The time to early peak untwisting rate was not different (50.9±9.6% vs 48.4±7.3%, P=0.12) but the late peak untwisting rate was significantly delayed in T1DM patients (80.4±12.5% vs 72.7±14.6%, P<0.05).

Table 6.1

Variables	T1DM	HC	P
Peak apical rotation, °	11.3±4.4	8.5±4	<0.01
peak basal rotation, °	-5.8±2.6	-4.9±2.5	0.09
Peak LV twist, °	15.3±4.4	11.3±6	<0.01
Peak LV torsion, °/cm	1.9±0.6	1.4±0.7	<0.01
Peak twist rate S, °/sec	12.7±5.1	10.9±4.8	0.08
Peak untwist rate E, °/sec	-11.9±4.6	-11.3±4.7	0.29
Peak untwist rate A, °/sec	-6.2±3	-5±3.9	<0.05
Time to untwist rate E, %*	50.9±9.6	48.4±7.3	0.12
Time to untwist rate A, %*	80.4±12.5	72.7±14.6	<0.05

LV rotation measurements in T1DM (whole group) as compared with HC expressed as mean ± standard deviation.

P<0.05 was considered as significant
* Expressed as % of RR interval.
LV – left ventricle, T1DM – type 1 diabetes mellitus, HC- healthy control.

LV volumes

The results from the cardiac MRI are summarized in Table 6.2. The LVEF and SV were similar in T1DM and HC. The early PFR was slightly but not significantly higher where as the late PFR was significantly higher in the T1DM as compared to HC. The LV filling patterns demonstrated a significantly increased LA contribution to LV filling in T1DM. Although the total diastolic period (as a percentage of the RR interval) was similar, T1DM patients spent significantly less time in early filling and significantly longer time in atrial filling.

Correlation and linear regression

LA contribution to LV filling correlated positively with LV torsion ($r=0.39$, $P<0.05$), trans mitral A wave($r=0.58$, $P<0.00$), A' ($r=0.56$, $P<0.00$), E/E' ($r=0.32$, $P<0.05$), and peak late filling rate ($r=0.80$, $P<0.000$) and negatively with E'/A' ($r=-0.54$, $P<0.000$) and E' ($r=-0.40$, $P<0.01$). On linear regression peak late filling rate ($r=0.60$, $P<0.000$), trans-mitral A wave ($r=0.25$, $P<0.05$) and A' ($r=0.30$, $P<0.01$) were predictors of LA contribution to LV filling.

Table 6.2

Variables	T1DM	HC	P
Peak emptying rate, ml/msec	0.35±0.1	0.31±0.1	0.08
Time to peak emptying rate, msec	148±30	166±0.2	**<0.05**
Peak early filling rate, ml/msec	0.44±0.14	0.40±0.10	0.19
Peak late filling rate, ml/msec	0.19±0.07	0.15±0.05	**<0.05**
Early filling contribution, %	75.6±8.9	80.7±7.1	**<0.05**
Late filling contribution, %	24.4±8.9	19.3±7.1	**<0.05**
Total systolic time, %	37.5±5.1	37.5±5.5	0.48
Total diastolic time, %	62.5±5.1	62.5±5.5	0.48
Total early filling time, % of diastole	71.6±7.2	76.9±4.8	**<0.01**
Total late filling time, % of diastole	28.4±7.2	23.1±4.8	**<0.01**
Ejection fraction, %	60.3±6.7	59.7±4	0.14
End diastolic volume, ml	117.1±29	113±20	0.30
End systolic volume, ml	47.4±17.1	45.9±10.9	0.36
Stroke volume, ml	69.6±14.6	67.1±11.4	0.26
Cardiac output, l/min	4.5±1.3	4.1±0.69	0.07
LV mass	98.2±27.2	95.3±22	0.33

LV volume results in T1DM (whole group) as compared with HC expressed as mean ± standard deviation.

P<0.05 was considered as significant
LV – left ventricle, T1DM – type 1 diabetes mellitus, HC- healthy control.

Discussion

The principal findings in this study are: a) LV untwist rate A and time to LV untwist rate A were increased in young patients with T1DM as well as other indices of left atrial function (trans mitral A wave and A'). b) Left atrial contribution to LV filling was increased in T1DM patients c) peak late filling rate, trans-mitral A wave and A' were predictors of the left atrial contribution to LV filling.

LV torsion is the net result of counter-clockwise rotation of the base with respect to clockwise rotation of the apex along the long axis of the LV. Normally LV torsion contributes significantly to an energy-efficient ejection during systole [14;20]. LV untwisting which follows LV twist is a key determinant of LV filling. It helps to generate the intra-ventricular pressure gradient during isovolumetric relaxation thus creating a suction effect to allow early diastolic filling to occur once the mitral valve opens [200]. In this study, we found that early LV untwist was preserved. This might be as a direct result of increased LV torsion which creates the potential energy for early untwisting. The late LV untwist was increased indicating augmented atrial contraction. In a previous study in diabetes patients the trans-mitral A wave was increased in subjects associated with impaired relaxation [196]. This is similar to our findings of increased indices of atrial function like increased trans-mitral A wave and A'. Also a recent study in T1DM patients demonstrated changes in LA transport function suggesting increased reliance on LA for LV filling [193].

Our study has demonstrated abnormalities in early diastolic filling. This is suggested by a reduced E' and E'/A' and an increased E/E' on tissue doppler analysis. Previously various studies have demonstrated early relaxation abnormalities as the precursors of heart failure in diabetes [61;196;275]. A recent study has demonstrated that E/E' is a predictor of mortality in

diabetic patients without heart failure who were followed up for more than 10 years [70]. The early relaxation abnormalities results in impaired early LV filling and probably represents one of the earliest functional changes in the left ventricle.

To the best of our knowledge this is the first study that investigates the LV filling patterns in T1DM patients with normal EF using LV volumes measured by cardiac MRI. The EF, SV, end-diastolic and end systolic volume were all similar in both T1DM patients and HC. However the late PFR was significantly increased in T1DM patients suggesting increased trans-mitral gradient produced by augmented LA contraction. This resulted in increased LA contribution to LV filling. This increased contribution of LA to LV filling helps to maintain adequate LV filling and hence generate appropriate SV. Hence the EF is maintained at this time despite abnormal early filling. The augmented LA function appears to be the key compensatory mechanism at this point. The early relaxation abnormality is likely to worsen on exercise and hence the reliance on LA for LV filling will only increase. The increase in LA function is noticed in early stages of heart failure. However, with worsening LV dysfunction LA dilates and this compensation is lost worsening the situation [123].

Clinical implications

Development of heart failure in diabetes is a complex process and is affected by many secondary factors like hypertension, CAD, renal disease and hyperlipidemia. We have shown in our study that LA plays a key functional role in compensating early relaxation abnormalities in patients who still have normal EF. Therefore assessment of LA function is an important step in evaluating these patients. Loss of atrial function may predict the onset of overt heart failure.

Study Limitations

One of the main drawbacks of the study was the small sample size of the study population. Also patients were studied at rest. It will be interesting to study LA function on exercise.

CHAPTER SEVEN

IMPACT OF PERHEXILINE ON GLUCOSE AND FAT METABOLISM IN PATIENTS WITH REFRACTORY ANGINA AND/OR REFRACTORY HEART FAILURE

Background

Perhexiline was a frequently prescribed anti-anginal agent in the 1970s and is effective at relieving symptoms of angina [95;277;281], improving exercise tolerance, and increasing workload needed to induce ischaemia [44]. Perhexiline use declined dramatically in the late 1970s and early 1980s following reports of hepatotoxicity [192;208] and peripheral neuropathy [28]. This was later demonstrated to occur in patients who are `slow hydroxylators` with a genetic variant of a cytochrome P-450 enzyme which metabolises the drug, resulting in drug accumulation, in turn leading to accumulation of phospholipids in liver and nerves [163]. Horowitz et al [96] demonstrated that the risk of toxicity could be virtually eliminated by maintaining plasma concentrations between 150 to 600 ng/ml without compromising efficacy of the drug and this was subsequently confirmed by Cole et al [44]. This has led to resurgence in the use of Perhexiline in some parts of the world, particularly Australia, for the treatment of refractory angina. At therapeutic plasma levels, Perhexiline is not negatively inotropic and does not alter systemic vascular resistance [121;190;191]. In studies funded by a BHF Project Grant we demonstrated salutary effects of Perhexiline in maximally treated heart failure patients of both ischaemic and non-ischaemic etiologies [132]. There was a large increase in the primary end point, VO2max (almost 3ml/kg/min), additionally LVEF increased by approximately 10 percentage points, and there was a substantial improvement in symptomatic status as assessed by the Minnesota Living with Heart Failure Questionnaire (all pre-defined secondary end points).

Perhexiline is currently used clinically in the treatment of refractory angina [95;277;281] and heart failure [132] in patients on maximum tolerated medical therapy. Perhexiline inhibits of CPT-1

and CPT-2, key enzymes in mitochondrial free fatty acid uptake [114;115], leading to increased myocardial glucose substrate utilization [182].

Previous animal studies have demonstrated that concentration-dependent inhibition of CPT-1 by Perhexiline in the liver or muscle [114]. This can produce variable effects on plasma glucose, insulin, triglycerides, FFA, glycerol and ketones depending on the site of action. The aim of the study was to assess the impact of Perhexiline on ketone body synthesis and fatty acid and glucose metabolism and assess whether this would throw light on CPT-1 inhibition patterns in humans. Plasma levels of glucose, insulin, ketone bodies, FFA, glycerol and triglycerides were measured at baseline and 1 and 4 weeks after commencement of Perhexiline.

Methods

30 patients who met the entry criteria indicated below and provided written informed consent were recruited to the study. Patients were recruited from cardiomyopathy clinics in Birmingham. Before commencement of Perhexiline patients underwent history, physical examination and filled in the Minnesotta Living with Heart Failure questionnaire. Following this, fasting blood samples were collected and patients were started on Perhexiline. After one week Perhexiline levels were taken and dosage adjusted to achieve therapeutic concentrations. At 4 weeks repeat fasting blood samples were collected and this completed the study. Patients continued on Perhexiline as this was commenced on a clinical basis.

Patient inclusion criteria

1. Patients with angina or heart failure
2. Being commenced on Perhexilene for clinical reasons i.e. refractory angina or heart failure.
3. Age > 18 years
4. Provide informed consent

Exclusion criteria

1. Patients < 18 years or who cannot provide informed consent
2. Women of child bearing age who are not using effective contraception (or if pregnancy test positive)
3. Abnormal liver function tests (LFT)
4. Clinically apparent peripheral neuropathy
5. Severe chronic renal failure (creatinine >250) or diabetic nephropathy
6. Concomitant use of Amiodarone, Quinidine, Haloperidol or Selective serotonin (5HT) uptake inhibitors such as Fluoxetine and Paroxetine which may inhibit the CYP2D6 enzyme

Insulin and ketone assay

Insulin and ketone assay was performed as described in detail in the methods chapter (chapter 2).

Results

27 patients completed the study. The other patients stopped taking Perhexiline due to side effects or did not have the second set of blood tests. Patients characteristic and treatments are shown in table 7.1. The patients were divided into those with and without diabetes before the analysis. The results were analysed on SPSS version 15.0. Paired students T test was used as test of significance. Spearmans rho rank tests were used as tests of correlation. The results are summarised in table 7.2.

Table 7.1

Parameter	Results	Parameter	Results
Age (years)	60.4 ± 13	*Diuretics*	18
Number (Male)	24 (16)	*Aldosterone antagonist*	10
MLHF score	63 ± 18.7	*B-Blockers*	17
EF%	31.35 ± 2.08	*ACE inhibitors*	16
Weight (kg)	96 ± 6.26	*ARB*	7
Diabetics	11	*Anticoagulants*	12
Ischaemic Heart Disease	15	*Antiplatelet*	13
Dilated Cardiomyopathy	9	*Nitrates*	7

Baseline characteristics and treatment regimen of patients in refractory angina and/or refractory heart failure.

The results are expressed as mean ± standard deviation

Table 7.2

Variable	Baseline (no-diabetes)	Post (no-Diabetes)	Baseline (Diabetes)	Post (Diabetes)
Glucose, mmol/L	5.1±0.5	5±0.6	8.4±2.4	6.7±2*
Insulin, µIU/ml	13±15.2	19.9±23.7*	41.4±37.4	23.1±16.4*
IS, %	187.5±178.4	112.2±125*	30.1±29.1	54.7±52.2*
IR	1±0.9	1.5±1.2*	5±4.6	3±2.3*
%B	85.2±56	124.9±84.4*	114.5±95.2	128.9±83.1
FFA, µmol/L	279±112	250±105	316±131	387±174*
Triglycerides, µmol/L	1184±655	1475±766	2025±552	2477±1496
Glycerol, µmol/L	99±44	99±22	99±40	90±50
Ketones, mmol/L	0.8±0.3	1.2±0.6*	1.4±0.7	1.6±0.8

The results at baseline and at 4 weeks following Perhexiline therapy. The patients are divided into those with diabetes and without diabetes. The results are expressed as mean ± standard deviation.

IS – insulin sensitivity, IR – insulin resistance, %B – percentage of beta cell activity, FFA – free fatty acids
*- Statistically significant as compared to baseline

Patients with diabetes (11 subjects)

There was a significant fall in fasting plasma glucose and insulin following Perhexiline therapy. There was a significant increase in insulin sensitivity as measured by HOMA (Figure 7.1). HOMA calculations also revealed that Perhexiline resulted in increased beta-cell function within the pancreas. The FFA concentration in the plasma significantly increased with a non significant increase in triglyceride concentration post Perhexiline therapy. Fasting plasma ketones increased non-significantly following Perhexiline therapy (Table 7.2).

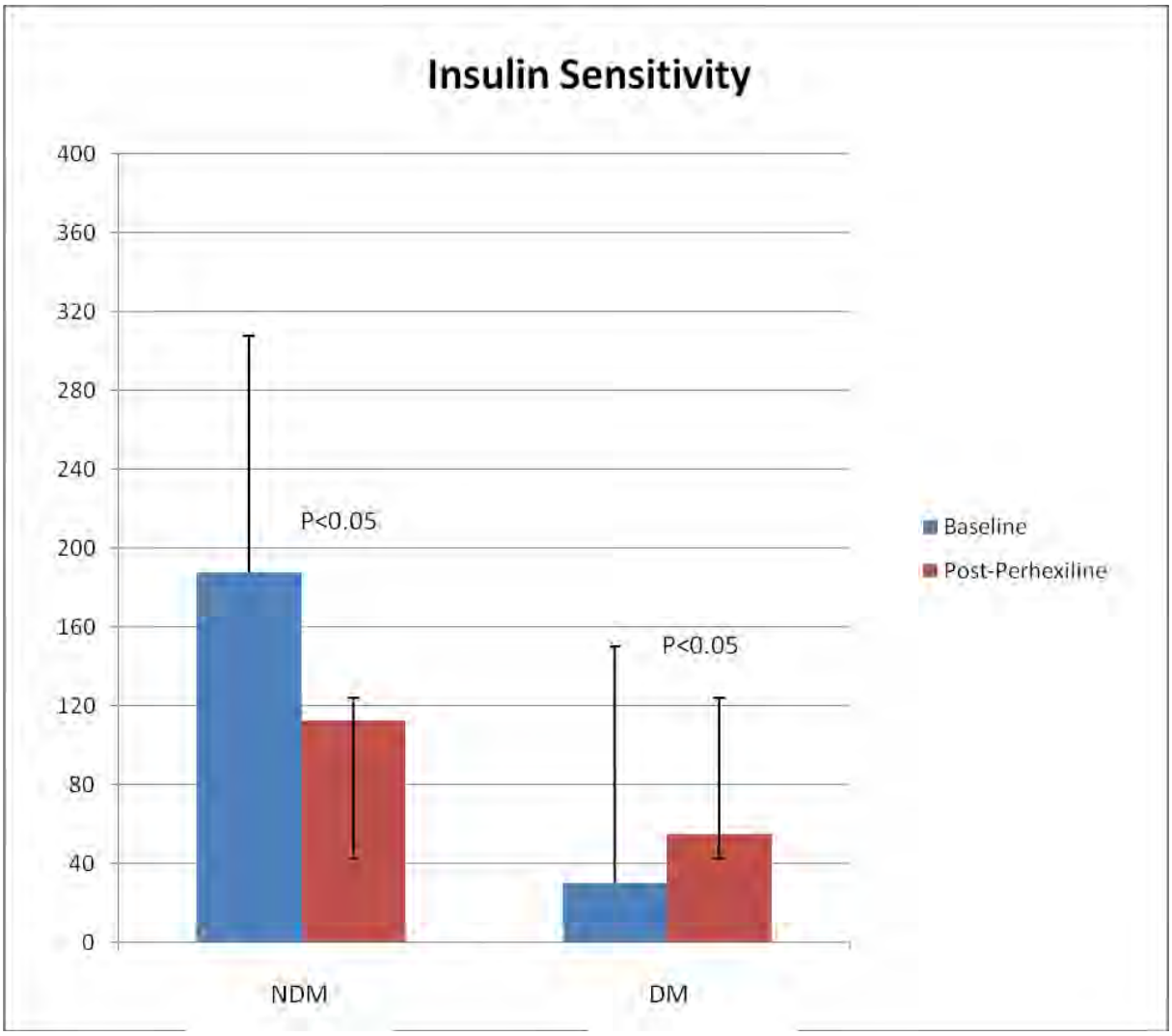
Patients without diabetes (16 subjects)

There was no change in fasting plasma glucose and a significant increase in plasma insulin following Perhexiline therapy. The HOMA calculated insulin sensitivity fell significantly. Interestingly HOMA also demonstrated that the beta cell function increased significantly as in the diabetic population. Perhexiline had no effect on plasma FFA and non-significantly increased the triglyceride levels. Interestingly there was a statistically significant increase in fasting plasma ketones post Perhexiline.

All subjects

There was a significant increase in plasma fasting triglyceride concentrations following Perhexiline therapy (1577±691 vs 1891±1151, $P<0.05$). Concurrently, there was a significant increase in plasma fasting ketone body levels (1.0±0.3 vs 1.4±0.7, $P<0.01$). Perhexiline caused a non-significant fall in fasting plasma FFA (303±131 vs 299±146) and glycerol (101±55 vs 87±39) (Table 7.3).

Figure 7.1



Changes in insulin sensitivity at baseline and following Perhexiline therapy (for 4 weeks).

NDM – patients without diabetes mellitus, DM – patients with diabetes mellitus.
P<0.05 was considered as statistically significant.

Table 7.3

	Baseline	Post-Perhexiline	P Value
Triglycerides, µmol/L	1577±691	1891±1151	<0.05
Ketones, mmol/L	1.0±0.3	1.4±0.7	<0.01
Free Fatty Acids, µmol/L	303±131	299±146	ns
Glycerol, µmol/L	101±55	87±39	ns

The results of fasting plasma levels at baseline and post Perhexiline therapy (4 weeks) in the whole patient group. The results are expressed as mean ± standard deviation.

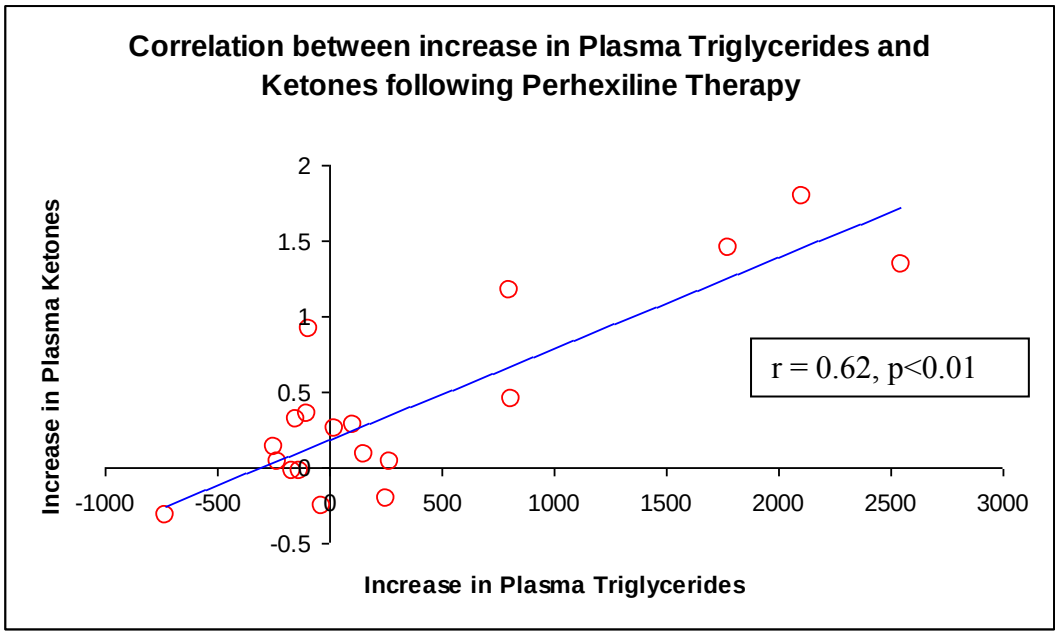
P<0.05 considered as statistically significant.

Spearmans rho Correlation

Perhexiline therapy resulted in increase of both fasting plasma ketones and triglycerides. In order to look for an association between the two, we conducted Spearmans Rank correlation tests. A significant positive correlation was noted between the increase in plasma ketones and triglycerides($r=0.62$, $P<0.01$) (Figure 7.2). The increase in ketones ($r=0.62$, $P<0.01$) and in triglycerides ($r=0.45$, $P<0.01$) both correlated positively with the plasma level of Perhexiline at 5 weeks. Interestingly, at Perhexiline levels of <0.50 mg/dl plasma ketones and triglycerides increased. Whereas at Perhexiline levels of >0.5 mg/dl there was only a modest increase in ketones with fall in triglyceride levels (Figure 7.3).

Post-Perhexiline levels of ketones correlated significantly with triglycerides ($r=0.66$, $P<0.01$) and FFA ($r=0.54$, $P<0.05$). But there was no association with either Perhexiline levels or plasma glycerol. Interestingly post perhexiline levels of FFA correlated with glycerol ($r=0.54$, $P<0.05$). However, no correlation was found between post-perhexiline triglycerides and either FFA or glycerol.

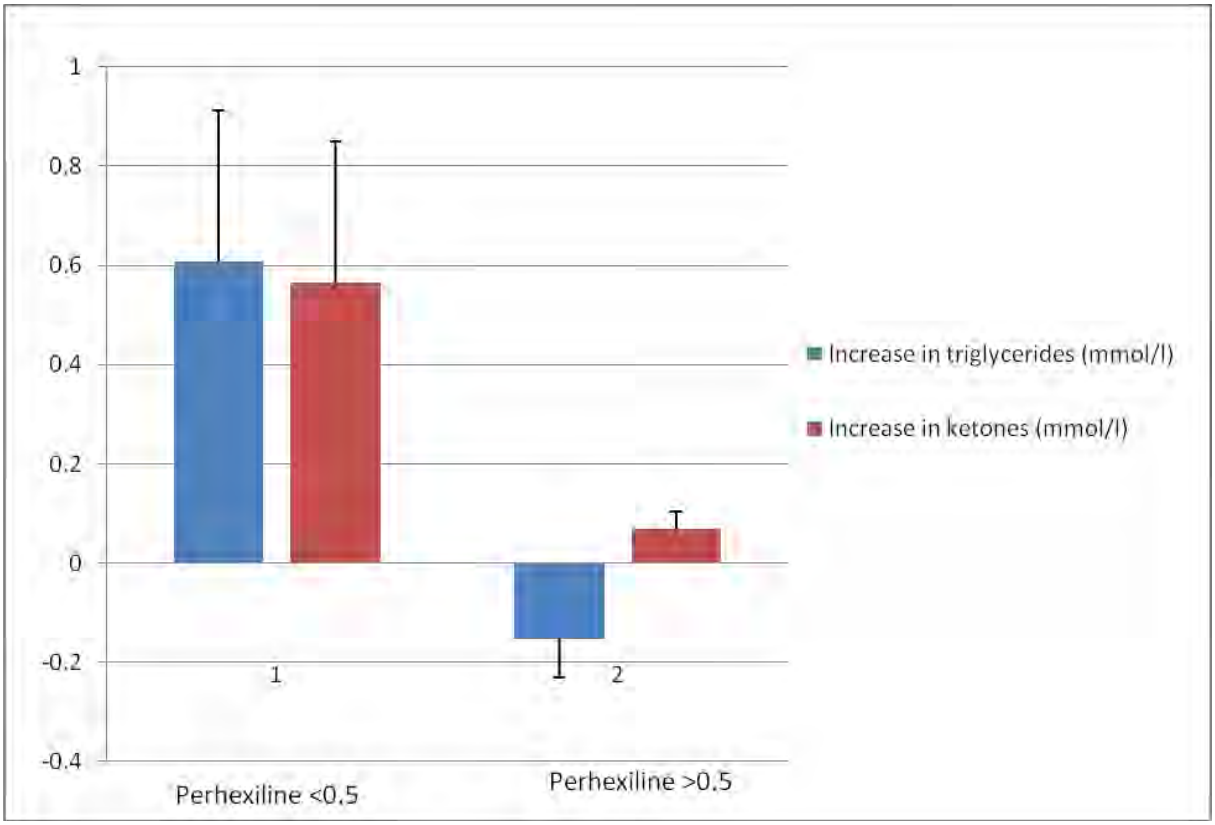
Figure 7.2



Scatter plot of increase in plasma triglyceride concentration against increase in ketones in the whole patient group. The blue line indicates the linear trend line.

Plasma triglycerides are measured in µmol/L and plasma ketones in mmol/L
P<0.05 considered as statistically significant.

Figure 7.3



Changes in plasma triglyceride and ketone concentration at different Perhexiline levels (<0.5 and >0.5). Perhexiline is measured in mg/L.

Discussion

In this study we have demonstrated a divergent response of Perhexiline on insulin sensitivity in patients with and without diabetes. Perhexiline inhibits the enzymes CPT-1 and CPT-2 preventing the entry of free fatty acids into the mitochondria and hence reducing fatty acid metabolism. This causes an increase in glucose utilisation. We expected Perhexiline to improve insulin sensitivity by driving glucose utilisation within the muscle in all subjects. However, unexpectedly and interestingly Perhexiline increased insulin sensitivity in diabetes patients whereas in non-diabetics it worsened the insulin sensitivity. There could be several explanations for these findings.

In diabetes patients insulin sensitivity is impaired as a part of disease process. Perhexiline improves glucose utilization within the muscles in these subjects improving the insulin sensitivity. However, the insulin sensitivity is normal to begin with in the patients without diabetes and increased glucose utilisation should not have any impact on the insulin sensitivity. Moreover, by preventing the entry of FFA into mitochondria Perhexiline may lead to accumulation of triglycerides and diacyl-glycerol in the cytosol resulting in increased insulin resistance at the cellular level. This effect may be overshadowed by the increased glucose utilization in patients with diabetes.

Although, there was a significant worsening of insulin sensitivity in the patients without diabetes following Perhexiline therapy, the fasting plasma glucose did not alter. The percentage of beta cell function increased resulting in higher levels of plasma insulin. The beta cell activity increased as a counter regulatory mechanism to worsening insulin sensitivity.

In this study, we also demonstrate that Perhexiline increases plasma levels of both triglycerides and ketones which is directly related to its plasma concentration. Previously, it

has been demonstrated that Perhexiline inhibits CPT-1 in rat liver and heart [115]. This inhibition is concentration-dependent with cardiac CPT-1 being inhibited at concentrations lower than that needed for inhibition of liver CPT-1. This leads to the possibility that inhibition of cardiac CPT-1 is related to its therapeutic action and that of liver CPT-1 may explain its hepato-toxicity at higher concentrations. However, no studies have been undertaken in humans to confirm this. This is the first study done to look at this possibility.

Under normal conditions, FFA generated from the adipose tissue is utilised by the liver for either beta-oxidation or synthesis of triglycerides. Acetyl Co-A generated from beta-oxidation of FFA either enters the Krebs cycle or is used to synthesise ketone bodies. In fasting conditions ketone synthesis and utilisation is increased due to lack of availability of glucose. We hypothesise that at therapeutic levels Perhexiline only inhibits skeletal and heart muscle CPT-1. This decreases FFA utilisation and hence more is available for the liver to generate ketones and triglycerides. However at supra-therapeutic levels Perhexiline also inhibits liver CPT-1. This prevents the uptake of FFA by the liver and hence we see only modest changes in the plasma ketone and triglyceride levels at higher plasma levels of Perhexiline.

Interestingly, previous studies in animal models (rats) have shown that the concentration of Perhexiline is 20 fold in the heart 3 hours after oral administration [115]. Hence, the plasma concentrations may not reflect the concentrations achieved in the tissue. The theurapeutic benefit of Perhexiline will be achieved only if it reaches adequate levels in the myocardium. This may be difficult to predict based only on the plasma concentration of Perhexiline, which does not necessarily predict tissue concentration. This is also important from the point of view of side effects. We cannot be sure that Perhexiline achieves high concentrations in the liver only at supra-therapeutic plasma levels. However, clinical studies previously have

demonstrated that Perhexiline can be used safely without producing hepatotoxic effects as long as the plasma concentration is maintained between 150-600 ng/ml [44].

Further studies measuring tissue concentrations and correlating this with plasma concentrations may help settle this issue. However, these studies would be extremely difficult to perform in humans. Animal studies will help to work out the underlying pathophysiological mechanisms as we are able to sample various tissues including liver, skeletal muscle and myocardium.

Conclusions

Perhexiline increases ketone and triglyceride synthesis which is concentration-dependent. A differential action on muscle and liver CPT-1 is a plausible explanation for this. Interestingly, Perhexiline increased the insulin sensitivity in patients with diabetes and decreased it in patients without diabetes.

CHAPTER EIGHT

MULTI-CENTRE EXPERIENCE ON THE USE OF PERHEXILINE IN CHRONIC HEART FAILURE AND REFRACTORY ANGINA: OLD DRUG, NEW HOPE

Publication

Dr G Nallur Shivu*, Dr T Phan ….. Prof M Frenneaux. Multi-Centre Experience on the use of Perhexiline in Chronic Heart Failure and Refractory Angina: Old Drug, New Hope. **European Journal of Heart Failure** 2009 Sep; 11(9):881-6. **(*Joint first author)**

Introduction

The prevalence of CHF is increasing and it is a leading cause of morbidity and mortality in developed countries and an emerging one in the developing world. The mortality of CHF at five years remains about 50%, which is similar to the prognosis for many cancers and is worse than this in more severe CHF [248]. Despite considerable advances in neuro-humoral modulation therapy [105], the limits of this approach have already been encountered [157]. Thus there is an urgent need for novel therapeutic approaches. Ironically, our search for a future therapy has led us to look to the past. An old drug, a metabolic modulator called perhexiline was first introduced into Europe nearly 30 years ago for the adjunctive treatment of angina [37].

Perhexiline was first introduced in the 1970s as an anti-anginal agent effective at relieving symptoms of angina, improving exercise tolerance, and increasing workload needed to induce ischemia. The drug works in part by modifying myocardial substrate utilisation from fatty acids to carbohydrates, which is energetically more efficient for the heart to metabolise [7]. By the early 1980s there were reports of hepatotoxicity [208] and peripheral neuropathy [28] with perhexiline use. These effects were later shown to occur in patients who were `slow hydroxylators` secondary to a polymorphic variant of the cytochrome P-450 enzyme (CYP2D6) which metabolises the drug [163]. However, this toxicity can be completely avoided by maintaining drug plasma concentration between 0.15-0.60 mg/L [96]. Perhexiline has acute (< 2 weeks) and chronic (> 3 months) potential toxicity as well as predictable effect on lowering blood glucose levels which, in diabetics, can potentially induce the development of hypoglycaemia. Both acute and chronic toxicity are essentially plasma-level related, but they are not confined to 'slow hydroxylators' only [94].

In the UK, perhexiline is available off licence, on a named patient and named consultant cardiologist basis. In some parts of the world, particularly Australia, perhexiline is quite widely used in the treatment of refractory angina and unstable angina, with excellent results [197]. In addition, recently, our group have demonstrated the beneficial short-term effects of perhexiline in patients with CHF (of both ischaemic and non-ischaemic aetiology) in a phase 2 double-blind, randomized, placebo-controlled trial [79]. This has led to our centres using perhexiline therapy in highly symptomatic otherwise optimally-treated patients with CHF and/or refractory angina. To date there is however very limited published long-term clinical data on the use of perhexiline in patients with CHF and/or refractory angina. In this study we aim to report on our five-years collective experience on the use of perhexiline in patients with CHF and/or refractory angina, with a focus on '*real-life*' drug side effects and toxicity, therapeutic drug level monitoring, five-year mortality outcomes and predictors of response to perhexiline therapy.

Methods

Study patients and outcome measures

The study population consisted of patients with CHF and/or refractory angina who were referred by specialist cardiologists from coronary care unit or outpatient cardiology practice at University Hospital of Birmingham and University Hospital of Wales. CHF was defined as LVEF <40% on echocardiography, on current optimal medical therapy with NYHA class IIB or worse symptoms. All refractory angina patients had evidence of significant CAD at coronary angiography and/or a documented history of acute myocardial infarction. Additionally, all patients had recurrent episodes of typical chest pain despite being on optimal medical therapy in the opinion of the treating cardiologist. Patients with refractory angina also

had coronary anatomy that rendered them unsuitable candidates for initial or repeat coronary artery bypass graft surgery or percutaneous transluminal coronary angioplasty.

A total of 151 patients were followed-up during the period Feb 2003 – Dec 2008. Data were retrospectively collated from two centralized perhexiline databases at the University Hospital of Birmingham (UK) and University Hospital of Wales (UK). Variables of interest such as patients' clinical characteristics, drug levels, side effects, response to therapy and mortality were analysed. All patients were followed-up until December 2008 for all-cause mortality analysis. Symptomatic responses to perhexiline therapy were assessed by our heart failure specialist nurse as well as cardiologist during follow-up outpatient consultations. Heart failure 'responders' would be typically those who self report improved exercise tolerance, reduced shortness of breath on activity of daily living, improved orthopnoea and paroxysmal nocturnal dyspnoea symptoms. Refractory angina 'responders' would be typically those who self report reduction in frequency and /or severity of angina and requiring less nitroglyerin use.
Abnormal aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin were defined as being twice the upper limit of the local laboratory reference range (AST (5-43 (U/L), ALP (70-330 (U/L) and bilirubin (1-22 μmol/L)). In addition, patients with renal impairment were defined as patients with urea and creatinine above the upper limit of local laboratory reference range (3.4-8.0 mmol/L and 60-126 mmol/L, respectively).

Therapeutic drug level monitoring

The two databases were maintained by specialist heart failure nurses with specialist heart failure cardiologist support. Patients were initially given 100mg of perhexiline twice daily after initial medical assessments for co-administered medication, peripheral neuropathy and

liver function testing. Serum perhexiline levels are assayed at approx. 1, 4 and 12 weeks after initiating perhexiline. Drug levels between 0.15-0.60 mg/L were considered to be therapeutic, sub-therapeutic levels were <0.15 mg/L and supra-therapeutic levels were >0.60 mg/L. Perhexiline level assays were performed at the Therapeutic and Toxicology Laboratory, University of Cardiff (UK) with blood samples sent either by hospital laboratory or by community general practice. Results are subsequently delivered to the cardiologist and the nurse to up-date the centralised databases. Patients were subsequently contacted by phone to be advised on any dose adjustment. After every dose adjustment the patients were scheduled to have a repeat perhexiline level testing. Table 8.1 outlines our perhexiline dose adjustment protocols with respect to corresponding serum perhexiline levels.

Statistics

Continuous variables were expressed as means ± standard deviation. P value of <0.05 was considered to indicate statistical significance. Comparisons between groups were performed with one-way ANOVA if the data were normally distributed. Variables of interest such as patients' age, gender, medical history, medications and presence of abnormal LFTs were included as independent variables in the multivariate analysis. Binary logistic regression was used to identify independent predictors of response to therapy. Survival curves were constructed by the Kaplan-Meier method and statistical differences between curves were assessed by the Tarone-Ware. Cox regression was used to assess significance of cumulative survival curves adjusted for age and gender. SPSS (v15.0) was used to perform the statistical operations.

Table 8.1: Perhexiline dosing schedule		
Time of blood sampling	**Perhexiline concentration (mg/L)**	**Recommended new daily dose (mgs)**
Dose planning based on Perhexiline assays result on day 7	0.00-0.05	300
	0.05-0.15	250
	0.15-1.00	200
	1.00-1.50	100
	1.50-2.00	50
	>2.00	Cease for 1 week then 50 mg on alternative days
Dose planning based on Perhexiline assays result at ≥ 4 weeks on therapy		
	<0.15	Double the daily dose
	0.15-0.60	No change
	0.60-0.90	Reduced dose by 25%
	0.90-1.20	Halve the daily dose
	>1.20	Cease for 1 week then reduce the daily dose to 25% of the previous dose

Results

Study population

Data on 151 patients on perhexiline therapy were analysed. Their mean age was 67±12 years with males making up 69.5% of the cohort. The average period of initial follow-up was 20±14 months. The indications for perhexiline therapy was refractory angina (54.3%), CHF (33.1%) or both (12.6%). In total, 41.6% had undergone coronary artery bypass grafting (CABG), 4.7% PCI, and 4.0% had both treatments. 62% of patients with CHF had NYHA class III symptoms and 52% of patients with refractory angina had Canadian Cardiovascular Society (CCS) [35] class II symptoms. Amongst patients with refractory angina, 69.5% were on a beta blocker, 50.0% on a calcium channel blocker, 66.7% on nitrates, 51.3% on nicorandil, 81.7% on a statin and 88.5% were on anti-platelet therapy. Amongst patients with CHF, 70.0% were on a beta blocker, 71.4% on an angiotensin converting enzyme inhibitor (ACEi), 26.5% on an angiotensin II receptor blocker (ARB) and 57.1% on an aldosterone antagonist. Table 8.2 provides a summary of patients' baseline clinical characteristics.

Perhexiline monitoring

The first drug level check was on average at 10±9 days with 44.8% within the therapeutic range and 20.3% above the therapeutic range. The time of the third level check was on average at 16±6 weeks with 68.8% of patients within the therapeutic range and 20.8% above the therapeutic range. At 3-4 months the mean dosage for patients was 189±84 mg/day (range of 19 -400 mg per day). For patients with CHF, refractory angina or both, the mean dosage were 194±98 mg/day, 179±72 mg/day and 236±90 mg/day, respectively (p=0.097). Figure 8.1 indicates the spectrum of dosages after 3-4 months on perhexiline therapy indicating the diversity of metabolizers within our cohort of patients. At the end of the long-

term mortality follow-up period which was on average 37±24 months, the percentage of patients achieving therapeutic range and those above the therapeutic level were 82.2% and 6.2%, respectively. (See table 8.3)

Side effects

The proportion of patients who developed abnormal AST, ALP and bilirubin were 4.6%, 4.0% and 3.3%, respectively. 23.8% of patients had transient side effects such as anorexia, weight loss and lethargy. Four patients (2.6%) had their perhexiline discontinued due to side effects such as nightmares and insomnia, and in one case due to peripheral neuropathy.

Table 8.2: Baseline clinical characteristics of patients	
	All patients
N	151
Male	105 (69.5)
Age, yrs	67±12
Male, No. (%)	105 (69.5)
Refractory Angina, No. (%)	82 (54.3)
CHF, No. (%)	51 (33.1)
CHF and refractory angina, No. (%)	18 (12.6)
NYHA classification, No. (%)	
IIb	20 (34.5)
III	36 (62.1)
IV	2 (3.4)
CCS classification, No. (%)	
I	17 (19.5)
II	45 (51.7)
III	16 (18.4)
IV	9 (10.3)
Diabetes, No. (%)	51 (33.8)
Renal Impairment, No. (%)	57 (37.7)
Revascularization, No. (%)	
Coronary artery bypass grafting	62 (41.6)
Percutaneous coronary intervention	7 (4.7)
Both	6 (4.0)
None	74 (49.7)
Liver function tests	
Aspartate aminotransferase, U/L	30±53
Alkaline phosphatase, U/L	130±114
Bilirubin µmol/L	12±8
Urea & Electrolytes	
Urea mmol/L	10±5
Creatinine mmol/L	144±53
Medications, No. (%)	
Beta Blockers	104 (68.9)
Calcium Channel Blocker	49 (32.5)
Angiotensin converting enzyme inhibitor	60 (39.7)
Angiotensin II receptor blocker	21 (13.9)
Aldosterone antagonists	43 (28.5)

Nitrates	65 (43)
Nicorandil	47 (31.1)
Statins	81 (53.6)
Anti-platelets	85 (56.3)
Diuretics	97 (64.2)

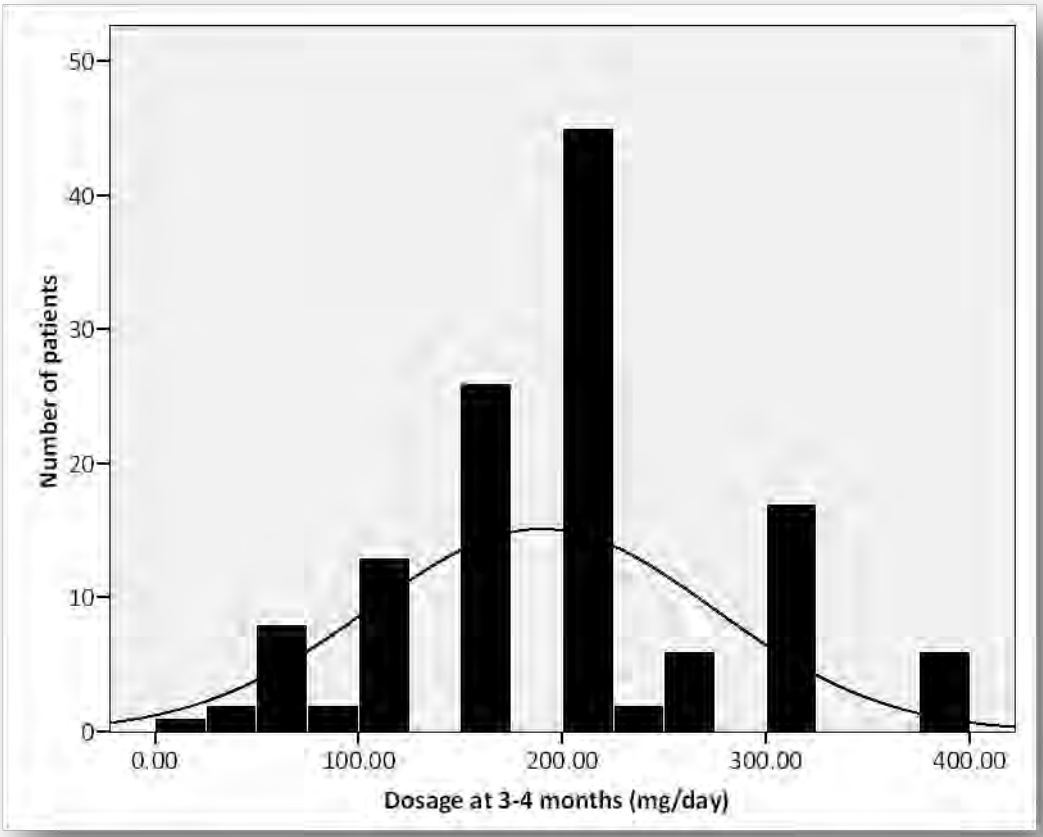
Data are presented as mean ± standard deviation or number (%) of patients.

CCS – Canadian Cardiovascular Society functional classification of angina, NYHA – New York Heart Association functional classification.

Table 8.3: Drug level safety monitoring	
	All patients
Time to first drug level assay – day	10±9
First drug level - mg/l	0.32±0.31
Sub-therapeutic – No. (%)	50 (35.0)
Therapeutic– No. (%)	64 (44.8)
Supra-therapeutic– No. (%)	29 (20.3)
Time to second drug level assay (Weeks)	7±4
Second drug level (mg/l)	0.53±0.37
Sub-therapeutic – No. (%)	18 (13.2)
Therapeutic– No. (%)	71 (52.2)
Supra-therapeutic– No. (%)	47 (34.6)
Time to third drug level assay (Weeks)	16±6
Third drug level (mg/l)	0.43±0.32
Sub-therapeutic – No. (%)	13 (10.4)
Therapeutic– No. (%)	86 (68.8)
Supra-therapeutic– No. (%)	26 (20.8)
Time to most recent drug level assay (months)	37±24
Most recent drug level (mg/l)	0.34±.22
Sub-therapeutic – No. (%)	17 (11.6)
Therapeutic– No. (%)	120 (82.2)
Supra-therapeutic– No. (%)	9 (6.2)

Data are presented as mean ± standard deviation or number (%) of patients.

Figure 8.1



A histogram demonstrating the distribution of dosage amongst all patients at 3-4 months.

Response to perhexiline therapy

58.9% of patients reported to have felt better on the perhexiline (responders). Amongst patients with only refractory angina 64.6% had a symptomatic response and amongst patients with only heart failure 48.0% had a symptomatic response. Amongst patients who had both heart failure and angina 61.1% had symptomatic response. Multivariate analysis (with logistic regression) was used to examine independent predictors of response to perhexiline therapy, with responders to perhexiline as the dependent variable, we found that the presence of refractory angina (OR 2.84, 95% CI 1.28-6.32, P=0.01) was an independent predictor of response to perhexiline therapy.

Mortality outcomes

Long term mortality data were collected for all 151 patients. The mean duration of follow-up for mortality data was 37±24 months. The all-cause five-year mortality was 27.7%. Amongst patients with refractory angina, CHF or both, the five-year mortality was non-significantly different at 20.5%, 31.0% and 38.4%, respectively (p=0.20 Cox regression, adjusted for age and gender). Amongst patients classified as responders or non-responders, the five-year mortality was similar at 26.1% and 32.2%, respectively (p=0.94 by Cox regression, adjusted for age and gender). The proportion of patients who had their perhexiline stopped was 19.2%.

Discussion

In this study we have demonstrated the efficacy and safety of perhexiline therapy which is the only metabolic agent currently in clinical use in the UK for CHF and refractory angina. This study, to date, represents the longest reported follow-up ever of patients with CHF and/or refractory angina treated with perhexiline. The principal findings are: a) a majority of patients

experienced symptom relief with perhexiline therapy, b) the use of perhexiline in patients was safe and only a small minority developed any side-effects or abnormal LFTs, c) an independent predictor of response to perhexiline is the presence of refractory angina, d) five-year mortality was non-significantly different in patients with CHF, refractory angina , or both, and were similar in responders and non-responders.

It has long been established that CAD is associated with impaired cardiac energetics [284], which has been reviewed elsewhere [7]. More recently however, there is a growing body of evidence to suggest that an impairment of cardiac energetic status may also play an important role in the pathophysiology of heart failure even in the absence of CAD. The hypothesis that heart failure is due to energy-starvation has been around for decades. Analysis of human biopsy specimens has shown a 25-30% reduction in ATP levels in the failing human heart [172]. This has been confirmed by cardiac MRS studies, which have shown that the reduction in PCr is greater in magnitude than the reduction in ATP. In the canine rapid pacing heart failure model a reduction in high energy phosphate status precedes objective evidence of impairment of LV systolic function [234]. In CHF there is strong evidence demonstrating deranged substrate metabolism and insulin resistance [202, 203]. Furthermore, there is a generalised down regulation of metabolism that contributes to the observed myocardial energetic deficiency [99]. There is also evidence to suggest oxygen wastage relating to insulin resistance and increased catecholamine secretion in failing hearts [15]. These observations provided the theoretical platform for the use of metabolic modulators such as perhexiline in CHF and CAD [79, 205].

Perhexiline works by modifying myocardial substrate utilisation from FFAs to carbohydrates [104]. Perhexiline inhibits both CPT-1 and CPT-2, which are involved in mitochondrial uptake

of long chain fatty acids. This results in a reduction in myocardial fatty acid β-oxidation, and an increase in glucose utilization at a reduced oxygen cost for energy production [7].

Studies have shown that in the presence of high plasma FFA the heart becomes less mechanically efficient (≈30%) than when carbohydrate metabolism predominates [124], the reason being that FFAs requires substantially more oxygen to produce a unit of ATP than carbohydrates (a theoretical difference of approximately 10%). In practice the increased oxygen requirement compared with glucose appears to be substantially greater (approximately 30%) [208-210], implying an 'oxygen wasting' effect. In addition, high levels of FFA can induce mitochondrial uncoupling that wastes energy [211].

The use of perhexiline in CAD has been extensively discussed in the literature over the years. In a systematic review of 26 randomized, double-blind, controlled trials including about 700 patients, perhexiline was found to be extremely potent at providing symptom relief as a monotherapy or as an adjunct to current best anti-anginal therapy [116]. Although all but one of the trials was of cross-over design, perhexiline led to 50% reduction in anginal symptoms over the use of nitroglycerin [116]. In addition to being excellent in chronic stable angina, perhexiline is also an effective therapy in acute coronary syndrome. Recently, our group have demonstrated the beneficial short-term effects of perhexiline in patients with CHF (ischaemics and non-ischaemics) in a double-blind, randomized, placebo-controlled trial [79]. Other studies have reported beneficial effects of trimetazidine, a metabolic modulator that inhibits FFA beta oxidation, in patients with heart failure [213].

The main focus of this study was to examine the acute and chronic toxicity of perhexiline, and the plausibility and effectiveness of the therapeutic drug level monitoring in a *real-life* clinical setting. In this study patients were started on an initial dose of 200mg which is similar to the eventual maintenance dose in many cases (and sometimes lower than the maintenance dose). Our incidence of side effects is similar to published data and depending on the manner and rate of perhexiline administration, the incidence of adverse events with perhexiline can vary between 0% and 60% [116]. The common transient side effects (acute toxicity) include symptoms such as dizziness, unsteadiness, as well as nausea, vomiting, headache, anorexia and weight loss (2 to 4kg). Other less common reported side effects include lethargy, tremor, diarrhoea, insomnia, and loss of libido. These side effects usually spontaneously resolve or abate with drug dose reduction and do not result in drug discontinuation. Only four patients (2.6%) had their perhexiline stopped secondary to nightmares and insomnia, and in one case it was due to peripheral neuropathy.

Chronic toxicity usually takes at least 3 months to develop. This includes debilitating neuropathy [129], papilloedema, and severe hepatotoxicity [208]. In 1983, Shah et al reported 80 cases of hepatotoxicity and 131 cases of neuropathy associated with perhexiline use in the United Kingdom [233]. Before drug level monitoring was introduced in 1988, perhexiline associated hepatotoxicity constituted between 5-9% of all serious adverse hepatic events in New Zealand and France [116]. Perhexiline also can lower blood glucose level which, in patients with diabetes, can potentially induce hypoglycaemia [49]. The mechanisms responsible have not been clearly elucidated.

In order to prevent these acute and chronic toxicities, perhexiline plasma level should be tightly monitored and stay within the therapeutic window (0.15-0.60 mg/L) [96]. Drug level monitoring is essential to identify patients who are slow metabolizers, which occurs in about 7% to 10% of U.S. and European Caucasians who harbour mutations in CYP2D6 [60]. In normal metabolizers, perhexiline's half-life is between 3-12 days, in slow metabolizers this half-life could be as long 30 days. In our study population the range of dosage after 3-4 months indicates the diversity of metabolizers from some ultra-rapid to slow metabolizers, which reinforces the necessity for therapeutic drug level monitoring.

In clinical practice drug interaction is also an important aspect to consider. The most obvious group of agents are the selective serotonin reuptake inhibitors (SSRIs) e.g., fluoxetine and paroxetine which interferes with the P450 CYP2D6 microsomal enzymes. In addition, in patients with existing liver impairment due to other causes, perhexiline metabolism may be impaired. For all the reasons outlined above it is mandatory for patient's liver function and co-administered drugs to be considered carefully.

In terms of responders, patients with refractory angina respond symptomatically better to perhexiline than patients with CHF and indeed from multivariate analysis the presence of refractory angina was an independent predictor of response to perhexiline therapy. The five-year mortality was similar in responders and non-responders. This study demonstrates the effectiveness of perhexiline in alleviating symptoms. However, the question of whether it also has a beneficial effect on mortality remains to be answered in a randomized placebo controlled trials.

This study has demonstrated promising results with the use of perhexiline in CHF and/or refractory angina in terms of both symptomatic relief and drug safety. We show that perhexiline can be safely started with 100mg twice daily for the adjunctive treatment of highly symptomatic patients with CHF and/or refractory angina. This should be commenced after a baseline medical assessment (including LFTs) with attention to co-administered medication for possible drug interaction. Therapeutic drug level monitoring should be undertaken after the initiation of the drug with subsequent dose adjustment to prevent acute and chronic toxicity and to achieve the therapeutic range.

Study limitations

We acknowledge that our measure of response to therapy is subjective and that it is possible that patients with refractory angina had more symptomatic improvement because improvements in angina may be more noticeable. Ideally we would have liked to present more objective measurements. However in the context of a clinical *real-life* database, such objective measurements such as LVEF or exercise tolerance pre and post perhexiline therapy were not completely available. However, the results and findings of this study will contribute significantly to the lack of *real-life* long-term mortality data and drug safety and monitoring of perhexiline in CHF and/or refractory angina as well as from a patient perspective some subjective measurement of symptomatic improvement such as breathlessness and/or chest pain. We would also have liked to define the causes of death in all our patients; unfortunately this information was not available. In this *real-life* analysis the percentage of patients on relevant optimal conventional medical therapy was not 100%, which could be due to intolerance, side-effects or the presence of contraindications.

Conclusion

Perhexiline therapy provides symptomatic relief in the majority of patients with minimal side effects or toxicity. Careful therapeutic level monitoring for dose titration is important to prevent acute and chronic toxicity. Patients with refractory angina were more likely to be responders. Five-year mortality was non-significantly different in patients with CHF compared to patients with refractory angina and was similar in responders and non-responders.

CHAPTER NINE

DISCUSSION

The myocardium is a metabolic omnivore and utilises fatty acids, glucose, ketones, amino acids and lactate to produce energy. Its main feature is the fact that it is able to alter its substrate use based on availability and various situations of stress. Metabolic conditions like diabetes result in specific changes in myocardial metabolism. Altered metabolism in itself results in cardiac muscle dysfunction and can play a potentially significant role in the development of heart failure in diabetes. Metabolic modulators like perhexiline are potentially significant new treatments in the management of heart failure and CAD.

In this work I have initially discussed the metabolic alterations in various diseases like heart failure, CAD and diabetes. Currently available metabolic modulators and the evidence base for their use are discussed. Then I explored the potential mechanisms for the development of altered cardiac energy status in diabetes. Using STE we studied the early changes in the myocardium in T1DM. In addition using cardiac MRI we explored the role of left atrial function in LV filling in these individuals and demonstrated the use of STE to complement data on left atrial function. In the study with Perhexiline we demonstrated its effects on carbohydrate and fatty acid metabolism in subjects treated with Perhexiline for clinical reasons. Finally we looked at clinical experience in using perhexiline for refractory heart failure and angina.

Cardiac MRS allows measurement of high energy phosphate kinetics in the myocardium. Previous studies in patients with type 1 and type 2 diabetes have demonstrated impaired cardiac energetics in asymptomatic patients with diabetes. However, these studies did not elucidate the underlying pathophysiological mechanisms. In particular I wanted to demonstrate the role of microvascular disease in the development of impaired cardiac

energetics. My work has shown that coronary microvascular disease probably plays little role in development of impaired cardiac energetics and therefore it is primarily metabolic in nature. Cardiac energetic impairment is an important factor in the development of diabetic cardiomyopathy. This raises the question as to whether metabolic modulators would be able to delay the onset of diabetic cardiomyopathy.

There have been great advances in cardiac imaging in the last decade. Advanced echocardiographic techniques like TDI and speckle tracking have allowed early detection of abnormalities in LV function. STE in particular has allowed measurement of LV rotation. I used speckle tracking and demonstrated that increased LV torsion occurs even prior to the onset of overt diastolic dysfunction. LV torsion and apical rotation could be measured quickly from the 2D images and potentially used clinically. If my findings are substantiated by large scale population studies LV torsion measurement could be a good screening tool to detect early diabetic cardiomyopathy.

Great advances have also been made in cardiac MRI in the last decade. I used cardiac MRI to make accurate measurement of LV volumes during the cardiac cycle. From this data I obtained accurate measure of LV filling characteristics. I demonstrate the importance of the atrial component of LV filling and how this compensates for reduced LV compliance in the early stages of diabetic cardiomyopathy. The onset of atrial fibrillation could be detrimental in these patients and hasten the development and presentation of heart failure. Therefore preventing the development of atrial fibrillation could be a potential way of preventing onset of symptomatic diabetic cardiomyopathy.

This part of my work raises many important questions and can form the basis for further studies to help us understand the pathogenesis of diabetic cardiomyopathy. Ideas for further studies are as follows –

1. Study of the effect of microvascular disease on cardiac energetic impairment in type 2 diabetes patients. Patients with type 2 diabetes tend to have higher incidence of associated risk factors including hypertension and hypercholesterolemia. Hence it will be interesting to study this group of patients.
2. Study of LV torsion and untwist patterns in patients with type 2 diabetes prior to onset of cardiomyopathy. It will be interesting to study the early changes in the myocardium in these subjects.
3. The HbA1C range in my subjects was 6 – 9%. In order to study the impact of glycemic control on cardiac energetic impairment and LV torsion subjects with different HbA1C ranges needs to be studied. These studies will also provide insights into the effect of glycemic control on the development of cardiomyopathy in diabetes patients.
4. It will be very interesting to conduct larger scale longitudinal studies in diabetes patients. Our subjects had early functional and structural abnormalities in the myocardium. Following these patients on a regular basis will provide insights into the progression of these early structural abnormalities and also provide us clues about the specific changes that herald the onset of overt cardiomyopathy. This can provide the basis for identifying the best screening tools in patients with diabetes to detect the development of cardiovascular complications and targeting specific therapies in these patients.
5. Perhexiline and other metabolic modulators increase glucose utilisation within the myocardium by various mechanisms as elucidated before (Chapter 1). Another

interesting study would be to assess the effect of the metabolic modulators on cardiac energetic impairment in diabetes patients. If the metabolic modulators are able to reverse the energetic impairment, they may have the potential to be used to prevent or delay the onset of cardiovascular complications in diabetes patients.

Metabolic modulators are a group of drugs that act by enhancing glucose utilisation within the myocardium and hence making it more oxygen efficient. Perhexiline is a metabolic modulator that prevents FFA uptake by inhibition of CPT1 and CPT2 resulting in enhanced glucose metabolism. I studied the effects of Perhexiline on carbohydrate and lipid metabolism in patients with intractable angina and/or heart failure. Perhexiline improved insulin sensitivity in patients with diabetes; however it made it worse in patients without diabetes. Also Perhexiline increased ketone and triglyceride synthesis which was directly dependent on its concentration. The differential action on muscle and liver CPT-1 is a plausible explanation for this.

Perhexiline is used currently for patients with intractable angina and /or heart failure with maximal medical therapy on a named patient basis. We analysed the database of perhexiline patients at two centres and reported on side effects, tolerance, mortality and experiences with drug level monitoring. Perhexiline therapy provides symptomatic relief in the majority of patients with minimal side effects or toxicity. Careful therapeutic level monitoring for dose titration is important to prevent acute and chronic toxicity. Patients with refractory angina were more likely to be responders. Five-year mortality was non-significantly different in patients with CHF compared to patients with refractory angina.

Studying myocardial metabolism provides insights into the patho-physiology of heart failure in diabetes. Ideas for further studies are as follows –

1. Many small scale studies have demonstrated benefits of metabolic modulators in angina and heart failure. There is an urgent need for large scale studies using metabolic modulators to confirm or refute their potential importance in providing symptomatic improvement in these patients. These large scale studies will also provide insights into the effects of these agents on long term morbidity and mortality in angina and heart failure.
2. My study on the effects of Perhexiline on carbohydrate and fat metabolism have provided interesting results and raised many important questions. In order to study the underlying pathophysiological mechanisms responsible for these changes animal studies can be conducted. Animal studies will provide us the opportunity to sample tissues including liver, skeletal muscle and myocardium and hence help to determine the tissue concentrations to work out the mechasims involved in the pathogenensis.
3. Of particular interest in my study was the divergent response of perhexiline on insulin sensitivity in patients with and without diabetes. In order to confirm or refute these findings the same study can be conducted by measuring insulin sensitivity via insulin clamping method. Insulin clamping provides a more robust and accurate measurement of insulin sensitivity as compared to HOMA method.

Limitations of the study

One of the main limitations of this study is the small sample size. Although the study provides insights into the patho-physiology of myocardial dysfunction in asymptomatic patients with T1DM, this has to be proven using large scale studies. The studies did not

collect longitudinal data and hence we cannot comment on the progression of these early changes in diabetic myocardium. All study participants were type I diabetes patients without a history of hypertension or hypercholesterolemia. These results cannot therefore be extrapolated to type II diabetes patients in whom hypertension and hypercholesterolemia frequently co-exist.

Appendix 1

THE UNIVERSITY OF BIRMINGHAM

Information sheet for participants

(Observational study only)

Study title: **Mechanisms responsible for cardiac and skeletal muscle energetic impairment in diabetes.**

Researchers: **Prof M Frenneaux, Prof M Stevens and Dr G Nallur Shivu.**

I am writing to invite you to take part in a study we are conducting. Your treatment will not be affected in any way if you decide not to take part in this study.

What is the purpose of this study?

We are planning to study the mechanisms involved in development of heart failure in diabetic patients. We plan to study the metabolism of heart and skeletal muscle in diabetics and compare it with healthy volunteers.

Why have I been chosen?

You have been chosen because you have diabetes which is currently well controlled, and you have no diabetic complications.

Who is organising the study*?*

This study is organised by the University of Birmingham and funded by the British Heart Foundation. Prof Michael Frenneaux (Head of the Department of Cardiovascular Medicine at University of Birmingham) is the chief investigator.

What will happen to me if I take part?

Following an overnight fast, you will be asked to arrive at 9:00 a.m. at the Department of Cardiovascular Medicine, which is on the site of Queen Elizabeth Hospital, Birmingham. You will be examined and asked to provide written informed consent. You will have the opportunity to ask questions and clarify any doubts before the start of the study.

Note: If you are a woman of child bearing age then we will ask you if you are using effective contraception. We will also do a pregnancy test before the start of the study. You will not be included in the study if you are not using effective contraception or if you have a positive pregnancy test.

The study will involve the following tests:
1.Blood Tests: Blood samples will be collected to measure your kidney function, haemoglobin, liver function, glucose and fat (lipids). This will involve inserting a small needle into your vein and drawing blood into various bottles.

2.Metabolic Exercise Test: You will be asked to exercise on a treadmill wearing a mask to measure the amount of oxygen you consume.

3.Echocardiography: An echocardiogram (a non-invasive ultrasound scan of the heart) will be performed. The above three tests will last a total of one hour.

4.NIRS: We will then conduct NIRS, which is test to determine the oxygen consumption by your forearm muscle. For this we will tie a tourniquet around your arm and place the NIRS probe on the forearm muscle. The tourniquet will be released after 1-2 minutes. The whole test will take about 10 minutes.
5.MRI: Then you will have the Magnetic Resonance studies carried out in the University of Birmingham Imaging Centre (BUIC). Scanners that are used for the Magnetic Resonance tests are shaped like a polo mint and some patients find this scanner slightly claustrophobic. The first test (Magnetic Resonance Imaging [**MRI**] scan of the heart) will measure the heart size and function at rest. For this test, you will lie comfortably on your back, and we will ask you to hold your breath for a few seconds during the scan. This test will involve inserting a small needle in your forearm and injecting a contrast agent called Gadolinium. About 15 minutes after the first scan we will inject a drug called adenosine and repeat the scan. The adenosine can make you feel tight in the chest –but this lasts for only a few seconds. The whole test will take approximately 40 minutes.

6.Cardiac MRS: This test (Magnetic Resonance Spectroscopy [**MRS**] of the heart) will look at the metabolism of the heart. We will ask you to lie on your stomach, and you do not need to hold your breath. This study will last about 45 minutes.

7.Skeletal MRS: Then you will have a similar scan (**MRS**) on your forearm and calf muscle. For this scan, you will be asked to lie on your back and we will apply a tourniquet around your arm for ten minutes. We will then ask you to exercise your calf muscle until you feel tired. The whole studies on your forearm and calf muscle will take approximately around 60 minutes.

What do I have to do?

Study day 1: Blood tests, exercise test, and echocardiography. Then you will be taken to BUIC (Birmingham) for MRI and MRS scan of the heart and MRS scan of calf muscle. We will pay your travel and any accommodation expenses.

What are the possible benefits of taking part?

There are no specific benefits to you by taking part in the study. This study will however help us to better understand how diabetic patients develop heart failure. This may allow us to find new treatments for heart failure in diabetics in the future.

Who will have access to the data and study results?

Prof M Frenneaux will be ultimately responsible for the data and study results. University of Birmingham and the University of Oxford will have control of the data. Ethics Committees, national and international regulatory authorities, which will review information on the drug and study, may also inspect them. These will be kept in a locked filing cabinet at the Department of Cardiovascular Medicine, University of Birmingham. In addition, information about you will be kept confidential, and your medical records will not be made public. Furthermore, if the results from this trial should be published, your identity will be kept completely confidential. If at the end of the study you would like a copy of the published paper, this can be sent to you on request.

Are there compensation arrangements if something goes wrong?

If the unlikely event of your health deteriorating in any way as a result of negligence, you may submit a claim to the University Hospital of Birmingham NHS Foundation Trust for compensation to be considered. If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal NHS complaints mechanisms should be available to you. If you are covered by Private Medical Insurance you should contact them to determine whether your cover would be affected in any way by taking part in this research.

What happens if I do not want to participate or I change my mind during the research study?

You do not have to participate in this study. If you decide not to participate or change your mind later on; you can withdraw at any time, without affecting your subsequent treatment in any way.

Are there other ways of treating my condition?

You are being considered for this study in the hope of finding new drug treatments to prevent heart failure in diabetics. Studies are underway looking at different drugs.

What if new information becomes available?

You would be informed by telephone of any new developments.

Will my GP be informed*?*

Yes. Your GP will receive a letter explaining the objectives of the study.

If you have any complaints during the study please do not hesitate to let me know.

If you are unhappy about our approach, manners, or the way we have dealt with any of your requests, please feel free to contact chief investigator Professor M Frenneaux in the Department of Cardiovascular medicine, University of Birmingham (Tel: 0121–4146926).

If you are interested in taking part or if you have more questions or do not understand something please contact one of the following members of our research team:

Dr Ganesh Nallur Shivu
Department of Cardiovascular Medicine
University of Birmingham
Edgbaston, Birmingham
B15 2TT
[redacted]

In cases of emergencies Dr G Nallur Shivu can also be contacted on his mobile phone (07789818670). Finally, thank you very much for taking the time to consider taking part in this study.

THE UNIVERSITY
OF BIRMINGHAM

Information sheet for participants (Healthy Volunteers)

Study title: **Mechanisms responsible for Cardiac and Skeletal Muscle Enegetic Impairment in Diabetes**

Researchers: Prof M Frenneaux, Prof M Stevens and Dr G Nallur Shivu.

I am writing to invite you to take part in a study we are conducting. We intend to assess the function and the energy status of the heart and limb muscle.

What is the purpose of this study?

We are planning to study the function and energy status of the heart and limb muscle. We would like to initially study healthy volunteers and then compare these results in healthy volunteers with results in diabetic patients. This potentially can be a useful tool to assess the mechanisms involved in the development of heart failure in diabetics.

Why have I been chosen?

You have been chosen because you are a healthy volunteer with no ongoing medical problems.

Who is organising the study*?*

This study is organised by the University of Birmingham and funded by a charity called the British Heart Foundation. Prof Michael Frenneaux (Head of the Department of Cardiovascular Medicine at University of Birmingham) is the chief investigator.

What will happen to me if I take part?

Following an overnight fast, you will be asked to arrive 9:00 a.m. at the Department of Cardiovascular Medicine, which is on the site of Queen Elizabeth Hospital, Birmingham. You will be examined and asked to provide written informed consent. You will have the opportunity to ask questions and clarify any doubts before the start of the study.

Note: If you are a woman of child bearing age then we will ask you if you are using effective contraception. We will also do a pregnancy test before the start of the study. You will not be included in the study if you are not using effective contraception or if you are pregnant.

The study will involve the following tests:
1.Blood Tests: Blood samples will be collected to measure your kidney function, haemoglobin, liver function, glucose and fat (lipids). This will involve inserting a small needle into your vein and drawing blood into various bottles.

2.Metabolic Exercise Test: You will be asked to exercise on a treadmill wearing a mask to measure the amount of oxygen you consume.

3.Echocardiography: An echocardiogram (a non-invasive ultrasound scan of the heart) will be performed. The above three tests will last a total of one hour.

4.NIRS: We will then conduct NIRS, which is test to determine the oxygen consumption by your forearm muscle. For this we will tie a tourniquet around your arm and place the NIRS probe on the forearm muscle. The tourniquet will be released after 1-2 minutes. The whole test will take about 10 minutes.
5.MRI: Then you will have the Magnetic Resonance studies carried out in the University of Birmingham Imaging Centre (BUIC). Scanners that are used for the Magnetic Resonance tests are shaped like a polo mint and some patients find this scanner slightly claustrophobic. The first test (Magnetic Resonance Imaging [MRI] scan of the heart) will measure the heart size and function at rest. For this test, you will lie comfortably on your back, and we will ask you to hold your breath for a few seconds during the scan. The whole test will take approximately 40 minutes.

6.Cardiac MRS: This test (Magnetic Resonance Spectroscopy [MRS] of the heart) will look at the metabolism of the heart. We will ask you to lie on your stomach, and you do not need to hold your breath. This study will last about 45 minutes.

7.Skeletal MRS: Then you will have a similar scan (MRS) on your forearm and calf muscle. For this scan, you will be asked to lie on your back and we will apply a tourniquet around your arm for ten minutes. We will then ask you to exercise your calf muscle until you feel tired. The whole studies on your forearm and calf muscle will take approximately around 60 minutes.

What are the possible benefits of taking part?

There are no specific benefits to you by taking the drug. This study will however help us to assess measurement of energetic status in healthy volunteers and to compare with these measurements in patients with diabetes. It will also help us to find new and better ways of treating heart failure patients and assessing the efficacy of these treatments.

Who will have access to the data and study results?

Prof M Frenneaux will be ultimately responsible for the data and study results. University of Birmingham and Birmingham University Imaging Centre will have control of the data. Ethics Committees, national and international regulatory authorities, which will review information on the study, may also inspect them. These will be kept in a locked filing cabinet at the Department of Cardiovascular Medicine, University of Birmingham. In addition, information about you will be kept confidential, and your records will not be made public. Furthermore, if the results from this trial should be published, your identity will be kept completely confidential. If at the end of the study you would like a copy of the published paper, this can be sent to you on request.

What happens if I do not want to participate or I change my mind during the research study?

You do not have to participate in this study. If you decide not to participate or change your mind later on; you can withdraw at any time.

What if new information becomes available?

You would be informed by telephone of any new developments.

Will my GP be informed?

Yes. Your GP will receive a letter explaining the objectives of this study.

If you have any complaints during the study please do not hesitate to let us know. If you are unhappy about our approach, manners, or the way we have dealt with any of your requests, please feel free to contact chief investigator Professor M Frenneaux in the Department of Cardiovascular medicine, University of Birmingham (Tel: 0121–4146926).

If you are interested in taking part or if you have more questions or do not understand something please contact one of the following members of our research team:

Dr Ganesh Nallur Shivu
Department of Cardiovascular Medicine
University of Birmingham
Edgbaston, Birmingham
B15 2TT
[redacted]

In cases of emergencies Dr G Nallur Shivu can be contacted on [redacted] Finally, thank you very much for taking the time to consider taking part in this study.

UNIVERSITY OF BIRMINGHAM

Patient Identification/Study Number: _____

CONSENT FORM

Mechanisms responsible for cardiac and skeletal muscle energetic impairment in diabetes.

Researchers: Prof M.P. Frenneaux, Prof M Stevens, Dr G Nallur Shivu

PLEASE INITIAL BOX:

1) I confirm that I have read and understand the information sheet dated …… (Version ….) for the above study and have had the opportunity to ask questions. ☐

2) I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason, and without my medical care or legal rights being affected in any way. ☐

3) I understand that sections of any of my medical records may be looked at by responsible individuals from the University of Birmingham or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

4) I agree to my GP being informed of my participation. ☐

5) I agree to take part in this study. ☐

_____	_____	_____
Patient Name	Date	Signature
_____	_____	_____
Researcher Name	Date	Signature
_____	_____	_____
Witness Name	Date	Signature

UNIVERSITY OF BIRMINGHAM

Patient Identification/Study Number: _____

CONSENT FORM
(Healthy Volunteers)

Mechanisms responsible for cardiac and skeletal muscle energetic impairment in diabetes.

Researchers: Prof M.P. Frenneaux, Prof M Stevens, Dr G Nallur Shivu

PLEASE INITIAL BOX:

6) I confirm that I have read and understand the information sheet dated …… (Version ….) for the above study and have had the opportunity to ask questions. ☐

7) I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason, and without my medical care or legal rights being affected in any way. ☐

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9) I agree to my GP being informed of my participation. ☐

10) I agree to take part in this study. ☐

Patient Name	Date	Signature
Researcher Name	Date	Signature
Witness Name	Date	Signature

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