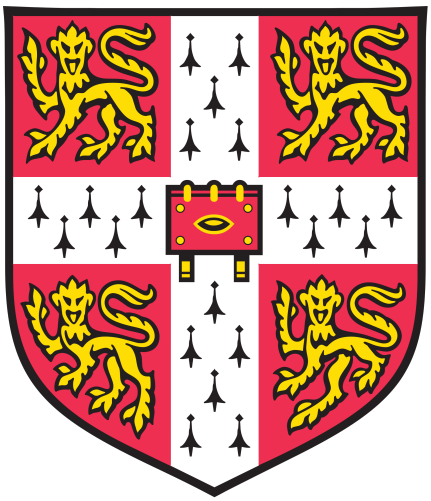
Cardioprotective effects of Glucagon-like Peptide 1 (GLP-1) and their mechanisms



This dissertation is submitted for the degree of

Doctor of Medicine (MD)

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Preface

This dissertation is the result of my own work and includes nothing that is the outcome of work done in collaboration except as declared and specified in the text. It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other university or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other university or similar institution except as declared in the text. It does not exceed the prescribed word limit of 60,000 words.

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Soli Deo gloria!

Summary

Background

Glucagon-like Peptide 1 (GLP-1) is a human incretin hormone that has been demonstrated to protect against non-lethal ischaemia reperfusion injury in the left ventricle in humans. It has been suggested from some animal research that this protection may be mediated through the pathway of ischaemic conditioning, of which the opening of the mKATP channel is a key step. Furthermore, it is uncertain whether the protection applies to the right ventricle. Finally, there is limited human evidence of a protective effect against lethal ischaemia reperfusion injury.

Methods

Two studies use non-lethal ischaemia to test whether GLP-1 protection is maintained despite blockade of the mKATP channel with the sulfonylurea, glibenclamide. A demand ischaemia study uses dobutamine stress echo to compare LV function. The other uses transient coronary balloon occlusion to generate supply ischaemia during GLP-1 infusion, assessed by conductance catheter. A further transient balloon occlusion is also used to assess the effect of supply ischaemia on RV function. Finally, the GOLD PCI study assesses whether GLP-1 protects against periprocedural myocardial infarction when administered during elective PCI in a randomised, placebo controlled double blind trial.

Results

Glibenclamide did not affect GLP-1 cardioprotection in either supply of demand ischaemia suggesting that GLP-1 protection is not mediated through the mKATP channel. The RV experienced stunning with RCA balloon occlusion but there was little evidence of cumulative ischaemic dysfunction with further occlusions. GOLD PCI is continuing to recruit patients. The nature of the study means results cannot be assessed until recruitment is complete.

Conclusions

GLP-1 is an agent with potential for clinical use as a cardioprotective therapy. It's mechanism of action in the heart remains uncertain.

Key Words

Cardioprotection; glucagon-like peptide 1; ischaemia-reperfusion injury; interventional

cardiology

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Abbreviations

ACS	Acute coronary syndromes
ATP	Adenosine tri-phosphate
BL1	Baseline 1
BL2	Baseline 2 (30-minute recovery)
BO1	Balloon occlusion 1
BO2	Balloon occlusion 2
BP	Blood pressure
CAD	Coronary artery disease
CCS	Canadian Cardiovascular Society
CMR	Cardiac magnetic resonance imaging
CO	Cardiac output
CsA	Cyclosporine A
CTIMP	Clinical trial of investigational medicinal product
DBP	Diastolic blood pressure
DMC	Data monitoring committee
DSE	Dobutamine stress echo
DPP4	Dipeptidyl peptidase-4
EDP	End diastolic pressure
EDPVR	End diastolic pressure volume relation

EDV	End diastolic volume
EF	Ejection fraction
ESP	End systolic pressure
ESPVR	End systolic pressure volume relationship
ESV	End systolic volume
GIK	Glucose, insulin, potassium infusion
GLP-1	Glucagon-like peptide 1 (7-36) amide
GLP-1R	GLP-1 receptor
GLP-1RA	GLP-1 receptor agonist
GPCR	G-protein coupled receptor
IC	Ischaemic conditioning
IHD	Ischaemic heart disease
IMP	Investigational medicinal product
IPC	Ischaemic preconditioning
IR	Ischaemia-reperfusion
IVC	Inferior vena cava
LAD	Left anterior descending
LAP	Left atrial pressure
LDL	Low density lipoproteins
LV	Left ventricle

MACCE	Major adverse cardiovascular and cerebrovascular events
MAP	Mean arterial pressure
MASV	Mitral annular systolic velocity
MI	Myocardial infarction
mKATP	Mitochondrial KATP channel
mPTP	Mitochondrial permeability transition pore
MRI	Magnetic resonance imaging
NEP	Neutral endopeptidase
NO	Nitric oxide
NSTEMI	Non ST elevation myocardial infarction
NYHA	New York Heart Association
PCI	Percutaneous coronary intervention
PI3K	Phosphoinositol-3-kinase
PPCI	Primary percutaneous coronary intervention
PV	Pressure-volume
RA	Right atrium
RAP	Right atrial pressure
RCA	Right coronary artery
RISK	Reperfusion injury survival kinases
RIPC	Remote ischaemic preconditioning

ROS	Reactive oxygen species
RV	Right ventricle
SAFE	Survival activating factor enhancement
STEMI	ST elevation myocardial infarction
SI	Strain index
SRI	Strain rate index
SV	Stroke volume
SW	Stroke work
TDI	Tissue Doppler imaging
WHO	World Health Organisation
2D-STE	Two dimensional – speckle tracking echocardiography

Chapter 1 Introduction

1.1 Introduction

1.1.1 Ischaemic heart disease

Ischaemic Heart Disease (IHD) remains the leading cause of death in the developed world. In the UK, it is responsible for 73,000 deaths each year, with about 1 in 6 men and 1 in 10 women likely to die from the disease (NHS Figures). IHD is caused by narrowing or blockage of the coronary arteries, primarily because of atherosclerosis, which supply blood to the myocardial tissue. There are a number of risk factors for development of atherosclerosis. These include family history, cigarette smoking, hypertension, hypercholesterolaemia and diabetes mellitus(1). Some risk factors are modifiable with lifestyle changes and drug therapy. Coronary artery disease (CAD) has a variety of clinical presentations including angina pectoris, heart failure, arrhythmia and acute coronary syndromes (ACS) such as myocardial infarction (MI).

1.1.2 Stable coronary artery disease

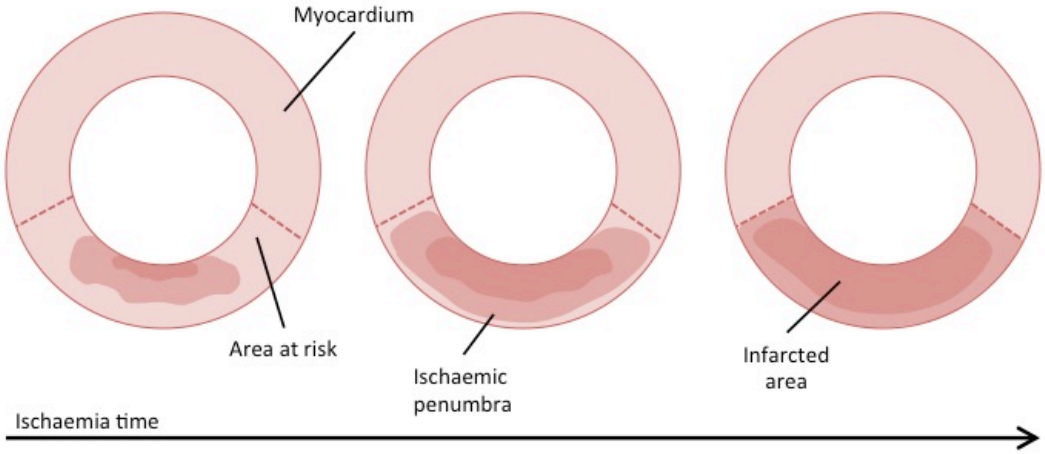
Atherosclerosis is an inflammatory process which involves recruitment of inflammatory cells into the wall of the artery(2). This results in uptake of oxidised low-density lipoproteins (LDL), which contain cholesterol. This deposition forms atherosclerotic plaques, which may gradually reduce the cross-sectional area of the coronary vessel. If this process continues it will eventually limit the flow of blood in the coronary artery. When the limited blood supply does not meet the metabolic demand of the myocardium, the result is angina pectoris (hitherto angina). Angina is chest pain, which is typically related to an increase in myocardial metabolic demand,

for example during exertion or emotional stress. Treatment of stable CAD depends upon both medical treatment and coronary revascularisation with either percutaneous treatment such as angioplasty and stenting, or surgical treatment. Medical treatment includes vasodilators to increase coronary blood flow, and beta-blockers to reduce myocardial oxygen demand.

1.1.3 Acute myocardial infarction

The core of an atherosclerotic plaque is shielded from the lumen of the artery by a fibrous cap. Those plaques with thin fibrous caps are vulnerable to rupture. Rupture causes the contents of the plaque core, necrotic tissue and cholesterol, to spill into the lumen of the vessel; triggering the formation of thrombus, which blocks of the vessel(3). The myocardial tissue supplied by the occluded coronary vessel is threatened with cell death. An ischaemic "wavefront" spreads from subendocardial to subepicardial tissue and unchecked this will result in MI and cell death(4). This is illustrated in Figure 1.1.

Figure 1.1 The wavefront of myocardial ischaemia and infarction



1.1.4 Current treatment of acute myocardial infarction

Rapid restoration of flow in the coronary artery is the cornerstone of treatment for acute MI (AMI). As recently as the 1970's there was little effective treatment for AMI other than analgesia. Patients would frequently suffer the consequences of untreated infarction – heart failure, cardiogenic shock, arrhythmia and the mechanical complications of MI (ventricular septal defect, free wall rupture and papillary muscle rupture leading to acute mitral valve regurgitation) – all of which contributed to high mortality and morbidity. From the 1980's onward, treatment involved the use of thrombolytic agents to break down the clot, hopefully restoring blood flow in the vessel. Streptokinase was replaced with recombinant tissue plasminogen activators but the benefits remained limited.

Balloon angioplasty – deployment of a balloon within the coronary artery to open a narrowed or blocked vessel – was developed for the treatment of intractable angina, and was trialed as a treatment of AMI. However, the high risk of abrupt closure of the vessel meant that mortality was high. The development of balloon expandable coronary stents allowed the swift opening of the occluded coronary vessel with a low risk of immediate reocclusion(5). Treatment of coronary artery disease with balloon angioplasty and stenting is collectively known as percutaneous coronary intervention (PCI). PCI can be performed during the first hours following thrombotic occlusion of the coronary artery. This is known as primary PCI (PPCI). The rapid restoration of coronary flow and reperfusion of ischaemic myocardium salvages tissue in the ischaemic penumbra, reducing final infarct size and improving both morbidity and mortality(6).

The *quantity* of myocardium saved during an AMI depends on this rapid reperfusion -

"time is muscle". However, despite ever diminishing PPCI door-to-balloon times, the mortality of patients with AMI has plateaued(7). The concept that reperfusion carries a risk of causing further damage to the myocardium may help to explain the "leveling-off" of mortality improvement.

1.2 Ischaemia-reperfusion Injury

1.2.1 What is ischaemia-reperfusion (IR) injury?

Ischaemia-reperfusion (IR) injury, damage attributed directly to the restoration of a blood supply to an ischaemic tissue, is observed during PCI in humans(8), and may account for up to 50% of the final infarct size(9). Restoring the blood supply to a tissue, whilst minimising IR injury, is required for high *quality* reperfusion and may be achieved by cardioprotection during reperfusion.

IR injury occurs minutes after tissue perfusion has been restored and contributes to the persistent death of viable myocytes(10). Myocyte death during IR injury occurs in three forms – necrosis, apoptosis and autophagy; all are interrelated but only apoptosis is tightly regulated. IR injury appears to be mediated by a mitochondrial membrane channel - the mitochondrial Permeability Transition Pore (mPTP)(11). Mitochondria are responsible for production of ATP, the energy currency of the cell, as well as coordinating other intracellular signaling processes. Experimental studies have shown that mPTP remains closed during ischaemia and opens during the first few minutes of reperfusion(12). mPTP opening activates several apoptotic and necrotic sub-cellular signaling mechanisms, including the release of cytochrome c, collapse of the mitochondrial membrane potential, depletion of ATP, and activation of caspases. This process is only partially understood but culminates in cell death(13-

15).

Reperfusion of ischaemic myocardium produces a turbulent restoration of intracellular physiology. Mechanisms of cellular homeostasis may be overwhelmed leading to elevated levels of intracellular calcium (Ca^{2+}) and abrupt changes in pH. Intracellular Ca^{2+} overload and reactive oxygen species (ROS) both open the mPTP, whilst the relationship between pH and mPTP kinetics is complex(9).

Cytosolic Ca^{2+} removal is tightly regulated under normal conditions within the cardiomyocyte. Under conditions of ischaemia, there is reversal of normal Na^{+}/Ca^{2+} exchange to maintain physiological intracellular pH. During reperfusion, extracellular pH returns to normal whilst intracellular pH initially remains low, driving further H^{+}/Na^{+} exchange that exacerbates the rise in cytosolic Ca^{2+}(16). Intracellular Ca^{2+} causes reversal of the mitochondrial Na^{+}/Ca^{2+} exchange with influx of Ca^{2+} triggering mPTP opening(17).

Rapid increases in the availability of oxygen may result in the formation of reactive oxygen species such as superoxide. ROS during ischaemia are low and of uncertain pathological significance. However, there is strong evidence that a burst of ROS at reperfusion is responsible for initiating mitochondrial membrane damage. Oxidative stress at reperfusion limits the availability of nitric oxide (NO), which is an intracellular mediator of cardioprotection. Whilst an association with myocardial stunning appears clear, the role of ROS in infarction is less certain. However it has been implicated in necrosis and apoptosis(18).

1.2.2 Clinical consequences of IR injury

PPCI has improved the prognosis of patients suffering from AMI. Nonetheless the

clinical consequences of IR injury are numerous, including myocardial stunning (transient myocardial dysfunction following restoration of coronary circulation), arrhythmias and no-reflow (impediment of blood flow to myocardial tissue despite relief of the epicardial coronary obstruction) that lead to further extended periods of secondary ischaemia and infarction(19). Lethal reperfusion injury contributes to apoptosis and necrosis of myocardial tissue and extends the ischaemic wavefront and MI in susceptible tissues. High quality reperfusion depends on limiting the damage attributed to IR injury. Patients most likely to suffer clinical consequences of IR injury are those with short ischaemic times and those with larger coronary territories affected (e.g. patients with anterior infarcts). These patients have a large volume of myocardium that can be salvaged with reperfusion.

1.3 Cardioprotection to limit IR injury

1.3.1 Cellular basis of conditioning

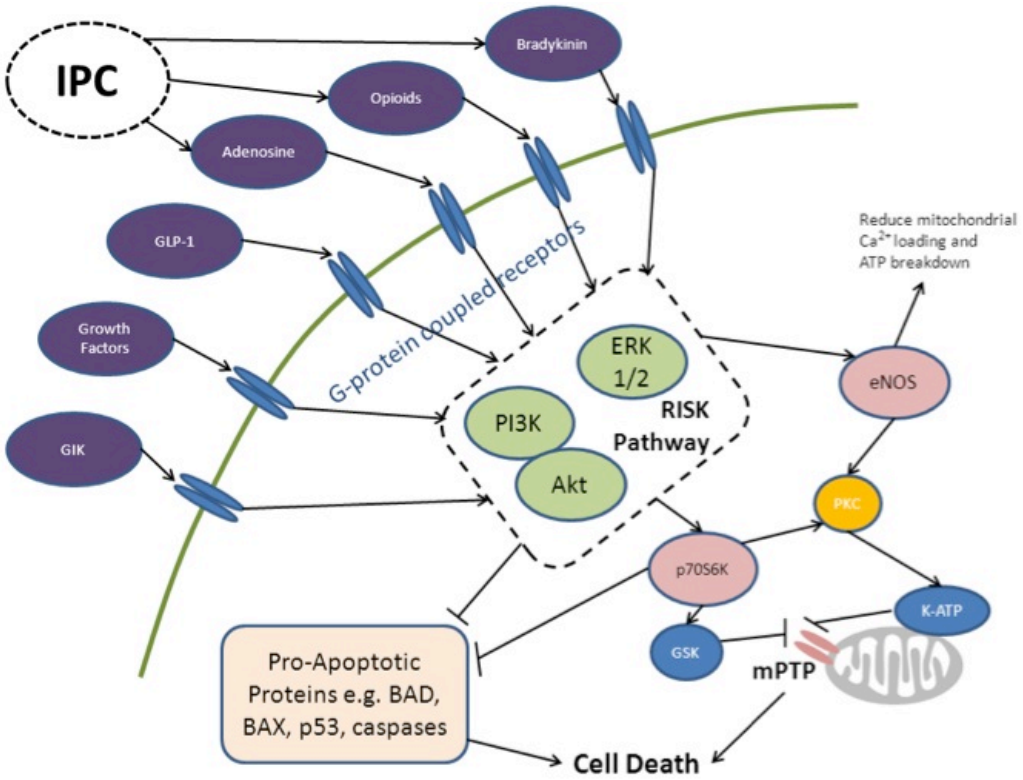
Ischaemic preconditioning (IPC) is a potent adaptive response triggered by brief periods of ischaemia that affords cardioprotection during episodes of more prolonged ischaemia. The effect was first described more than 25 years ago by Murry *et al* where brief circumflex coronary artery occlusion prior to IR limited infarct size in a canine model. Infarct size was not reduced when the duration of coronary occlusion was prolonged to three hours indicating that timely reperfusion is required for IPC cardioprotection to occur(20).

IPC provides maximal protection as soon as its triggering threshold has been met. This “all or nothing” effect is likely to represent a steep dose-response curve where maximal protection is rapidly achieved and further stimulation provides little or no

additional benefit(21). Typically, in humans, this threshold is a coronary balloon occlusion for longer than 60-90 seconds. IPC reaching this threshold can attenuate markers of ischaemia (ST segment elevation, chest pain, lactate, CKMB) during PCI(22). Early “classical” protection in IPC is transient, lasting 1 – 4 hours.

The sub-cellular signal transduction of early IPC is summarized in **Figure 1.2.** G protein coupled receptors (GPCRs) on the cell surface activate PI3K-Akt and ERK1/2 cascades, the so called Reperfusion Injury Survival Kinase (RISK) pathway that must be active for cardioprotection to occur(23). These pathways converge in the mitochondrial KATP (mKATP) channel. Opening of this channel prevents mPTP opening, abrogating IR injury.

Figure 1.2 - Simplified pathway for activation of IPC



There is also a late, second window of protection (SWOP) conferred by IPC. This

occurs between 12 - 72 hours after the IPC stimulus and is mediated by altered gene expression and new protein synthesis (23,24). Demand ischaemia models have demonstrated a SWOP in humans(25) and daily IPC with pneumatic cuff inflations caused sustained protection from IR induced endothelial dysfunction in healthy volunteers(26).

IPC is difficult to translate into clinical practice as it requires access to the coronary blood supply *before* ischaemia occurs; the onset of ischaemia is usually unpredictable. There is also an associated risk from additional coronary instrumentation. However, brief ischaemia in more ischaemia-resistant distant vascular beds (such as skeletal muscle) has been shown to confer similar protection(27). This is termed remote ischaemic preconditioning (RIPC), acting via neuro-endocrine and/or systemically released mediators that have yet to be fully characterised(28). Furthermore, intermittent reperfusion, known as postconditioning, applied after prolonged ischaemia also mimics the cardioprotection of IPC with clear translational benefits to treat unheralded ischaemic events as they occur(29). Remote postconditioning may offer the best of both worlds: a bedside treatment that can be implemented after the onset of acute ischaemia whilst ensuring protective potency by accessing multiple signaling pathways that amplify the cardioprotective effect. However, in practice, the successful translation of any form of ischaemic conditioning (IC) has proved to be less than straightforward(30).

1.4 Translation of conditioning mechanisms to clinical practice

1.4.1 Pre and postconditioning

A number of studies have demonstrated the benefits of RIPC in elective PCI to limit MI4a. MI4a is PCI-related myocardial infarction; defined in the 3rd Universal definition of myocardial infarction "as a rise in troponin I to 5 times the 99th percentile of the upper reference limit, or an increase from baseline by 25% post-PCI"(31). Protection is inducible with 3-4 alternating cycles of sphygmomanometer pneumatic cuff induced forearm ischaemia for 5 minutes with 5 minutes of reperfusion, optimally less than one hour prior to PCI(32). RIPC also reduced Major Adverse Cardiovascular and Cerebrovascular Events (MACCE) rate at 6 months, sustained at 6 years of follow-up(32,33). Subsequent trials with similar protocols have elicited results which support these findings(34). As discussed above, the all or nothing nature of IPC creates a threshold dose at which protection occurs. In the case of RIPC, this threshold is a number of cycles of ischaemia and reperfusion. The threshold for IPC in humans varies depending upon a number of factors. It is increased in patients with diabetes mellitus(35). An increased threshold can be overcome by additional cycles of IR. In some clinical studies, the choice of conditioning protocol has also been dictated by patient tolerance(32).

A small trial of pre-hospital RIPC (after onset of symptoms but prior to reperfusion) for STEMI demonstrated improvement in myocardial salvage index (defined as the difference between the final infarct size and area at risk, expressed as a percentage of the ventricular mass) on CMR. However, no improvement in final infarct size, left ventricular (LV) ejection fraction (EF), biomarker rise or clinical endpoints was

observed(36). Conditioning using sphygmomanometer pneumatic cuff inflation ten minutes prior to PPCI in STEMI showed improvements in ST segment resolution and attenuated the troponin rise. These changes were greater in a subgroup also given intravenous morphine infusion, a preconditioning-mimetic (see later)(37).

The results of postconditioning PPCI trials have largely been disappointing following initial encouraging results, which suggested a reduction in infarct size(38-41). The most effective induction protocol for postconditioning in humans is still not established and ranges from 4 to 6 cycles of between 30 and 60 seconds of coronary occlusion/reperfusion. A recent meta-analysis suggested that there was a small benefit in favour of postconditioning in biomarker studies but CMR study results were neutral(42). A randomised study assessing postconditioning during PPCI in 700 patients showed no improvement in ST segment resolution at 30 minutes or MACCE(43). A further study investigating remote postconditioning in 360 stable or unstable patients undergoing PCI found no effect on periprocedural troponin rise(44). However, this study was confounded by failure to reach a threshold for protection until 25 minutes after reperfusion(45).

Despite the potential benefits of IC: safe, cost effective and potent protection due to signal amplification via multiple pathways, challenges abound when applying conditioning. This is particularly true during PPCI when timing of protection application is so crucial, and so difficult. The pitfalls of these clinical trials are discussed further below. Pharmacological treatments that mimic conditioning or directly close the mPTP efficiently may be more attractive.

1.4.2 Conditioning mimetics

There are a number of substances that may mimic IC, targeting different aspects of its pathway. Here a number of these are reviewed together with the evidence supporting their use. Overall, no agent has successfully translated into routine clinical practice for cardioprotection.

Cyclosporine A (CsA) is an immunosuppressant drug which predominantly inhibits T cell activation but also directly inhibits opening of mPTP, and has the potential to prevent IR injury(46). Most animal studies have suggested that CsA reduces infarct size and patients receiving a bolus of CsA before PPCI also had a reduction in infarct size with sustained LV improvement on CMR at six months(47). Unfortunately, the recent large scale CIRCUS trial of CsA administered prior to PPCI showed no difference in infarct size assessed with both biomarkers and cardiac MRI, or any impact on MACCE or mortality(48). A number of reasons for the failure of CIRCUS have been proposed. A different pharmaceutical preparation of CsA was used compared to the earlier study, which used a lipid emulsion as a carrier vehicle. Furthermore, patients with an ischaemic time of up to 12 hours were included in the study. Finally, in order to reduce the required sample size, the composite primary endpoint included worsening of heart failure (before discharge) and adverse LV remodeling. The sample size is not large enough to detect important endpoints such as death or rehospitalisation with heart failure.

Some agents target the IC pathways upstream of the mPTP. Treatment with adenosine receptor antagonists have been shown to block the cardioprotective effects of postconditioning in animal models(49). The AMISTAD trial demonstrated a reduction in infarct size on SPECT when intravenous infusion of adenosine was

given with thrombolysis(50). The AMISTAD 2 trial, a larger trial of adenosine during reperfusion (mechanical or thrombolytic) did not demonstrate a clinical benefit, although a SPECT imaging sub-study did show a reduction in anterior infarct size consistent with the earlier study(51). Further post-hoc analysis suggested that adenosine given early, before reperfusion, had a mortality benefit (52). Nevertheless studies with intracoronary boluses of adenosine at the time of PCI have not reproduced these findings (53). It should be noted that the half-life of adenosine *in vivo* is only seconds. It is implausible to presume that isolated boluses would produces significant effects on infarct size. Longer infusions may be needed to show protective benefit.

Blockade of the opioid receptor has been shown to abrogate cardioprotection whilst morphine induces mKATP-dependent cardioprotection in rats(54). Human studies of opioid-mediated cardioprotection are sparse although the use of opiates during PCI is widespread. Evidence from CABG studies supports a cardioprotective role for opiates(55) and in PPCI per-conditioning, the addition of intravenous morphine during reperfusion reduced troponin release and increased ST segment resolution(37).

Nicorandil is a hybrid molecule of an ATP-sensitive potassium channel opener and a nitrate. Nicorandil reduced infarct size in rabbits if given during ischaemia but before reperfusion(56). Human studies have been heterogeneous with evidence of reduction of in heart failure and arrhythmia but no impact on mortality or biomarker measures of infarction(57).

Glyceryl Trinitrate (GTN) is an NO donor with vascular and myocardial effects. It is both an exogenous trigger of preconditioning and part of the intracellular signal

cascade for cardioprotection. Nitrate use is ubiquitous in PPCI and its role as a cardioprotective agent has been difficult to study. A meta-analysis in the 1980's demonstrated a significant mortality benefit when given during treatment of AMI(58) but this has not been confirmed in contemporary practice.

Chronic inhibition of the RAAS with ACE inhibitors or angiotensin receptor blockers (ARB) following myocardial infarction has been demonstrated to prevent left ventricular negative remodeling and improves morbidity and mortality(59,60). Recent animal evidence has suggested it may have a role in prevention of IR injury, particularly in elderly populations(61). However, there is little human evidence for the introduction of ACE inhibition or ARB before reperfusion to prevent IR injury.

1.5 Alternative methods of cardioprotection

1.5.1 Anti-oxidant and anti-inflammatory targets

ROS are present during the first moments of reperfusion and low levels appear to be responsible for initiating cell survival pathways. However, higher levels seen later during reperfusion are responsible for myocardial stunning and lethal IR injury(62). The ability of anti-oxidants to mediate cardioprotection has not been universal; a large study demonstrated that Trimetazidine (a metabolic agent that reduces oxidative stress(63,64)) did not improve mortality following thrombolysis(65) but oral loading of Trimetazidine did reduce troponin rises following elective PCI(66). Human superoxide dismutase given during PPCI has no mortality benefit(67) but Vitamin C (Ascorbic Acid) may reduce oxidative stress during IR injury although this has not been explored during PCI(68). It seems that Vitamin C abrogates IPC in animal models which could limit its application in humans(69).

Anti-inflammatory agents targeting CD18, CD11/CD18 integrin receptor, P-selectin, C5 and IL-1 have all been investigated for cardioprotective effects to prevent IR injury. Disappointingly, large trials such as APEX-AMI (Pexelizumab - a monoclonal antibody to C5 complement fragment - for AMI) have shown no mortality benefit(70). However, infarct size measured in a CMR sub-study was reduced(71). The inflammatory process surrounding myocardial infarction continues to provide potential targets for cardioprotection but no therapy has yet emerged with a proven benefit in PCI.

Statins lower LDL-cholesterol by inhibition of HMG-CoA reductase but may also have early pleiotropic anti-inflammatory and cardioprotective effects. Atorvastatin reduces

infarct size in a PI3K-Akt dependent manner(72), but the STATIN STEMI trial showed no difference in MACCE rate in patients given statin prior to PPCI(73). A meta-analysis has shown that statins given before cardiac procedures (PCI and CABG) significantly reduce post-procedure MI4a with a trend towards mortality benefit(74).

1.5.2 Blockade of calcium overload

The rise in intracellular Ca^{2+}, responsible for mPTP opening, can be blocked by indirectly inhibiting Na^{+}/ Ca^{2+} exchange via Na^{+}/H^{+} exchange (NHE) inhibition. NHE-1 inhibitors (eniporide and cariporide) did not demonstrate a mortality benefit in PCI, although in one study cariporide reduced myocardial infarction following CABG at the cost of an increased stroke risk(75). Ranolazine is a late current sodium channel blocker used as an anti-anginal agent. It may reduce Na+-dependent intracellular calcium overload during ischaemia. Ranolazine has been shown to be protective when given prior to elective PCI(76) although a trial of ranolazine given up to 48 hours after ACS showed no clinical benefit(77). The Ranolazine Cardioprotection in PCI study was terminated due to failure of recruitment (ClinicalTrials.gov Identifier: NCT01767987). This may reflect the difficulty of performing large, high quality trials of cardioprotective agents, often with little industry support.

1.5.3 Therapeutic hypothermia

Mild hypothermia introduced before the onset of reperfusion has been shown to limit infarct size in a number of animal models(78). The mechanisms are overlapping but not identical to those of IC. Evidence for reduction in infarct size in humans is weaker. Studies such as COOL-MI and ICE-IT have been negative although subgroup analysis showed benefit in anterior infarcts in both studies(79). Pooled

analyses have suggested that achieving a target temperature of below 35^{o}C before initiation of reperfusion was associated with reduced infarct size(80). CHILL MI assessed an endovascular cooling system against the standard of care in 120 patients and did not show a reduction in infarct size. Incidence of heart failure was significantly reduced and there was a possible infarct size benefit in patients with early anterior infarcts(81). Feasibility studies have suggested pre-hospital cooling is possible(82).

1.5.4 Manipulation of myocardial metabolism

Glucose-Insulin-Potassium (GIK) cardioprotection may share signaling pathways with IC. DIGAMI demonstrated a reduction in mortality when a GIK regime was given to hyperglycaemic diabetic patients during AMI, aiming to restore normoglycaemia (83). DIGAMI 2 compared GIK and either insulin based, or standard long-term glucose therapy against placebo, but did not achieve its glycaemia targets in any of its intervention arms. It showed no mortality benefit(84). There have been large numbers of further clinical trials looking at the effect of GIK and these are reviewed elsewhere(85). The IMMEDIATE trial of pre-hospital GIK infusion in patients with suspected ACS did not show any mortality benefit, nor was there a reduction in progression to MI (the primary endpoint) but there was reduction in infarct size in STEMI patients receiving GIK (86).

1.5.5 Summary

A large number of clinical trials have attempted to harness the IC pathways and other intracellular mechanisms to effect cardioprotection in the LV. Broadly speaking these have been unsuccessful. This is in part due to a failure to apply the rules of

cardioprotection, as well as incomplete understanding of the biology of IR injury and IC. Nonetheless, some promising therapies have been accompanied by disappointing trial results. The difficulties of conducting human clinical trials in cardioprotection are discussed in **Chapter 1.7**.

1.6 Cardioprotection in the right ventricle

Almost all of the studies discussed in the section above have focused on protecting the LV from IR injury. However, the clinical relevance of the right ventricle (RV) in the pathophysiology and treatment of heart disease is gaining increased recognition(87). Involvement of the RV in inferior myocardial infarction increases the risk of cardiogenic shock and increases mortality, even when treated with PPCI(88). The development of structural interventions such as transcatheter aortic valve replacement (TAVR) means that large ischaemic insults to the RV such as rapid pacing (RP) occur frequently in clinical practice(89). Acute changes in RV function may have important hemodynamic and clinical consequences.

The blood supply to the RV depends upon the prevailing coronary anatomy. In a right dominant system the right coronary artery (RCA) supplies most of the ventricle(90) whilst in left dominant system perfusion is shared with the left circumflex artery. The RV is believed to be relatively resistant to ischaemia compared to the LV(91). Coronary balloon inflation during PCI provides a model of supply ischemia. The impact of brief occlusion of the RCA on the RV is a reduction in stroke volume and stroke work during the deployment of the balloon(92). However, the response of the RV after reperfusion is unknown. The studies of brief coronary occlusion in the LV suggest that, after a brief improvement in function resulting from hyperemia, there is residual stunning of the ventricle, which is still present at thirty minutes after reperfusion(8).

Limited work has been undertaken to apply the principles of cardioprotection to the RV. This is likely to result from a combination of factors including its perceived

resistance to ischaemia and rapid recovery from MI, as well as the smaller numbers of patients presenting with RV infarction. Nonetheless, some animal studies have shown that IPC reduces infarct size in the RV(93,94). This protection is KATP dependent and remains despite hypertrophy, although it is abolished in the failing RV. Further work examining the effect of IR on the RV in humans is needed, particularly given the large ischaemic insults to which it is subjected as a part of modern interventional practice. Understanding the response of the RV to ischaemia will allow new treatments to be formulated.

1.7 Why are cardioprotection trials unsuccessful?

In sections 1.4 and 1.5 a large number of therapeutic interventions have been discussed. The field of cardioprotection is littered with promising interventions, almost all which have failed to translate into clinical practice. The reasons for failure were numerous. A number of key themes appear and are worth briefly visiting. These are summarized in **Table 1.1**.

The appropriate selection of patients for cardioprotection trials is important and frequently overlooked. Patients with ischaemic times of several hours are likely to have completed their MI and, as the initial landmark study of conditioning confirmed(20), are unlikely to benefit. Cardioprotective interventions cannot resurrect infarcted tissue. Cardioprotection trials need to target those patients arriving at hospital with short ischaemic times or those situations where the ischaemic event is planned.

Patients are heterogeneous and often elderly with comorbidities and medications that will affect cardioprotection. For example, Yellon and colleagues have demonstrated a decline in the effectiveness of RISK pathway signaling with aging(95). Co-morbidities such as diabetes mellitus also affect signaling pathways responsible for IC. Avoidance, or stratification, of these groups needs to be considered in the design of studies(35). This has led some to assert that conditioning is a “healthy heart phenomenon” that can only be demonstrated in juvenile animal models(96). This is unlikely but does emphasize that human studies should focus on interventions with large effects in animal models. This will maximize the chance of successful translation since the effect in humans is likely to be smaller.

A failure to acknowledge the time-dependence of cardioprotection is a common flaw of many negative trials. Initiation of therapies after the window for cardioprotection has closed will be unsuccessful, regardless of the promise of the intervention in animal studies (where timing is often also easier to control)(97). The challenge for future studies is in accessing cardioprotective cellular pathways before or in the moments immediately following reperfusion without unduly delaying reperfusion therapy.

Selection of endpoints in trials remains a difficult issue. Trials focused on clinical endpoints such as mortality or MACCE need to ensure that they have statistical power whilst not broadening the entry criteria to include individuals that are unlikely to benefit. Other important endpoints such as infarct size (the most commonly used primary endpoint in PPCI trials of cardioprotection) have their own inherent problems. Infarct size must control for the area-at-risk (AAR) to ensure that comparisons are valid. T2-weighted CMR (the gold standard) relies on myocardial oedema to estimate AAR. However, post-conditioning has recently been shown to reduce myocardial oedema during STEMI(29,98), leading to the overestimation of the percentage infarction in the conditioning group(99). Furthermore, the distribution of myocardial oedema changes over time, possibly adopting a bimodal pattern that may confound the endpoint(100). Endpoints should be chosen that reflect the biology of cardioprotection, which is predominantly to limit lethal IR injury. Endpoints should focus on infarct size, and heart failure, rather than angina and the need for un-planned revascularization.

Table 1.1 Why have cardioprotective trials failed

Why have trials failed?	Explanation
Patient Selection	• Heterogeneous patient groups including patients that may be resistant to conditioning (e.g. elderly, diabetics) • Medication effects that block or mimic cardioprotective effects • Application after a long ischaemic time and completed MI • Application when effect e.g. infarct size, might be small (non-anterior MI) and as a result protection is undetectable.
Timing	• Conditioning must be activated *before* or in the first moments of reperfusion. • Conditioning mimetics have similar requirements. • Cooling must be to the desired temperature *before* reperfusion for maximum effect. • It may be challenging to deliver conditioning or cooling protocols without delaying reperfusion.
Dose	• Conditioning protocols are not consistent between trials. • The threshold for protection may not have been attained.
Endpoints	• Multiple endpoints have been selected without consistency. • Biomarker release e.g. troponin have consistently shown to diminish after conditioning but are not the gold standard for measuring infarct size reduction. • Infarct size reduction *must* control for the size of the area at risk (AAR). This is challenging to define *in vivo,* and even the gold standard T2W CMR oedema to quantify AAR may be diminished by cardioprotection.

Finally, it is worth commenting on the choice of intervention itself. Many interventions target a single aspect of IC, or alternatively access a different signaling pathway. There may prove to be multiple redundancies in the signaling pathways leading to both protection and cell death. Treatment that is able to target multiple pathways of protection, either through a single agent, or by combining multiple cardioprotective strategies may be more likely to protect against infarction. Additionally, clinical trials should only be undertaken where there is consistent evidence of protection across a number of animal models, as well as “proof of concept” studies in humans. These smaller human studies are particularly important in the field of cardioprotection. Identification of problems with translation to a clinical setting at this stage will allow better study design to overcome such obstacles, or early abandonment of strategies which are no longer considered viable.

1.8 Glucagon-like Peptide 1

1.8.1 Introduction

GLP-1 (7-36) amide is a 30 amino-acid cleavage product of proglucagon secreted by enteroendocrine L-cells in the gut(101). Oral ingestion of a meal is the primary physiological stimulus to GLP-1 secretion(102). GLP-1 is not secreted in response to an intravenous glucose infusion.

GLP-1 has receptor-dependent and independent actions. It causes glucose-dependent insulin release through binding the GLP-1 receptor (GLP-1R) on pancreatic beta cells. GLP-1 does not cause hypoglycemia as its insulinotropic effect does not occur at blood glucose concentrations less than 4.0 mmol/l (103). GLP-1 has a number of other physiological effects, which serve to lower plasma glucose levels. These include stimulation of insulin gene transcription in the beta cell(104), and reduced gastric emptying(105). Enhancement of peripheral insulin sensitivity remains unproven with conflicting evidence(106).

The GLP-1R is a 463-amino acid, GPCR found on the cell surface membrane of numerous tissues throughout the body. Its presence within the myocardium has remained controversial. Both mouse and primate studies have suggested that the GLP-1R remains confined to the atria, and possibly just the sino-atrial node(107,108). No study, using specific antibodies or other validated techniques, has demonstrated either the presence or absence of the GLP-1R in human ventricular cardiomyocytes. The location of the receptor is important in elucidating the mechanism of GLP-1 cardioprotection. Evidence of receptor-independent effects suggest that there may an alternative receptor, or perhaps actions that do not require a receptor(109,110) – these are discussed further below.

GLP-1 is cleaved by the enzyme dipeptidyl peptidase-4 (DPP4) to GLP-1 (9-36) amide with a half-life of approximately 2-minutes(111). The biological role of this breakdown product is uncertain but it has reduced incretin activity. GLP-1 is further degraded by neutral endopeptidase (NEP) to GLP-1 fragments whose biological activity is the subject of ongoing research.

A number of pharmaceutical products have been developed to utilize the incretin effect of GLP-1 whilst avoiding the difficulties associated with its rapid breakdown to an apparently inactive form. These include DPP4 inhibitors such as Sitagliptin (Januvia, Merck, NJ, USA), Saxagliptin (Onglyza, AstraZeneca, UK) and Vildagliptin (Galvus, Novartis, Switzerland) all of which increase levels of native GLP-1; and DPP4 resistant GLP-1 receptor agonists (GLP-1RA) such as Exenatide (Byetta, AstraZeneca, UK) and Liraglutide (Victoza, Novo Nordisk, Denmark).

1.8.2 Pre-clinical evidence of GLP-1 cardioprotection

GLP-1 protects cardiomyocytes from cell death. *In vitro,* GLP-1 averted cell death in HL-1 murine adult cardiomyocytes treated with the pro-apoptotic agent staurosporine(112). Both wild type and GLP-1R homozygous knockouts experienced cardioprotection from GLP-1 (7-36) and (9-36), suggesting the protective effect occurred independent of receptor binding (113). GLP-1 protection was similarly associated with reduced activation of pro-apoptotic molecules in rats, and occurred regardless of whether it was given prior to ischaemia or in early reperfusion(114,115). *In vivo,* GLP-1 infusion given to dogs with a pacing-induced cardiomyopathy improved LV function with systemic changes such as reduced heart

rate and systolic blood pressure(116). Furthermore, GLP-1 infusion reduced infarct size after IR injury (117).

GLP-1 RA's have also been shown to protect against IR injury. Albiglutide (Eperzan, GlaxoSmithKline, UK) and lixisenatide (Lyxumia, Sanofi, France) both reduced final infarct size in rat models(118,119) whilst pretreatment with liraglutide protects in mice(120). In a porcine model of IR injury, treatment with exenatide for three days reduced infarct size and improved LV function(121). However, initiation of exenatide after onset of ischaemia did not result in cardioprotection suggesting that, at least for GLP-1RA's, there may be a time-dependent element to the cardioprotection(122).

1.8.3 Clinical evidence of GLP-1 mediated cardioprotection

GLP-1 mediated cardioprotection is conserved in a number of animal species including humans. The evolutionary reason for this cardioprotection is speculative. It may simply be conserved by association with IC-mediated cardioprotection and this may confer a survival advantage by limiting birth-induced fetal ischaemia. Similarly, GLP-1 mediated protection may have evolved to protect our prehistoric ancestors when eating - the immobility that ensues may have increased their vulnerability to predators. Whatever the reason, it now provides a target, which may be exploited for clinical benefit, particularly in the setting of cardiac ischaemia.

A number of human studies have shown evidence of GLP-1 mediated cardioprotection. These are summarized in **Table 1.2**. Protection may limit non-lethal forms of IR injury such as myocardial stunning or post-infarct remodeling. Endpoints in clinical studies have focused more heavily on these forms of IR injury. Future clinical trials during elective or emergent percutaneous coronary revascularisation

offer an ethically acceptable method of assessing GLP-1 protection from lethal IR-injury.

Table 1.2 Studies addressing GLP-1 mediated cardioprotection in humans

Author	Year	Cardioprotective agent	Number of participants	Protocol	Result
Nikolaidis *et al*.(123)	2004	GLP-1	21	72 hour GLP-1 infusion initiated within 4 hours of PPCI in patients with severely impaired LV function vs. saline control	Improved global and regional LV function on echocardiography
Sokos *et al*.(124)	2006	GLP-1	21	Subcutaneous infusion of GLP-1 given for 5 weeks in patients with NYHA III/IV heart failure vs. saline control	Improved quality of life score, 6-minute walk tests and LV EF
Read *et al*.(125)	2010	Sitagliptin	14	Patients with CAD given single dose of sitagliptin / placebo followed by DSE. Patients acted as there own control.	Sitagliptin improves EF and regional tissue Doppler indices at peak stress and 30 minute recovery
Read *et al*.(126)	2011	GLP-1	20	GLP-1/saline infusion initiated after coronary balloon occlusion in patients undergoing PCI with further balloon occlusion at 30 minutes	GLP-1 infusion protects against stunning and cumulative ischaemic dysfunction on PV loop measurement
Read *et al*.(127)	2012	GLP-1	14	Patients with CAD given infusion of GLP-1 / placebo followed by DSE. Patients acted as there own control.	GLP-1 infusion improves EF and regional tissue Doppler indices at peak stress and 30 minute recovery
Lonborg *et al*.(128)	2012	Exenatide	172	GLP-1 infusion initiated 15 minutes before intervention in PPCI patients and continued for 6 hours	Reduced final infarct size on CMR. No change in biomarker rise or overall LV EF. No difference in clinical outcome.

Woo *et al.(129)*	2013	Exenatide	58	Twice daily subcutaneous exenatide injection for 72-hour compared to placebo in patients receiving PPCI	Reduced infarct size on CMR and reduced biomarker rise (CKMB and Troponin-i)
McCormick *et al.*(130)	2014	Sitagliptin	20	DM patients with CAD underwent DSE after 4 weeks of sitagliptin	Sitagliptin improves EF and regional tissue Doppler indices at peak stress and 30 minute recovery compared to baseline
McCormick *et al.*(131)	2015	GLP-1	20	GLP-1/saline infusion initiated prior to coronary balloon occlusion in patients undergoing PCI, monitored with pressure-volume loops	GLP-1 infusion protects against ischaemic dysfunction and myocardial stunning when given prior to coronary balloon occlusion
McCormick *et al.*(132)	2015	GLP-1	10	GLP-1 infusion given during DSE, concurrently with hyperglycaemic, hyperinsulinaemic clamp in type 2 DM, who acted as their own control	GLP-1 infusion EF and regional tissue Doppler indices at peak stress and 30 minute recovery
Roos *et al.*(133)	2015	Exenatide	91	GLP-1 infusion initiated immediately before PPCI and continued for 72-hours	No change in final infarct size, EF or biomarker rise

EF = ejection fraction, LV = left ventricle, DSE = dobutamine stress echocardiogram, PV = pressure-volume, DM = diabetes mellitus

GLP-1 has been shown to protect against ischaemic LV dysfunction following periods of demand ischaemia induced with dobutamine stress echocardiography (DSE) (127). This effect has recently been shown to be maintained even during a hyperglycaemic, hyperinsulinaemic clamp(132). Similar benefit has been derived through the administration of the DPP4 inhibitor sitagliptin in both diabetic and non-diabetic patients(125,130).

GLP-1 protects against cumulative myocardial stunning following coronary balloon occlusion during elective PCI when assessed using an LV conductance catheter (126,131). The protection is present whether GLP-1 administration precedes or follows supply ischaemia. This contrasts with IC, which does not protect the ventricle against stunning in the same manner(134). Protection from myocardial stunning may have particular benefit in those patients with cardiogenic shock where ischaemic contractile dysfunction adds to the cascade of haemodynamic failure associated with the condition. A 72-hour infusion of GLP-1 initiated following AMI produced improvements in global and regional wall motion on echocardiography(124). Myocardial stunning is reversible and the mechanisms underlying this protection are almost certainly different to those underlying infarction(135).

Patients treated with exenatide during ST-elevation myocardial infarction (STEMI) had a 15% reduction in infarct size on cardiac MRI (CMR) although there was no reduction in peak troponin levels or LV EF assessed at 90 days(128). Of note, all the benefit seen in this study occurred in patients with short ischaemic times(136). A further study of 58 primary PCI patients treated with exenatide prior to reperfusion showed a reduction in infarct size (on CMR and biomarker measures) as well as improvement in LV systolic function at six-month follow-up(129). In contrast, early results of the EXAMI trial have shown no benefit (on MRI infarct size or biomarkers)

from a 72-hour exenatide infusion initiated immediately prior to primary PCI for STEMI(133).

GLP-1 has been administered to patients undergoing CABG. Whilst it did not result in significant differences in LV dysfunction assessed on echocardiography, there was an overall reduction in the need for inotropes and vasoactive infusions compared to control(137). The study also highlighted a reduction in ventricular arrhythmia - a surrogate for ischaemia. A further double-blind trial of GLP-1 infusion during on-pump cardiac surgery resulted in improved glycemic control. No assessment of whether this intervention also had a cardioprotective effect was reported(138).

Large-scale studies of DPP4 inhibitors (SAVOR-TIMI 53, TECOS and EXAMINE) have demonstrated their cardiovascular safety in diabetic patients(139-141). It is worth noting that there was an increased incidence of hospitalisation for heart failure. This finding may be the result of post-hoc testing for multiple endpoints. It has not been seen in the other large-scale DPP4 studies, nor replicated in large-scale retrospective cohort analyses(142). The primary endpoints of these studies were cardiac events following long-term therapy. Despite large numbers they were not designed to assess possible cardioprotection during an acute ischaemic event, and the results cannot be used to assess the cardioprotective efficacy of GLP-1 or GLP-1RAs.

1.8.4 Mechanism of GLP-1 mediated cardioprotection

Many of the above studies have demonstrated aspects of cardioprotection, providing clues as to how GLP-1 protects against IR injury. Several mechanisms have been proposed for GLP-1 mediated cardioprotection. A change in myocardial glucose

utilisation may result in increased metabolic efficiency and myocardial resistance to ischaemia, thus limiting infarction. Vasodilation and reduction in systemic and/or pulmonary vascular resistance can also reduce cardiac work and ATP demand during ischaemia. Finally, the pathways of IC may be activated to increase cellular resistance to IR injury. One, or more, of these pathways may overlap, limiting both lethal and non-lethal IR injury to varying extents. **Figure 1.3** offers an overview of a number of proposed mechanisms for GLP-1 mediated cardioprotection. Evidence for these mechanisms is reviewed below.

Figure 1.3 Possible mechanisms of GLP-1 cardioprotection

1.8.5 Myocardial glucose utilisation and GLP-1

A change in myocardial glucose utilisation is consistent with the physiological role of an incretin hormone. Fatty acids are the main fuel for energy production in the heart(143), although the omnivorous myocyte will utilise a number of different sources for ATP production(144). Glucose is the most oxygen efficient source of ATP, and is the preferred substrate when insulin and glucose levels are high, as in the post-prandial state(145). Glucose is preferentially metabolised in ischaemic tissues and this is facilitated by augmentation of glucose uptake through up-regulation of the glucose transporters GLUT1 and GLUT4 to the cell-surface membrane(146). Ischaemic myocardium relies increasingly on anaerobic respiration through glycolysis and if ischaemia is prolonged, intracellular levels of lactate and Ca^{2+} rise, pH falls, and ultimately the apoptotic cascade is initiated. Increasing substrate availability through increased concentrations of glucose and/or insulin can protect by delaying the initiation of this cascade – the so-called glucose-insulin-potassium (GIK) effect. GIK was amongst the earliest cardioprotective interventions to be investigated but its benefit remains uncertain and controversial(85). Some data has shown a reduction in infarct size when given during AMI(86). However, these benefits may have been mitigated by clinical problems with administration of insulin during PPCI including a risk of hypoglycemia and hyperkalemia. Lack of a clear cardioprotective benefit in subsequent larger studies such as DIGAMI II has limited the translation of GIK into clinical practice(147).

Increased glucose uptake by the myocardium has been shown to be beneficial in IR injury(148). In a canine model of dilated cardiomyopathy, GLP-1 was shown to increase myocardial glucose uptake. This was associated with an improvement in myocardial performance(149). GLP-1 reduced levels of lactate and pyruvate in a

porcine model of IR injury suggesting alteration of myocardial glucose utilisation. Of note, infarct size was not affected by GLP-1 in this model(150). Additionally, Albiglutide produced an increase in myocardial glucose uptake and a shift from fatty acid to carbohydrate oxidation during IR injury in rats(118). In an *ex vivo* rat model chronic treatment with DPP4 inhibitors reduced infarct size in a glucose dependent manner(151).

A recent study in rats has shown that GLP-1 appears to induce a switch to carbohydrate oxidation in the area *not at risk* following IR injury, as well as an increase in anaerobic glycolysis in the area subjected to IR injury(152). An increase in glucose utilisation in the area not at risk may allow increased overall contractility of the ventricle during ischaemia. Additionally, a rise in glycolysis may represent an increased number of viable cardiomyocytes within the ischaemic area.

Conversely, a number of recent human studies have cast doubt on changes in myocardial glucose utilisation as the mechanism behind GLP-1 mediated cardioprotection. A study of 20 non-diabetic patients given a 48-hour subcutaneous infusion of GLP-1 did not show any significant change in metabolic parameters(153). Furthermore, coronary sinus sampling in humans subjected to brief coronary artery occlusion demonstrated no significant change in myocardial glucose extraction when GLP-1 was infused(131). In healthy volunteers, GLP-1 did not affect overall myocardial glucose utilisation in both normo- and hypoglycaemic states. In this study, the sub-group of volunteers with a high baseline level of glucose utilization had a reduced level with GLP-1. In contrast, those with low baseline level were found to have increased glucose utilisation(154).

Subtle changes in metabolism may have profound effects on the cardiomyocyte in IR injury but remain difficult to detect in human studies. Careful design of human studies is needed to disentangle the metabolic changes induced by GLP-1 and those responsible for cardioprotection.

1.8.6 Conditioning pathways and GLP-1

IC is tissue resistance to prolonged ischaemia, induced by repeat cycles of transient ischaemia and reperfusion, either locally or in a remote tissue. Discussed earlier, it has been demonstrated to be beneficial in both animal models(20) and some human clinical trials(32,33). IC is mediated through the reperfusion injury survival kinase (RISK) and survivor activating factor enhancement (SAFE) pathways, which are complex but have been well described(155). A number of cell surface GPCRs(156) initiate these survival cascades utilizing intracellular signaling molecules including PI3K, Akt, p70s6k, p38MAPK and ERK1/2. These pathways converge upon the mKATP(157,158) – the final common step in conditioning pathways that inhibit the opening of the mPTP, preventing mitochondrial electrochemical uncoupling, loss of the membrane potential, depletion of ATP, release of cytochrome C, hypercalcemia and ultimately cell death(15,159).

Evidence that GLP-1 may activate IC mechanisms has accumulated(160). GLP-1 is known to activate the PI3K-Akt pathway in the pancreas(161) and GLP-1 mediated cardioprotection in an isolated rat heart model was associated with the RISK pathway. Inhibition of p70s6 kinase, a downstream target of PI3K, inhibited the cardioprotective effect of GLP-1. Whilst PI3K and Akt are consistently involved in GLP-1 cardioprotection (109,114), phosphorylation of ERK 1/2 occurred in some

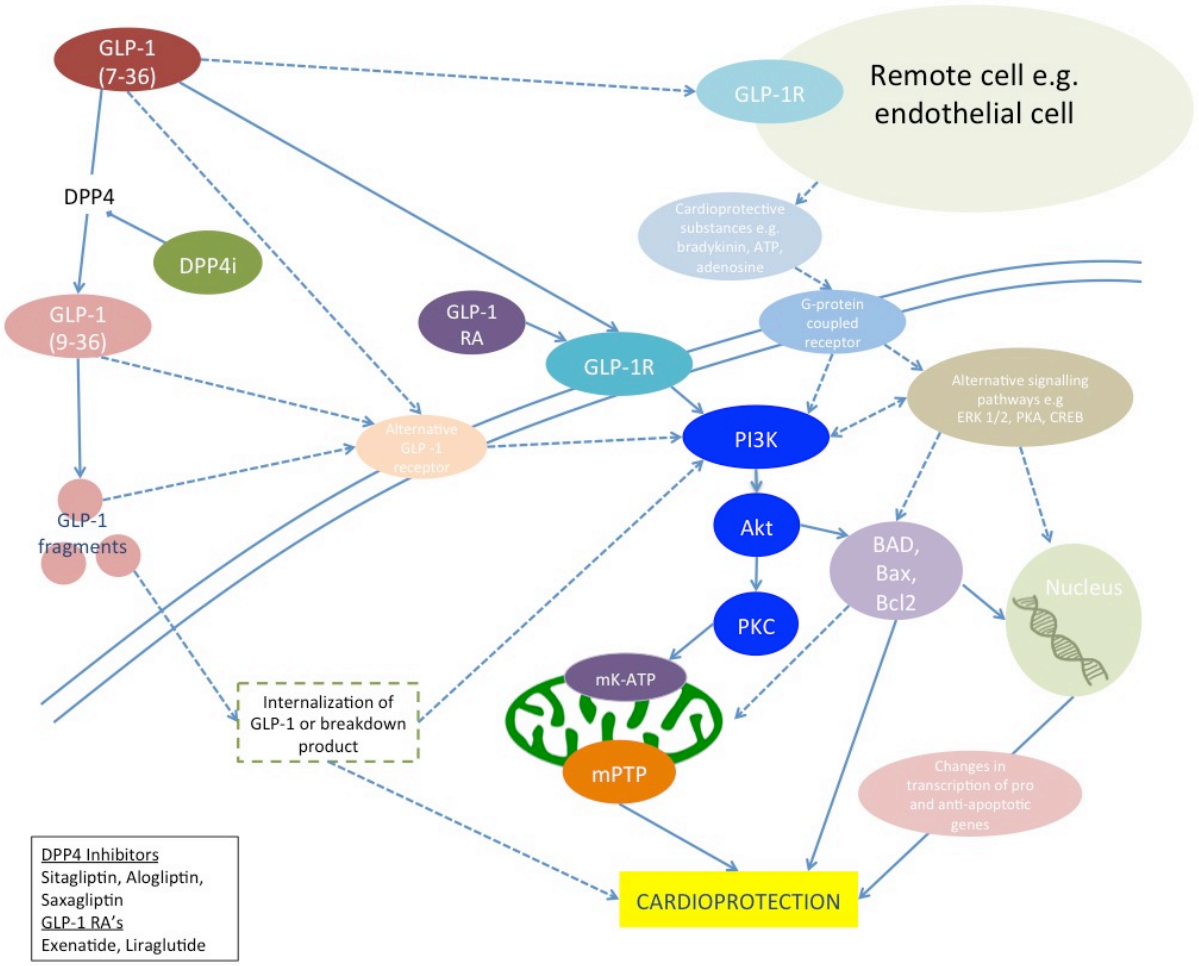
studies (110) but not others(162). Pigs exposed to IR injury had reduced infarct size when pretreated with exenatide. Levels of phosphorylated Akt and Bcl-2 were elevated after treatment with exenatide suggesting activation of conditioning pathways(121).

The role of the GLP-1R in the activation of these sub-cellular signaling pathways is unclear with inconsistent evidence in animal models. In the glucose-dependent rat model discussed above, the protective effect of DPP4 inhibition was lost by blocking the GLP-1 receptor (151). However, GLP-1R knockout mice still experienced cardioprotection with GLP-1(113). Recently, mice with cardiomyocyte specific disruption of the GLP-1R were shown to still experience robust protection from liraglutide(122). Recent evidence suggests that the GLP-1R may not be present in ventricular cardiomyocytes at all (107). Either another, as yet unidentified, receptor is mediating the GLP-1 effect on the RISK pathway of the cardiomyocyte, or indirect activation is occurring, with GLP-1 binding a remote GLP-1R on endothelial or smooth muscle cells. This could trigger paracrine release of cardioprotective agents that act on the adjacent cardiomyocyte.

Activation of IC is associated with adenosine signaling pathways(163,164). Recent work in anesthetized dogs showed that alogliptin-induced cardioprotection was abolished with blockade of adenosine A_1 receptor, despite continued activation of RISK pathways, suggesting an interaction between adenosine and GLP-1 signaling. There may be cardioprotective “redundancy” at play(162). A simplified subcellular signaling pathway through which GLP-1 may act to effect cardioprotection is proposed in **Figure 1.4**.

Some actions of GLP-1 have been shown to be dependent upon the mKATP channel. This includes concentration-dependent relaxation of rat aorta, which was inhibited by the mKATP blocking agent, glibenclamide(165). Glibenclamide also inhibited flow-mediated dilatation of human arteries in forearm blood flow studies with GLP-1(166).

Figure 1.4 Proposed schema of GLP-1 mediated cardioprotection



1.8.7 Vasodilatory and haemodynamic effects of GLP-1

Reduction in systemic vascular resistance decreases afterload and venodilation reduces preload - both reduce cardiac work and increase myocardial resistance to ischaemia, as does reduction in heart rate. Coronary vasodilation also reduces ischaemia by augmenting blood supply and aiding collateral recruitment to the myocardium.

GLP-1 produces coronary vasodilation and microvascular recruitment in animal models(167,168). Murine studies show that GLP-1 vasodilates constricted coronary arteries by increasing NO mediated cGMP release in smooth muscle cells. This is associated with reduced IR injury. Vasodilation was present in GLP-1R knockout mice, suggesting it was a receptor-independent effect. Vasodilation occurred with the cleavage product GLP-1 (9-36), but not with the GLP-1RA Exendin-4(113). GLP-1 mediated concentration-dependent relaxation of ex vivo rat aorta was eliminated when KATP channels were blocked, linking the haemodynamic effects of GLP-1 to conditioning pathways as discussed above (165). Research involving healthy human volunteers demonstrated that GLP-1 infusion caused recruitment of microvasculature in cardiac muscle assessed with myocardial contrast echocardiography (169).

Studies examining the impact of GLP-1 on heart rate and blood pressure have been limited to GLP-1RA. Liraglutide reduced diastolic and systolic blood pressure in mice by binding the GLP-1R - the effect was lost in GLP-1R knockout mice and was mediated by atrial natriuretic peptide (ANP)(108). In humans with diabetes mellitus, administration of liraglutide did not result in an increase in ANP although it was associated with a small and possibly detrimental rise in heart rate(170). A recent meta-analysis including unpublished data from pharmaceutical companies suggest that GLP-1RA are associated with a rise in heart rate(171). Liraglutide also produced

an elevated heart rate in an open label trial of patients comparing it to sitagliptin in type 2 diabetics. Sitagliptin was not associated with a heart rate rise suggesting that native GLP-1 may not have the same effect or perhaps implicating the cleavage product of GLP-1 in it's haemodynamic effects(172).

1.8.8 Understanding the mechanism of GLP-1 mediated cardioprotection

A clear understanding of the mechanism by which GLP-1 elicits cardioprotection has proved elusive. Differences between animal models make studies difficult to compare. It is not clear that murine models reflect the biology of larger mammals. The relationship between animal and human physiology, particularly with regard to the location and presence of the GLP-1 receptor, mean that firm conclusions are difficult to draw. Even within published literature, there is variability in cardioprotective potency largely attributable to differences in study design.

Physiological studies in humans, whilst often harder to perform, and limited by ethical and practical considerations may provide clarity over the mechanism by which GLP-1 exerts its cardioprotective effect. Particular importance should be placed on limiting the confounders implicit in human studies (such as medications, diabetic status, comorbidities and counter-regulatory hormones) to ensure that useful information can be gleaned. Where these factors cannot be controlled, allowance should be made in discussion of results. Areas of useful endeavor may include assessment of the effects of GLP-1 in the human coronary microcirculation and the interaction of RISK/SAFE pathways with GLP-1. GLP-1 may act through multiple pathways of protection, including some not yet described, increasing its potential usefulness but also the complexity of understanding its mechanism.

1.8.9 The mechanism of GLP-1 cardioprotection in human ischaemia studies

A number of human studies performed at this institution have provided insight into the mechanism of GLP-1 protection against non-lethal ischaemia. GLP-1 is protective against demand ischaemia when administered directly and when levels of native GLP-1 are elevated through a DPP4 inhibitor(125,127). This finding suggests the GLP-1 (7-36) amide is responsible for the cardioprotection, as breakdown products such as GLP-1 (9-36) amide are not increased by treatment with DPP4 inhibitors. Demand ischaemia studies performed during a hyperinsulinaemic, hyperglycaemic clamp showed that elevated insulin levels had a neutral effect on GLP-1 cardioprotection(132). This finding is surprising given the evidence for changes in myocardial glucose utilisation in animal studies. An environment that promotes glucose uptake and metabolism should support a metabolic switch from free fatty acid to glucose oxidation. If this were the mechanism behind protection, then improvement in myocardial performance would be expected.

Furthermore, during supply ischaemia studies, sampling of glucose from the coronary sinus and coronary artery allowed measurement of transmyocardial glucose concentration. An increase in glucose uptake or utilisation would lead to an increased gradient. However, the study demonstrated no difference in glucose gradient when GLP-1 was infused during supply ischaemia(131).

The apparent absence of an effect on myocardial glucose utilisation in these studies suggests an alternative mechanism may be more likely. There is considerable evidence for activation of subcellular IC pathways in animal models(109,114,115). Against this, it should be noted again that IC does not protect against non-lethal

ischaemia whilst GLP-1 does. This is a fundamental difference between the two. Additionally GLP-1 does not appear to exhibit the same time-dependence as IC, with protection occurring regardless of whether treatment is initiated before or after reperfusion in supply ischaemia studies(126,131).

Given the apparently contradictory evidence, further studies are warranted to investigate whether KATP dependent cellular pathways are implicated in GLP-1 cardioprotection against non-lethal IR injury.

1.9 Hypotheses

The work in this thesis investigates the cardioprotective effect of GLP-1 further. The discussion above raised questions about the role of mKATP dependent pathway as a mediator of GLP-1 cardioprotection. The availability of the KATP blocker glibenclamide (discussed further in Chapter 2) provides an apparently elegant way to interfere with these conditioning pathways during GLP-1 infusion and ischaemia. This lead to the first hypothesis, which was that:

1. GLP-1 cardioprotection in the LV is mediated through the mKATP channel during both demand and supply ischaemia.

Whilst the effect of GLP-1 on non-lethal ischaemia in the LV has been well documented, its effect in the RV is unknown. During inferior MI, the RV may be heavily involved. Infarction of the RV has significant haemodynamic consequences for patients, particularly those presenting with cardiogenic shock. Since the response of the RV to brief coronary occlusion is unknown, a study was designed to address whether the RV responded to brief coronary balloon occlusion in the same way as the LV, with stunning and cumulative dysfunction. Furthermore, the study was designed to investigate whether GLP-1 provides the same protection to the RV as it had during balloon occlusion in the LV. These findings have consequences for both mechanistic understanding of GLP-1 protection, and clinical utility. In this thesis, only the first of these questions will be addressed. The second hypothesis was therefore:

2. The RV experiences stunning and cumulative ischaemic dysfunction following serial right coronary artery balloon occlusion

If GLP-1 is to prove a useful therapy, it needs to be efficacious in larger clinical trials. In the final hypothesis, we sought to investigate whether GLP-1 was a useful treatment for lethal IR injury. A stable cohort was felt to be an appropriate choice for the first phase 2 clinical trial of GLP-1 for cardioprotection and therefore patients undergoing elective PCI were selected. The third hypothesis was therefore:

3. GLP-1 cardioprotection reduces the incidence of PCI-associated myocardial infarction when administered prior to the procedure

Since the study investigating this hypothesis underwent a complex regulatory approval and the recruitment target is 400 patients, the results are not complete. As a double blind study, an interim analysis of the results is not possible. This thesis will discuss the design of the study, the blinded demographic and procedural characteristics of the first 100 patients and endpoint data in a blinded fashion.

The methods section, which follows, details the techniques used to assess the hypotheses listed above. As a set, these hypotheses will lead to a deeper understanding of the mechanism of GLP-1 mediated cardioprotection and its clinical utility.

Chapter 2 Materials and Methods

2.1 Introduction

The methods for this thesis were designed to test the hypotheses discussed in Chapter 1. Since GLP-1 mediated cardioprotection against non-lethal IR injury has been demonstrated in humans using DSE, and coronary balloon occlusion, the methods in this thesis are based upon the same techniques. The first hypothesis was that GLP-1 utilises pathways of IC. Glibenclamide was selected as an agent that interferes with this pathway but is safe to use in humans. The design of the studies reflects the need to prioritize patient safety. The second hypothesis is that the effect of GLP-1 is similar in the RV to that already demonstrated in the LV. In this case, the method chosen reflects previous work in the LV to allow comparison with previous studies in the discussion.

The materials and methods in this section cover the broad methodology for the three mechanistic studies included within this thesis. These are:

1. The effect of glibenclamide on GLP-1-mediated cardioprotection during demand ischaemia

2. The effect of glibenclamide on GLP-1-mediated cardioprotection during supply ischaemia

3. The effect of GLP-1 on right ventricular function during supply ischaemia

Detailed protocols are included in the respective chapters for each study. The two agents investigated in this thesis are discussed. Following this, a discussion of the treatment of blood tests taken during the studies is included.

The first of these studies assesses the effect of glibenclamide on GLP-1-mediated cardioprotection using a DSE model. Principles of DSE to assess LV function during stress are discussed. Both supply ischaemia studies use a conductance catheter to assess ventricular function during supply ischaemia. The conductance catheter is discussed in general terms, with detailed protocols included in the relevant chapters.

2.2 Infusions and medications used in the studies

2.2.1 Glucagon-like Peptide 1 (7-36) amide acetate

Glucagon-like Peptide 1 (7-36) amide acetate, hereafter simply referred to as GLP-1 unless otherwise indicated was supplied from Bachem Distribution Services GmbH (Weil am Rhein, Germany). GLP-1 was prepared in the same manner in all studies in this thesis.

GLP-1 is supplied as a lyophilised white powder in a glass vial containing 100 µg of the product. GLP-1 was dissolved into 1ml of 0.9% normal saline. This was then further diluted into 50ml normal saline. This was delivered to the patient via a syringe pump.

The selected rate was 1.2 pmol/kg/min in all studies, which corresponds to a dose at which expected effects have been seen in previous work(126,127,131). The dose is also lower than some studies where nausea has been a problem(137). GLP-1 (7-36) amide is rapidly degraded by the enzyme DPP4 to GLP-1 (9-36) amide. The half-life of GLP-1 is 1-2 minutes in healthy volunteers(173). Continuous infusion is needed to ensure a steady level of GLP-1 during the studies. A study with an infusion rate of 0.9 pmol/Kg/min showed steady state levels of GLP-1 achieved within 30 minutes(174). In these studies a 30 minute GLP-1 infusion has been used. Studies at this institution have demonstrated protective effects supra-physiological levels of GLP-1 with these timings. In the dobutamine stress studies discussed below, the GLP-1 was started 15 minutes prior to the commencement of dobutamine for practical reasons. Again, this was approximately 30 minutes prior to peak ischaemia.

2.2.2 Glibenclamide

Glibenclamide is an oral hypoglycemic agent belonging to the family known as sulfonylureas. It acts on the sarcolemmal KATP channel in the pancreatic beta cell to increase release of insulin(175). Conventional sulfonylureas such as glibenclamide have also been shown to bind to KATP channels in the myocardium, specifically the mKATP channel(176). This channel is responsible for the protective effects of IC, as discussed in the introduction. Glibenclamide has been shown to mitigate the effects of IC and other IC-mimetics in both human and animal studies(177-180). Furthermore, the abrogative effect of glibenclamide is different to that of glimepiride, a more recent sulfonylurea, which does not interfere with IC(181).

It is on this basis that glibenclamide was selected as an appropriate agent to investigate whether GLP-1 is an IC-mimetic. Previous studies have suggested that glibenclamide does not have an independent negatively inotropic effect, or increase the effect of ischaemia. In the demand ischaemia study this was not retested but a glibenclamide only control arm was included in the supply ischaemia study.

Glibenclamide is rapidly absorbed from the gastrointestinal tract. There is no significant first pass metabolism meaning that almost 100% of the oral dose is bioavailable. Previous studies have shown that glibenclamide blocks IPC in the myocardium after 60 minutes(179). A dose of 5mg was used in forearm blood flow studies in humans during which IPC was abrogated(182). Pharmacokinetic studies with 5mg oral glibenclamide have shown the mean time of maximal serum concentration (T_{max}) to be 3.6±2.3 hours after administration(183). Glibenclamide was administered in a similar timeframe, given a minimum of 60 minutes prior to

initiation of GLP-1 in the stress echo studies. In conductance catheter studies, the glibenclamide was administered between 60 minutes and 180 minutes prior to the first balloon occlusion.

Glibenclamide is a powerful oral hypoglycemic and when given to non-diabetic patients there is a significant risk of hypoglycemia. These effects may be magnified in combination with administration of GLP-1, a stress test or PCI, and a fasted patient(184). For this reason a 5mg oral dose was selected. This dose is lower than that used in some cardiovascular studies (which used 10mg). However, one forearm blood flow study showed abrogation of IPC with a 5mg dose(182).

In all studies using glibenclamide a dextrose infusion at a low (1.5mg/Kg/min) background level was given. This was designed to avoid stimulation of pancreatic beta cells to release insulin(185). This could be up titrated to maintain blood glucose levels if needed. The prolonged biological half-life of glibenclamide (up to 10 hours) meant that following administration patients needed a significant oral carbohydrate meal and continued dextrose infusion until this was possible. Close monitoring of patients following completion of the investigatory portion of the protocol was performed with frequent blood glucose monitoring. Precise details of the safety steps taken are included with each protocol below.

2.3 Biochemistry

In all studies in this thesis, a number of blood tests were performed that fall outside routine clinical investigations. These include plasma GLP-1 (7-36) amide, insulin and free fatty acid level. These tests require specific collection techniques described

here. Samples were analyzed in the Core Biochemical Assay Laboratory (CBAL) on the Cambridge Biomedical Campus.

2.3.1 GLP-1 levels

GLP-1 is rapidly degraded in plasma from the active GLP-1 (7-36) amide to GLP-1 (9-36) amide by the enzyme DPP4. This degradation continues after blood samples have been withdrawn and therefore this process needs to be halted to allow accurate assessment of plasma levels of GLP-1.

Blood for GLP-1 assays was drawn up into pre-prepared 2ml syringes containing 20µL DPP4 inhibitor (Milipore, UK). These syringes were chilled prior to collection and the blood sample was immediately transferred to 2.5ml EDTA tubes, which had also been prepared, containing the protease inhibitor aprotinin (Trasylol). These samples were stored on ice until they were spun and stored at -20^{o}C.

2.3.2 Insulin and free-fatty acids

Samples for insulin and free fatty acids were also collected. Bloods was collected into lithium-heparin tubes, which were also stored on ice prior to centrifugation and storage at -20^{o}C. All samples were spun within 1 hour of collection.

2.4 Dobutamine stress echocardiography

2.4.1 Introduction

DSE is widely used in clinical practice for the detection of ischaemia, for assessment of the impact of known coronary stenoses and for the assessment of functional reserve in patients with heart failure or valvular pathology. The test allows rapid quantification of both ischaemia and viability within the regions of the LV(186).

Myocardial ischaemia can be assessed with a number of criteria. Overall assessment of global LV function can be made through measures such as EF and mitral annular systolic velocity (MASV). Visual assessment of individual regions of the myocardium can be made through assessment of wall motion. These can be scored on the number of segments affected and the severity of the wall motion abnormalities.

Visual assessments are useful in clinical practice but are subject to a high degree of both intra- and inter-observer variability – even amongst experts with a high volume practice – with inter-observer agreement as low as 43% when image quality was sub-optimal(187). An alternative is a quantitative assessment of individual segments of myocardium through tissue Doppler imaging (TDI).

2.4.2 Tissue Doppler velocity imaging

Colour TDI is a standard function of modern cardiac ultrasound machines. It has been validated to measure myocardial velocity in order to assess global and regional myocardial function. Myocardial velocities are usually low (<20 cm/s) and lower in ischaemic or diseased myocardium. The amplitude of the signal is significantly higher than the surrounding blood (40dB) allowing a clear distinction between the myocardial tissue and blood inside the heart.

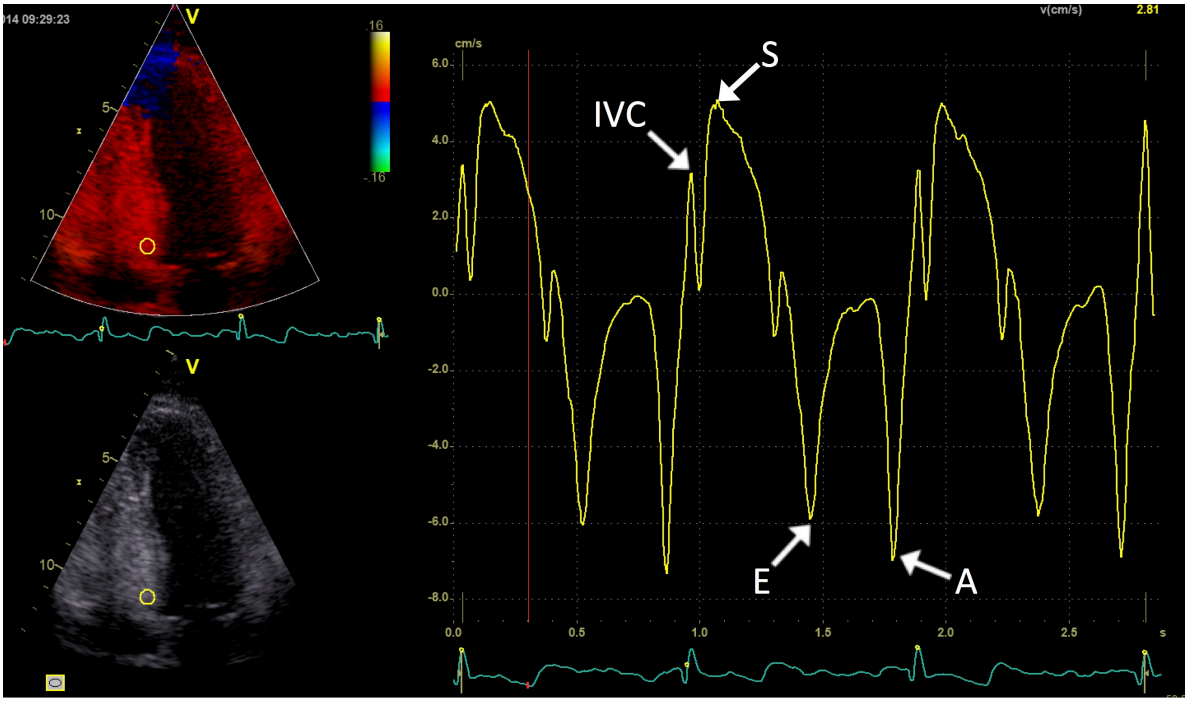
Colour TDI utilises the Doppler effect in order to distinguish between tissues moving towards and away from the probe. As tissue moves away from the ultrasound beam, the reflected signal has an increased wavelength whilst those segments moving towards the probe produce the opposite (a shortened wavelength). Myocardial velocities are represented by red (positive velocities) and blue (negative velocities) colour superimposed on a greyscale 2D ultrasound image of the myocardium. Colour TDI is superior to pulsed wave TDI (for this analysis) as it improves spatial resolution of the regions under investigation and the assessment of multiple regions captured during a single cardiac cycle. Optimization of the ultrasound frame-rate by using the minimum sector width possible ensures the most reliable assessment of myocardial velocities.

Images taken during stress echocardiography can be recorded digitally and transferred to an offline workstation for storage and analysis. Regional myocardial velocities are analysed by selecting the area of interest. Tracking of the region of interest throughout the cardiac cycle avoids sampling of the LV blood pool, which may introduce interference to the myocardial velocity profile generated.

The normal profile consists of a peak at maximum systole, preceded by a peak representing isovolumetric contraction. A transient fall in velocity between these two peak corresponds to the opening of the aortic valve(188). Two diastolic peaks (negative velocities) represent early and late diastolic relaxation – the late velocity augmented by atrial contraction. An example of a normal profile is shown in **Figure 2.1**.

Tissue Doppler assessment of regional wall motion has been shown to reproducible and consistent between observers(189,190). It has been validated in-vitro and against ventriculography and M-mode measurements(191). In animal studies, changes in tissue velocity occur rapidly after onset of ischaemia(192). Using TDI during DSE allows rapid assessment of these changes, ensuring that the effect of ischaemia can be measured.

Figure 2.1 - Tissue velocity profile



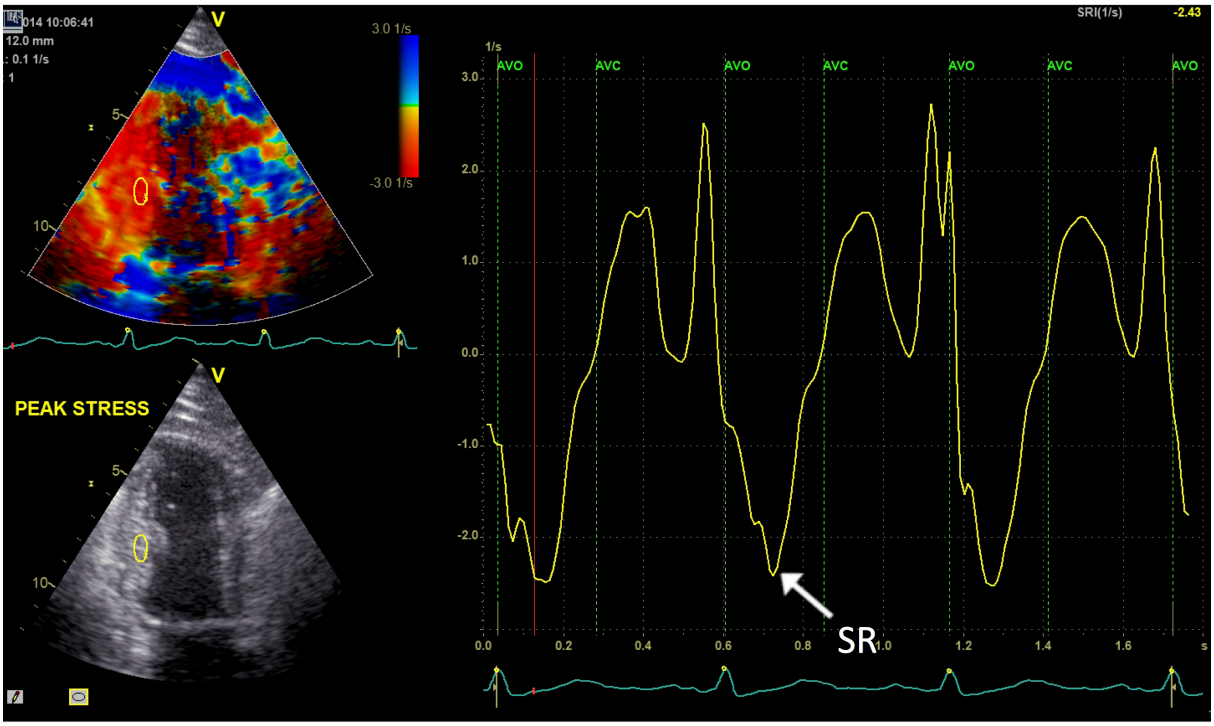
The peak of isovolumetric contraction (IVC) is followed by the opening of the aortic valve. A further peak represents peak systolic velocity (S). Following peak systole the closure of the aortic valve is seen as a shock wave before two diastolic troughs (E and A) representing passive filling and atrial contraction respectively.

2.4.3 Myocardial strain

A disadvantage of tissue Doppler velocity as a measure of myocardial function is the effect of extra-cardiac movement (e.g. with breathing) or tethering from adjacent segments on the analysis. These effects are known as translational effects. Myocardial strain and strain rate are measures of myocardial deformation derived from TDI, which avoid these translational effects by taking into account local velocities(193).

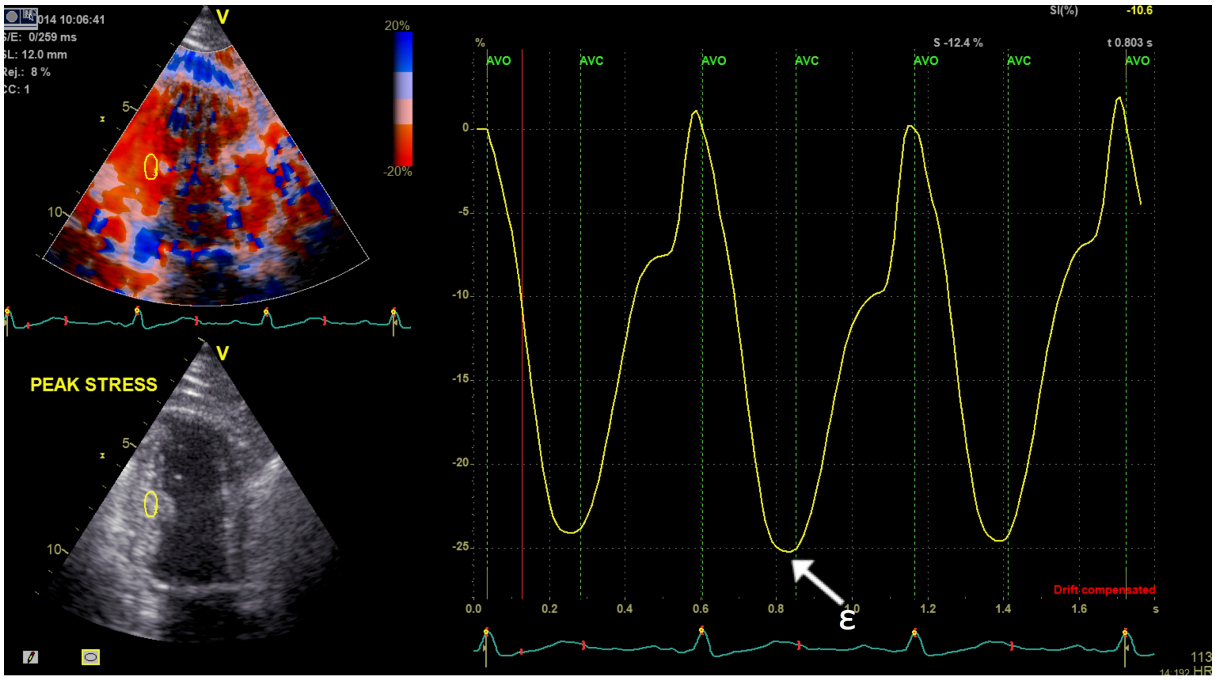
Myocardial strain is defined as the deformation of a section of myocardium in comparison to its original state. Strain rate is measured in s^{-1} and correlates well with dP/dt_{max} (the maximum rate of isovolumetric contraction) – a measure of contractility and systolic function(194). Peak systolic strain rate is the maximum rate of deformation during systole. These measures have been validated against 3D CMR of the LV in patients with normal and infarcted myocardium(195). Strain is given as a percentage change in the strain from baseline. It is derived as the integral product of strain rate for time. Contraction of the ventricle produces a negative strain. Infarcted tissue does not contract and therefore shows minimal or no change during contraction. Examples of strain and strain rate profiles are shown in **Figure 2.2** and **Figure 2.3**.

Figure 2.2 - Strain rate profile



Peak systolic strain rate is the most negative point on the strain rate curve during systole (SR). In this case the peak systolic strain rate measured in the mid inferolateral segment as seen in the apical 2-chamber view during peak stress is shown.

Figure 2.3 – Strain profile



Peak systolic strain (ε) is the most negative point on the curve during systole. In this case the peak systolic strain measured in the mid inferolateral segment as seen in the apical 2-chamber view is shown.

In order to accurately calculate the peak strain and strain rate during systole it is important to define the systolic period of the cardiac cycle clearly. Systole can be identified on the tissue Doppler profile by the opening and closing of the aortic valve which produces a brief negative deflection after the spike of isovolumetric contraction (opening) and a shockwave before the E wave of diastole(closing)(196). These are marked in **Figure 2.1** above.

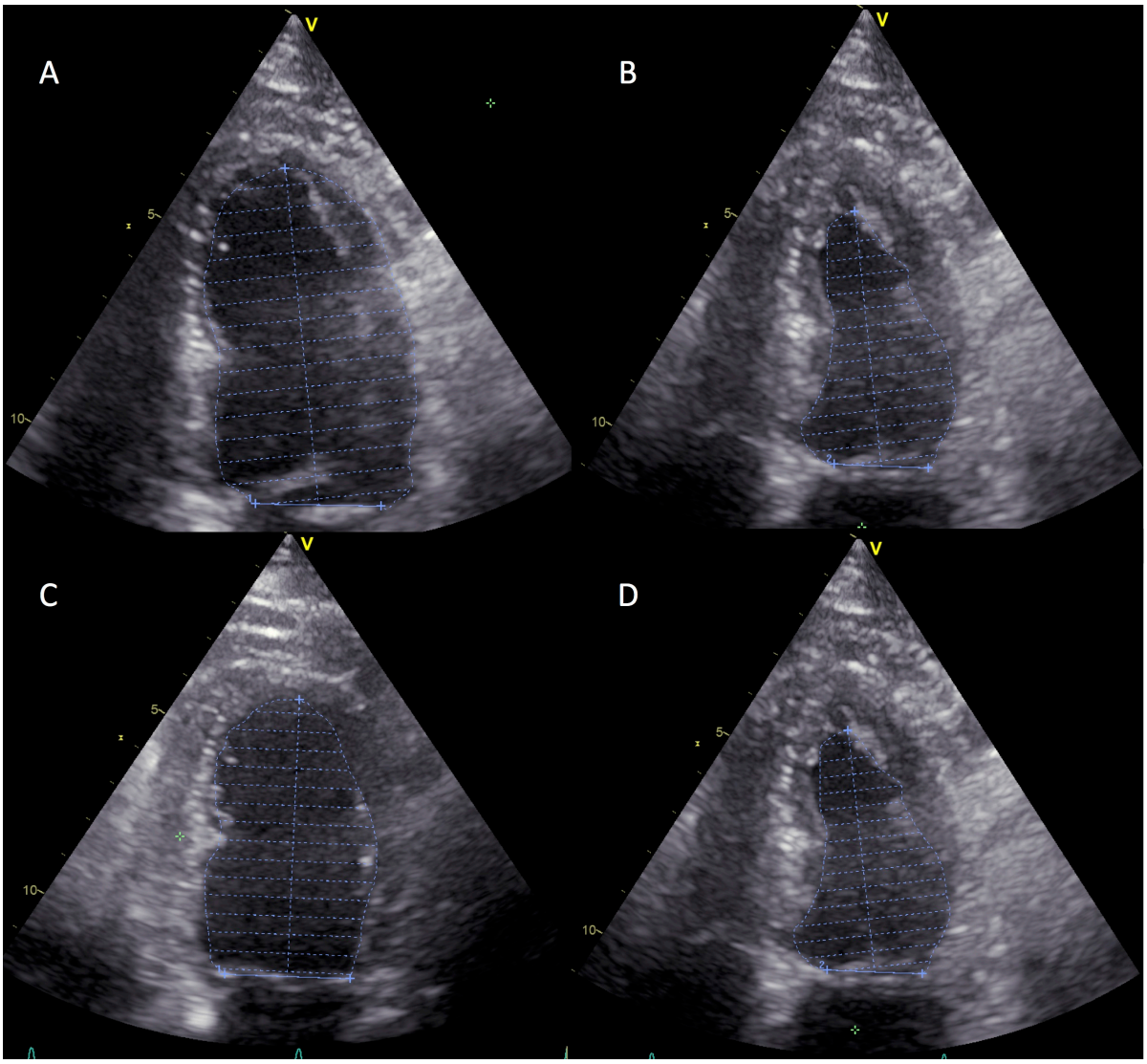
Despite the benefits of strain imaging it continues to have some disadvantages. It remains angle-dependent and, like most measures of contractility, it is load-dependent. In clinical practice, strain imaging is rarely performed due to its time-consuming nature. A non-invasive alternative to Doppler echocardiography is 2D-speckle-tracking echocardiography (2D-STE). This technique can overcome some of the disadvantages of Doppler echocardiography, particularly the angle dependent, and one-dimensional nature of the technique. However, using 2D-STE to assess myocardial deformation is less accurate than Doppler strain imaging during DSE in the RCA and circumflex territories. These difficulties are mainly due to undersampling at the elevated heart rates achieved during peak stress. This thesis does not use 2D-STE as this disadvantage outweighs its benefits given the need to assess all areas of the myocardium that are ischaemic at peak stress.

2.4.4 Global assessment of systolic function

EF is the most commonly used measure of LV systolic function used in routine clinical practice. Assessment of LV EF with echocardiography can be made in a number of different ways. The most basic assessment is a visual estimate, performed by an experienced operator, usually after visualizing the LV in the apical 2, 3 and 4 chamber views. Whilst this may be a useful assessment in clinical practice, its

variability and subjectivity mean that it is not a useful for a research study. A quantitative assessment of EF is to use a Simpsons Biplane Method, in which the LV volume is calculated from two apical views at both end-systole (maximum inward excursion of the endocardial border) and end-diastole (closure of the mitral valve). Change in volume can then be used to calculate EF. Accurate assessment of EF using this technique depends upon clear visualization of the endocardial border (to allow its tracing) on a 2D greyscale image. An example of a Simpsons Biplane method to assess EF is shown in **Figure 2.4**.

Figure 2.4 - Simpsons biplane assessment of EF



Example of a Simpson Biplane measurement. A 4-chamber view in diastole (A) and systole (B), with a 2-chamber view also shown (C and D).

2.4.5 Mitral annular systolic velocity as a global measure of systolic function

Mitral annular systolic velocity (MASV) is a TDI-derived index of global myocardial contractility that is well correlated with EF and dP/dt_{max}. It is the velocity of the mitral

annulus (averaged from 6 sites seen in three apical views) towards the ultrasound probe in peak systole(197). The index is more sensitive than EF to changes in systolic function during dobutamine infusion.

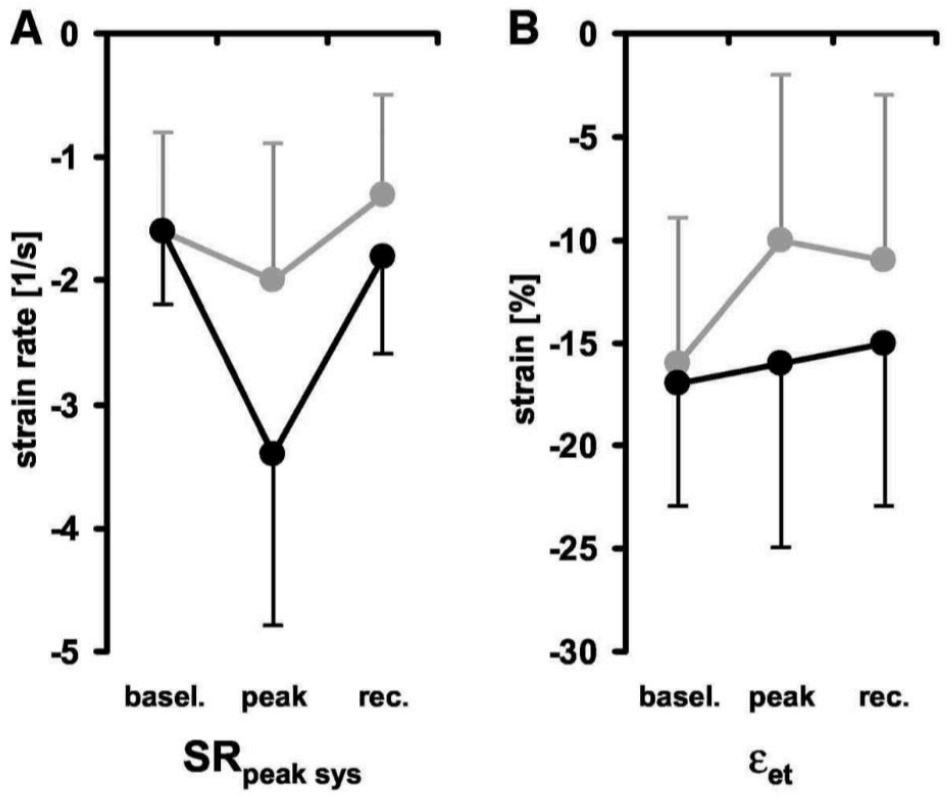
2.4.6 Tissue Doppler in stress echocardiography

Stress echocardiography is used to assess the presence or absence of ischaemia, the contractile reserve of the ventricle in heart failure, or in the case of this thesis, the effect of known ischaemia-inducing coronary stenoses on the function of the LV.

Tissue velocity, strain and strain rate have been shown to be useful modalities during stress echocardiography with both dobutamine and exercise(193,198). Using these modalities allows interpretation of stress echocardiography with less expertise and increased reproducibility compared to visual wall motion analysis. In addition, the quantitative nature of the assessment improves its usefulness as a research tool. Ischaemia can be demonstrated within 15 seconds of onset with TDI.

The ability to distinguish between ischaemic and non-ischaemic segments is an important aspect of TDI. Tissue velocity usually rises with stress (either dobutamine or exercise). A fall or failure to rise indicates an ischaemic segment. Changes related to the onset of ischaemia are detected rapidly. Overall strain rate also rises in magnitude with stress, whilst this rise is blunted or absent in ischaemic segments. Strain initially rises in response to dobutamine but falls to baseline levels at peak stress – effectively meaning that no significant change is seen at peak stress in the normal heart. In ischaemic segments there is a decline in strain at peak stress(199). Expected changes in strain and strain rate are summarised in **Figure 2.5**.

Figure 2.5 - TDI Profiles in ischaemic and non-ischaemic Segments



Strain and strain rate in ischaemic (grey) and non-ischaemic segments (black) - adapted from Read et al(127)

2.4.7 Dobutamine stress echocardiography in this thesis

DSE were performed according to a standard clinical protocol used at Addenbrooke's hospital. Dobutamine (1mg/ml) was administered using an infusion pump at escalating doses. An initial dose of 10 μg/Kg/min was given for 6 minutes followed by escalation to 20, 30 and 40 μg/Kg/min every three minutes. Up to 2mg of atropine could also be given if needed. The maximum length of dobutamine stress was therefore 15 minutes. Criteria for discontinuation of dobutamine were:

- completion of test (15 minutes of dobutamine stress)
- achievement of target heart rate of (220-Age) * 0.85 BPM
- ischaemic changes on 12-lead ECG (ST segment deviation of > 2mm)
- angina
- severe arrhythmia
- increase in systolic blood pressure to >240mmHg
- reduction in systolic blood pressure to <100mmHg
- severe, symptomatic hypoglycemia
- patient request

Transthoracic echocardiography (Vivid E9, GE Healthcare, UK) was performed with the patient in the left recumbent position. Images were acquired at rest, peak stress and 30 minutes recovery. At least three cardiac cycles in 2-, 3- and 4-chamber apical views were recorded in mid-expiration – this minimised the impact of respiration on tissue velocities. Cardiac cycles were captured with TDI and 2D greyscale for

comparison. Sector widths were minimised to increase the frame-rate. All images were transferred to a workstation for offline analysis.

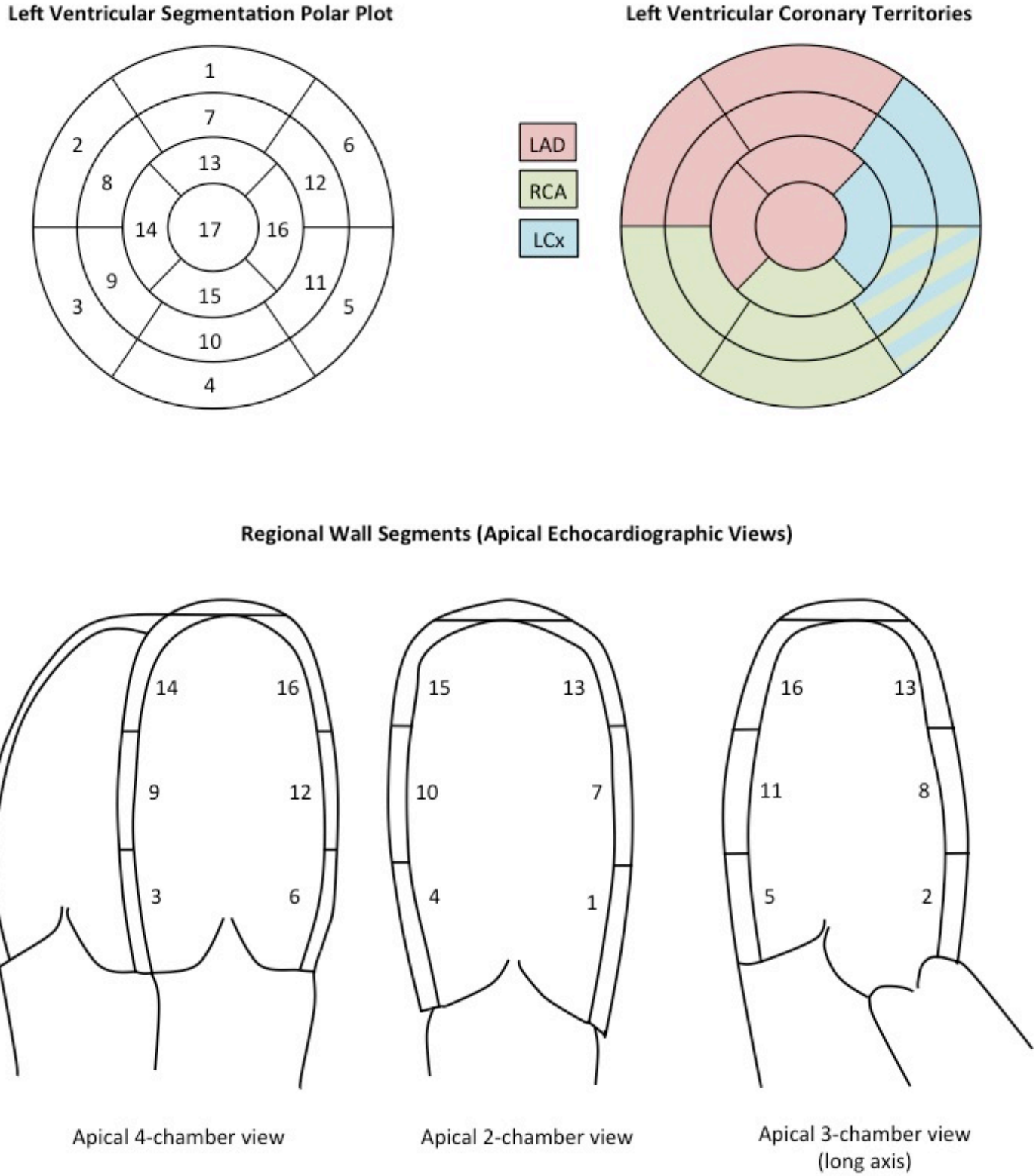
Offline analysis was performed on an Echopac workstation (GE Healthcare, UK). EF was calculated according to the Simpsons Biplane method described above. MASV was calculated from six sites, averaged over three cardiac cycles.

A 17-segment model was used for regional analysis. Since apical segments produced less reliable velocities (due to the technical difficulty of achieving a Doppler parallel to the segment, and increased tethering effects from basal and mid segments) they have been excluded(189). Therefore the remaining 12 segments have been used in this thesis.

All patients underwent recent coronary angiography for angina symptoms. A diameter stenosis of 50% or greater was considered to be a significant stenosis. Each segment in the analysis was assigned a coronary supply, allowing ischaemic and non-ischaemic segments to be compared(200). These are demonstrated in **Figure 2.6**.

Segmental analysis was performed for tissue velocity, strain and strain rate. Samples areas were placed in the basal and mid segments. A circular sample area was used for velocities with an ellipse for strain and strain rate. Sample areas were tracked throughout the cardiac cycle to ensure that the area remained within the same layer, and did not drift into the ventricular cavity.

Figure 2.6 - 17-segment model of left ventricle



1. Basal anterior
2. Basal anteroseptal
3. Basal inferoseptal
4. Basal inferior
5. Basal inferolateral
6. Basal anterolateral
7. Mid anterior
8. Mid anteroseptal
9. Mid inferoseptal
10. Mid inferolateral
11. Mid inferolateral
12. Mid anterolateral
13. Apical anterior
14. Apical septal
15. Apical inferior
16. Apical lateral
17. Apex (cap)

Polar plot and echocardiographic representation of regional segments. The coronary territories are variable although they can be ascribed as below. The inferolateral segments are assigned depending upon the coronary dominance.

2.5 Quantification of ventricular function during supply ischaemia – the conductance catheter

2.5.1 Introduction

Assessment of ventricular performance on a “beat-by-beat” basis during supply ischaemia can be performed with a conductance catheter, which can be placed in either the LV or the RV via the femoral artery or femoral vein respectively. This allows generation of pressure-volume (PV) loops (plots of pressure against volume within the ventricle) from which multiple parameters of cardiac performance can be generated(201).

2.5.2 Pressure-volume Loops

The cardiac cycle is made up of 4 phases relating to changes in pressure and volume within the ventricle. The cardiac cycle in the LV is described.

At the end of diastole, electrical activation of the ventricle initiates contraction of the myocardium. The pressure inside the ventricle rises quickly and when the pressure exceeds that of the left atrium then mitral valve closes. Because the pressure in the aorta remains greater than that of the LV (and hence the aortic valve is closed) the pressure continues to rise whilst the volume of blood within the chamber remains unchanged.

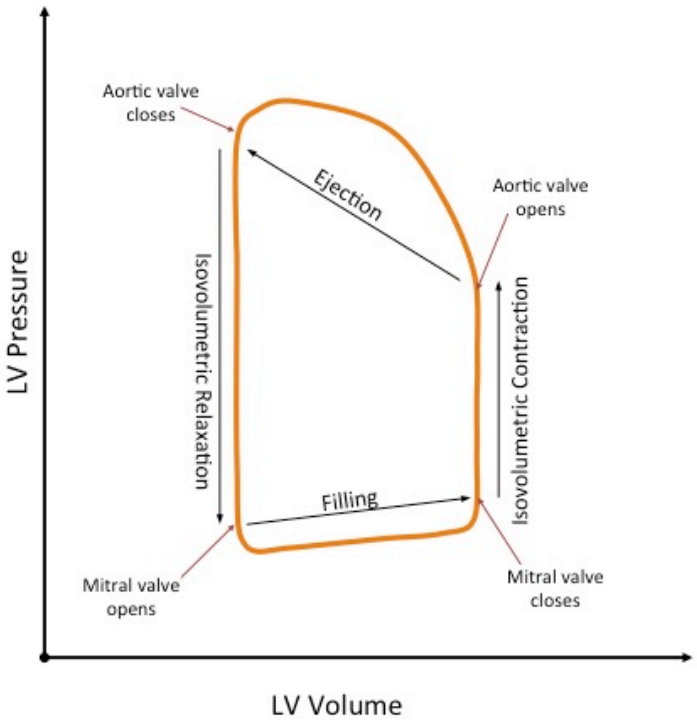
As the LV pressure exceeds that of the aorta, the aortic valve opens and blood is ejected from the chamber. LV pressure reaches a peak and as the myocardium begins to relax, the ventricular pressure falls.

When the pressure falls below that of the aorta, the aortic valve closes and the ejection of blood is complete. The pressure continues to fall without changes in volume until the pressure is below that of the atria. This is known as isovolumetric relaxation.

At this point filling of the ventricle occurs, as the mitral valves open and blood enters (atrial pressure is greater than ventricular pressure). Ventricular filling is augmented by atrial contraction, which further elevates atrial pressure.

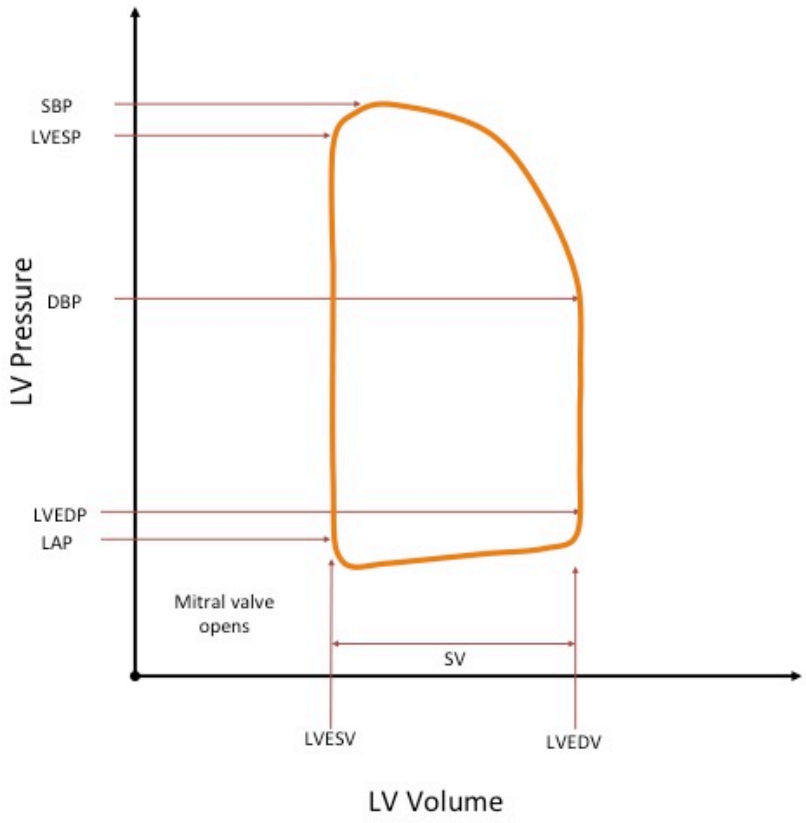
These changes in volume and pressure can be plotted on a graph to create a pressure-volume loop (PV loop). **Figure 2.7** shows an illustration of a PV loop. The changes in the volume can be seen on the x-axis whilst the y-axis shows changes in LV pressure.

Figure 2.7 - Left ventricular pressure-volume Loop



The PV loop demonstrates a number of haemodynamic parameters, which are immediately visible. These are shown in **Figure 2.8** and include pressure measurements such as systolic blood (SBP), diastolic blood pressure (DBP), LV end-systolic pressure (LV ESP), LV end-diastolic pressure (LV EDP) and left atrial pressure (LAP). Volume parameters such as stroke volume (SV), end-systolic volume (ESV) and end-diastolic volume (EDV) can also be read directly from the loop.

Figure 2.8 - Haemodynamic measurements displayed on a pressure-volume loop



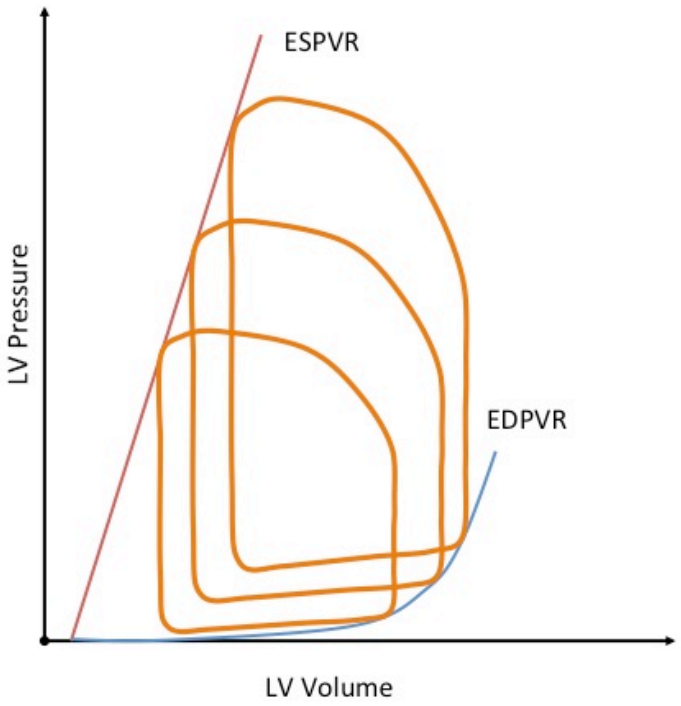
2.5.3 Pressure Volume Relationships

The PV loop allows an assessment of the relationship between pressure and volume. This provides for a more detailed analysis of systolic and diastolic ventricular function. There are two important relationships that can be derived which have been shown to be load-independent – i.e. they are not dependent upon either the preload (the atrial filling pressure) of the afterload (the systemic resistance to ejection – in effect the blood pressure). These relationships are known as end-systolic pressure volume relationship (ESPVR) and the end-diastolic pressure volume relationship (EDPVR)(202).

The ESPVR relationship can be expressed on the PV loop as a line providing a boundary to the upper left corner of the PV loop. The stiffness (end systolic elastance) of the ventricle is the slope of the line. The EDPVR is also expressed as a non-linear curve on the PV loop bounding the lower right corner of the loop. The diastolic compliance is the reciprocal of EDPVR. When EDPVR rises and there is reduced diastolic compliance in the LV, patients may experience diastolic heart failure. This is often described as a stiffening of the ventricle.

The ESPVR and EDPVR can be calculated in a patient by producing multiple PV loops with different load conditions – for example by alteration of patient fluid status or by balloon occlusions in the inferior vena cava (IVC) to adjust right atrial filling pressure (the preload). An example of these loops and the ESPVR and EDPVR are illustrated in **Figure 2.9**.

Figure 2.9 - ESPVR and EDPVR in the left ventricle

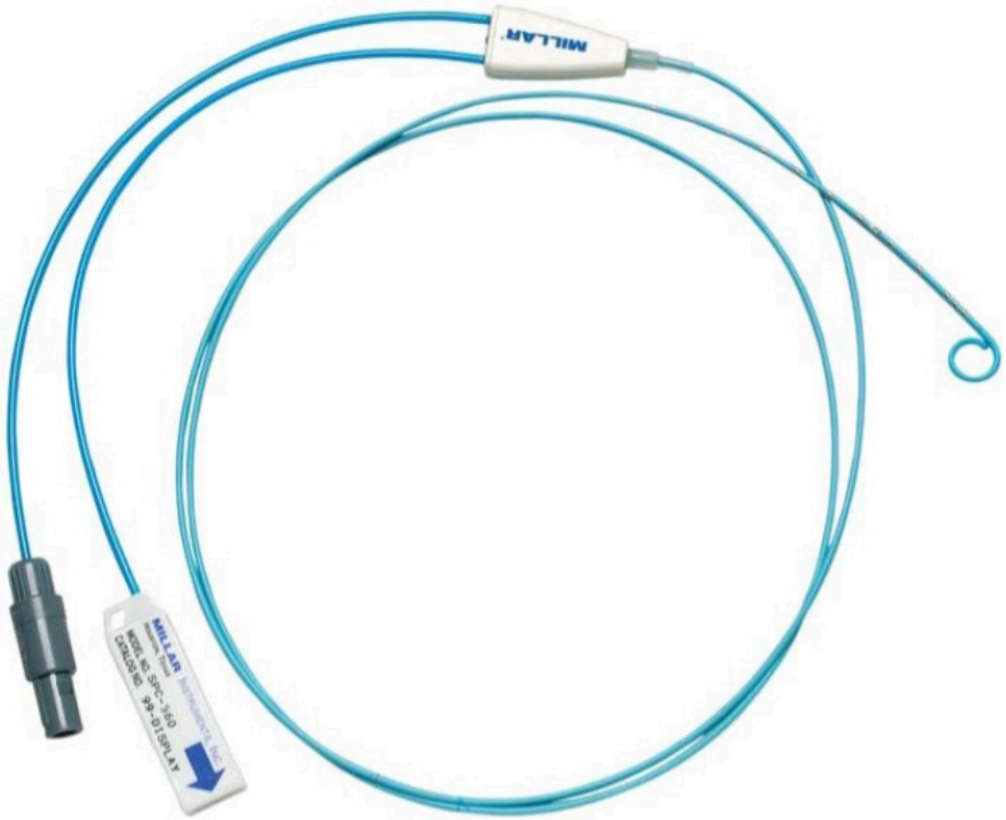


In the studies included within this thesis, ESPVR and EDPVR have not been calculated for ethical reasons. Additionally it was impractical to produce multiple PV loops at different preloads during short coronary balloon occlusion.

2.5.4 Use of conductance catheter to measure time-varying ventricular volume

The conductance catheter system (Millar Instruments, USA) used in the studies for this thesis is a closed ended pigtail catheter (7 French) with pressure sensors and 8 electrodes for simultaneous pressure and volume recording. It is shown in **Figure 2.10**. It is connected to a signal conditioning unit (Millar Instruments, USA).

Figure 2.10 Conductance catheter



The catheter measures volume according to a technique described by Baan et al. The time varying conductance, G(t), of a 20kHz current applied to the proximal and distal electrodes is recorded by the 6 remaining electrodes. It is calculated as the sum of the 5 measures of conductance between pairs of electrodes. LV volume, varying with time, V(t), is therefore calculates as:

$$V(t) = (1/\alpha) \times (L^2/\sigma) \times G(t) - V_c$$

α is the ratio of conductance derived volume to the true ventricular volume, L is the inter-electrode distance, σ is the specific conductance of blood measured at the

beginning of the study and V_c is a volume correction accounting for parallel conductance through surrounding structures outside the ventricular cavity.

$$V_c = (1/\alpha) \times (L^2/\sigma) \times G_p$$

G_p is parallel conductance, and is measured by hypertonic saline technique(201). Rapid injections of 10% saline (5-10ml each) into either the pulmonary artery or IVC (depending upon either left or right ventricular assessment respectively). These small injections increase ventricular conductivity without significantly affecting volume. A regression analysis of EDV versus ESV during transient increased conductivity leads to a linear relationship. This relationship can be extrapolated to the point of zero blood conductivity (LVEDV=LVESV), with the residual volume giving an estimate of the volume outside the ventricular cavity. The α coefficient is the ratio between the conductance catheter derived cardiac output and the Fick method calculated cardiac output.

2.5.5 Calculating indirect Fick cardiac output

The Fick principle is that the flow of blood to an organ (in this case the lungs) can be calculated if the amount of a marker substance taken up per unit time, the arterial concentration of the substance and the venous concentration of the substance are all known. In the case of cardiac output this can be considered equivalent to pulmonary blood flow if there are no shunts present. Cardiac output is therefore:

$$CO = (VO_2)/(C_{arterial} - C_{venous})$$

The patients VO_2 is an estimate calculated by multiplying the body surface area by estimated basal O_2 consumption rate (125mlO_2/min/m2). A direct Fick study would measure oxygen consumption to calculate this. $C_{arterial}$ and C_{venous} are calculated from the oxygen saturation of blood (SO_2), using the fact that each gram of haemoglobin carries 1.34ml 0_2. The arterial sample is taken from the femoral artery whilst the venous sample is taken from the pulmonary artery. For either sample:

$$C = Hb \times SO_2 \times 1.34$$

2.5.6 Analysis of pressure-volume loops

Offline analysis of PV loops will be performed using Labchart software (AdInstruments, New South Wales, Australia). Assessment of systolic function will include the parameters of SV, SW, EF and dP/dt_{max} (the maximal rate of isovolumic pressure rise). For diastolic dysfunction, the parameters assessed will be dP/dt_{min} (the maximal rate of isovolumic pressure fall), EDP and Tau (time constant of isovolumic relaxation). Assessment of ESPVR and EDPVR will not be assessed for practical and ethical reasons.

Tau is a calculated value. The conductance catheter derived P_t (time constant of pressure relaxation) is measured from the time of peak rate of pressure decline (dP/dt_{min}) to 5 mmHg above end diastolic pressure. Tau is derived from the monoexponential decay of the pressure waveform:

$$P_t = K\, e^{-t/Tau}$$

Tau is the slope of the log P_t versus t relation (Tau = -1/slope, assuming $P_\infty = 0$).

2.5.7 Use of LV conductance catheter based PV loop analysis in humans

The use of the conductance catheter as a research tool to assess LV function has been established in human studies(8,203). As discussed in the introduction it has been used to demonstrate the protective effect of GLP-1 against non-lethal ischaemia in humans(126,131). The calculations from the conductance catheter are well validated against echocardiography, cardiac MRI and post-mortem ventricular casts(204,205). The advantage of using conductance catheter technology is the generation of “beat-to-beat” data, which allows assessment of rapid changes in contractile function in a practical fashion.

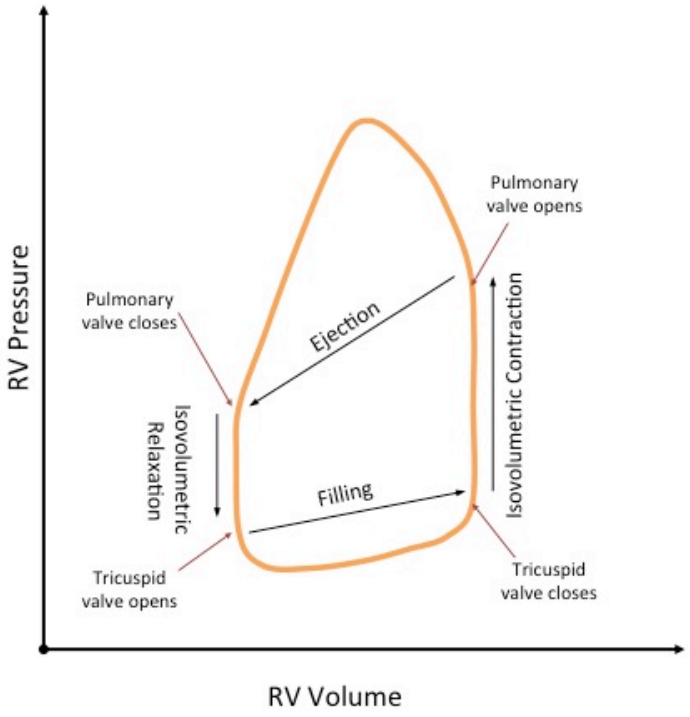
2.5.8 Conductance catheter assessment of the right ventricle

In this thesis, both LV and RV assessment using conductance catheter techniques will be employed. The description offered above refers to the LV conductance catheter measurements but the principles of assessment of the RV are similar(206). However there are important caveats to its use.

The advantage of conductance catheter assessment of the RV is the ability to obtain beat-to-beat changes in RV function. In addition, the complex geometry of the RV is difficult to assess with other methods such as echocardiography. Although the mathematical assumptions behind volumes in the conductance catheter are based upon the cylindrical geometry of the LV, there is a considerable body of evidence that they can also be applied to the RV, including close agreement with measurements taken at post-mortem(207) and with cardiac MR(208).

In addition to its complex geometry, the RV also differs from the LV at it is, under normal circumstances, connected to a low impedance pulmonary vascular system. The pulmonary vasculature is associated with lower resistance than the systemic circulation, and greater arterial distensibility – arterial capacitance. These differences result in a different shape to the pressure-volume loops - more triangular than those derived from LV conductance catheter recordings. This is because the low impedance and high capacitance of the pulmonary circulation allows the ejection phase to continue even as pressure continues to decline (**Figure 2.11**). The isovolumic relaxation phase of the cycle is significantly shortened or even eliminated. Where a patient has increased afterload on the RV (significant pulmonary hypertension or pulmonary stenosis) the PV loop is similar to that of the LV (with an end-systolic shoulder) because of reduced capacitance in the pulmonary vasculature. It has been noted that in patients attending for diagnostic cardiac catheterisation, a large proportion will have an end-systolic shoulder on their RV loops consistent with elevated afterload(209).

Figure 2.11 - Right ventricular pressure-volume loop



2.5.9 Use of RV conductance catheter based PV loop analysis in humans

The RV has a more irregular, crescentic shape compared to the cylindrical LV. Furthermore, the thin-walled RV provides less resistance to conductance and increases the likelihood of error in assessment of parallel conductance(210). This lead to concerns over the applicability of the conductance catheter to assessment of the RV, in particular with regard to volume assessment. However, the use of the conductance catheter to assess RV function has been validated against cardiac MRI and ventricular casts despite its complex geometry (207,208). Although conductance catheter studies have been shown to be poor determinants of absolute ESV and

EDV in the RV, accurate assessment of SV was possible. RV function during RCA balloon has been assessed in a previous human study, although the effect of ischaemia on stunning and cumulative dysfunction was confounded by the failure to assess the RV beyond the moments after reperfusion (see the discussion of the effect of hyperaemia during reperfusion later)(92).

2.5.10 Assessment of ischaemic dysfunction and stunning using conductance-catheter

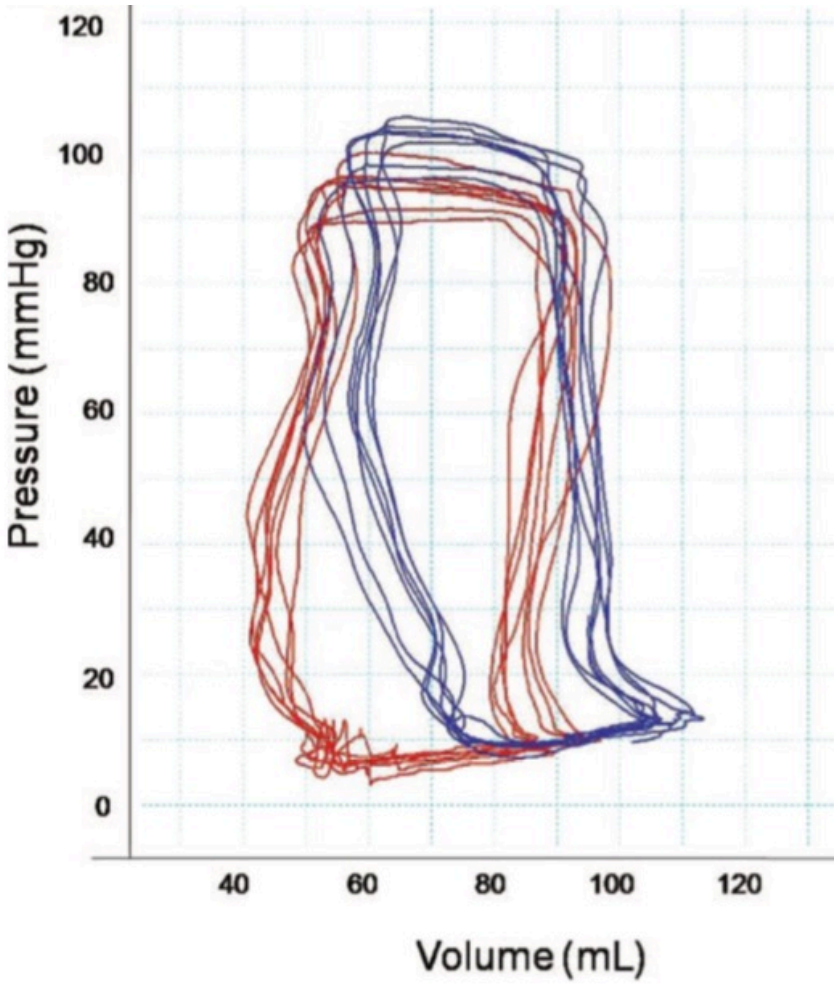
In the studies included in this thesis, conductance catheters are used to assess the degree of ventricular dysfunction during and after ischaemia. Balloon occlusion of the coronary artery (either left anterior descending or right coronary artery depending upon the study) is the source of supply ischaemia.

In previous conductance catheter studies, measurements were taken before and after 1-minute low-pressure coronary balloon occlusion. These measurements were repeated before and after further coronary balloon occlusion at 30 minutes. Coronary balloon occlusion leads to ischaemic dysfunction in the LV and there is absence of complete recovery at 30 minutes (stunning). Further occlusions lead to an increase in ventricular dysfunction, compared to the first occlusion(8). This is termed cumulative stunning. PV loops shown in **Figure 2.12** demonstrate changes associated with ischaemic dysfunction in the LV whilst **Figure 2.13** shows cumulative LV measured by dP/dt_{max}, EF, dP/dt_{min} and Tau.

Conductance catheter evidence of stunning in the RV is limited although animal models using LV conductance catheters have suggested that RV performance

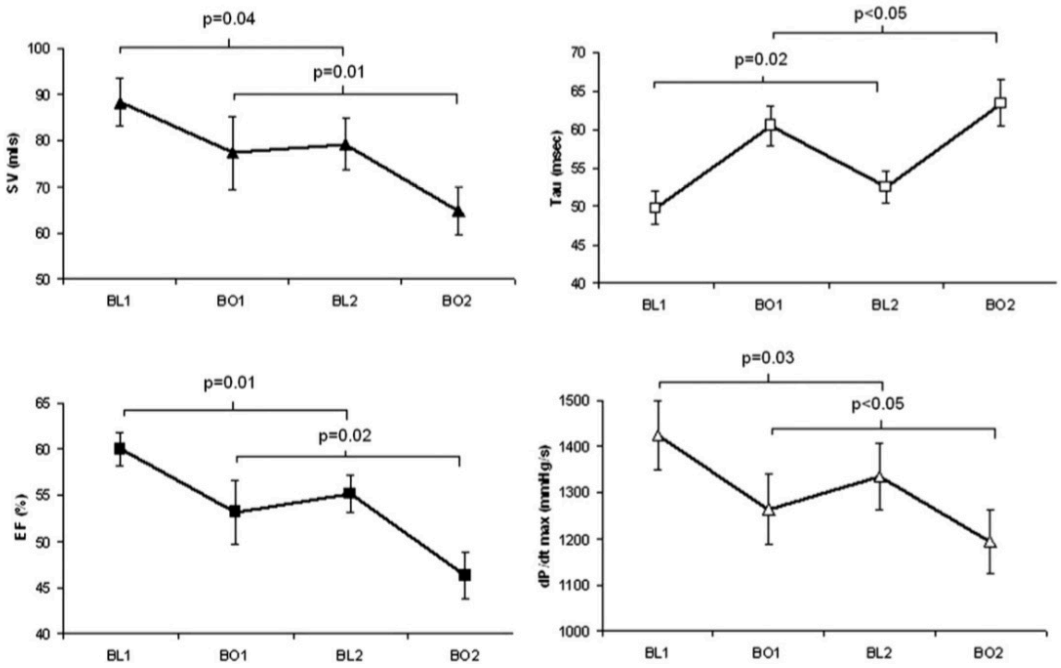
deteriorates with multiple balloon occlusions in the RCA(211). Human studies have shown a decline in RV stroke volume with single coronary balloon occlusion of the LAD or RCA(92). Other studies have suggested rapid improvement in RV function with reperfusion(212) although this may be confounded by the effect of hyperaemia (discussed later).

Figure 2.12 - Example pressure-volume loops at rest and during ischaemia



PV loops at rest (red) and after a one-minute balloon occlusion (blue). There is a shift to the right with a rise in end diastolic pressure, and fall in stroke volume and stroke work. From Read et al(126).

Figure 2.13 - Pilot data demonstrating stunning and cumulative dysfunction of the left ventricle



Stunning and cumulative LV dysfunction following coronary balloon occlusion in humans. **BL1** = baseline. **BO1** = balloon occlusion 1. **BL2** = baseline 2 (30-minutes after balloon occlusion). **BO2** = balloon occlusion 2. SV = stroke volume. EF = ejection fraction. Tau = time-constant of pressure relaxation. dP/dt_{max} = maximal rate of pressure increase. Mean±SEM. Adapted from Hoole *et al*(8).

Early human studies into cumulative stunning were confounded by the Gregg effect. This occurs when reactive hyperaemia following ischaemia causes stretch-activated channels in the microvasculature to open. An influx of calcium leads to an augmentation of myocardial contractility. LV function may briefly exceed baseline levels. The effect occurs in the first few moments after restoration of flow, and can be illustrated with conductance catheter measurements at 30 seconds, or 1 minute, after balloon deflation. This effect meant that human evidence of stunning was initially missed(92,203).

Chapter 3 Mechanism of GLP-1 induced protection against ischaemic left ventricular dysfunction and stunning in demand ischaemia

3.1 Introduction

GLP-1 has been shown to protect against ischaemic LV dysfunction during demand ischaemia and myocardial stunning during recovery from that ischaemia. As discussed in the introduction, considerable evidence has emerged from animal studies that the pathways of IC are responsible for GLP-1 mediated cardioprotection. The aim of this study was to establish whether the cardioprotection offered during demand ischaemia in humans is mediated through these pathways. Understanding the pathway of cardioprotection will provide insight into how best to target cardioprotection. This may be with combinations of treatment to a variety of different targets or with drugs that are more specific.

Glibenclamide is a sulfonylurea, which blocks KATP channels. Sulfonylureas are used to treat type 2 diabetes mellitus. They bind to KATP channels on the sarcolemma of pancreatic beta cells precipitating the release of insulin. Whilst modern sulfonylureas such as gliclazide are more specific to this KATP channel, glibenclamide (which is no longer routinely used in modern UK clinical practice) has non-specific binding to KATP channels including the mKATP channel, which is responsible for mediating the protective effects of the RISK and SAFE pathways.

We therefore set out to establish whether pretreatment with the sulfonylurea glibenclamide abrogated the cardioprotective effect of GLP-1. The pharmacology of glibenclamide is discussed further in Chapter 2.

The study was approved by the Cambridge East Research Ethics Committee and was compliant with the declaration of Helsinki. The hypothesis for this study was that pretreatment with glibenclamide before a DSE during GLP-1 infusion would reduce EF at peak stress.

3.2 Methods

3.2.1 Patient selection

Patients requiring coronary revascularisation following diagnostic angiography at Addenbrooke's Hospital were included in the study. The inclusion criteria were:

- coronary artery disease requiring PCI or CABG
- normal LV systolic function (assessed by echocardiography or left ventriculography) – ejection fraction > 50%
- aged over 18 years with capacity to give consent for procedure

Exclusion criteria for the study were:

- significant valvular pathology or previous valve surgery
- presence of cardiac pacemaker or similar device
- diabetes mellitus
- significant co-morbidity with life expectancy of <6 months

3.2.2 Study design

Patients were allocated to undergo two DSE in random order, performed at least one week apart. Patients attended in the morning following an overnight fast. Patients received a GLP-1 infusion during both DSE, with and without oral glibenclamide 5mg given 60 minutes prior to the GLP-1 infusion. The DSE were otherwise identical.

It would have been preferable to increase the number of DSE that each patient in the study had to perform control and glibenclamide only DSE. However, the study was limited to two for ethical, practical and recruitment purposes.

3.2.3 Pre-procedure

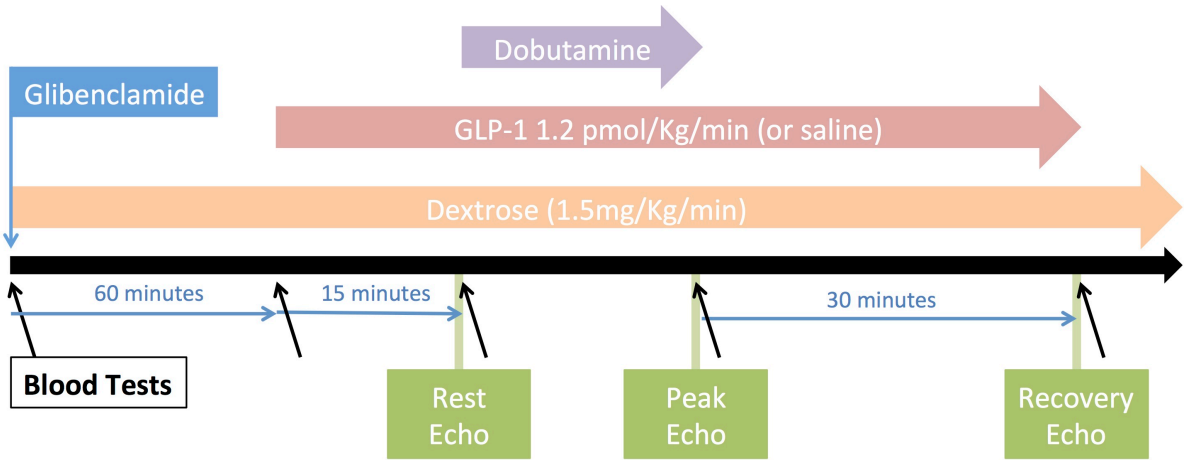
Patients were asked to abstain from caffeine, alcohol, sublingual or oral nitrates, and nicorandil for 24 hours prior to the procedure. Patients were fasted for a minimum of 6 hours prior to the procedure. Patients attended the clinical research facility at 0800 hours. Prior to the procedure a full history and clinical examination was performed.

3.2.4 Dobutamine stress echocardiograms

A timeline for the DSE protocol is shown in **Figure 3.1**. Baseline blood tests were taken. Glibenclamide 5mg orally was administered on the glibenclamide visit. A 20% dextrose infusion was initiated at 1.5mg/Kg/min. After 60 minutes a GLP-1 infusion was infused at 1.2 pmol/Kg/min. The delay of 1 hour before GLP-1 administration was based upon the discussion above (section 2.2.2) with peak activity of glibenclamide occurring between 1 and 3 hours after administration. Timings were therefore set to ensure that the DSE was completed within this window.

After infusion of GLP-1 for 30 minutes, a dobutamine stress echocardiogram was undertaken according to the protocol described in Section 2.4.7. GLP-1 infusion was continued for 30 minutes recovery. After completion of the DSE, protocol the dextrose infusion was continued until after the patient had eaten a carbohydrate rich meal.

Figure 3.1 - Timeline for DSE protocol



The timeline shows the administration of dextrose, GLP-1, glibenclamide and dobutamine during the study. The relationship of blood tests (black arrows) and echocardiography is shown.

3.2.5 Safety

The risks of this protocol reflect the combination of drugs used in the procedure.

Glibenclamide is a powerful oral hypoglycemic agent, which increases insulin secretion from the pancreas. In this study, a 20% dextrose infusion at a low 1.5mg/Kg/min background level was administered. This was designed to avoid stimulation of pancreatic beta cells to release insulin(185) and was up-titrated to maintain blood glucose levels if the patient experienced symptomatic or biochemical evidence of hypoglycemia. Blood glucose was monitored every 15 minutes over the first 4 hours following administration of glibenclamide and half-hourly after this. The frequency of this monitoring could be adjusted depending upon the clinical state of the patient. Following the procedure, the patients were given a carbohydrate rich meal and the dextrose infusion was weaned when possible. Patients were observed for a number of hours prior to discharge.

Patients gave written consent for risks associated with DSE, which included angina and arrhythmia.

3.2.6 Biochemistry

Baseline assessment of full blood count, urea, creatinine and electrolytes, lipid profile and HbA_1C was performed prior to the procedure on the patients first visit. These test were processed through the Addenbrooke's pathology service.

Venous samples were collected from the patient at multiple time points throughout both procedures:

- baseline
- prior to GLP-1 infusion
- prior to DSE
- peak stress
- 30-minute recovery

Samples were taken for GLP-1 (7-36) amide. As GLP-1 (7-36) amide is rapidly cleaved to GLP-1 (9-36) amide by the enzyme DPP4, the blood for GLP-1 assays was drawn up into pre-prepared 2ml syringes containing 20 µL DPP4 inhibitor (Milipore, UK). These syringes were chilled prior to collection and the blood sample was immediately transferred to 2.5ml EDTA tubes, which had also been prepared, containing the protease inhibitor aprotinin (Trasylol). These samples were stored on ice until they were spun and stored at -20^{o}C.

3.2.7 Statistics

Previous data has shown that a GLP-1 infusion caused an additional increase in EF at peak stress from 70.8±4.96% to 77.0±4.39% in a paired study(127). We therefore performed a power calculation based on this data to establish a sample size for the study. The study was powered to detect a 5% change in ejection fraction at 80% power between the two DSE in both groups. A paired t-test at the 5% level of significance was to be used.

A group number of 7 patients was calculated. In order to allow for missing data it was planned to collect 10 patients. Each patient acted as their own control with comparisons made between the two DSE. A paired sample Students t-test was used to compare the DSE with a p-value of <0.05 considered statistically significant. The primary endpoint was EF at peak stress. Secondary endpoints were MASV, peak systolic tissue velocity, strain and strain rate (at baseline, peak stress and recovery), as well as EF at baseline and recovery.

Inter-observer variability and intra-observer variability were assessed using images from three randomly selected patients. This was expressed as an Interclass Correlation Coefficient (ICC) with 95% confidence interval(213).

3.3 Results

3.3.1 Demographics

Ten patients were recruited into the study. Two patients did not go on to have both DSE (one patient withdrew and one needed urgent revascularisation prior to second DSE). Detailed demographic data are summarized in **Table 3.1**.

Overall median age was 65.5 years (57.5-71) with 9 (90%) male participants. There was CAD in 2.4 vessels. Patients had a number of baseline blood tests performed during their first visit. These results are shown in **Table 3.2**.

Table 3.1 Demographics of demand ischaemia population

	n=10
Age, median [IQR]	65.5 [57.5-71]
Male Sex, n (%)	9 (90)
BMI, Kg/m^2 (mean±SD)	30.7±4.89
Previous MI, n (%)	1 (10)
Active or Ex Smoker, n (%)	6 (60)
Hypertension, n (%)	4 (40)
Hypercholesterolemia, n (%)	4 (40)
Planned Revasc. (CABG), n (%)	5 (50)
Medication	
Beta Blocker, n (%)	6 (60)
ACE inhibitor, n (%)	6 (60)
Ca Channel Blocker, n (%)	0 (0)
Long acting nitrate, n (%)	4 (40)

Table 3.2 - Baseline blood test results prior to first DSE

Blood Test	n=10 (mean±SD)
Haemoglobin, g/L	144.5±11.6
Creatinine, µmol/L	77.2±17.2
Total Cholesterol, mmol/L	3.79±0.66
HDL Cholesterol, mmol/L	1.21±0.39
LDL Cholesterol, mmol/L	1.87±0.93
Triglycerides, mmol/L	1.40±0.68
HbA_1c, mmol/mol	37.7±4.36

3.3.2 Haemodynamics

There were no significant differences between the haemodynamics at peak stress with and without glibenclamide pretreatment. Importantly, GLP-1 infusion did not increase heart rate during the 15 minutes prior to the DSE in the GLP-1 only DSE (57±3.8 vs. 58±3.9, p=0.44).

Table 3.3 - Haemodynamics during dobutamine stress echo

	GLP-1	GLP-1 & Glibenclamide	p
Heart Rate (Baseline), BPM	60±10.5	64±8.6	0.23
Heart Rate (Peak Stress), BPM	115±11.5	119±14.2	0.12
Heart Rate (Recovery), BPM	71±11.9	67±9.1	0.34
Peak Systolic Blood Pressure, mmHg	159±35	157±32	0.82
Peak Diastolic Blood Pressure, mmHg	75±10	77±9	0.62
Peak Mean Arterial Pressure, mmHg	103±16	104±16	0.89
Peak Rate-Pressure product, mmHg.BPM	18059±2562	18675±3647	0.59

3.3.3 Biochemistry

During both DSE, GLP-1 levels at peak stress were 50-fold higher than pre-DSE levels. Insulin levels rose in both DSE but were twice as high for the GLP-1 + Glibenclamide group (p=0.04). There was increased suppression of free fatty acids with GLP + Glibenclamide. Biochemistry data for the study are tabulated in **Table 3.4** and shown in **Figure 3.2**, which includes historical control data for illustrative purposes**.**

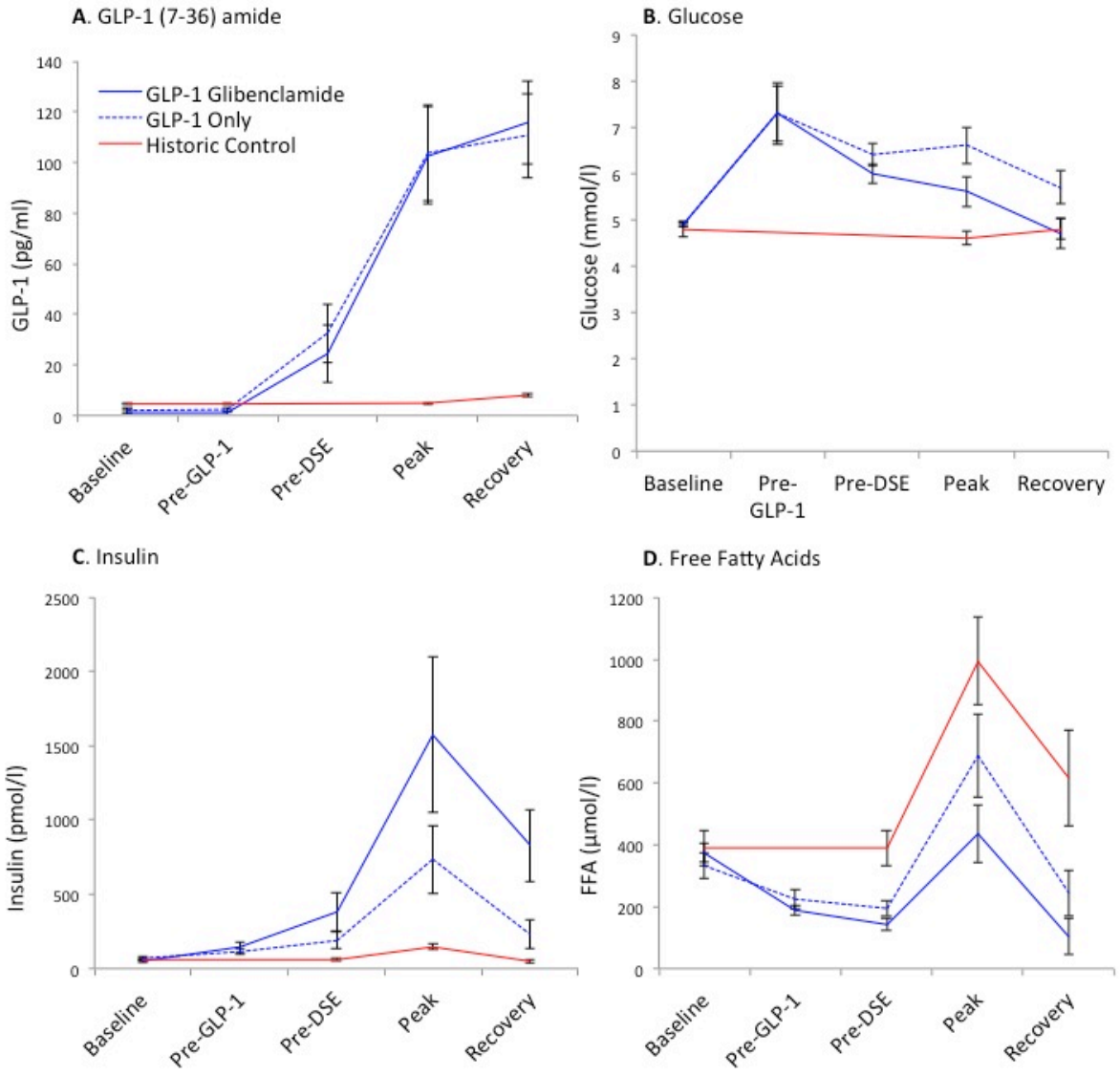
Table 3.4 Metabolic data from DSE protocol

	Baseline	Pre GLP-1	Pre-DSE	Peak Stress	30-minute recovery
GLP-1 (7-36) amide, pg/ml (mean±SD)					
GLP-1 Only	2.0±2.6	2.2±2.9	32.4±32.4	103.7±57.5	110.6±46.6
GLP-1 Glibenclamide	1.2±1.3	1.1±0.65	24.2±20.3	102.6±26.2	115.8±59.4
p-value	0.94	0.67	0.15	0.96	0.25
Insulin, pmol/L (mean±SD)					
GLP-1 Only	68±28	118±65	191±173	732±679	230±117
GLP-1 Glibenclamide	50±19	141±78	379±292	1577±1174	828±527
p-value	0.10	0.86	0.22	0.04	0.07

Glucose, mmol/L (mean±SD)					
GLP-1 Only	4.9±0.11	7.3±1.9	6.4±0.7	6.6±1.1	5.7±1.0
GLP-1 Glibenclamide	4.9±0.16	7.3±1.7	6.0±0.6	5.6±0.9	4.7±0.9
p-value	0.82	0.98	0.12	0.06	0.02
Free Fatty Acids, µmol/L (mean±SD)					
GLP-1 Only	332±126	223±95	195±74	687±402	243±179
GLP-1 Glibenclamide	374±66	188±35	144±45	435±210	103±79
p-value	0.07	0.80	0.46	0.14	0.31

Dextrose Infusion, $mg.Kg^{-1}.min^{-1}$ (mean±SD)					
Glibenclamide Only	1.5±0	1.5±0	1.5±0	1.5±0	1.5±0
GLP-1 Glibenclamide	1.5±0	1.5±0	1.5±0	1.5±0	1.9±0.6
p-value	-	-	-	-	0.17

Figure 3.2 Metabolic results for DSE study



Mean±SEM. Historic control data is based on Read et al(127). Note that in historic data, glucose was not infused. In the GLP-1 Glibenclamide arm, the dextrose infusion was increased at the recovery timepoint.

3.3.4 Global measures of systolic function

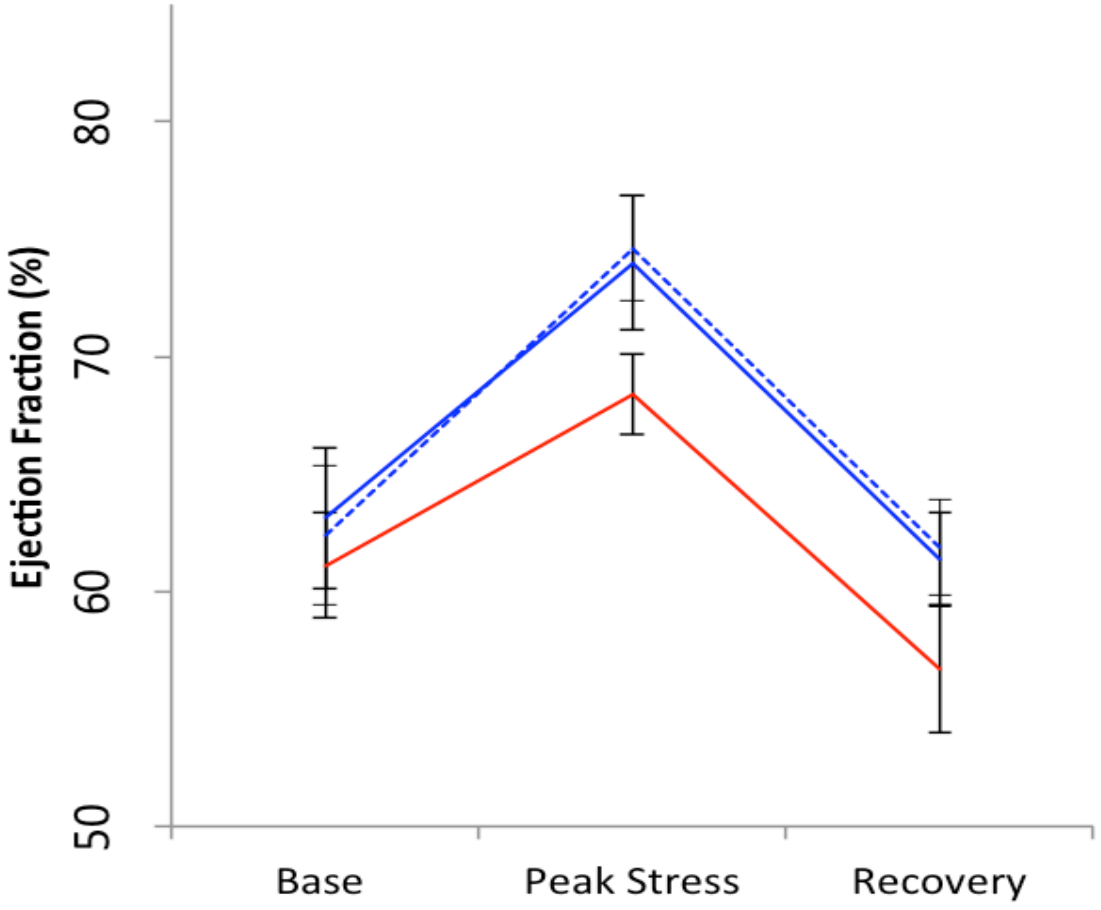
Only the eight patients who completed the full protocol (the two withdrawals are documented above) have been included in the paired analysis of systolic function. Of

note, the mean time from administration of glibenclamide to peak stress was 97.3±7.9 minutes. Results are tabulated in **Table 3.5**. Historical control data is shown in the figures for illustrative purposes. Historical data for *GLP-1 Only* overlapped the GLP-1 curve in this study and is not shown for clarity.

Ejection Fraction

EF was unchanged at baseline between the GLP-1 Only and GLP-1 + Glibenclamide DSE (62.3±8.4 vs. 63.1±8.5, p=0.45). At peak stress there was no change in EF with glibenclamide pretreatment (74.6±6.4 vs. 74.0±8.1, p=0.76). There was also no significant difference between EF in the two DSE at 30-minute recovery (61.9±5.7 vs. 61.4±5.6, p=0.74). There was no significant difference in EF between baseline and recovery in either DSE – i.e. there was no evidence of post-ischaemic LV stunning. **Figure 3.3** shows the change in ejection fraction for each DSE in the study.

Figure 3.3 Global LV function assessed with ejection fraction



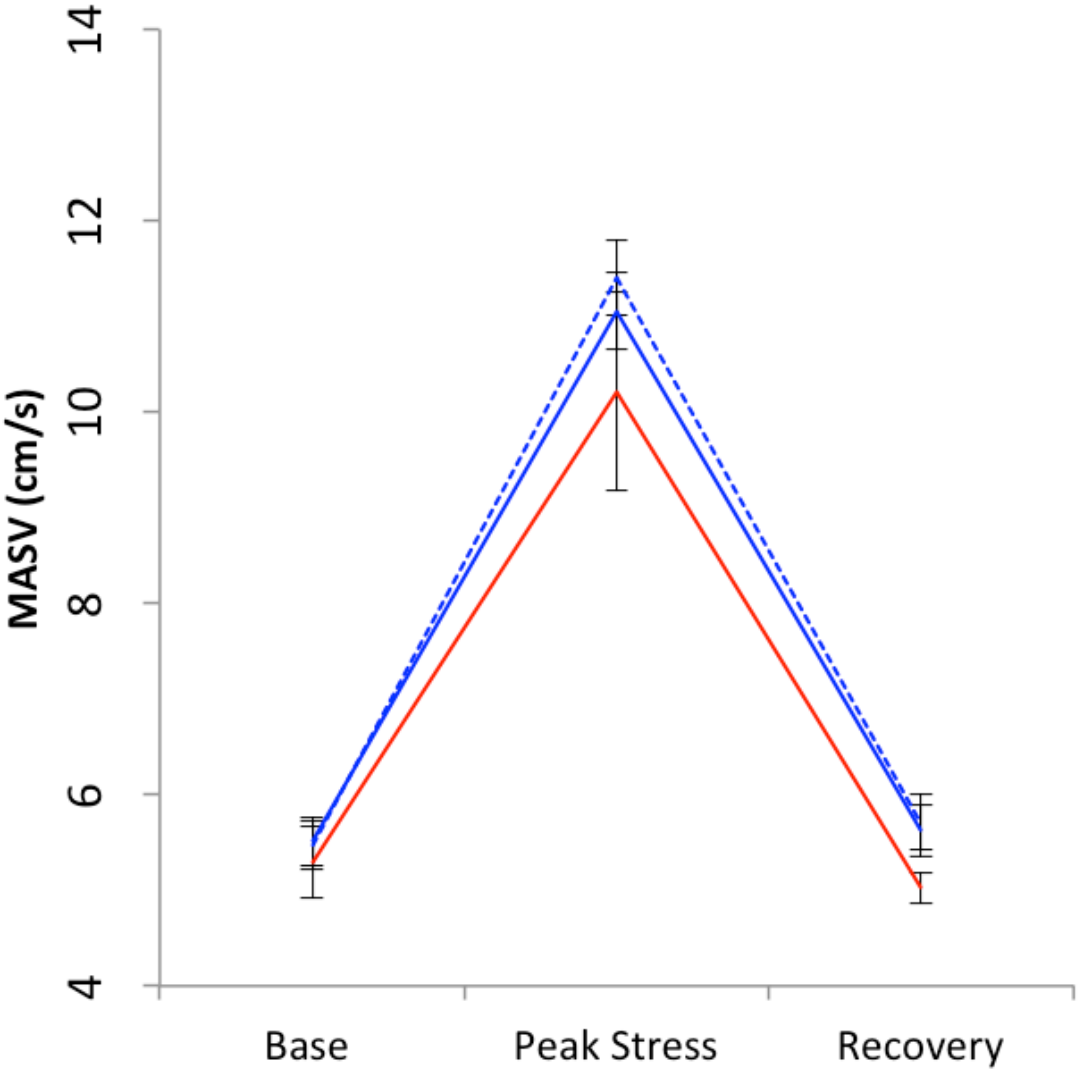
Mean±SEM. Dotted Blue = GLP-1 Only, Solid Blue = GLP-1 + Glibenclamide. Red = historical control data

Historic control data based on Read et al(127).

Mitral annular systolic velocities

There was no difference in MASV at baseline between GLP-1 Only and GLP-1 + Glibenclamide DSE (5.47±1.78 vs. 5.51±1.78, p=0.82). MASV was also identical at peak stress (11.4±2.69 vs. 11.1±2.80, p=0.24) and 30-minute recovery (5.70±1.99 vs. 5.62±1.75, p=0.51). Of note, there was no significant difference between baseline and recovery in either DSE. **Figure 3.4** shows MASV during each timepoint of the study.

Figure 3.4 Global LV function assessed with mitral annular systolic velocity



Mean±SEM. Dotted Blue = GLP-1 Only, Solid Blue = GLP-1 + Glibenclamide. Red = historical control data

Historic control data based on Read et al(127).

Table 3.5 Global LV function during DSE study

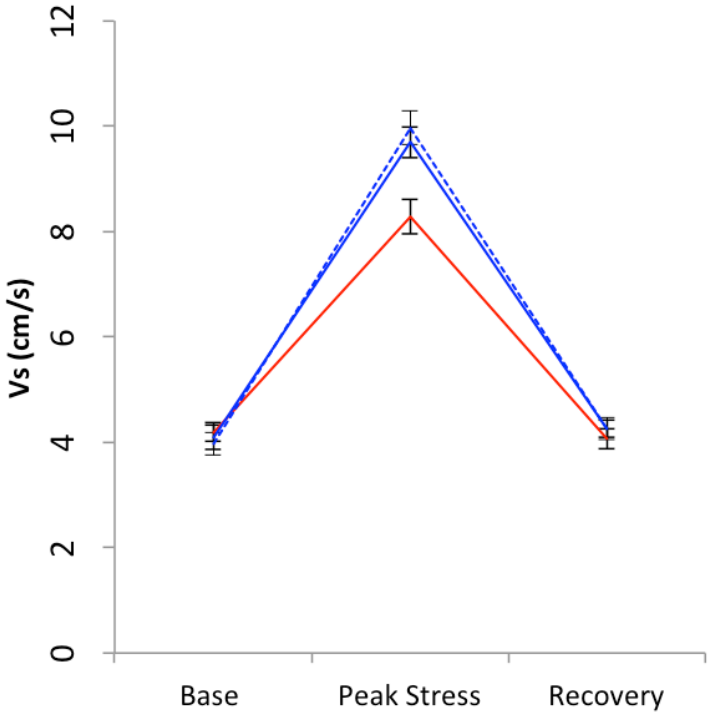
	GLP-1 Only	GLP-1 Glibenclamide	p-value
Ejection Fraction, % (mean±SD)			
Baseline	62.4±8.4	63.1±8.5	0.45
Peak Stress	74.6±6.4	74.0±8.1	0.76
Recovery	61.9±5.7	61.4±5.6	0.74
MASV, cm/s (mean±SD)			
Baseline	5.47±1.79	5.51±1.78	0.82
Peak Stress	11.40±2.69	11.06±2.80	0.24
Recovery	5.70±1.99	5.62±1.75	0.51

3.3.5 Regional assessment of systolic function

Peak systolic tissue velocities

There was no difference between GLP-1 Only and GLP-1 + Glibenclamide DSE at baseline (3.97±2.05 vs. 4.09±2.21cm/s, p=0.45). At peak stress there was no difference between DSE (9.97±3.07 vs. 9.69±2.87cm/s, p=0.17). At 30-minute recovery the DSE were identical (4.26±2.01 vs. 4.26±1.60cm/s, p=0.58). There was no evidence of stunning in either study (**Figure 3.5**).

Figure 3.5 Peak tissue velocity during DSE



Mean±SEM. Dotted Blue = GLP-1 Only, Solid Blue = GLP-1 + Glibenclamide. Red = historical control data

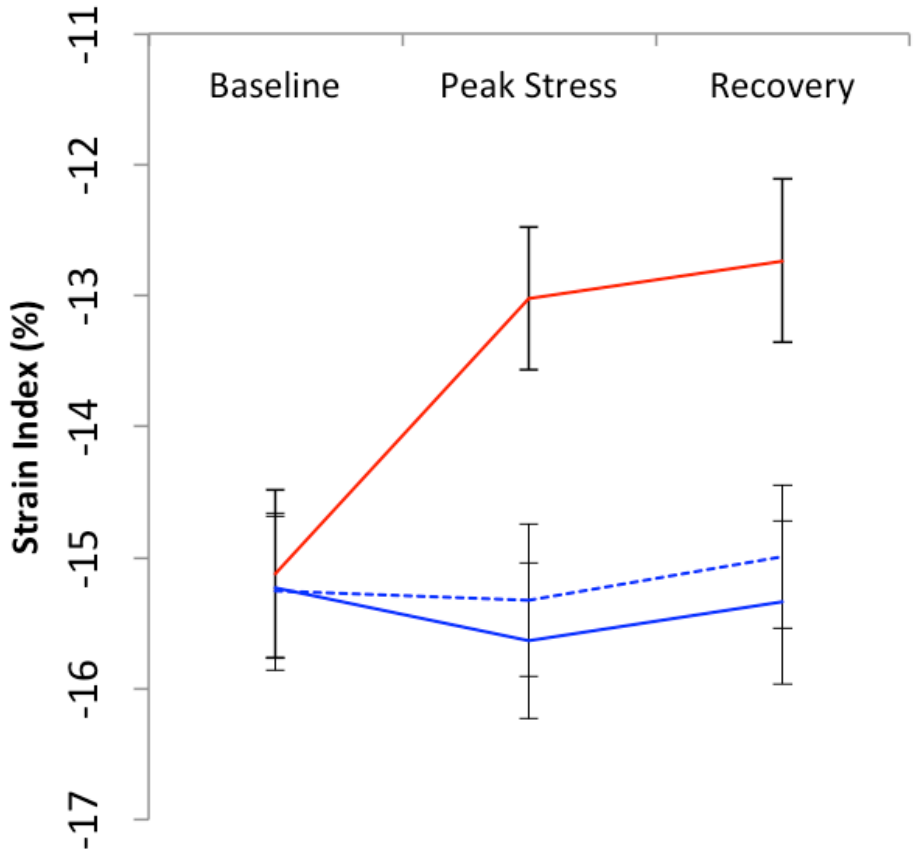
Historic control data based on Read et al(127). Historical data for *GLP-1 Only* overlapped the GLP-1 curve in this study and is not shown for clarity.

Peak systolic strain and strain Rate

There was no significant difference between GLP-1 Only and GLP-1 + Glibenclamide DSE at baseline (-15.3±5.79 vs. -15.2±5.24%, p=0.91). Strain did not significantly change at peak stress with no difference between DSE (-15.3±5.66 vs. -15.6±5.79%, p=0.65). In recovery, there was also no difference between the two DSE (-15.0±5.27 vs. -15.3±6.06%, p=0.59). Results are shown in **Figure 3.6**

There was no significant difference in strain rate at baseline (-1.18±0.40 vs. -1.22±0.40, p=0.38), peak stress (-2.72±1.11 vs. -2.70±0.91, p=0.81) and 30-minute recovery (-1.26±0.52 vs. -1.25±0.53, p=0.86). There was no evidence of stunning in either DSE. Results are shown in **Figure 3.7**. Regional indices of systolic function are also tabulated in **Table 3.6**.

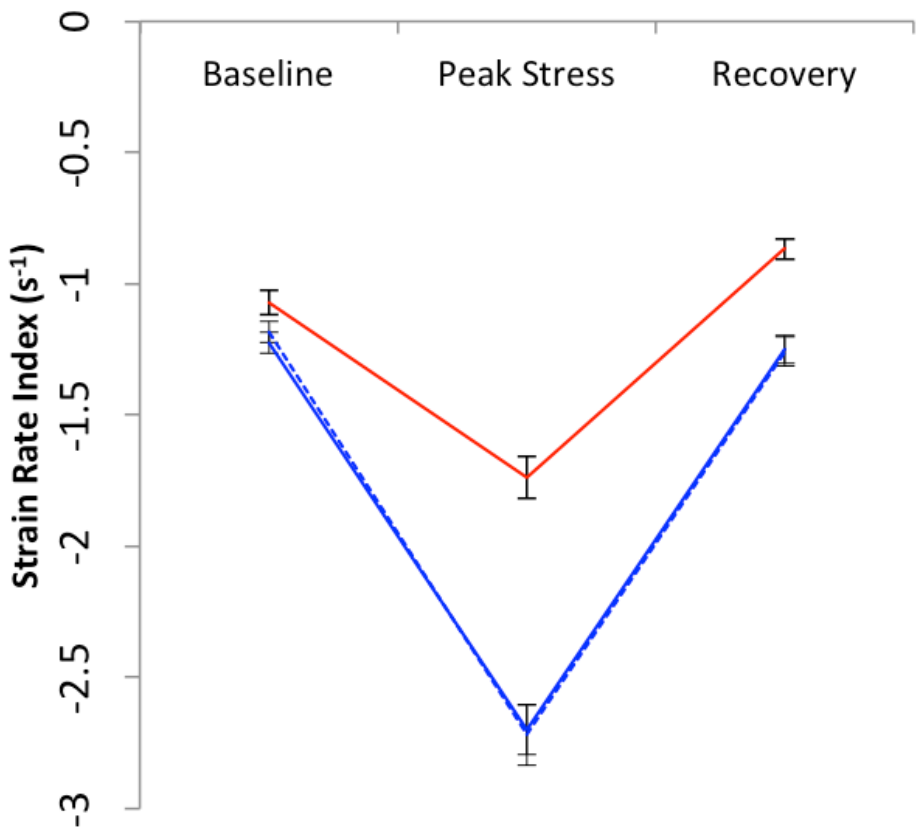
Figure 3.6 Strain index during DSE



Mean±SEM. Dotted Blue = GLP-1 Only, Solid Blue = GLP-1 + Glibenclamide. Red = historical control data

Historic control data based on Read et al(127). Historical control data is shown in the figures for illustrative purposes. Historical data for *GLP-1 Only* overlapped the GLP-1 curve in this study and is not shown for clarity.

Figure 3.7 Strain rate index during DSE



Mean±SEM. Dotted Blue = GLP-1 Only, Solid Blue = GLP-1 + Glibenclamide. Red = historical control data

Historic control data based on Read et al(127). Historical control data is shown in the figures for illustrative purposes. Historical data for *GLP-1 Only* overlapped the GLP-1 curve in this study and is not shown for clarity.

Table 3.6 Regional wall function during DSE protocol (all segments)

	GLP-1 Only	GLP-1 Glibenclamide	p-value
Peak Systolic Velocity (Vs), cm/s (mean±SD)			
Baseline	3.97±2.05	4.09±2.21	0.45
Peak Stress	9.97±3.07	9.69±2.87	0.17
Recovery	4.26±2.02	4.26±1.60	0.58
Strain Index (SI), % (mean±SD)			
Baseline	-15.3±5.79	-15.2±5.24	0.91
Peak Stress	-15.3±5.66	-15.6±5.79	0.65
Recovery	-15.0±5.27	-15.3±6.06	0.59

Strain Rate Index (SRI), s^{-1} (mean±SD)			
Baseline	-1.18±0.40	-1.22±0.40	0.38
Peak Stress	-2.72±1.11	-2.70±0.91	0.81
Recovery	-1.26±0.52	-1.25±0.53	0.86

Ischaemic segments

Previous work has shown that the cardioprotective effect of GLP-1 is limited to those segments that become ischaemic during the DSE(127). Therefore, further analysis, concentrated exclusively on those segments, was needed. As with the all-segment analysis there was no change in measures of tissue velocity, strain or strain rate between the two groups. These results are tabulated in **Table 3.7**.

Table 3.7 Regional wall function during DSE protocol (ischaemic segments)

	GLP-1 Only	GLP-1 Glibenclamide	p-value
Peak Systolic Velocity (Vs), cm/s (mean±SD)			
Baseline	3.76±1.89	3.92±2.07	0.38
Peak Stress	9.86±3.08	9.59±2.81	0.18
Recovery	3.95±1.95	4.04±1.56	0.98
Strain Index (SI), % (mean±SD)			
Baseline	-15.3±5.83	-15.1±5.30	0.68
Peak Stress	-15.5±5.52	-15.6±6.09	0.92
Recovery	-14.8±5.35	-15.3±6.21	0.48
Strain Rate Index (SRI), s^{-1} (mean±SD)			
Baseline	-1.19±0.74	-1.25±0.51	0.29

Peak Stress	-2.68±1.69	-2.79±1.17	0.65
Recovery	-1.24±0.92	-1.25±0.69	0.71

3.4 Discussion

In this study, the addition of glibenclamide did not alter the changes in global and regional systolic function during the DSE or at recovery. Since other studies have clearly demonstrated the protective effect of GLP-1 in ischaemic segments undergoing dobutamine stress(125,127,130), it may be assumed that GLP-1 mediated cardioprotection was maintained. As glibenclamide acts to blockade the mKATP channel, this data suggests that GLP-1 action on ischaemic myocardium is not mediated via KATP channels.

IC acts via KATP dependent subcellular signaling pathways downstream of the prosurvival kinase pathways of cardioprotection. These have been implicated in the protection offered by GLP-1 as discussed in Chapter 1. Whilst IC does not protect against ischaemia during dobutamine stress(134), GLP-1 has been shown to offer improvement in regional and global measures of LV function during DSE. Here, blockade of the mKATP channel with glibenclamide has not lead to a change in any measure of ischaemic contractile dysfunction. This suggests that the cardioprotection afforded by GLP-1 is not dependent on the KATP channel. This is a surprising result given the findings of a number of animal studies which implicate activation of a large number of intracellular signaling molecules involved in IC (including PI3K, AKt and PKC) in GLP-1 cardioprotection(109,114,115,162). Most animal studies assess infarct size resulting from lethal ischaemia rather than contractile dysfunction(117). Protection against lethal ischaemia may therefore remain KATP dependent. Alternatively there is considerable crosstalk between intracellular signaling pathways(214) and it is possible that mKATP is involved but is one of a number of redundant pathways through which GLP-1 operates. This would still be

fundamentally different to IC in cellular pathways in which mKATP is a final common step.

Of note, the addition of glibenclamide distorted the metabolic conditions during that DSE. Whilst glibenclamide did not affect GLP-1 levels, it did produce an increase in insulin release, with correspondent suppression of free fatty acids compared to the GLP-1 only DSE. In both DSE, a glucose infusion was also given, which would act to produce similar effects. These metabolic changes are similar to those in the supply ischaemia protocol discussed in Chapter 4 of this thesis. The implications of these metabolic changes are potentially confounding. They are discussed in more detail in both Chapter 4 and the limitations section below.

An important additional finding of the study was the absence of heart rate rise. This was particularly noteworthy during the administration of GLP-1 prior to the GLP-1 only DSE (see Section 3.3.2), where confounding factors were minimal. Whilst chronic use of GLP-1RA have been reported to increase heart rate(171), no change was detected here. This is in line with studies of DPP4 inhibitors, which increase levels of native GLP-1 without evidence of rising heart rate(141).

3.4.1 Study limitations

This study was conducted with glibenclamide given in addition to GLP-1. This risked significant hypoglycaemia. The administration of a low-dose dextrose infusion was intended to counter this. Nonetheless, the combination of increased insulin secretion with dextrose may have introduced a protective effect on the myocardium in both DSE. Research within our group provides evidence against this being an important factor in our model. These studies show that high levels of insulin and glucose do not

influence myocardial performance during dobutamine stress, either with or without GLP-1(132). It is worth noting the wide variability in the insulin response to glibenclamide, as seen in the wide standard deviation at peak stress and recovery. The time window and dose were chosen based on previous studies of RIPC (as discussed in the methods) but serum high performance liquid chromatography to assay glibenclamide levels was not performed, since it was unavailable from the Core Biochemical Assay laboratory.

Optimally, one or two further DSE would have been performed on each patient – a control DSE, and a DSE with glibenclamide only. This was not possible for a number of practical and ethical reasons. Most important of these was the need to avoid delaying patients from receiving the revascularisation they required. However, we attempted to address this limitation through the supply ischaemia protocol used in Chapter 4.

3.5 Conclusions

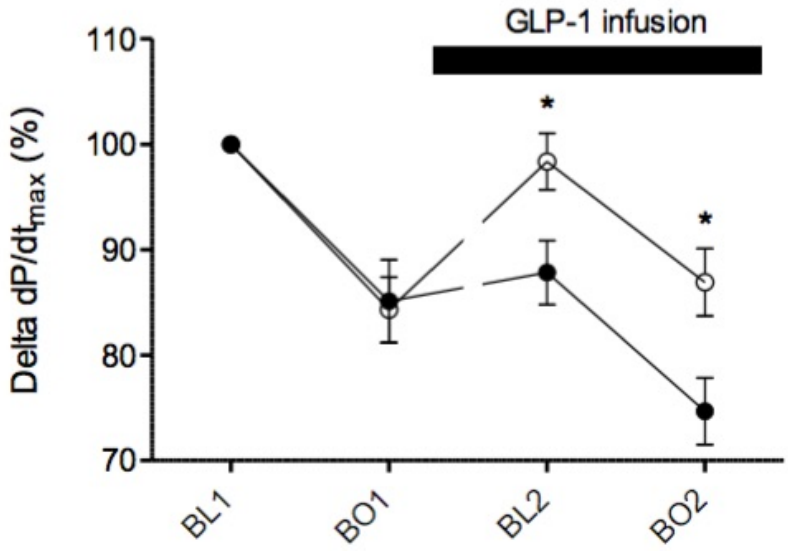
Blockade of mKATP channels with glibenclamide did not appear to affect GLP-1 cardioprotection during dobutamine stress. Further work is needed to robustly demonstrate the absence of an effect, possibly by demonstrating similar results during supply ischaemia.

Chapter 4 Mechanism of GLP-1 induced protection against cumulative myocardial dysfunction in supply ischaemia

4.1 Introduction

GLP-1 protects against LV stunning and cumulative dysfunction during supply ischaemia(126,131). This has been discussed in detail in in Chapter 1. The protection occurs regardless of whether the GLP-1 is initiated before or after a first balloon occlusion. **Figure 4.1** shows the effect of a GLP-1 infusion initiated after a balloon occlusion (BO1) on systolic function at 30-minute recovery (BL2) and after further balloon occlusion (BO2).

Figure 4.1 - The effect of GLP-1 on left ventricular function during serial balloon occlusion



Change in dP/dt_{max} with (white circles) and without (black circles) GLP-1 infusion. Adapted from Read *et al*(126).

Understanding the mechanism behind this protection is important, in order to develop targeted therapies which will protect the myocardium in a clinical situation. A number of different cellular pathways have been implicated in GLP-1 protection against non-lethal ischaemia. Many studies have been undertaken in animal models, which may not accurately reflect human biology. Whilst IC does not protect against non-lethal ischaemia such as myocardial stunning, a number of animal studies have implicated the pathways of IC in GLP-1 protection(109,114). As discussed in Chapter 1, the mKATP channel is an important effector of IC. Administration of agents that block the channel have been shown to interfere with IC in both animal and human studies. Glibenclamide (discussed in Chapter 2) has been shown to block the mKATP channel in humans and abrogate IC.

The aim of this study was to establish whether co-administration of the glibenclamide with GLP-1 abolished protection against stunning and cumulative dysfunction. The absence of cardioprotection when these agents are administered together would provide powerful evidence that the RISK/SAFE pathways are involved in GLP-1 mediated protection against myocardial stunning. The study used supply ischaemia through coronary balloon occlusion in human subjects to assess stunning and cumulative dysfunction measured with the conductance catheter technique described in chapter 2.

4.2 Methods

4.2.1 Patient selection

Patients attending for elective angioplasty and stenting (PCI) at Papworth Hospital were included in the study. The inclusion criteria were:

- coronary artery disease requiring PCI in the LAD artery
- normal LV systolic function assessed by echocardiography or left ventriculography (EF > 50%)
- aged over 18 years with capacity to give consent for procedure
- adequate vascular access to enable placement of arterial and venous sheaths for conductance catheter study

Exclusion criteria for the study were:

- significant region wall motion abnormality affecting the anterior wall of the LV
- significant valvular pathology or previous valve surgery
- presence of cardiac pacemaker or similar device
- diabetes mellitus
- significant co-morbidity with life expectancy of < 6 months

4.2.2 Pre-procedure

Patients were asked to abstain from caffeine, alcohol, sublingual or oral nitrates, and nicorandil for 24 hours prior to the procedure. Patients were fasted for a minimum of

6 hours prior to the procedure, and were reloaded with dual-antiplatelets (aspirin 300mg and clopidogrel 300mg). Patients were assigned to one of four groups. These were:

- Control
- GLP-1 Only
- Glibenclamide Only
- GLP-1 & Glibenclamide

Blood samples for plasma GLP-1 (7-36) amide, insulin, and free fatty acid levels were taken prior to any intervention. Patients in the Glibenclamide only group and the GLP-1 & Glibenclamide group were administered glibenclamide 5mg orally 1 hour prior to the predicted start time of the procedure. An infusion of dextrose 10% was started in glibenclamide-treated groups at a rate of 1.5mg/Kg/min.

4.2.3 Cardiac catheterisation

Sheaths were placed in the right femoral artery (7F), right radial artery (6F) and right femoral vein (6F). Where a sheath could not be placed in the right radial artery the left radial artery or left femoral artery was used at the operators discretion. Heparin (70-100IU/Kg) was given to maintain an activated coagulation time > 250 seconds throughout the procedure. Blood samples for GLP-1 (7-36) amide, insulin, and free fatty acids were taken from the right femoral vein.

An 8-electrode, 7F conductance catheter (Millar Instruments, USA) was advanced across the aortic valve and placed at the apex of the LV, across the longitudinal axis of the ventricle. A multi-purpose catheter was advanced into the pulmonary artery from the right femoral vein. Right atrial, right ventricular and pulmonary artery pressures were measured. Samples for measurement of pulmonary and peripheral arterial oxygen saturations were taken and used to estimate cardiac output according to the indirect Fick method.

The conductance catheter was connected to a signal-conditioning unit and calibrated according to the technique established by Baan et al and described above(201). Calibration was performed by injection of 5ml of hypertonic (10%) saline through the pulmonary artery catheter. Conductance catheter measurements were taken in held mid-inspiration.

A guide catheter was advanced through the right radial artery and the left coronary system was intubated. A coronary wire was passed across the lesion in the LAD artery. Baseline recording of PV loops were taken at this stage (**BL1**). A semi-compliant coronary balloon (Emerge, Boston Scientific, USA), appropriate to the size of vessel was inflated to low pressure (4-6 atmospheres) within the lesion for 1 minute. Fluoroscopy was performed to ensure occlusion of the vessel. PV loops were recorded at 1-minute occlusion (**BO1**).

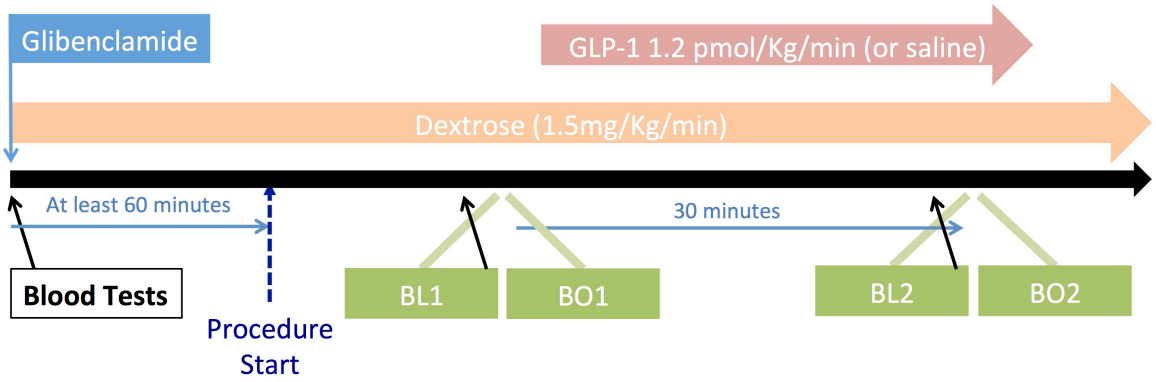
Following this, a GLP-1 or matched 0.9% saline infusion was initiated. No further coronary interventions were performed for 30 minutes. At 30 minutes further samples for GLP-1 (7-36) amide, free fatty acids, glucose, and insulin were measured from the femoral venous sheath. Further baseline pressure-volume loops were recorded

(**BL2**) followed by a coronary balloon occlusion in the same fashion as previously. PV loops were again recorded after 1-minute occlusion (**BO2**).

Following the conductance catheter study, the patient proceeded to have PCI performed to the vessel as required. The conductance catheter was removed at the end of the case under fluoroscopic guidance. Haemostasis for the radial access site was with a TR band as per hospital protocol. The femoral arterial access was sealed with a collagen plug (Angioseal, St. Jude Medical, MN, USA) following fluoroscopic examination to ensure this was safe. The femoral venous sheath was removed with manual compression on the ward after the case was complete.

A timeline for the study is shown in **Figure 4.2**

Figure 4.2 Study timeline



Black arrows indicate the relationship of blood tests to the balloon occlusions.

4.2.4 Safety

The additional risks of this protocol, over and above those of the PCI itself, related to the additional interventions and the combination of drugs used in the procedure.

Glibenclamide is an oral hypoglycemic agent, which increases insulin secretion from the pancreas. This runs the risk of hypoglycemia when administered to a non-diabetic patient. This effect may be exacerbated by the co-administration of GLP-1. Patients taking part in this protocol were therefore administered a dextrose 10% infusion throughout the procedure and afterwards in recovery. This was started at a low rate but could be up titrated if the patient experienced symptomatic or biochemical evidence of hypoglycemia. Patients had blood glucose measured every 15 minutes over the first 4 hours following administration of glibenclamide and half-hourly after this. Following the procedure the patient was given a carbohydrate rich meal and the dextrose infusion was weaned.

The use of additional sheaths in the femoral artery increased the small risk of vascular damage. Patients gave consent with knowledge of this increased risk, but care was taken to minimize the risk by ensuring that the femoral arterial sheath was correctly sited using fluoroscopic guidance.

4.2.5 Statistical analysis

Analysis of non-diabetic patients who had previously received saline or GLP-1 infusion after a balloon occlusion in a similar protocol to this study had a delta dP/dt_{max} at BL2 of -13.4±9.3% and -1.4±8.5% respectively. We estimated that to

detect a difference of 8% at BL2 (α=0.05, β=0.2) a sample size of 8 per group was required.

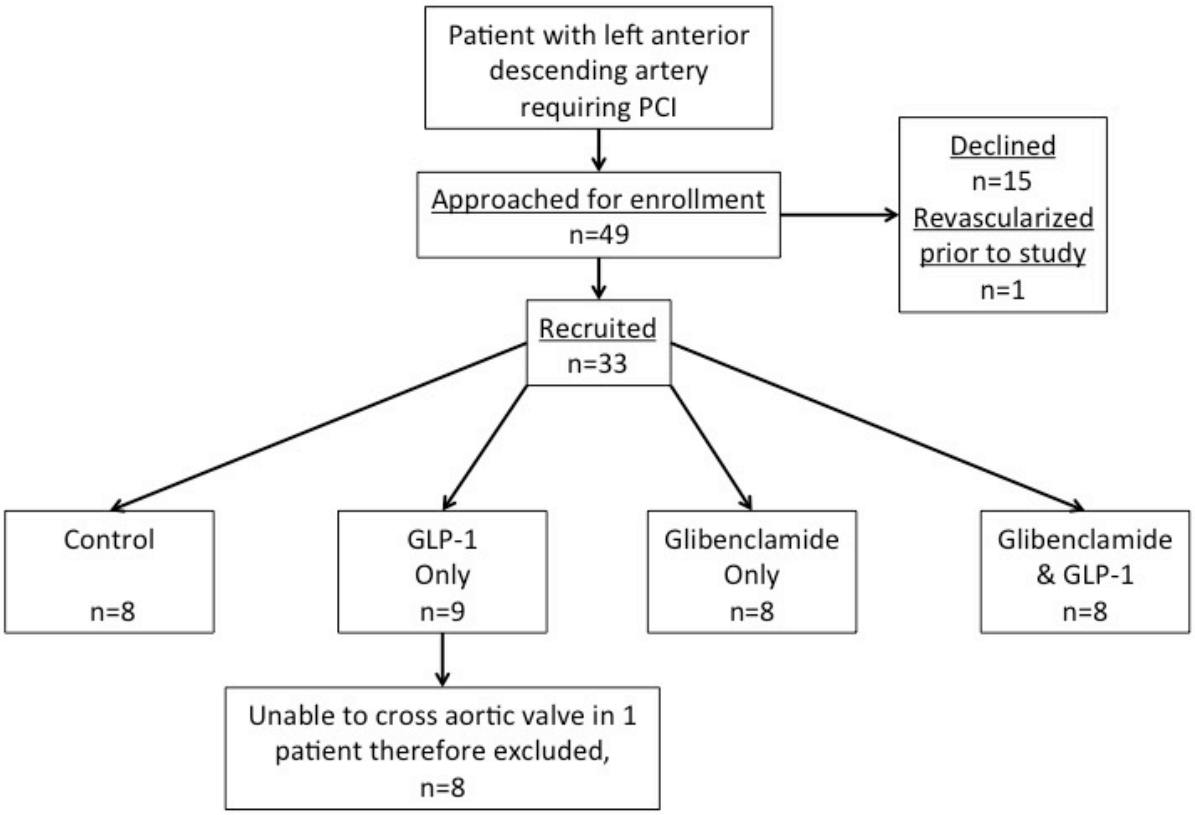
Continuous data are presented as mean±SD unless otherwise stated. Paired and unpaired Student's t-test or Mann-Whitney U test were used to compare continuous variables as appropriate, and Chi-Square test was used for categorical variables. 4-way comparisons between groups were carried out with a one-way independent ANOVA. A p-value of < 0.05 was deemed statistically significant.

4.3 Results

4.3.1 Demographics

Thirty-three patients were recruited into the four groups (Saline Control, GLP-1 Only, Glibenclamide Only, GLP-1 and Glibenclamide). In one instance, the operator was unable to cross the aortic valve with the conductance catheter and this patient was excluded. A consort diagram in **Figure 4.3** shows the allocation.

Figure 4.3 CONSORT diagram for LV conductance catheter study



The demographics and baseline blood results were well matched between all four groups. These results are summarized in **Table 4.1**. There were also no significant differences between baseline haemodynamic data in the four groups (**Table 4.2**).Time between administration of glibenclamide to the first balloon occlusion was also measured. There was no significant difference between the Glibenclamide only and the GLP-1 Glibenclamide group (116±31 v. 124±34 mins, p=0.63).

Table 4.1 Demographics of LV conductance catheter study

	Control	GLP-1	GLP-1 Glibenclamide	Glibenclamide	p-value
Age, years, median [IQR]	58 [55.5-63.5]	62.5 [59.5-78.5]	63 [57.5-77]	67 [56-71]	0.26
Male Sex, n (%)	7 (87.5)	7 (87.5)	7 (87.5)	7 (87.5)	1.00
BMI, $Kg.m^{-2}$ (mean±SD)	32.2±2.9	31.2±5.4	28.6±2.1	33.1±5.2	0.25
Previous MI, n (%)	3 (37.5)	2 (25)	1 (12.5)	2 (25)	0.72
Active or Ex Smoker, n (%)	4 (50)	6 (75)	3 (37.5)	4 (50)	0.50
Hypertension, n (%)	3 (37.5)	4 (50)	4 (50)	2 (25)	0.70

Medication					
Beta Blocker, n (%)	7 (87.5)	7 (87.5)	7 (87.5)	5 (62.5)	0.48
ACE inhibitor, n (%)	4 (50)	4 (50)	1 (12.5)	4 (50)	0.54
Ca Channel Blocker, n (%)	2 (25)	2 (25)	2 (25)	2 (12.5)	0.91
Long acting nitrate, n (%)	2 (25)	3 (37.5)	4 (50)	3 (37.5)	0.79
Baseline Bloods (mean±SD)					
Haemoglobin, g/L	145±8.1	128±23.5	143±11.6	138±9.2	0.17
Creatinine, µmol/l	94.8±29.0	97.0±23.4	86.2±35.4	89.6±19.9	0.97
Cholesterol, mmol/L	3.61±0.8	4.20±0.90	3.99±1.1	4.16±1.5	0.73
Blood Glucose, mmol/l	5.1±0.3	5.0±0.3	5.3±0.3	5.5±0.3	0.87

*p-value for 4-way inter group comparison

Table 4.2 Baseline haemodynamic data during LV conductance catheter

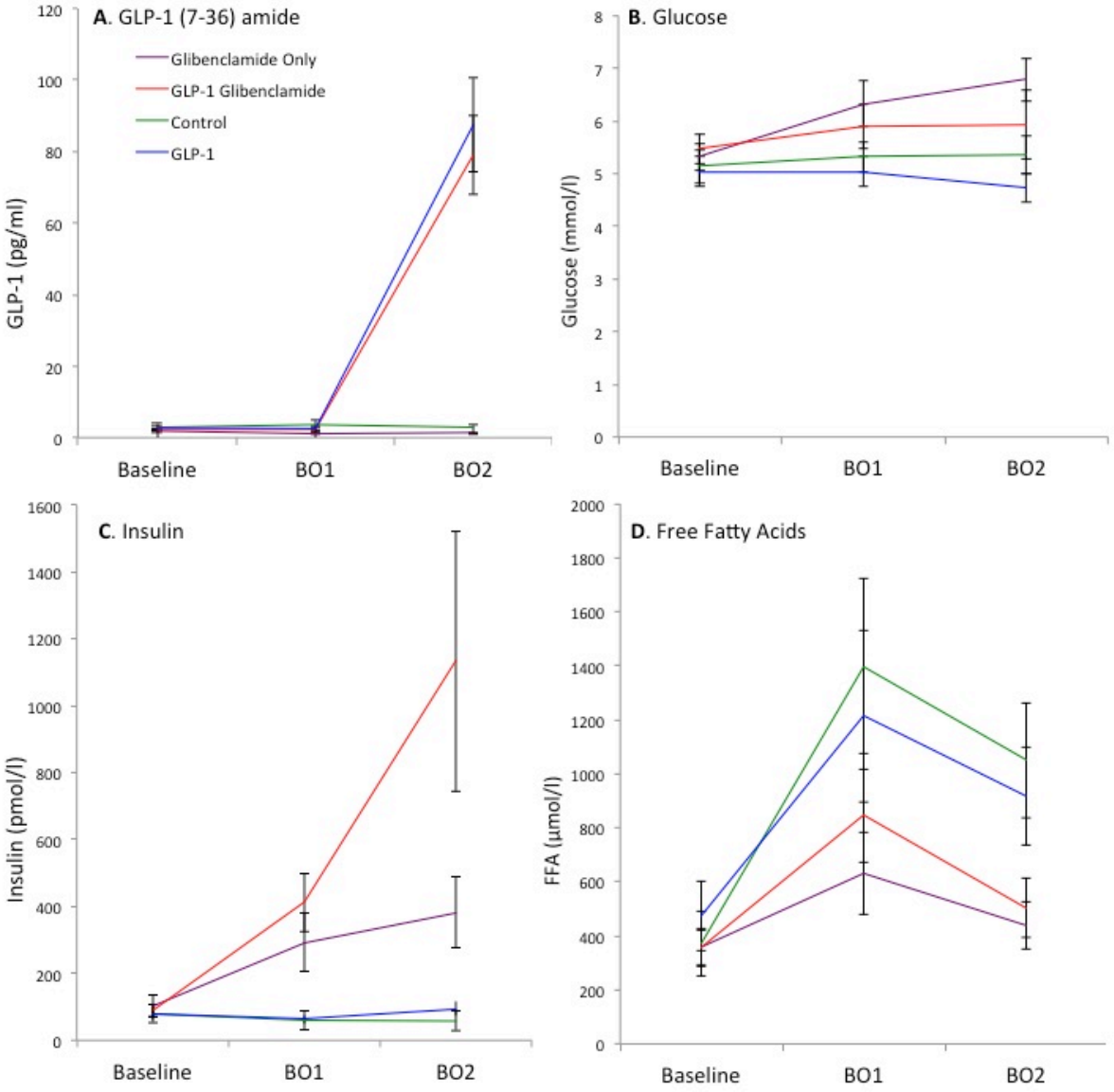
	Control,	GLP-1 Only	Glibenclamide Only	GLP-1 Glibenclamide	p-value*
Heart Rate, BPM	58±8	70±14	61±8	61±8	0.13
Mean Arterial Pressure, mmHg	93±9	88±17	88±12	88±17	0.31
LV EDP, mmHg	14.3±2.5	19.7±7.6	16.8±2.9	13.8±2.9	0.27
Stroke Volume, ml	74.7±19.8	75.2±21.2	79.5±23.1	87.1±30.1	0.69
Ejection Fraction, %	60.6±8.7	60.8±7.26	57.3±8.4	64.2±4.7	0.51
Cardiac Output (Fick), L/min	4.6±0.9	5.1±1.3	5.5±1.1	5.6±0.7	0.24
dP/dt_{max}, mmHg/s	1646±259	1465±313	1457±515	1486±306	0.77
dP/dt_{min}, mmHg/s	-2134±315	-2051±380	-1828±719	-1853±417	0.53
Tau, ms	50.0±11.2	58.9±10.0	55.4±10.0	48.1±5.9	0.14

*p-value for 4-way intergroup comparison. All values are mean±SD

4.3.2 Biochemistry

Biochemistry data is summarized in **Figure 4.4**. Levels of plasma GLP-1 (7-36) amide were below 3.0 pg/ml at baseline and during BO1 confirming no significant difference between groups. Plasma levels of GLP-1 (7-36) amide were significantly higher at BO2 after GLP-1 infusion compared with those groups administered only saline, with no significant difference between the GLP-1-treated groups (p=0.41). Plasma levels of insulin were significantly elevated at BO2 in patients treated with glibenclamide. The combination of GLP-1 and glibenclamide caused an even greater rise although the response was highly variable between individuals. Patients treated with glibenclamide also had significant suppression of free fatty acids at BO1 and BO2. Plasma glucose levels were higher at BO2 in glibenclamide treated groups, compared with control, despite the rise in insulin levels, reflecting the co-administration of dextrose alongside glibenclamide. Results are tabulated in **Table 4.3**.

Figure 4.4 Metabolic changes during the LV conductance catheter study



Mean±SEM

Table 4.3 Metabolic data in LV conductance catheter study

	Baseline		Balloon Occlusion 1 (BO1)		Balloon Occlusion 2 (BO2)	
		p-value*		p-value*		p-value*
GLP-1 (7-36) amide, pg/ml						
Control	2.9±3.57	-	3.6±4.13	-	2.9±1.84	-
GLP-1 Only	2.8±1.43	0.51	2.5±2.04	0.49	87.5±37.1	<0.001
Glibenclamide Only	1.9±1.66	0.46	1.1±0.77	0.11	1.3±0.54	0.07
GLP-1 Glibenclamide	2.2±0.59	0.57	2.3±1.32	0.37	78.9±31.1	<0.001
Insulin, pmol/L						
Control	79±26	-	59±29	-	58±29	-
GLP-1 Only	77±52	0.91	63±85	0.82	95±64	0.16
Glibenclamide Only	103±93	0.51	292±245	0.02	382±297	0.02
GLP-1 Glibenclamide	90±51	0.63	412±244	<0.01	1133±1089	0.03
Glucose, mmol/l						

Control	5.1±0.3	-	5.3±0.3	-	5.4±0.4	-
GLP-1 Only	5.0±0.3	0.83	5.0±0.3	0.51	4.7±0.3	0.20
Glibenclamide Only	5.3±0.3	0.66	6.3±0.4	0.06	6.8 ±0.4	0.02
GLP-1 Glibenclamide	5.5±0.3	0.44	5.9±0.4	0.27	5.9±0.7	0.45
Free Fatty Acids, µmol/L						
Control	371±364	-	1399±973	-	1050±637	-
GLP-1 Only	473±367	0.57	1213±897	0.69	917±516	0.64
Glibenclamide Only	358±201	0.93	633±434	0.06	439±248	0.02
GLP-1 Glibenclamide	356±183	0.91	846±490	0.16	503±307	0.04
Dextrose Infusion, $mg.Kg^{-1}.min^{-1}$						
Glibenclamide Only	-	-	1.69±0.53	-	1.69±0.53	-
GLP-1 Glibenclamide	-	-	2.25±1.6	-	2.44±1.59	-

*p-value compared to control group. All values are mean±SD

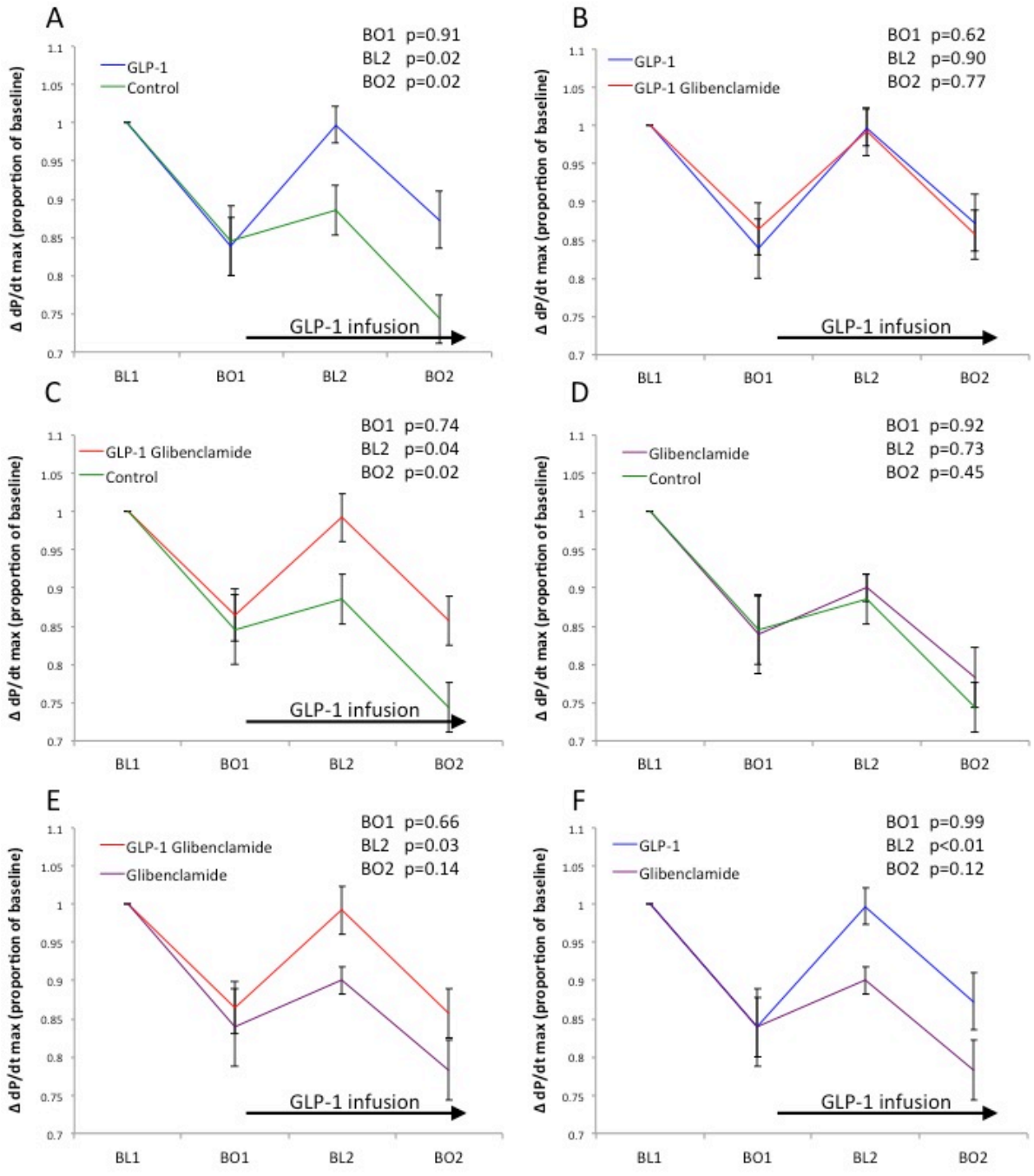
4.3.3 Left ventricular function

Table 4.4 shows the change in parameters of systolic and diastolic function during the study for each group. All groups suffered ischaemic LV dysfunction during BO1 compared to BL1. There was a significant deterioration in all measured parameters of systolic and diastolic function. There was no significant difference between any groups at BO1. At BL2 there was recovery of dP/dt_{max} and dP/dt_{min} to BL1 levels in both GLP-1 treated groups. There was impaired recovery of dP/dt_{max} and dP/dt_{min} in control and glibenclamide only groups with significant stunning compared to BL1. Glibenclamide had a neutral effect on systolic and diastolic function during balloon occlusion and recovery.

Cumulative dysfunction after the second balloon occlusion was only observed in groups not receiving GLP-1; systolic and diastolic function did not deteriorate below the level observed at BO1 in GLP-1 treated groups. Change in dP/dt_{max} (**Figure 4.5**) and dP/dt_{min} (**Figure 4.6**) are shown for each group at the specified time points.

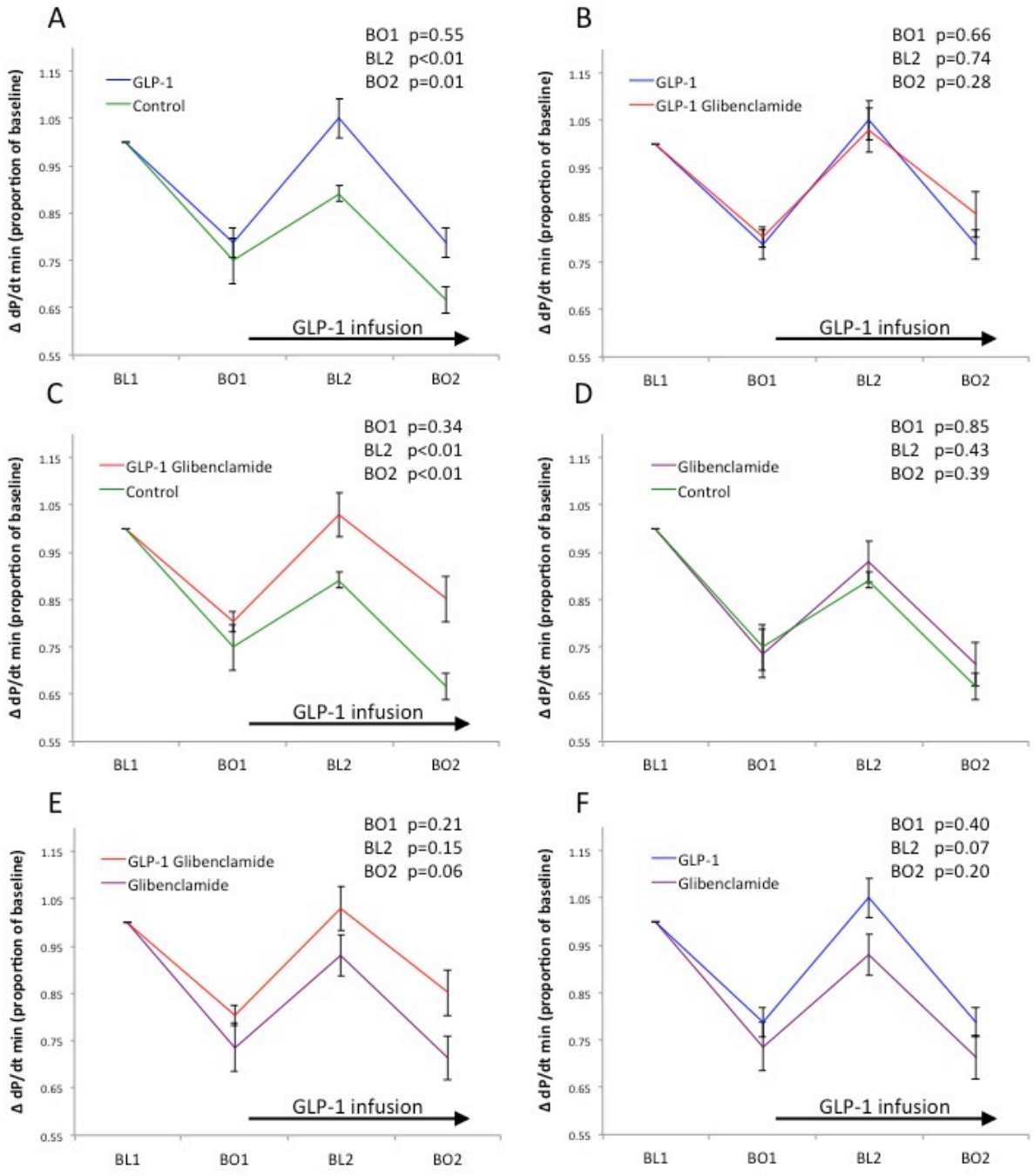
GLP-1 infusion also improved EF at BL2 and BO2 compared to control although this was only statistically significant at BO2. Tau appeared to improve with GLP-1 but this was not statistically significant. There was no significant change in heart rate ($p=0.14$) or mean arterial pressure ($p=0.67$) between balloon occlusions, or between individual groups.

Figure 4.5 Group by group changes in systolic function (dP/dt_{max})



Mean±SEM

Figure 4.6 Group by group changes in diastolic function (dP/dt_{min})



Mean±SEM

Table 4.4 Change in measures of systolic and diastolic function during conductance catheter study

	Baseline 1 (BL1)	Balloon Occlusion 1 (BO1)		Baseline 2 (BL2)		Balloon Occlusion 2 (BO2)	
	%	Δ%	p-value	Δ%	p-value	Δ%	p-value
dP/dt_{max}							
Control	100	-15.4±13.6	0.012	-11.5±10.0	<0.01	-25.7±9.6	<0.001
GLP-1 Only	100	-16.1±10.2	<0.01	-0.3±6.8	0.92	-12.8±10.5	0.011
Glibenclamide Only	100	-16.1±14.4	0.011	-10.0±4.7	<0.01	-21.7±10.5	<0.01
GLP-1 Glibenclamide	100	-13.5±9.7	<0.01	-0.8±9.0	0.82	-14.9±9.2	<0.01
Ejection Fraction							
Control	100	-18.3±14.1	0.01	-13.8±15.5	0.03	-31.1±16.0	<0.001
GLP-1 Only	100	-17.9±13.3	0.01	-8.4±12.7	0.10	-11.7±17.8	0.10
Glibenclamide Only	100	-17.2±13.4	0.01	-12.7±19.6	0.14	-26.9±14.2	<0.001
GLP-1 Glibenclamide	100	-16.9±13.8	0.01	-8.4±4.8	<0.01	-13.1±9.5	<0.01

Stroke Volume							
Control	100	-19.6±18.1	0.03	-16.4±16.3	0.02	-31.2±16.6	<0.001
GLP-1 Only	100	-19.8±17.5	0.01	-5.8±27.1	0.56	-12.6±28.8	0.26
Glibenclamide Only	100	-16.8±17.8	0.08	-12.6±18.2	0.12	-23.2±22.1	0.04
GLP-1 Glibenclamide	100	-14.8±11.1	0.01	-0.2±7.3	0.96	-5.6±4.4	0.03
dP/dt_{min}							
Control	100	-25.1±14.7	<0.01	-10.9±4.7	<0.001	-33.3±8.6	<0.001
GLP-1 Only	100	-21.3±8.3	<0.001	+5.0±11.6	0.26	-21.3±8.7	0.04
Glibenclamide Only	100	-26.4±14.3	<0.001	-7.1±12.5	0.17	-28.6±13.1	<0.001
GLP-1 Glibenclamide	100	-19.6±6.1	<0.001	+2.9±13.0	0.55	-14.3±13.6	0.02

Tau							
Control	100	+34.7±16.9	<0.01	+12.7±7.8	<0.01	+46.4±15.4	<0.001
GLP-1 Only	100	+27.3±11.6	<0.001	+8.0±18.6	0.26	+31.0±36.7	0.05
Glibenclamide Only	100	+30.5±25.8	<0.01	+13.1±19.9	0.14	+36.2±26.3	<0.001
GLP-1 Glibenclamide	100	+31.3±27.2	<0.001	+5.5±10.2	0.17	32.5±13.2	<0.001

*p-value compared to BL1 for each group. All values are mean±SD

4.4 Discussion

The study confirms that the LV experienced stunning and cumulative dysfunction when subjected to serial coronary balloon occlusions. GLP-1 infusion protected against both stunning and cumulative ischaemic dysfunction. Addition of glibenclamide to the GLP-1 treated group did not abrogate protection, as would be expected if the protection were KATP dependent. Glibenclamide alone had no effect on either stunning or cumulative ischaemic dysfunction compared to control. The implications of these findings are that GLP-1 protection against non-lethal IR injury is not mKATP dependent.

4.4.1 Ischaemic conditioning mechanism for GLP-1 cardioprotection

GLP-1 consistently activates the subcellular signaling apparatus of IC in animal models. These mediators include PI3K and Akt and are components of the RISK/SAFE pathways(109,114). In IC, these pathways act through the mKATP channel to deliver protection against lethal IR injury. Furthermore, a recent study showed that blockade of the GLP-1 receptor protected against RIPC in rats(215). Whilst IC does not protect against myocardial stunning (134), the activation of these pathways suggested a possible role for mKATP in GLP-1 cardioprotection.

This study shows that GLP-1 cardioprotection against non-lethal IR injury is not mKATP dependent, and is unlikely to be related to IC. Protection must be mediated through an alternative pathway. Although this finding contradicts the findings of some animal studies, there are good reasons to believe the results from this thesis. Firstly, the studies were performed in humans rather than small mammals, which may have different receptor distribution and physiology. Secondly, as discussed above, IC does not produce protection against myocardial stunning, in the manner of GLP-1. Finally,

IC has consistently been abrogated with glibenclamide but GLP-1 protection was not affected in this study.

Animal studies that characterize GLP-1 as the humoral mediator of RIPC are implausible(215). GLP-1 is rapidly degraded by DPP4, but humoral mediators of RIPC can be transferred in dialysate from one animal to another suggesting that they are long-lasting. Additionally, changes in GLP-1 levels in response to limb ischaemia are biologically trivial, despite a statistically significant rise in animal studies. Evidence of a KATP sensitive role was modeled in ex vivo rat aorta and pancreas rather than the human cardiomyocyte, which may not even have a GLP-1 receptor(165).

Despite these criticisms, animal evidence of mKATP dependent protection may still be applicable to lethal IR injury in humans, which was not assessed in this study. mKATP channel independent aspects of subcellular pro-survival kinase pathways may be involved in GLP-1 cardioprotection, or channel could be one of a number of redundant mechanisms of protection.

4.4.2 Metabolic mechanism for GLP-1 cardioprotection

Both GLP-1 and glibenclamide caused a rise in insulin levels and a fall in free fatty acids. Only GLP-1 produced cardioprotection. Therefore, the rise in insulin levels was not responsible for the GLP-1 cardioprotection. This finding is consistent with human hyperinsulinaemic clamp studies that showed a neutral effect when insulin levels were elevated during demand ischaemia(132).

It has also been suggested that GLP-1 protection is caused by a switch in cardiomyocyte metabolism toward increased myocardial glucose utilisation(216). The combination of glibenclamide, dextrose and GLP-1 were associated with the highest levels of insulin in the study but did not lead to additional protection. Therefore, despite a metabolic environment that was highly favourable to enhanced glucose utilisation (increased availability of glucose, high levels of insulin and reduced free fatty acid levels), no evidence of improved function was seen. A metabolic explanation for GLP-1 cardioprotection, involving a straightforward increase in glucose metabolism is therefore unlikely. This conclusion is supported by previous conductance catheter studies, which were unable to demonstrate a change in transmyocardial glucose gradients during ischaemia when GLP-1 was administered(131).

4.4.3 Haemodynamic mechanism for GLP-1 cardioprotection

The blood pressure and heart rate remained stable throughout the procedure in all groups. The absence of significant haemodynamic changes suggests that the protective effect of GLP-1 is not the result of significant alteration in the tone of the systemic vasculature. Whilst large-scale cardiovascular outcome studies have shown rises in heart rate with chronic use of GLP-1RA's, these changes have been highly variable with large changes in some individuals resulting in a small mean change. These changes were not seen during this study and do not explain the protection of GLP-1. Furthermore, they have not been seen to occur with native GLP-1. In this study, assessment in coronary flow and microcirculatory function was not performed.

It is plausible that GLP-1 is a coronary vasodilator, which in turn could augment ventricular function through the Gregg effect.

4.4.4 Alternative mechanism for GLP-1 cardioprotection

Since there is evidence that the GLP-1 receptor is not present on the human ventricular cardiomyocyte(107), GLP-1 may bind to an as yet unidentified receptor, or act remote to the myocardium. Studies have suggested that adenosine-dependent pathways may mediate some of the observed GLP-1 effects(162). Adenosine released from remote endothelial cells in response to GLP-1 receptor binding may elicit an inotropic effect through coronary vasodilation, or alternatively through a direct protective effect on cardiomyocytes(49).

Evidence of effects independent of the known GLP-1R pathway have also been described(109,110,113). Since our study investigated native GLP-1 rather than a stable GLP-1RA such as exenatide, it is possible that breakdown products such as GLP-1 (9-36) amide may be responsible for some of the observed cardioprotection. GLP-1 (9-36) has been implicated in cardioprotection in some animal studies(110,113), although, again, these studies investigated lethal IR injury.

4.5 Limitations

Some limitations of this study are similar to those identified in the demand ischaemia study. The blockade of the mKATP channel was not confirmed. However, previous studies have shown that this dose of glibenclamide was sufficient to interfere with IC (182). The biochemical data shows that glibenclamide was insulinotropic – this effect is mediated through the blockade of sarcoplasmic KATP channels on the pancreatic

beta cell(217). This insulinotropic effect necessitated the co-administration of a dextrose infusion with glibenclamide. Although this has the possibility of confounding the result, the analysis of the biochemical data above suggests it is unlikely to affect the conclusions of the study.

The study did not use load-independent measures of ventricular function such as the ESPVR described in chapter 2. This was a practical and ethical decision. Generating a family of PV loops to produce this relationship requires further catheterisation of the patient. Patients already had three sheaths in major vessels. Furthermore, the need for preload reduction during brief myocardial ischaemia presented an additional safety risk. Despite this limitation, blood pressure and heart rate remained constant throughout the procedure. This suggests that there was no significant change in preload or afterload during the study, allowing a valid comparison of the load-dependent parameters studied. Tau is particularly heart rate sensitive, and this did not change significantly throughout the protocol. No assessments of coronary or microvascular function were undertaken as part of this protocol. Changes in these parameters may be key to understanding the mechanism of GLP-1 protection. Measurement of these changes will form part of future work addressing the effect of GLP-1 during IR injury.

Non-lethal ischaemia was investigated in this study. It was not possible to investigate the effect of mKATP blockade on lethal ischaemia in humans. However, the GOLD PCI study described in Chapter 6 of this thesis investigates the effect of GLP-1 on lethal IR injury.

4.6 Conclusion

GLP-1 cardioprotection is not mediated through a KATP dependent mechanism. This study also provides powerful supporting evidence that the mechanism is also not explained by a simple metabolic switch from fat to carbohydrate metabolism. The mechanism remains elusive but these findings suggest new avenues for investigation.

Chapter 5 Assessment of the effects of right coronary balloon occlusion on right ventricular function

5.1 Introduction

The effect of GLP-1 on systolic and diastolic function in the LV have been shown in previous studies and reiterated in the findings of earlier chapters of this thesis. The importance of the RV in the pathophysiology of heart disease is of increasing clinical relevance(87). Involvement of the RV in inferior myocardial infarction increases the risk of cardiogenic shock and increases mortality, even when treated with PPCI (88). The RV is supplied predominantly from the right coronary artery (RCA) in those patients with a right-dominant coronary system(90).

Multiple studies have shown the dramatic effect of ischaemia on RV performance(218-220). RV infarction can be associated with acute haemodynamic compromise(221). Whilst the RV has been shown to be comparatively resistant to the long-term effects of ischaemia and infarction(222), in the acute situation the effect of deteriorating RV function may result in significant morbidity and mortality(223). GLP-1 is protective of LV function during LAD occlusion but its protective effect in the RV during RCA occlusion has not been investigated.

This study was set up to evaluate two hypotheses. The first is that RV stunning and cumulative dysfunction occurs following balloon occlusion of the RCA, in a similar fashion to that previously shown in the LV. The second hypothesis was that GLP-1 would abrogate stunning in a similar manner to the protection offered to the LV. In this thesis, the overall design of the study is presented but the results presented only address the first of these hypotheses.

The study used supply ischaemia through coronary balloon occlusion in human subjects to assess RV dysfunction measured with the conductance catheter technique described in Chapter 2. The study was approved by the Cambridge East Research Ethics Committee and was compliant with the declaration of Helsinki. The study was registered on clinicaltrials.gov.

5.2 Method

5.2.1 Patient selection

Patients attending for elective angioplasty and stenting (PCI) at Papworth Hospital were included in the study. The inclusion criteria were:

- coronary artery disease requiring PCI in the proximal RCA (above the level of the RV branch)
- normal RV and LV systolic function (assessed by echocardiography or cardiac MRI) – ejection fraction > 50%
- aged over 18 years with capacity to give consent for procedure
- adequate arterial access to enable placement of arterial and venous sheaths for conductance catheter study

Exclusion criteria for the study were:

- significant valvular pathology or previous valve surgery
- presence of cardiac pacemaker or similar device
- treatment with insulin, DPP4 inhibitor, GLP-1 RA or sulfonylurea.
- significant co-morbidity with life expectancy of < 6 months

5.2.2 Pre-procedure

Patients were asked to abstain from caffeine, alcohol, sublingual or oral nitrates, and nicorandil for 24 hours prior to the procedure. Patients were fasted for a minimum of 6 hours prior to the procedure, and were reloaded with dual-antiplatelets (aspirin 300mg and clopidogrel 300mg). Patients were assigned to either control or GLP-1.

5.2.3 Cardiac catheterisation

Sheaths were placed in the right femoral vein (7F) and right radial artery (6F). Where a sheath could not be placed in the right radial artery the left radial artery or right femoral artery was used at the operators discretion. Heparin (70-100IU/Kg) was given to maintain an activated coagulation time > 250 seconds throughout the procedure. Blood samples for GLP-1 (7-36) amide, insulin, and free fatty acids were taken from the right femoral vein.

A multi-purpose catheter was advanced into the pulmonary artery from the right femoral vein. Right atrial, RV and pulmonary artery pressures were measured. Samples for measurement of pulmonary and peripheral arterial oxygen saturations were taken and used to estimate cardiac output according to the Fick principle. The multi-purpose catheter was removed and an 8 electrode, 7F conductance catheter (Millar Instruments, USA) was advanced across the tricuspid valve and placed at the apex of the RV, across the longitudinal axis of the ventricle.

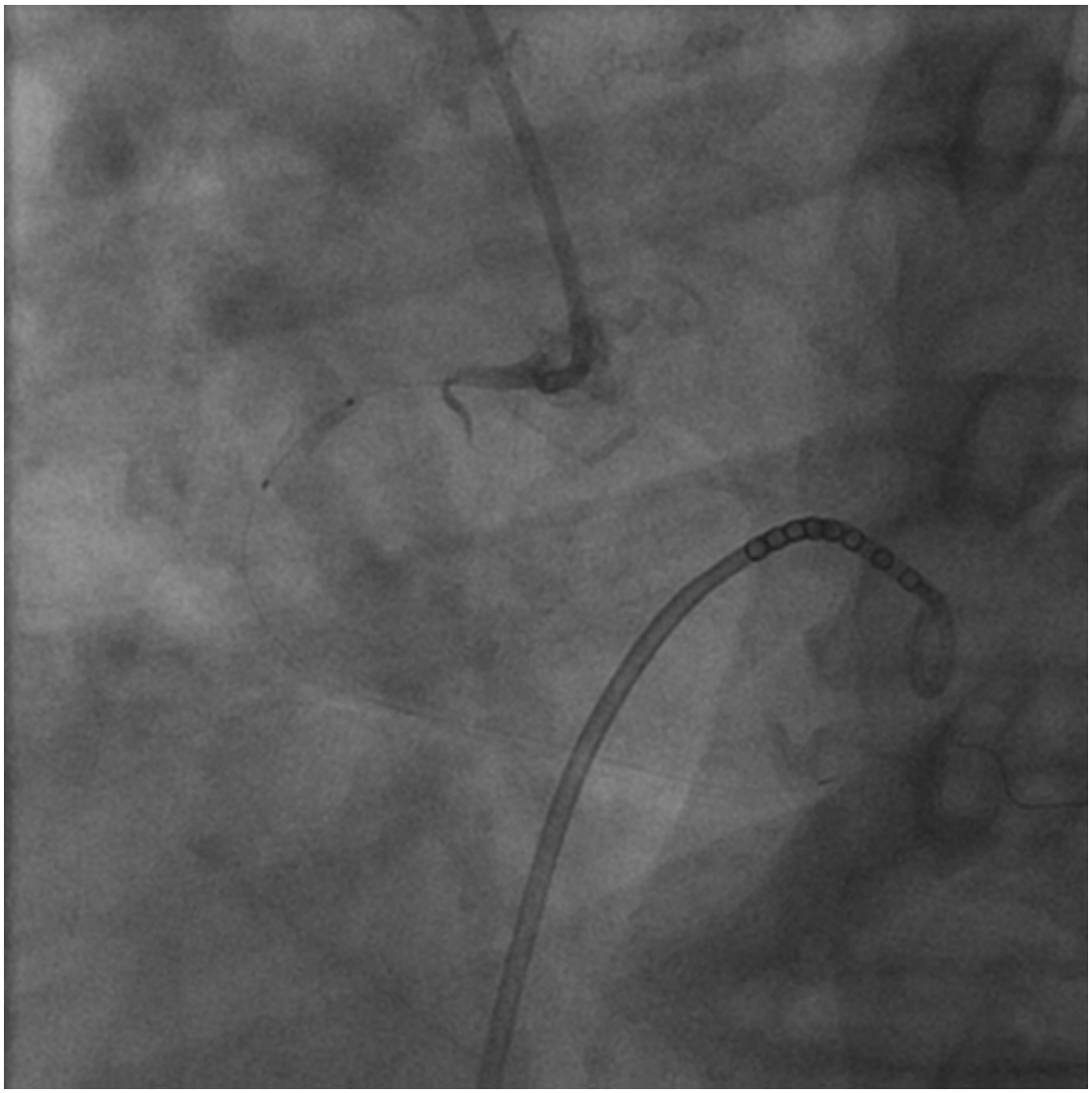
The conductance catheter was connected to a signal-conditioning unit and calibrated according to the technique established by Baan et al(201) and described in Chapter 2. Calibration was performed by injection of 5ml of hypertonic (10%) saline through

the sidearm of the venous sheath. Conductance catheter measurements were taken in held mid-inspiration.

A guide catheter was advanced through the right radial artery and the right coronary artery was intubated. A coronary wire was passed across the lesion. Baseline recording of PV loops were taken at this stage (**BL1**). A semi-compliant coronary balloon (Emerge, Boston Scientific, USA), appropriate to the size of vessel was inflated to low pressure (4-6 atmospheres) within the lesion for 1 minute. Fluoroscopy was performed to ensure occlusion of the vessel. PV loops were recorded at 1-minute occlusion (**BO1**).

Following BO1, a GLP-1 or matched 0.9% saline infusion was initiated. No further coronary interventions were performed for 30 minutes. Recording of PV loops continued during this time. At 30 minutes further biochemistry samples were taken from the femoral venous sheath. Repeat baseline PV loops were recorded (**BL2**) followed by a coronary balloon occlusion in the same fashion as previously. PV loops were again recorded after 1-minute occlusion (**BO2**). Angiographic pictures of the conductance catheter during RCA inflation are shown in **Figure 5.1**.

Figure 5.1 Conductance catheter during RCA occlusion



After the conductance catheter study, the patient proceeded to have PCI to the vessel as required. The conductance catheter was removed at the end of the case under fluoroscopic guidance. A TR band was applied to the radial access site to achieve haemostasis. The femoral venous sheath was removed with manual compression on the ward after the case was complete.

5.2.4 Safety

The additional risks of this protocol, over and above those of the PCI itself, related to the additional interventions. The additional risk of hypoglycemia through GLP-1 use alone was judged to be very low. The use of an additional sheath in the femoral vein increased the risk of vascular damage. Patients gave consent on the basis of this increased risk.

5.2.5 Statistical analysis

Patients were allocated to this study in two blocks – the control group followed by the GLP-1 treated group. This design allowed the testing of the first hypothesis - that the RV would experience stunning and cumulative dysfunction - prior to assessment of whether GLP-1 abrogated this. With a limited number of catheter devices available for research, it was important to establish that there was a clear effect in the control group. This would then provide justification for the second portion of the study. In this thesis the data from this control group will be presented. Additionally, the complexity of obtaining GLP-1 for this study meant that it was useful to begin the study as soon as possible rather delay whilst awaiting delivery.

Since the study is unblinded to both participant and researchers during the case, and all operators had experience in PV loop data acquisition using the conductance catheter in the RV (the learning effect), it was felt that this block allocation would not significantly impact on the study conclusions. Furthermore, all endpoint data was quantitatively measured rather than relying on a subjective assessment. This was agreed with the local ethics committee in approving this allocation of patients.

A power calculation was undertaken based on data from previous LV conductance catheter studies. The power calculation was based on a predicted reduction in tau of 10±8ms at BL2 compared to BL1 ($\alpha = 0.05$, $\beta = 0.2$). We estimated that 11 subjects per arm would be needed to detect this difference.

Continuous data are presented as mean±SD unless otherwise stated. Paired and unpaired Student's t-test or Mann-Whitney U test were used to compare continuous variables as appropriate, and Chi-Square test was used for categorical variables. A p-value of < 0.05 was deemed statistically significant.

5.3 Results

A power calculation demonstrated that 11 patients were needed in each group. 13 patients were recruited in the control group to allow for incomplete data. Patient demographic data for all patients recruited is presented in **Table 5.1**. Since patients acted as their own control for this portion of the study, no comparison is made.

Data for 12 of the 13 patients were included in the conductance catheter analysis. One patient was excluded, as the patient developed ventricular bigeminy during the procedure and data could not be accurately analysed. In one patient data could not be used during BO1 for technical reasons and this timepoint has also been excluded from the analysis. It was decided that this patient should not be completely excluded. However, since most of the statistical analysis in this study is paired, the removal of the BO1 data point excludes this patient from a number of these analyses. The means given at BL1, BL2 and BO2 include the patient. Although aware that this risks skewing the mean value displayed, the author felt that the greater risk of bias was to remove patients with missing data in an arbitrary fashion. **Table 5.2** contains baseline haemodynamic data for patients included in the PV loop analysis.

Table 5.1 Demographics of RV conductance catheter study

	n=13
Age, median [IQR]	65.5 [57.5-71]
Male Sex, n (%)	11 (84.6)
BMI, Kg/m^2 (mean±SD)	28.3±4.0
Previous MI, n (%)	3 (23.1)
Active or Ex Smoker, n (%)	9 (69.2)
Hypertension, n (%)	4 (30.8)
Hypercholesterolemia, n (%)	6 (46.2)
NYHA Heart Failure Score (0-4)	
≤ 2, n (%)	12 (92.3)
3, n (%)	1 (7.7)
CCS Angina Score (0-4)	
≤ 2, n (%)	6 (46.2)
3, n (%)	7 (53.8)
4, n (%)	4 (40)

BMI = body mass index, NYHA = New York Heart Association, CCS = Canadian Cardiovascular Society

Table 5.2 Baseline haemodynamic data

	n=12
MAP, mmHg (mean±SD)	91±13
mRAP, mmHg (mean±SD)	6.3±3.4
mPAP, mmHg (mean±SD)	19.3±5.5
CO (Fick), L/min, (mean±SD)	5.1±1.0

MAP = mean arterial pressure (systemic), mPAP = mean pulmonary artery pressure, mRAP = mean right atrial pressure, CO = estimated cardiac output using indirect Fick calculation

5.3.1 Effect of balloon occlusion on RV systolic function

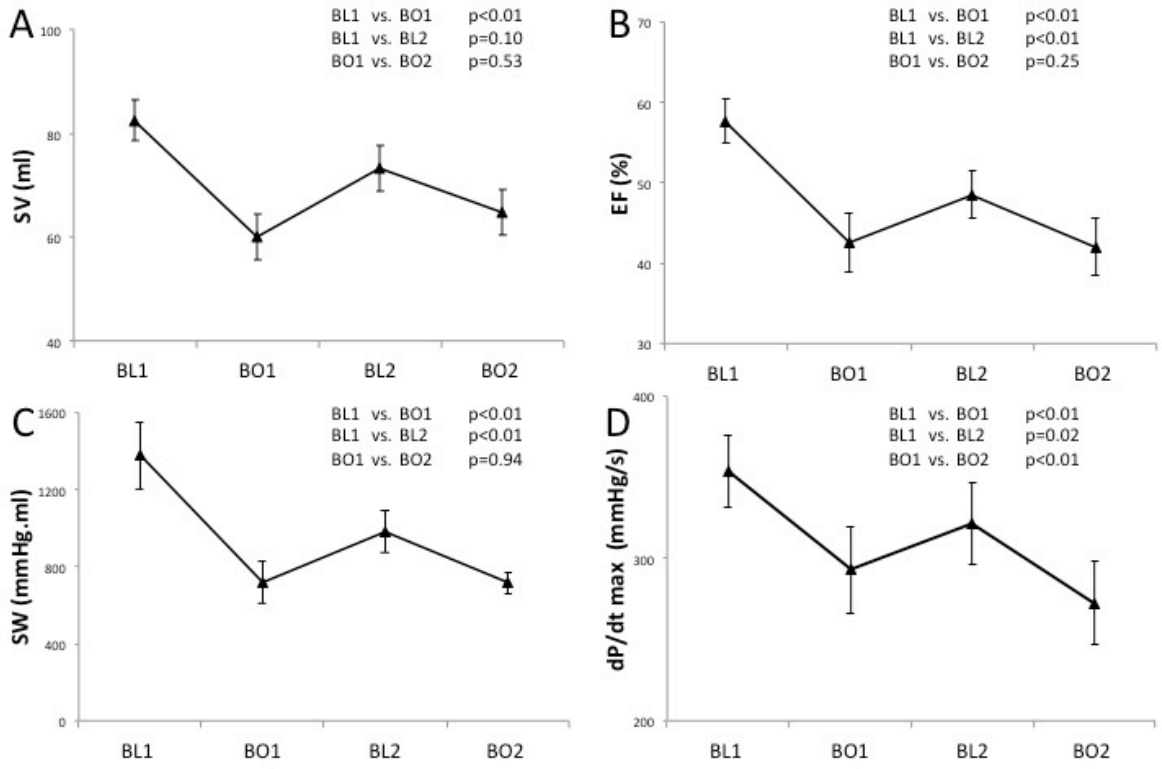
Table 5.3 shows the change in parameters of systolic function throughout the protocol. Occlusion of the RCA was associated with a deterioration in systolic parameters of RV function. Both pressure and volume measures of RV systolic function fell at BO1 compared to BL1. Declines were seen in stroke work (SW), stroke volume (SV), ejection fraction (EF) and dP/dt_{max}. The load independent measure effective arterial elastance (Ea) rose during balloon occlusion indicating reduced contractility.

At 1-minute after the balloon was deflated there was a rapid improvement in parameters of systolic function. dP/dt_{max} was significantly elevated above BL1 (423±131 vs. 353±77, $p<0.01$). Volume indices of systolic function (SW, SV, EF) were numerically elevated above BL1 levels but did not reach statistical significance.

At 30-minute recovery (BL2) this initial improvement was lost and markers of systolic function were reduced compared to BL1. dP/dt_{max}, SW and EF were all significantly reduced. SV and CO were also reduced compared to BL1 but did not reach statistical significance. Ea remained elevated at BL2 but this was not statistically significant.

Further balloon occlusion caused ischaemic dysfunction in both volume and pressure parameters of systolic function compared to BL2. However there was no significant difference between volume measures of systolic function compared to the first balloon occlusion (SW, EF, SV). dP/dt_{max} was reduced further compared to BO1. Ea was unchanged between the two balloon occlusions. **Figure 5.2** shows the changes in parameters of systolic function through the protocol.

Figure 5.2 Change in RV systolic function during protocol



Mean ± SEM. All for panels show a different indicator of systolic function at baseline (BL1), during balloon occlusion 1 (BO1), at 30-minute recovery (BL2) and during balloon occlusion 2 (BO2). Panel A shows Stroke Volume (SV). B shows ejection fraction (EF), C shows stroke work (SW) and D shows dP/dt_{max}.

Table 5.3 Change in measures of systolic function during RV conductance catheter study

	BL1	BO1	1-minute recovery	BL2	BO2
dP/dt_{max} (mmHg/s)	353±77	293±93.3**	424±132**$^{++}$	321±87*	272±89^{+}
EF (%)	57.7±9.4	42.5±12.1**	60.0±10.6^{++}	48.5±10.2**	41.9±12.3
SV (ml)	82.6±14.5	60.0±14.8**	84.7±12.3^{++}	73.3±15.5	64.8±15.1
SW (mmHg.ml)	1376±601	719±362***	1477±714^{++}	984±382**	714±194
Ea (mmHg/ml)	0.35±0.09	0.52±0.19**	0.37±0.11^{+}	0.44±0.20	0.49±0.18

*p<0.05 vs. BL1, **p<0.01 vs. BL1, ***p<0.001 vs. BL1, $^{+}$p<0.05 vs. BO1, $^{++}$p<0.01 vs. BO1, $^{+++}$p<0.001 vs. BO1

Mean±SD. SV=stroke volume, SW = stroke work, EF = ejection fraction, Ea = effective arterial elastance

5.3.2 Effect of balloon occlusion on RV diastolic function

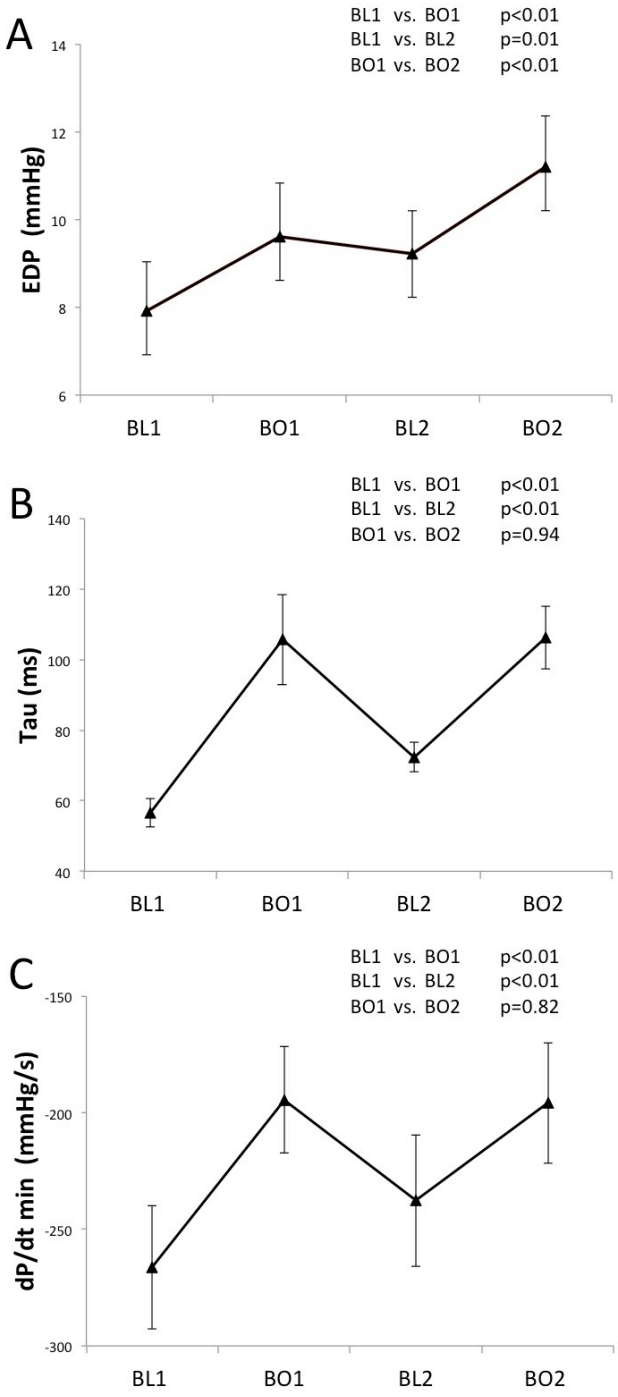
Table 5.4 shows the changes in parameters of diastolic function throughout the protocol. During BO1, both dP/dt_{min} and Tau rose compared to BL1 indicating declining diastolic function. This was associated with a rise in end diastolic pressure (EDP). At 1-minute recovery, EDP was still significantly elevated compared to BL1 (8.43±4.1 vs. 7.91±3.9mmHg, p=0.01). However, it did fall compared to BO1 (8.43±4.1 vs. 9.61±4.2mmHg, p<0.01). Tau also improved compared to BO1 but did not return to BL1 levels at 1-minute. dP/dt_{min} was not significantly different to BL1 but improved compared to BO1.

At 30-minute recovery (BL2), the markers of diastolic function had not returned to baseline for any of the measures. In addition, EDP remained elevated. A second balloon occlusion (BO2) caused a further rise in EDP beyond that seen at BO1. dP/dt_{min} and Tau both rose again with BO2 but did not worsen beyond the level seen during BO1. Changes in diastolic function throughout the study are shown in **Figure 5.3**.

5.3.3 Transient hyperaemic effect on systolic and diastolic function

Review of PV loop data recorded throughout the whole period of recovery allows the change in indices to be plotted. This is shown in **Figure 5.4**. This figure shows that the improvement seen immediately after reperfusion in both systolic and diastolic function was transient.

Figure 5.3 Change in RV diastolic function during protocol



Mean ± SEM. All four panels show a different indicator of systolic function at baseline (BL1), during balloon occlusion 1 (BO1), at 30-minute recovery (BL2) and during balloon occlusion 2 (BO2). Panel A shows end diastolic pressure (EDP). Panel B shows Tau. Panel C shows dP/dt_{min}.

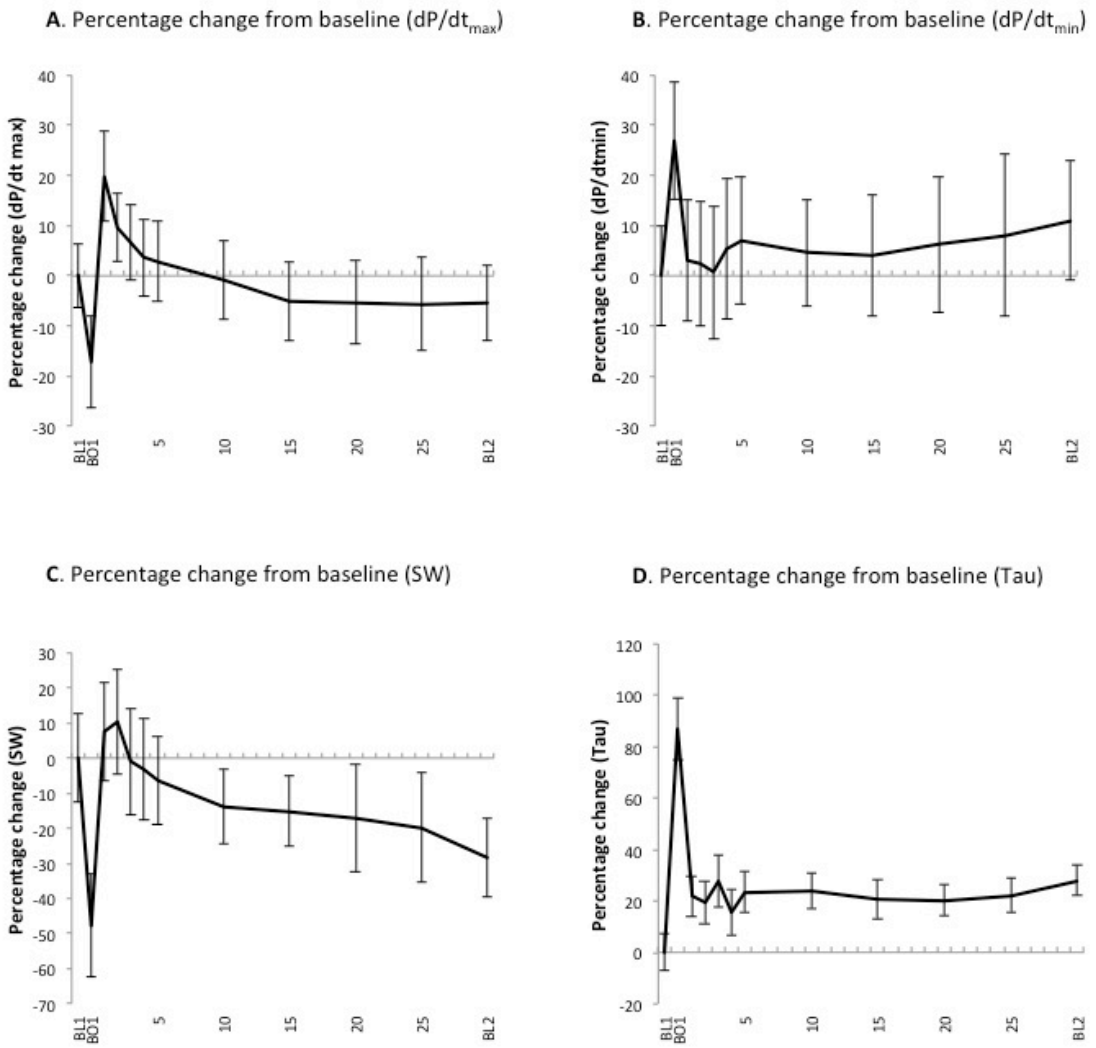
Table 5.4 Change in measures of diastolic function during RV conductance catheter study

	BL1	BO1	1-minute recovery	BL2	BO2
dP/dt_{min} (mmHg/s)	-266±92	-194±79***	-258±108^{+}	-238±98**	-196±81**
Tau (s^{-1})	56±14	105±44***	69±18***$^{++}$	72±14***	106±31***
EDP (mmHg)	7.92±3.9	9.62±4.2***	8.44±4.1*$^{+}$	9.23±3.3*	11.2±4.0***$^{++}$

*p<0.05 vs. BL1, **p<0.01 vs. BL1, ***p<0.001 vs. BL1, $^{+}$p<0.05 vs. BO1, $^{++}$p<0.01 vs. BO1, $^{+++}$p<0.001 vs. BO1

Mean±SD. EDP = end diastolic pressure

Figure 5.4 Temporal changes in systolic (A,C) and diastolic (B,D) function during recovery



Mean±SEM. SW = stroke work, BL1 = baseline 1, BO1 = balloon occlusion 1, BL2 = baseline 2

5.4 Discussion

One-minute balloon occlusion caused ischaemic RV dysfunction, affecting both systolic and diastolic parameters. Release of the balloon was associated with initial recovery in systolic function to supra baseline levels, with some improvement in diastolic function although not to baseline. At 30-minute recovery there was residual dysfunction in parameters of both systolic and diastolic function indicating that the RV was stunned. Only dP/dt_{max} and EDP deteriorated beyond the level of BO1 during BO2, suggesting that there was no cumulative ischaemic dysfunction for some indices of RV performance.

Ischaemic dysfunction following balloon occlusion of the LV has been demonstrated in a number of studies including within this thesis (Chapter 4) (126,131,224). These studies have shown stunning affecting both systolic and diastolic function at up to thirty minutes. Previous investigation into the effect of coronary balloon occlusion on the RV only looked at immediate ischaemic dysfunction and recovery in the first minute(92). These studies did not show evidence of RV stunning. Early conductance catheter studies missed myocardial stunning because the timing of post-occlusion measurement was within the hyperaemic phase of the coronary vasculature(203). In this study, there was rapid recovery of RV systolic function at 1-minute. This is known as the Gregg effect and has been described in response to ischaemia. It reflects a ventricular response to hyperemia(225). Increased volume of the microvasculature following reperfusion causes stretch-activated calcium channels to open. The resultant influx of calcium increases myocyte contractility and briefly masks the effect of stunning – this confounds investigation of stunning at 1-minute after reperfusion.

The RV has long been considered more resistant to ischaemia than the LV(88). By continuing to observe the RV after hyperaemia has resolved we see that the RV still experiences significant contractile dysfunction during occlusion of the RCA and stunning is unmasked in the minutes following supply ischaemia.

The absence of cumulative ischaemic dysfunction in several measures may support the hypothesis that the RV is more resistant to ischaemia than the LV. Alternatively, it may be that no further RV dysfunction could be produced with a single one-minute balloon occlusion. Longer balloon occlusions may be needed to produce further deterioration in RV function. If the values of Tau, SV and dP/dt_{min} are maximally impaired, then the residual function of the RV may depend upon LV contraction or the supply of some territory from another coronary artery. However, dP/dt_{max} fell and EDP continued to rise with further ischaemia suggesting that there was some continued deterioration in both systolic and diastolic function with a second occlusion.

The findings are valuable in clinical practice. PCI to the RCA is a common procedure in interventional cardiology practice. Understanding the response of the RV to balloon angioplasty may help clinical practice, particularly when there is haemodynamic instability in the aftermath of PCI. Furthermore, patients presenting with inferior myocardial infarction and haemodynamic instability often have evidence of RV involvement after much longer occlusion times. This study suggests that after reperfusion there is still residual dysfunction at 30-minute recovery. Whether this recovery takes longer if occlusion is prolonged is a matter of speculation.

The study confirms the first hypothesis; RCA balloon occlusion leads to myocardial stunning and ischaemic dysfunction in the RV. There is cumulative dysfunction seen

in some measures of RV function (EDP and dP/dt_{max}). These findings justify the second part of the protocol – assessment of whether GLP-1 protects against stunning and cumulative dysfunction in the RV. Recruitment of patients to this second group is ongoing and results will not be reported in this thesis.

5.4.1 Study limitations

The complex shape of the RV makes accurate assessment of the volume difficult as discussed in Chapter 2. The study has not been randomised. However, sequential patients eligible to enter this study are approached, avoiding selection bias between GLP-1 and control. Regardless, the conclusions of this thesis are unaffected as patients acted as their own control. Since the study is not complete, analysis of the biochemistry has not yet taken place. This will be completed once all participants in the study have undergone their procedure.

5.5 Conclusions

Brief occlusion of the RCA causes RV systolic and diastolic dysfunction. There is stunning at 30-minute recovery. However, cumulative ischaemic dysfunction was limited to some indices, with others equal to the dysfunction caused by the first balloon occlusion.

Chapter 6 GLP-1 loading during percutaneous coronary intervention (GOLD PCI) trial

6.1 Introduction

The pursuit of ever faster door-to-balloon times in modern interventional cardiology has resulted in dramatic improvements in morbidity and mortality in patients with AMI. Nonetheless, there is a limit to how much further improvement in patient outcome can be achieved through increasing efficiency in the healthcare system(226) and changes to stent or scaffold design. However, reperfusion injury may be responsible for up to 50% of final infarct size(9). This makes cardioprotective interventions such as GLP-1 attractive, since interfering with IR injury offers the promise of further reductions in infarct size. This may translate into further mortality benefit, but also into reduced heart failure following MI. Since protection in earlier human studies, including those in this thesis, has been directed against myocardial stunning and ischaemic dysfunction, it has also been proposed that GLP-1 may be of particular benefit in the treatment of cardiogenic shock.

Furthermore, there are considerable consequences to IR injury during elective PCI. Inflation of coronary balloons, combined with the loss of side-branches, and the scattering of debris to distal vessels mean that elective PCI is often associated with a substantial period of ischemia. Evidence for embolization during PCI is extensive(227,228). There is evidence of transient microcirculatory dysfunction following elective angioplasty and stenting. This results from embolization and the release of vasoactive substances from the disrupted endothelium. A number of procedural factors including stent length, lesion complexity and x-ray screening time

have been associated with the degree of myonecrosis(229). Patient factors also impact on the likelihood of significant ischemia. IR injury is often clinically silent at the time of stenting, but may still result in considerable morbidity. In other cases IR injury leads to ventricular arrhythmias such as ventricular tachycardia or fibrillation. These are associated with further myocardial ischemia. Microcirculatory dysfunction, as a result of the factors discussed above, is associated with the no-reflow phenomenon, leading to MI and increased procedural mortality.

The degree of ischemia during elective PCI can be assessed using a number of different endpoints including periprocedural MI. The 3rd universal definition of MI, published by the World Health Organization (WHO) defines a PCI associated MI (MI4a) as a rise in troponin to greater than 5x 99th centile of the reference range(31). This rise is greater than that needed to diagnose a spontaneous MI. The prognostic value of biomarker evidence of myonecrosis after elective PCI has been extensively debated(230). Troponin rise is associated with irreversible myocardial injury on cardiac magnetic resonance imaging (CMR)(231), whilst post-procedural troponin rise has been shown to be predictive of major adverse cardiovascular events (MACE), particularly when levels exceeded the WHO criteria for MI4a(232). Troponin has also been shown to be predictive in the absence of CK-MB rise(233). Other studies have suggested that post-procedural troponin release is not or only weakly associated with increased long-term mortality(234-236), but these have been confounded by the use of lower thresholds to define troponin elevation, highlighting the importance of the WHO definition. There is therefore considerable evidence that a therapy targeted at reducing the frequency of MI4a may provide clinical benefit to patients.

The GLP-1 Loading During Percutaneous Coronary Intervention (GOLD PCI) study set out to establish whether GLP-1 provided clinically relevant cardioprotection against lethal IR injury in patients undergoing elective PCI. The methodology is summarized below. Whilst the benefit of a cardioprotective intervention in elective PCI may be lower than if administered during PPCI, there are a number of advantages to performing a phase 2 trial in this patient group. The CRISP-Stent study demonstrated reduction in periprocedural troponin rise during elective PCI(32). This was associated with a reduction in MACCE at 6-year follow-up(33). Although there are fundamental differences between GLP-1 protection and IC, this finding suggests that an upfront protective strategy has the potential to confer benefit to patients treated with elective PCI.

GLP-1 has been shown to reduce infarct size in lethal IR injury in animal models but this has not been studied in humans. Many small animals have been shown to have the GLP-1R present on the cardiomyocyte(237). Evidence suggests that this receptor is not on the human ventricular cardiomyocyte(107), meaning that GLP-1 may not protect in a similar fashion in humans. Nonetheless, pilot studies have demonstrated evidence of protection with GLP-1RAs (238). GLP-1 clearly protects against other aspects of IR injury in humans, such as stunning and cumulative ischaemic dysfunction, as demonstrated in studies performed at our institution(126,127,131,132), and in Chapters 3 and 4 of this thesis. This study will provide insight into whether GLP-1 protects against lethal IR injury in humans.

Furthermore, GLP-1 has not previously been used in a phase 2 trial in patients with CAD. A strong rationale for the study is to demonstrate the safety of the technique

when used in a stable cohort of patients before considering the rationale to proceed to studies in patients with cardiogenic shock or AMI.

6.2 Methodology for GLP-1 loading during elective percutaneous coronary intervention (GOLD PCI) trial

6.2.1 Trial summary

Patients attending Papworth Hospital for elective PCI were recruited to this study. They were randomly assigned to receive either GLP-1 infusion or placebo during the PCI procedure. The PCI was performed without other deviation from standard practice. The allocation to GLP-1 or placebo was blinded from the patient, operators and the research team. At six hours post-procedure a Troponin I blood test was taken and compared to a baseline sample. Follow up of patients was also planned for 6, 12 and 60 months to look for evidence of major adverse cardiovascular and cerebrovascular events (MACCE).

6.2.2 Patient selection

Patients attending Papworth Hospital for elective PCI were recruited to this study. Inclusion criteria were broad to allow generalisability of the results. The inclusion criteria were:

- patients aged over 18 years
- attending for elective PCI to a native coronary artery
- able to give informed consent.

Exclusion criteria were:

- severe co-morbidity (defined as a life expectancy less than 6 months)
- MI within the preceding 3 months
- women of child bearing age
- chronic renal impairment (a serum creatinine > 200 μmol/L).

- pre-specified medications:
    - nicorandil
    - sulfonylureas
    - DPP4 inhibitors such as sitagliptin
    - GLP-1 receptor agonists such as exenatide
    - insulin.

Following initial recruitment to the study the exclusion criteria were modified to MI within 6 weeks rather than 3 months. This decision was taken after analysis suggested the patients undergoing staged procedures were receiving these procedures at a 6-8 week time-point.

Patients were recruited from the waiting lists for elective PCI at Papworth Hospital. They were approached via telephone or at a pre-admission clinic.

6.2.3 Assignment, randomisation and blinding

Patients were randomised after signing consent forms. The randomisation was blinded to the research team. The randomisation was performed in the hospital pharmacy according to a computer-generated randomisation list provided by an independent statistician (Oxford University Consulting, UK). The type of randomisation used was a stratified random permuted block randomisation. Patients were stratified by presence or absence of diabetes mellitus. The randomisation was to receive either GLP-1 or placebo in a 1:1 fashion.

The GLP-1 infusion or placebo infusion was prepared by two unblinded research nurses in a closed preparation room and provided to the research team for

administration. There was no visible difference between placebo and GLP-1. Emergency unblinding (if needed) was performed by the pharmacist responsible for the allocation at the request of the consultant operator responsible for the PCI.

6.2.4 Trial infusion

The Investigational Medicinal Product (IMP) in this trial was Glucagon-Like Peptide-1 (7-36) amide acetate (Bachem AG, Switzerland), hereafter referred to as GLP-1. This GLP-1 is the same as that used in the physiological studies described in earlier chapters but the sourcing was complicated by the registration of the trial as a Clinical Trial of an Investigational Medicinal Product. GLP-1 was purchased from Bachem Distribution Services GmbH (BDS, Weil am Rhein, Germany). The trial packaging and release of the product for use as an IMP was performed by Catalent Pharma Solutions Ltd (Catalent, Bolton, UK).

GLP-1 was supplied as a lyophilised powder in a glass vial. The trial infusion was made up by diluting 100μg (the vials contents) into 50ml of 0.9% saline solution. GLP-1 was administered at the standard rate of 1.2 pmol/Kg/min, as used in physiological studies. The placebo comparator used was 50ml 0.9% saline solution which was administered as though it contained GLP-1 (i.e. the same rate).

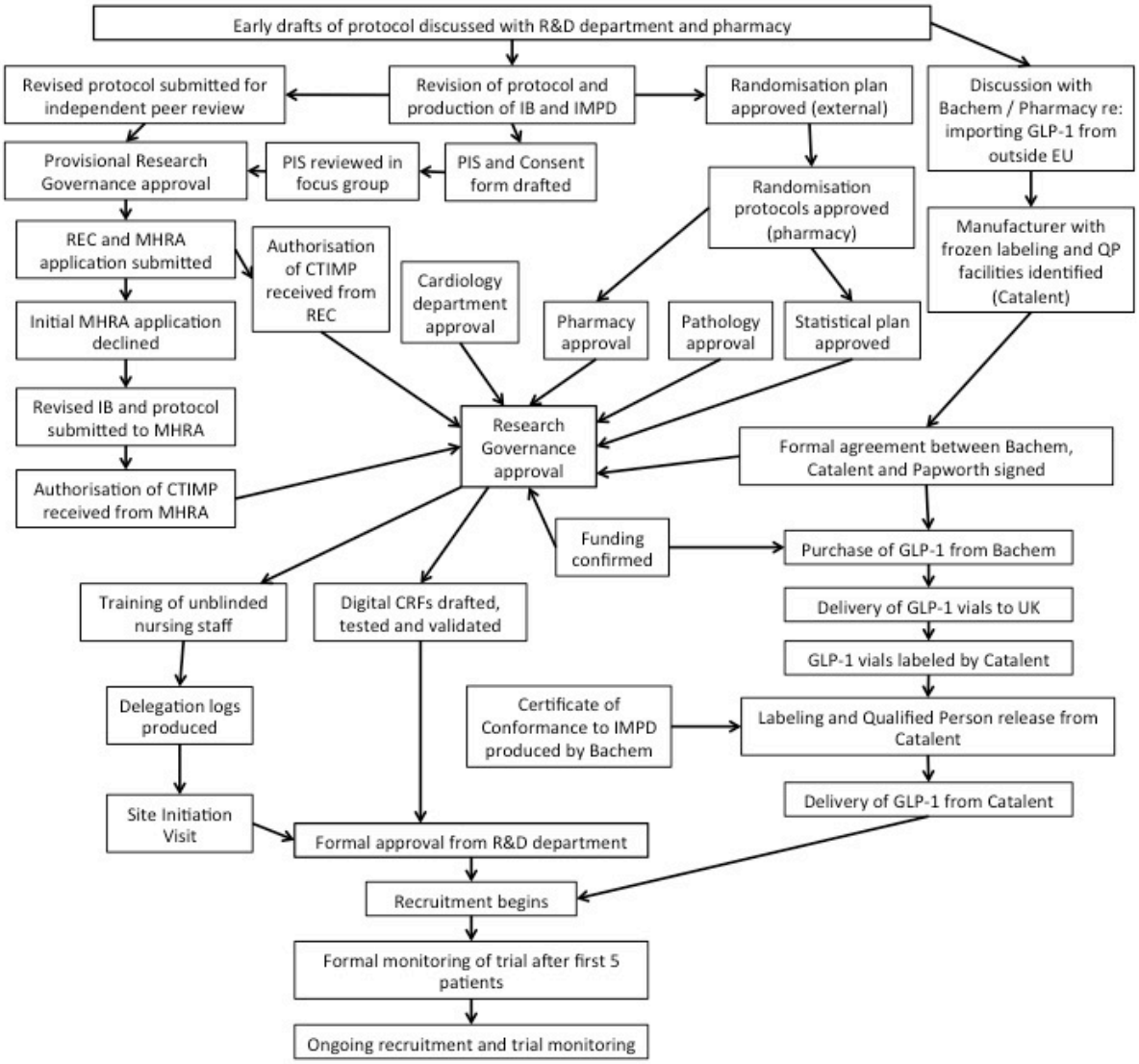
6.2.5 Regulatory approval

The regulatory steps to approval of GOLD PCI were complex. Early drafts of the protocol were reviewed internally by the R&D department of Papworth Hospital NHS Foundation Trust, who acted as the sponsor. The protocol underwent numerous revisions to ensure that it was compliant with UK and European Union (EU) law, as

contained within the “Grey Guide” to CTIMPs. Amongst the most complex steps was the import and labeling of the GLP-1 for use in the trial. Since the *drug substance* GLP-1 is produced in Switzerland by Bachem AG and its contractor Baccinex AG, documentary evidence of compliance with EU Good Manufacturing Practice was required for approval. Furthermore, final preparation of the GLP-1 for sale into the EU as a *medicinal product* was performed by BDS, a subsidiary company based in Germany. The GLP-1 was purchased from this company, rather than it’s Swiss parent. However, BDS could not perform product labeling and a Qualified Person (QP) clinical trial release compliant with the requirements of the regulator. The product was therefore imported to the UK via Catalent, a company with a UK manufacturing licence and the capability to perform trial specific labeling of a frozen product. A technical agreement covering the product and QP release of the GLP-1 for the CTIMP was negotiated and signed by Bachem AG, Catalent and Papworth Hospital.

Investigator Brochure and Investigational Medicinal Product Dossiers were produced as part of the clinical trial application. As a product without marketing authorization for any indication within the EU, these documents were constructed by the author with assistance from staff at BDS. These were reviewed and accepted by the MHRA after revision. Further revision of the IMPD was required by both Catalent and BDS to ensure that a certificate of conformance could be issued prior to QP release of the product to the trial. **Figure 6.1** shows a simplified schematic of the process for approval of GOLD-PCI.

Figure 6.1 Approval process for GOLD-PCI



6.2.6 Administration of trial infusion

Baseline blood tests were taken for Troponin I, myocardial bound- creatine kinase (CK-MB), creatinine, GLP-1 (7-36), free fatty acids (FFA), glucose and insulin. Baseline full blood count and biochemistry panels were also recorded although these are routinely performed prior to PCI at this institution.

The trial infusion was started approximately thirty minutes prior to the estimated start time of the procedure. Procedures were always timed so that GLP-1 was infused for at least 30 minutes before the start of the procedure. If the procedure was significantly delayed then the infusion was paused and initiated again based on the revised procedure time. If the procedure was delayed by longer than 4 hours then a fresh infusion was required. If this was not possible, the patient returned to routine care only but data was still collected for analysis on an intention to treat basis. If the procedure was cancelled then the patient received the infusion during the rescheduled visit. The trial infusion continued throughout the PCI procedure and in recovery afterwards. The infusion was allowed to continue until 50ml had been administered, or until the 6-hour blood tests had been taken.

6.2.7 PCI procedure

The PCI procedure was performed as per normal care. Blood pressure and heart rate were recorded at the beginning and end of each case. A blood glucose measurement was recorded at the start of the procedure. Blood samples were also taken at the beginning of the case for GLP-1 (7-36), free fatty acids (FFA), glucose and insulin.

Assessment of coronary flow and microcirculatory function were carried out before and after treatment of the coronary lesion. Coronary flow was assessed by TIMI Epicardial Flow Grade (TFG) (239). Contrast is injected into the coronary artery following selective coronary catheterization. The contrast is visualized with cineangiography and a score is determined based on the flow seen in the vessel:

0. The absence of antegrade flow beyond a coronary occlusion.
1. Penetration of the lesion by contrast but incomplete filling of the distal vessel.

2. Delayed but complete opacification of the distal vessel with contrast.
3. Anterograde flow to the distal bed occurs as promptly as flow proximal to the coronary occlusion.

Microcirculatory function was assessed with the TIMI Myocardial Perfusion Grade (TMPG). This measurement is sometimes called a myocardial blush score(240). This is a visual score based on the flow of contrast through the microcirculation, which in normal circumstances has a transient ground-glass (blush) appearance. TMPG images were taken by cineangiography in an orientation least likely to superimpose uninvolved territories. A frame rate of 25-30 frames per second (dependent upon the equipment available) was selected. Contrast was injected into the coronary artery at the start of cineangiography. Duration of cineangiography was required to be at least three cardiac cycles of the washout phase. The appearance of the microcirculation was sored as follows:

0. Failure of contrast to enter the microvasculature. Either minimal or no blush or opacification of the myocardium in the distribution of the culprit artery, indicating lack of tissue-level perfusion.
1. Contrast slowly enters but fails to exit the microvasculature. There is blush or opacification of the myocardium in the distribution of the culprit lesion that fails to clear from the microvasculature, and contrast is present on the next injection (~30 seconds between injections).
2. Delayed entry and exit of contrast from the microvasculature. There is blush or opacification of the myocardium in the distribution of the culprit lesion that is strongly persistent at the end of the washout phase (ie, dye is strongly

persistent after 3 cardiac cycles of the washout phase and either does not or only minimally diminishes in intensity during washout).

3. Normal entry and exit of contrast from the microvasculature. There is blush or opacification of the myocardium in the distribution of the culprit lesion that clears normally and is either gone or only mildly/moderately persistent at the end of the washout phase.

Choice of treatment and PCI technique was at the discretion of the operator. The study did not mandate stent selection or use of intra-coronary imaging. Multi-vessel PCI could be undertaken. In this instance, TFG and TMPG were collected for each vessel. Lesion characteristics including site, severity, degree of calcification and length were collected. Procedural data including stent choice, balloon time, contrast use, procedure length and radiation dose were also noted.

Patients were asked whether they experienced any ischaemic symptoms during the procedure, rating the severity on a score from 0-10. ST deviation of >1mm on procedural ECGs was noted.

6.2.8 Trial endpoints

The primary endpoint for this trial was rate of MI4a. This was defined as patients with a Troponin I level of 5 x 99th centile of the normal reference range at 6 hours following the procedure(241).

Secondary endpoints were:

- absolute change in Troponin I from baseline
- change in CKMB from baseline

- ischaemic symptoms during the procedure
- ECG changes during the procedure
- TIMI Epicardial Flow Grade
- TIMI Myocardial Perfusion Grade
- MACCE rate at 6 months
- MACCE at 12 months
- MACCE at 60 months

Exploratory sub-group analysis of these endpoints in diabetic and non-diabetic subgroups were planned prior to commencement of the trial.

6.2.9 Data monitoring committee

A Data monitoring committee (DMC) was set up to monitor trial participants for safety. The DMC also reviewed event rates in the control arm of the study to ensure that it was adequately powered in order to meet its endpoints. The DMC was composed of three senior clinicians from within Papworth Hospital who were independent of the department of interventional cardiology. In addition, the committee also had a chair, an external interventional cardiologist, who was independent of both the investigators and the sponsor (Papworth Hospital NHS Foundation Trust). Finally, the committee was supported by an external statistician who was unblinded to patient allocation in the study.

6.2.10 Biochemistry

Troponin I is a biomarker released during injury to cardiomyocytes. It is commonly used in clinical practice for the detection of MI since it is highly specific to cardiac

injury. This contrasts with other biomarkers that have been used historically but are less specific. A high-sensitivity troponin assay was used for the study (Siemens Loci, Germany).

The WHO 3rd Universal definition of MI includes PCI-associated myocardial infarction (MI4a). This is defined as a rise in troponin I to 5 x 99th centile of the reference range following a PCI(241). By comparison, the definition of spontaneous MI (outside a procedural setting) is a rise above the 99th centile in conjunction with relevant symptoms or ECG criteria.

The assay used for troponin I in this study as a limit of detection of 17 ng/L with the 99th centile of the reference range corresponding to 56 ng/L. For this assay, MI4a (5 x 99th centile of the reference range) is defined as a rise to greater than 280ng/L. This value has been taken as the cut off for the primary endpoint in the study.

GLP-1, free-fatty acids and insulin levels have been collected for patients involved in the study at two time points. The first is at baseline, prior to administration of the trial infusion. These measurements are then repeated at the beginning of the PCI procedure after the infusion has been initiated and allowed to reach a steady-state level. The procedure for obtaining these samples is the same as that described earlier in the thesis.

6.2.11 Statistical methods

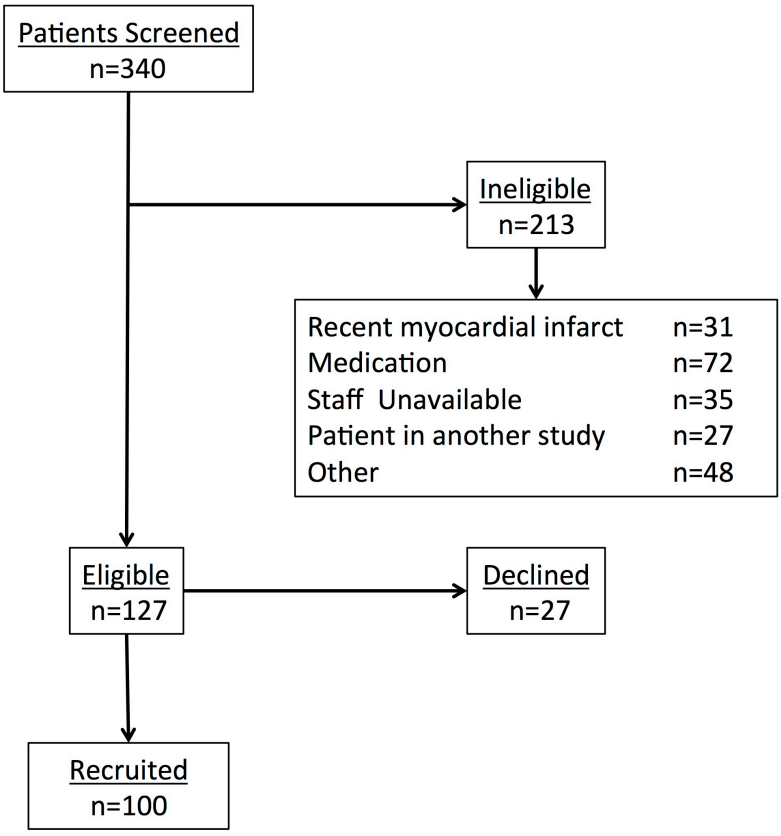
The nature of this study as a CTIMP necessitated the involvement of a statistician and the drafting of a formal statistical analysis plan.

The study is powered using a comparison of proportions to show a 12.5% absolute reduction in the frequency of MI4a in the treated group. Pilot data from PCI with 2nd generation drug eluting stents shows an incidence of a MI4a of up to 25% and the study is therefore powered for a 50% relative reduction in the frequency of the events. Power has been calculated with alpha 0.05 and beta 0.2 giving a group size of 168 – a total of 336 patients. Recruitment is planned for 400 patients to allow for incomplete data. This power calculation will be reviewed as the trial progresses by the DMC to ensure adequate statistical power.

6.3 Results

GOLD-PCI is a blinded clinical trial, which will continue to run with an estimated recruitment period of at least two more years (from March 2016). As a result, it is not possible to provide data that compares GLP-1 with placebo for this thesis. Presented are total group data, which include demographic and procedural data, incidence of adverse events and overall data regarding the endpoints studied as of March 2016. A consort diagram (**Figure 6.2**) shows the recruitment for the study.

Figure 6.2 – Consort diagram for the GOLD-PCI study



Demographic data is summarized in **Table 6.1**. 100 patients were recruited between March 2015 and March 2016. There were 76% male with a median age of 67.5.

Table 6.1 Demographics of the GOLD-PCI study

Demographics	n=100
Age	67.5 [64 – 74]
Male Sex	76 (76%)
Diabetes Mellitus	12 (12%)
Chronic Kidney Disease	5 (5%)
Previous MI	28 (28%)
Heart Failure	2 (2%)
Previous PCI	29 (29%)
Previous CABG	4 (4%)
Hypertension	53 (53%)
Hypercholesterolemia	29 (29%)
BMI, Kg/m^2 (mean±SD)	29.0±5.36

Of the patients recruited to the study, 93% had a PCI procedure with 7 patients not proceeding to an angioplasty or stent. This was due to either a change in clinical circumstance between initial referral and the procedure, or the elective decision of the operator not to undertake the procedure. Procedural data including quantitative coronary angiographic analysis of those lesions treated with angioplasty and stenting are shown in **Table 6.2**.

Table 6.2 Procedural characteristics of the GOLD-PCI study

Procedural data	n=93
Dual-Antiplatelet therapy	
Aspirin	93 (100%)
Clopidogrel	83 (89.2%)
Ticagrelor	10 (10.8%)
Multi-vessel PCI	14 (15.1%)
Vessel (First treated)	
LAD (including Diagonals)	46 (49.5%)
RCA	26 (28.0%)
CX (including Obtuse Marginals)	19 (20.5%)
LMS	0 (0%)
Intermediate	2 (2.2%)
Lesion type (First treated)	
A/B1	27 (29.1%)
B2/C	66 (70.9%)
Lesion length, mm (mean±SD)	25.9±14.9
Min Lumen diameter, mm (mean±SD)	0.87±0.46

Reference diameter, mm (mean±SD)	2.93±0.56
Diameter stenosis, % (mean±SD)	70.3±14.5
Area stenosis, % (mean±SD)	88.1±8.9
Acute gain, mm (mean±SD)	1.82±0.67
Lesion success	91 (97.8%)
Total balloon time, secs (mean±SD)	120±83
Screening time, mins (mean±SD)	12.7±6.3
Contrast dose, ml (mean±SD)	170±68
Stent data	**n=132**
Stent length	23 [18-32.25]
Stent diameter	3.0 [2.5-3.5]
Stent type	
Drug-eluting stent	127 (96.2%)
Bare metal stent	4 (3.0%)
Bioresorbable vascular scaffold	1 (0.8%)

Overall, there was a mean of 1.4 stents per patient, with 1.2 stents per lesion. Where a procedure took place – such as further coronary angiography, or pressure wire assessment of the vessel – endpoints are recorded. Overall, the incidence of MI4a

was 8.2%. This rate is below the predicted rate based on previous data. **Table 6.3** shows this data.

Table 6.3 Endpoints of GOLD-PCI study

Endpoints	
MI4a	8 (8.2%)
Post procedure Troponin I	39 [17-103]
Ischaemic Symptoms	63 (77%)
ST-segment deviation	37 (40.2%)
TIMI Flow Grade ≤ 2	0 (0%)
TIMI Myocardial Blush Grade ≤ 2	3 (3.2%)

Follow-up data as well as a number of biochemical markers (including CKMB and GLP-1 levels) will be processed as a batch at the completion of recruitment to the study. Safety information was also collected during the study. There were no severe unexpected serious adverse reactions (SUSAR) reported to the MHRA during the first 100 patients. No episodes of hypoglycaemia reached the study criteria for seriousness. A summary of all adverse events reported up until patient discharge is included in **Table 6.4**.

Table 6.4 Adverse events in GOLD-PCI

Adverse Event	Numbers	Relationship to GLP-1
Hypoglycaemia	3	Probable (all)
Haematoma	3	Not related (all)
Vasovagal response	2	Unlikely (both)
Severe chest pain	2	Not related (both)
Bradycardia	2	Not related (all)
Swollen lips	1	Unlikely
Headache	1	Unlikely
Shivering	1	Unlikely
Arm pain	1	Unrelated
Nausea/vomiting	1	Possible
Hospitalisation following high volume of contrast	1	Unrelated

6.4 Discussion

GOLD PCI was designed to evaluate the efficacy of GLP-1 as a protective agent during elective PCI. The design of the study paid attention to the difficulties inherent in trial design for cardioprotection. The time-dependence of cardioprotection is reflected in the choice of an elective setting for this early evaluation of the agent. Whilst an acute MI population would perhaps have greater potential to demonstrate clinical benefit, the stable nature of this subset of patients makes it an attractive target for a “proof-of-concept” study.

GLP-1 has no licensed indication within the EU, whilst in the United States it has only been given an Investigational New Drug license. As a result, the IMP will be subject to intense scrutiny, for efficacy, but also patient safety. Studies in which IMPs are used to treat AMI can struggle to obtain informed consent. Whilst consent can be obtained verbally, or in short written form prior to PPCI, patients may not have time to weigh the risk and benefit of involvement. This makes GOLD PCI an ideal early phase 2 study, providing safety data that could support an application for a clinical trial in an unstable patient group such as PPCI or in cardiogenic shock. Regional and national ethics committees have provided permission to other researchers to “presume” consent, such as in the case of HEAT PPCI(242). However in these situations there has often been equipoise between two licensed and appropriate treatments(243). Positive results may provide data to ethically justify a more extensive PPCI study, whilst safety during PCI can be shown regardless of efficacy. The data does not show an excessively high rate of adverse events, particularly adverse reactions. This safety data can be better assessed once the study has unblinded.

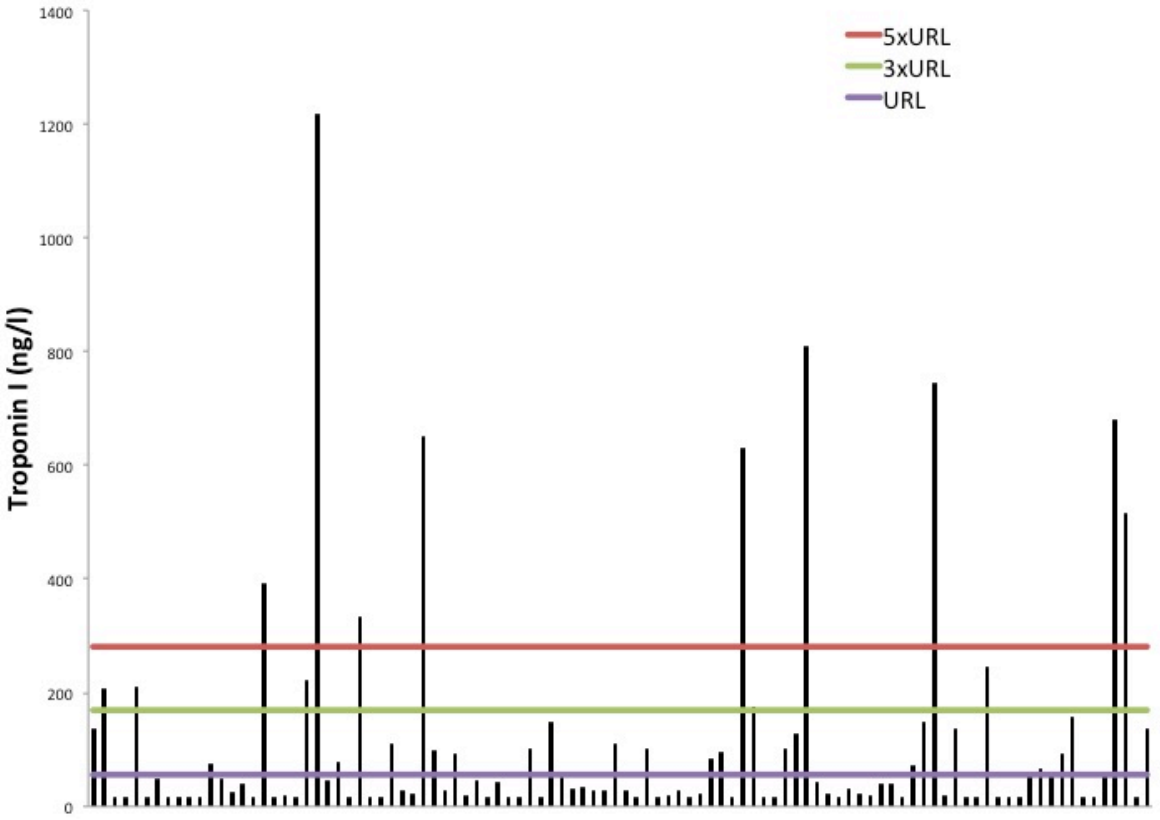
Another clear advantage of this stable population is that the time-dependence of cardioprotection does not limit either the recruitment or the conduct of the study. Since elective PCI, by its nature, is a planned period of ischaemia, the GLP-1 can be initiated in advance of stenting. Whilst time dependence does not seem to be important for GLP-1 cardioprotection against non-lethal ischaemia, its impact upon lethal ischaemia is not clear. Since the lesions to be treated are known in advance, the study is likely to experience a low dropout rate from the study since almost all patients recruited will be treated with a stent.

By including a pre-specified analysis of the subset of patients with diabetes, this particular heterogeneity can be investigated. The role of GLP-1 may be different in diabetic patients. Additionally, the threshold for IC is higher in diabetics and therefore one may expect a dose-dependent effect if the pathway for GLP-1 protection against lethal IR injury is similar.

Several comments can be made on the data already presented. A low rate of MI4a is seen in this early data. A number of factors may explain this. Firstly, the data used to predict the power of the study was based on second-generation drug-eluting stents and bare metal stents. Newer stents and bioresorbable scaffolds may have a lower profile or produce less downstream emboli, resulting in less ischaemia and infarction around the elective procedures. The definition of MI4a has also changed since the pilot data was recorded. The 3rd universal definition of an MI4a raised the Troponin I threshold from 3x to 5x the 99th centile of the reference range. This reduced the number of patients diagnosed with MI4a. Under the older definition, the number with an MI4a was 13 (13.3%), whilst the number with a troponin rise above the 99th centile

(the criteria for diagnosis of an MI in the appropriate clinical situation) was 31 (31.6%). **Figure 6.3** shows a bar chart with all Troponin values plotted and shows the threshold for the current WHO definition, the older definition of MI4a and the threshold for diagnosis of spontaneous MI, in the present of appropriate clinical features.

Figure 6.3 Chart displaying distribution of post-procedural Troponin I throughout first 100 patients in GOLD PCI



All patients in the study received dual-antiplatelet therapy, but a number received ticagrelor rather than clopidogrel, as they were staged procedures following an ACS presentation. Additionally, techniques employed by operators continue to improve,

and, as a high volume centre, operators at Papworth Hospital have consistently outperformed national and international statistics for mortality and other safety outcomes. This may translate into a reduced incidence of MI4a. Finally, although there are no specific exclusion criteria that preclude them, there are few highly complex procedures within the data set, including no rotational atherectomy cases and low numbers of complex bifurcation or chronic total occlusion cases. These cases are far more likely to be associated with a high level of post-procedural Troponin I elevation. At a recent meeting of the trial steering committee, it was agreed to attempt to enrich the cohort with more of these higher risk patients.

It may be that the statistical calculation underpinning the trial has led to an underpowered design. As the trial is double blind, it is not possible to perform a side-by-side analysis of control and GLP-1 treated to groups to properly assess this. However, the DMC have the power to recommend changes to planned recruitment number to ensure the study is adequately powered. If the MI4a rate is of the order of 10%, a figure suggested by the early data, a revised power calculation (using a comparison of proportions) could be made for a 50% reduction in MI4a rate to 5%. These revised figures produce a required sample size of 435 patients per group. It needs to be considered whether this remains an achievable sample size for a single centre study.

6.5 Conclusion

GOLD-PCI is a study targeted at assessing the efficacy of GLP-1 as a cardioprotective strategy in the elective setting. It has been designed to avoid a

number of the failings of other cardioprotective studies. The interim analysis performed here highlights the difficulty of moving from focused preclinical studies into larger scale clinical trials. Whilst this study has attempted to apply lessons learned from the failure of previous studies in cardioprotection, translation research remains extremely challenging. Limited patient recruitment, because of carefully chosen inclusion and exclusion criteria, has slowed the progress of the study. At the same time, the low rate of endpoints has reduced its statistical power, which, with limited resources, may make it impossible to test the hypothesis for which it was designed. The results of the study are awaited once recruitment has been completed but will not be reported in this thesis.

Chapter 7 Conclusions

7.1 Summary

GLP-1 is an incretin hormone that has potential for therapeutic use as a cardioprotective agent. The work in this thesis has examined three hypotheses relating to its function in the myocardium. In the demand ischaemia study (Chapter 3) it was shown that the addition of glibenclamide did not interfere with the protective effect of GLP-1 during DSE. Similarly, in the LV supply ischaemia study (Chapter 4), GLP-1 mediated protection against LV stunning and cumulative dysfunction was not affected by the addition of glibenclamide. Glibenclamide alone did not have independent effect on LV stunning or dysfunction. The findings of these two studies together suggest that GLP-1 cardioprotection is not mediated through a KATP dependent pathway. These findings may reflect the operation of an alternative pathway, redundancies within the RISK/SAFE pathways that allow continued protection despite interference, or a combination of the above.

The findings of the RV supply ischaemia study (Chapter 5) addressed the response of the RV to non-lethal ischaemia. Whilst the RV was stunned in a similar fashion to the LV, the addition of a further balloon occlusion did not result in cumulative ischaemic dysfunction. The degree of ischaemic dysfunction and stunning in the RV after a single balloon occlusion suggests the RV is an attractive therapeutic target for the short-term administration of a protective agent. This is particularly relevant for the treatment of patients suffering from cardiogenic shock after RV infarction. At this stage, the study is still to be completed, and the effect of GLP-1 on RV stunning remains the subject of this ongoing work.

The GOLD-PCI trial continues to recruit patients. The early demographic data suggests that the study reflects a real world population although the low rate of MI4a suggests that it may ultimately be underpowered, but changes to study recruitment targeting higher risk PCI patients may mitigate this. Furthermore, if the rate of MI4a continues decline, the number needed to treat for any therapy looking to mitigate this troponin rise will continue to rise and the clinical relevance of any therapy may be lost.

Recent studies addressing the role of GLP-1 as a cardioprotective agent have been sparse. Cardiovascular outcome trials for GLP-1 RA's are beginning to be published. Whilst the Exenatide Study of Cardiovascular Event Lowering Trial (EXSCEL) (ClinicalTrials.gov Identifier: NCT01144338) is due to complete in 2018, the Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER) study has recently been published. The latter study confirmed a statistically significant reduction in major adverse cardiovascular events, and cardiovascular death, when administering liraglutide to type 2 diabetic patients with a high cardiovascular risk indicating a possible cardioprotective effect(244). The FIGHT trial (ClinicalTrials.gov Identifier: NCT 01800968) presented at the AHA annual meeting in 2015 showed that liraglutide did not negatively affect outcomes when administered to patients with advanced heart failure(245). These large-scale studies suggest an important role for GLP-1, whilst indicating that more detailed work on its role in cardioprotection is needed.

7.2 Future directions

The mechanism by which GLP-1 mediates it protective effect in both lethal and non-lethal ischaemia remains uncertain. Whilst there remains strong evidence for the RISK/SAFE pathways, the mechanism of activation, and the final mediator of protection remain unknown. An alternative hypothesis, which remains to be tested, is that GLP-1 is a coronary or microvascular vasodilator. The effect of hyperaemia on indices of cardiovascular function is well documented. If GLP-1 were to induce hyperaemia in the coronary bed, this may result in improvement in indices of ventricular function such as those seen in the studies in this thesis. Since the GLP-1R has been reported to be absent from the ventricular cardiomyocyte of large mammals, such a mechanism would be consistent with current evidence. Confirmation of the absence of the GLP-1R from the ventricular cardiomyocyte in humans is also needed. Changes in myocardial metabolism may still account for some of the protection seen. However, the evidence from this thesis suggests that simple metabolic switching is not responsible. Differential changes between ischaemic and non-ischaemic segments of myocardium could still be responsible, with no overall effect on effluent of metabolites at the coronary sinus. This hypothesis may need testing in a large animal model as practical and ethical issues may interfere with human research.

The GOLD-PCI study may show a protective effect. However, regardless of the outcome, if it demonstrates that GLP-1 is safe to use during PCI, then further studies investigating the effect of GLP-1 when administered during PPCI, or to patients with cardiogenic shock may be considered. Studies in patients with cardiogenic shock are

justified based on the protection against non-lethal ischaemia seen with GLP-1 infusion during supply and demand ischaemia. Shock and PPCI studies will need to be designed and powered adequately to ensure that they can produce definitive results.

7.3 Conclusions

GLP-1 protection against non-lethal ischaemia in the LV is not KATP channel dependent. Non-lethal ischaemia in the RV produces stunning, and further investigation into whether GLP-1 protects in this situation is merited. GLP-1 remains an agent with significant promise for the treatment of myocardial IR injury and continued investigation is needed.

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Appendix A Conduct of the work in this thesis

Ethics approvals and other trial numbers

All work in this thesis was carried out with the approval of the listed ethics committees. All patients gave informed written consent to be involved in the studies contained within this thesis. The research was carried out in accordance with the Declaration of Helsinki.

Demand ischaemia protocol

Ethics committee	Cambridge East
Ethics approval number	08/H0304/68
Date of ethics approval	20/08/2013
ISRCTN Registration	ISRCTN69686930

LV supply ischaemia protocol

Ethics committee	Cambridge East
Ethics approval number	14/EE/0056
Date of ethics approval	02/04/2014
Clinicaltrials.gov registration	NCT02128022

RV supply ischaemia protocol

Ethics committee	Cambridge Central
Ethics approval number	14/EE/0141
Date of ethics approval	18/6/2014
Clinicaltrials.gov registration	NCT02236299

GOLD-PCI

Ethics committee	Cambridge Central
Ethics approval number	14/EE/0218
Date of ethics approval	16/07/2014
Clinicaltrials.gov registration	NCT02127996
EudraCT Number	2013-001967-22
Date of MHRA approval	05/08/2014

Contributions to this thesis

All work in this thesis is the author's. Research has been performed in collaboration and where others have been involved, their contribution is noted below. The author was involved in all aspects of the work from the inception to publication. The author wrote the protocols for all the studies. The author also wrote the ethics and MHRA applications for the studies. The DSE study application was submitted as an amendment to an existing authorisation. Furthermore, the author obtained permissions from the R&D departments for all studies and composed the IMPD and IB for use in GOLD PCI.

The author identified all patients and approached them for the conductance catheter and DSE studies. The author collaborated with the staff of the R&D department to identify and approach patients for GOLD PCI. Written consent was taken for all patients in the studies. In the case of the of DSE and conductance catheter studies this was performed by the author, whilst in GOLD PCI written consent was obtained by the author, or, in his absence, Dr Stephen Hoole.

For the DSE studies, the author recruited all patients, performed all DSE scans and analysed all echocardiographic data. The preparation and administration of intravenous dobutamine and GLP-1 (7- 36) amide was also performed by the author. The author administered the glibenclamide tablets. The nursing staff on the Clinical Research Facility assisted with the preparation of syringes for GLP-1 samples, and with the spinning and aliquoting of blood samples. The blood work was analysed in the University Core Biochemical Assay Laboratory by University and CUHP staff under the supervision of Mr Keith Burling, biochemist.

For the conductance catheter studies, the author supervised each of the procedures to ensure compliance with the protocol. The interventional procedures were performed by the Consultant Interventional Cardiologist responsible for the care of the patient. The author prepared the syringes for GLP-1 samples pre-procedure, took the blood samples, and was responsible for the preparation and administration of GLP-1 (7-36) and glibenclamide for each study. The spinning and aliquoting of blood samples post-procedure were performed by the Papworth Hospital tissue bank. The acquisition and analysis of the conductance catheter data was performed by the author with the assistance of Professor Paul White, Clinical Engineering Consultant.

In GOLD PCI, the author was involved at all stages of planning and authorisation of the study. The Papworth Hospital statistician produced the statistical analysis plan for the study (as per MHRA guidelines) in conjunction with the author. Recruitment was performed by the author and other staff in the R&D department. The preparation of the GLP-1 or saline infusions was performed in a double blind fashion by a team of research nurses. Administration of this infusion was performed by either the author or Dr Hoole.

All statistical analyses and the presentation of data in this thesis were conducted by the author.

Dr David Dutka DM FRCP, University Lecturer and Honorary Consultant Cardiologist

Dr Dutka supervised the work contained in this thesis. In particular, he contributed to the design and planning of all the research studies and assisted with the interpretation of the results.

Dr Stephen Hoole MA DM FRCP, Consultant Interventional Cardiologist

Dr Hoole co-supervised all the work contained in this thesis. He contributed to the design and conduct of the supply ischaemia studies and the GOLD PCI trial for which he acted as Principal Investigator. Dr. Hoole proofread the chapters of this thesis.

Professor Martin Bennett, BHF Professor of Cardiovascular Sciences

Professor Bennett agreed to formally supervise the author during the final months of this thesis after the retirement of Dr. Dutka.

Dr Liam McCormick MD MRCP, Consultant Interventional Cardiologist

Dr. McCormick contributed to the design and planning of the interventional studies and particularly the early development of the GOLD PCI protocol.

Ms Sophie Clarke BSc, PhD student

Ms. Clarke assisted with the design and planning of the studies as well as assisting with the interpretation, particularly of the metabolic data

Dr Johannes Reinhold MD MRCP, Clinical Research Fellow

Dr Reinhold assisted with the conduct of the dobutamine stress echocardiograms.

Professor Paul White PhD, Consultant in Clinical Engineering

Professor White provided technical assistance with the acquisition and interpretation of the conductance catheter data.

Mr Richard Axell MEng, Clinical Engineer

Mr Axell also provided technical assistance with acquisition and interpretation of conductance catheter data

Dr Nick West MD FRCP, Consultant Interventional Cardiologist

Dr West performed the interventional procedures for the supply ischaemia studies.

Dr Michael O'Sullivan PhD FRCP, Consultant Interventional Cardiologist

Dr O'Sullivan performed the interventional procedures for the supply ischaemia studies.

Location of the studies

The DSE studies were performed in the Wellcome Trust Clinical Research Facility in the Addenbrooke's Centre for Clinical Investigation, which is located in the main building at Addenbrooke's Hospital. The echocardiographic images acquired during the DSE scans were uploaded from the echo machine onto University of Cambridge computers. Blood samples taken during the studies were centrifuged, aliquoted and stored in freezers within the Clinical Research Facility. They were analysed in the Core Biochemical Assay Laboratory on the Addenbrooke's Hospital site.

The interventional studies were performed in the Cardiac Catheterisation Laboratories at Papworth Hospital, which is a tertiary cardiac centre in Cambridgeshire. The conductance catheter data were analysed on a trust computer on the Addenbrooke's site. Blood samples taken during the interventional procedures were centrifuged, aliquoted and stored in freezers in the Tissue Bank at Papworth Hospital. They were transferred to the Core Biochemical Assay Laboratory on the Addenbrooke's Hospital site for analysis.

Publications and Presentations arising from this thesis

Publications

- Giblett JP, Axell RG, White PA, Clarke SJ, McCormick LM, Read PA, Reinhold J, Brown AJ, O'Sullivan M, West NEJ, Dutka DP, Hoole SP. Glucagon-like Peptide-1 derived cardioprotection does not utilize a KATP-channel dependent pathway: mechanistic insights from human supply and demand ischemia studies. Cardiovascular Diabetology. July 2016; 15 (99).
- Giblett JP, Clarke SJ, Dutka DP, Hoole SP. Glucagon-like Peptide-1: a promising agent for cardioprotection during myocardial ischaemia. *JACC: Basic to Translational Science*. June 2016; 1 (4) p267-76
- Giblett JP, West NEJ, Hoole SP. Cardioprotection for Percutaneous Coronary Intervention–Reperfusion Quality as well as Quantity. *International Journal of Cardiology*. 2014; 177 (3) p786–793

Presentations

- Giblett JP, Axell RG, Clarke SJ, West NEJ, O'Sullivan M, McCormick LM, Read PA, White PA, Dutka DP, Hoole SP. GLP-1 cardioprotection from myocardial stunning during supply ischaemia is not mediated by a mitochondrial KATP dependent pathway in humans. ESC 2016.
- Giblett JP, Axell RG, Clarke SJ, West NEJ, O'Sullivan M, McCormick LM, Read PA, White PA, Dutka DP, Hoole SP. GLP-1 cardioprotection against ischemic dysfunction in humans is not mediated by a mitochondrial K-ATP dependent pathway in left ventricular conductance catheter studies. TCT 2016.

- Giblett JP, Reinhold J, Clarke SJ, McCormick LM, Read PA, Dutka DP, Hoole SP. GLP-1 enhances left ventricular function during demand ischaemia and mitigates stunning in humans, but its effect is not mediated through the mitochondrial KATP channel. ESC 2016.
- Giblett JP, Axell RJ, Clarke SJ, McCormick LM, Read PA, Reinhold J, O'Sullivan DM, West NEJ, White, PA, Hoole SP. GLP-1 Cardioprotection is not mitochondrial KATP dependent. ACI 2016.

Publications during research fellowship not directly related to thesis

- Giblett JP, Brown AJ, Keevil H, Jaworski C, Hoole SP, West NEJ. Implantation of bioresorbable vascular scaffolds following acute coronary syndrome is associated with reduced early neointimal growth and strut coverage. *Eurointervention*. 2016; 12(6) p724-733.
- Giblett JP, Brown AJ, Hoole SP, West NEJ. Early disarticulation of a Bioresorbable Vascular Scaffold: an underreported consequence of repeat imaging. Under review with *Catheterization and Cardiovascular Interventions.*
- McCormick LM, Brown AJ, Ring LS, Gajendragadkar PR, Dockrill SJ, Hansom SP, Giblett JP, Gilbert TJ, Hoole SP, West NE. Direct stenting is an independent predictor of improved survival in patients undergoing primary percutaneous coronary intervention for ST elevation myocardial infarction. *European Heart Journal: Acute Cardiovascular Care*. 2014. p2048872614530864