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Insulin resistance, chronic heart failure and potential treatment

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Insulin Resistance, Chronic Heart Failure And Potential Treatment

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INDEX OF ABBREVIATIONS

CHF   Chronic Heart Failure

HF   Heart Failure

IR   Insulin Resistance

NYHA   New York Heart Association

DM   Diabetes Mellitus

T2DM   Type 2 Diabetes Mellitus

SD   Standard Deviation

OR   Odd Ratio

CI   Confidence Intervals

LVEF   Left ventricular Ejection Fraction

HbA1c   Glycated Haemoglobin A1c

FIRI   Fasting Insulin Resistance Index

HOMA-IR   Homeostasis Model Assessment for Insulin Resistance

ACEi   Angiotensin Converting Enzymes Inhibitor

TZD   Thiazolidinediones

eGFR   Estimated Glomerular Filtration Rate

ED   Endothelial Dysfunction

CVD   Cardiovascular Disease

NO   Nitric Oxide

SNS   Sympathetic Nervous System

RAS   Renin Angiotensin Aldosterone System

MRA   Minerocorticoids Receptors Antagonist

CPET   Cardiopulmonary Exercise Testing

AMPK   5'-AMP-activated protein kinase

AICAR   5-aminoimidazole-4-carboxamide riboside

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DECLARATION

I declare that this work has not previously been submitted for a higher degree. The design on the work presented in this thesis was that of the author and his supervisors, Professor Chim Lang and Professor Allan D Struthers. The author performed all research works unless acknowledged otherwise. Statistical support was provided by Dr Simon Ogston and Dr Donald Ang.

Ms Lesley McFarlane and Dr Velerie Godfley conducted all the laboratory analyses. The initial data of metformin use and mortality in CHF and glycaemic control in CHF study were collected and analysed by Dr Dougie Elder.

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Aaron KF Wong 2013

THESIS OUTLINE

Diabetes Mellitus (DM) and insulin resistant (IR) are highly prevalent among heart failure (HF) patients. There is now increasing evidence to suggest a bi-directional relationship between IR and HF. DM and IR not only lead to heart failure, but heart failure can also lead toward the development of DM or IR.

The degree of IR also correlates with the severity and mortality of CHF. The pathophysiology of IR in CHF has yet to be fully defined. Activation of sympathetic nervous system, abnormal regulation of adipocytokines systems, activation of inflammatory and coagulation cascade, accumulation of glycated products, endothelial dysfunction and hyperinsulinaemia are potential explanations of the development of IR in CHF. Additionally, it remains to be determined if IR is merely a marker reflecting the severity of CHF or whether it contributes to the disease in CHF. If IR is truly a culprit that worsens CHF, reversing IR may potentially be a new target for treatment in CHF, which may result in an improvement in symptoms and even mortality in patients with CHF. However, there are concerns over the use of certain insulin sensitizers, most notably, the thiazolidinediones (TZDs), which has been linked with increased risk of hospitalizations for CHF and concerns regarding its association with increased myocardial infarction. Despite previous concerns of lactic acidosis, there is now evidence that metformin may not only be safe but could potentially be useful in the setting of CHF. We have conducted a randomised double-blind, placebo-controlled trial testing the hypothesis of reversing IR with metformin in insulin-resistant CHF will have beneficial effects. If IR is a possible target for the treatment of CHF, what are the new and

potential treatment modalities? We have now had better understandings of the adipocytokines systems, which may prove to be a therapeutic option to improve IR in CHF. AMP-activated protein kinase (AMPK) pathway has become the focus of research as a novel therapeutic target in cardio-metabolic disease. It has been shown to mediate, at least in part, the effects of a number of physiological and pharmacological factors that improve IR. It also exerts beneficial effects on the vasculature and the heart. There have been some new AMPK activators that are currently being tested in vivo setting or phase 1-2 trials, and the early results are somewhat promising.

Increased understandings and refreshed insights of IR and CHF have opened a new horizon and encouraged us to explore more therapeutics options in CHF.

CHAPTER 1: DIABETES AND CHF

INTRODUCTION

Diabetes and CHF often co-exist with an inter-relationship such that each condition may impact on each other in terms of causation and outcome (Figure 1). The Framingham Study highlighted the co-existence of diabetes and CHF (1). Kannel et al reported that 19% of patients with CHF in the Framingham Study have diabetes and that the risk of CHF increases by 2-8 folds in the presence of diabetes (1). The prevalence of DM is around 4-7% in the general population and 0.5% of the general population has both DM and HF (2, 3). From population-based studies and in CHF trials, the prevalence of T2DM is estimated to be between 11% and 28% and increased to 25-30% among all patients hospitalized for CHF (4-6) (Table 1).

FIGURE 1: Bi-directional relationship between DM and HF

Study and date	Type of study	Number of participants	Age range	Mean age (years)	Definition of LVSD	Prevalence of LVSD	Prevalence of symptomatic/ asymptomatic LVSD	Population with LVSD— prevalence of DM	Population without LVSD— prevalence of DM
ECHOES, England[1] 2001	Epidemiological— primary care	3960	>45	61	LVEF <40%	n = 72 (1.8%)	Symptomatic, n = 38 (1%), asymptomatic, n = 34 (0.8%)	Symptomatic, n = 9 (24%), asymptomatic, n = 2 (6%)	n = 146 (3.8%)
Copenhagen[119] 2003	Epidemiological— primary care	764	50–89	66 (Median)	LVEF ≤40%	n = 36 (4.7%)	33% Asymptomatic	n = 5 (7.2%)	n = 43 (5.9%)
Poole, England[120] 1999	Epidemiological— primary care	817	70–84	76	Qualitative assessment	n = 61 (7.5%)	79% Asymptomatic	n = 6 (10%)	n = 43 (6%)
Glasgow[2] 1997	Epidemiological	1640	25–74	50	LVEF ≤30%, LVEF ≤35%	n = 43 (2.9%), n = 113 (7.7%)	77% of participants with LVEF ≤35% were asymptomatic	n = 14 (12.4%)	n = 34 (2.5%)
Vasteras, Sweden[121] 2001	Epidemiological	412	75	75	LVWMI <1.7%	n = 28 (6.8%)	46% asymptomatic	n = 6 (22%)	n = 27 (7%)
Olmsted, USA[122] 2003	Epidemiological	1888	>45	63	LVEF ≤50%, LVEF ≤40%	n = 123 (6.5%), n = 40 (1.8%)	–	n = 21 (17%), n = 6 (15%)	n = 130 (6.8%)
Copenhagen[123] 2005	Prospective, hospital clinic	188	–	69	LVEF <45%	All had LVSD	All were symptomatic	n = 48 (25.5%)	–

LVSD, left ventricular systolic dysfunction; DM, diabetes mellitus; LVEF, left ventricular ejection fraction; LVWMI, left ventricular wall motion index.

TABLE 1: The prevalence of DM in populations with and without LVSD.

MacDonald MR et al. Eur Heart J 2008;29:1224-40

As stated earlier, there has been a pathophysiological linkage between T2DM and HF. There are numbers of independent risk factors that were identified as predictors of the development of HF in DM. These include increased body mass index (BMI), age, the presence of coronary artery disease, New York Heart Association functional class and glycaemic control measured by HbA1c (6-8).

NYHA FUNCTIONAL CLASS – PREDICTOR OF RISK OF DEVELOPING DM IN CHF

In an Italian population-based study of 1,339 elderly subjects with a mean (+/- SD) age of 74.2 +/- 6.4 years. CHF has been shown to be a strong predictor of the development of DM independently of age, sex, family history of diabetes, BMI, waist/hip ratio, systolic and diastolic blood pressure, and treatment for CHF (OR = 1.4, 95% CI = 1.1-1.8)(9). This strongest association was observed in patients with more severe HF (NYHA III and IV) than patients with milder HF (NYHA I and II). Similar observation was found in the Bezafibrate Infarction Prevention study. Incidence of diabetes was determined by baseline NYHA functional classification. 2616 non-diabetic patients aged 45 to 74 years were divided into three groups according to New York Heart Association (NYHA) criteria: class I (n = 1986 patients), class II (n = 518), and class III (n = 112). The detection of a fasting blood glucose level ≥7 mmol/L during follow-up was defined as the criterion for the development of diabetes. 259 patients (13%) in NYHA class I developed DM, 76 (15%) in class II, and 22 (20%) in class III (P for trend = 0.05) during 8 years follow up of this study. NYHA class III were twice as likely (17% [n = 19]) to have fasting blood glucose levels of ≥7 mmol/L than those in NYHA class I (7.8% [n = 154]) or class II (8.7% [n = 45]) (P = 0.005) (Figure 2). In a multivariate analysis, NYHA class III was found to be the strongest predictor of the development of DM associated with a 1.7-fold (95% confidence interval [CI]: 1.1 to 2.6) increase of the rate of

development of diabetes, but not NYHA class II (hazard ratio = 1.0; 95% CI: 0.8

to 1.3) (10).
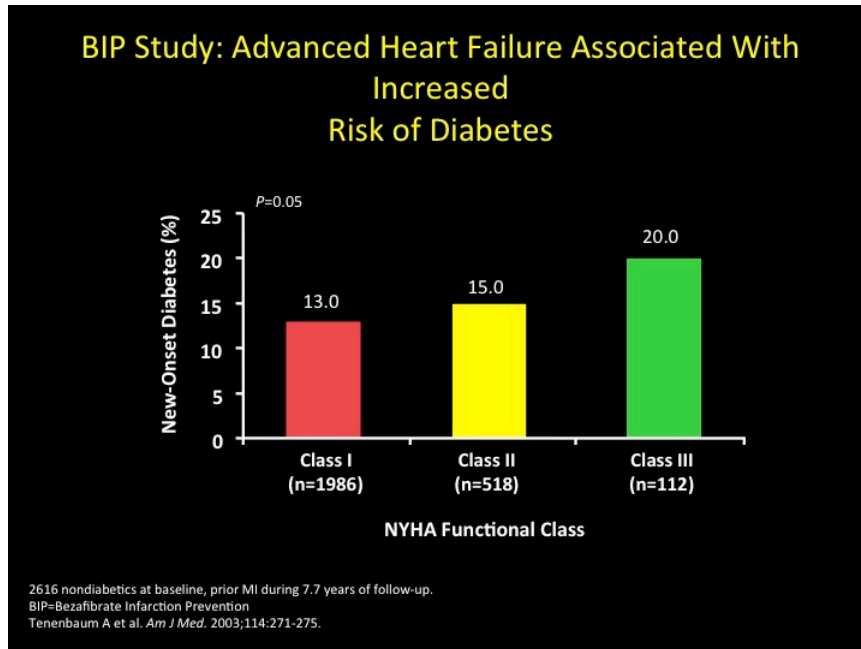
FIGURE 2 Bezafibrate infarction prevention study.

Advanced HF associated with increased risk of DM. Tenenbaum A et al. Am J Med 2003;114:271-275

GLYCAEMIC CONTROL- PREDICTOR OF RISK OF DEVELOPING HF IN DM

The risk of CHF appears to be related to the blood sugar control in patients with diabetes. Iribarren and colleagues demonstrated that a 1% increase in HbA1C was associated with an 8% increased in risk of CHF independent of blood pressure, body mass index, age and presence of coronary artery disease (1). Conversely, the UKPDS study showed that a 1% reduction of HbA1c was associated with a 16% reduced risk of developing CHF (2). The presence of diabetes mellitus is also associated with worse outcome in CHF trials. In the Left Ventricular Dysfunction (SOLVD) trial, diabetes mellitus was an independent predictor of mortality and morbidity in patients in CHF (3). Similarly, in the Beta-Blocker Evaluation of Survival Trial (BEST), patients with DM were associated with more severe HF and adverse outcome compared to CHF patients without DM (4). Held et al (5) showed each millimols per litre increased in fasting plasma glucose in patients with diabetes was associated with a 1.10-fold-increased risk of CHF hospitalization after adjustment for age and sex. All these findings showed a clear and important link between diabetes and CHF.

However, we are not certain of the association between the degree of dysglycaemia and risk of HF. If the degree of dysglycaemia does associate with increased risk of HF, then the next question naturally asked is what is the degree of dysglycaemia carries the highest risk of developing HF in patients with T2DM? We would have as a matter of course thought that more severe

dysglycaemia is associated with a higher incidence of HF than patients with normoglycaemia. However, there have been some controversies regarding the degree of dysglycaemia and incident HF. Recent retrospective studies have achieved different conclusions.

The benefit of improved glycaemic control on microvascular complications in T2DM is well established (7,14), and recent trials have attempted to clarify the role of glycaemic control on macrovascular outcomes (15,16). These data suggested that improved glycaemic control has the potential to reduce the risk of both micro- and macrovascular disease significantly when instigated early in the disease course, but in more advanced T2DM, the benefits of improved control were less evident (17). Furthermore, recent studies suggested that tight glycaemic control can sometimes be associated with a poorer macrovascular outcome (18,19) than standard care. In patients with co-existing CHF and T2DM, the relevance of good glycaemic control is a critical issue not only as the combination may be associated with a significantly poorer outcome but also as the choice of drugs available to manage hyperglycaemia in CHF are perhaps more limited (20,21). There are conflicting reports of the importance of glycaemic control in patients with T2DM and CHF (22-26). The relationship between glycaemic control and outcomes has been reported to be "U" shaped (26), "J" shaped (25), linear (24), and even inverse (23). The main findings from relevant trials regarding incident HF and degree of dysglycaemia are summarised as followed:

UKPDS—HBA1C, A PROGRESSIVE RISK FOR ADVERSE OUTCOME (2)

In year 2000, UKPDS 35 trial examined the relationship between long-term glycaemic control and micro- and macrovascular outcome in patients with Type 2 diabetes. The primary endpoints of the study were diabetes related death and all cause mortality. Secondary endpoints were myocardial infarction, stroke, amputation (including death related to peripheral vascular disease), and microvascular disease (predominantly retinal photo-coagulation). Other outcome measures were incident heart failure and cataract extraction. Adverse outcome was associated with the degree of dysglycaemia. The authors concluded that each per cent of mean HbA1c reduction was associated with 21% reduction of diabetes related endpoint (95% confidence interval 17% to 24%, P<0.0001), 21% for deaths related to diabetes (15% to 27%, P<0.0001), 14% for myocardial infarction (8% to 21%, P<0.0001), and 37% for microvascular complications (33% to 41%, P<0.0001). The risk of HF increased by 16% for each per cent increased of mean HbA1c overtime (p=0.021), adjusted for age of diagnosis of DM, sex, ethnic group, history of smoking, presence of hypertension and microalbuminuria, and dyslipidaemia. No threshold of risk was observed for any end point. The risk of adverse outcome was the lowest in patients with HbA1c within the normal range (<6%) (2).

VETERANS AFFAIRS STUDY—U SHAPE CURVE RELATIONSHIP (6)

At the Veterans Affairs medical centres, Aguilar et al conducted a retrospective study to determine the relationship between HbA1c and CHF by assessing the association of different quintiles of HbA1c and CHF-related outcome (mortality and risk of HF hospitalization). 5815 veterans with CHF and T2DM treated in ambulatory clinics were included in the study. After periods of two years follow up, a U shape curve relationship was found between HbA1c and mortality. Death occurred in 25% of patients in Quintile1 (HbA1C ≤6.4%), 23% in Quintile 2 (6.4% 9.0%). The middle quintile was found to have the lowest mortality after adjustment for potential confounders when compared with the lowest quintile (risk-adjusted hazard ratio: 0.73, 95% confidence interval: 0.61 to 0.88, p = 0.001). Conversely, it was a linear relationship for HF hospitalization with increasing quintiles of HbA1C (Q1: 13.3%, Q2: 13.1%, Q3: 15.5%, Q4: 16.4%, and Q5: 18.2%). However, this association was not statistically significant when adjusted for potential confounders (26)(Figure 3), suggesting that the differences in baseline demographics and treatments may be accounting for the increased rate of heart failure hospitalization This highlighted the complex relationship between HbA1c and mortality in patients with diabetes and heart failure. Patients in the lower and higher quintiles of HbA1c have a higher mortality than patients with modest glycaemic control. They postulated that the increased mortality in patients with the higher quintile of HbA1c levels was likely multifactorial secondary to the direct and indirect effects of hyperglycaemia. The adverse effects of hyperglycaemia include increased oxidative stress, endothelial dysfunction, increased protein kinase C

activation, and ultimately accelerate atherosclerosis. Chronic hyperglycaemia is associated with accumulation of advanced glycation end products, which lead to increased myocardial stiffness, and dys-regulation of various cellular signaling pathways, and eventually cellular dysfunction. Elevated HbA1c is also linked with increased insulin resistance and may also be related to poor compliance with medications, which in turn may be associated with a poor outcome. The potential explanations of increased mortality in patients with the lowest quintile of HbA1c are likely to be secondary to the hazardous effects of intensive glucose control, and possibly related to protein malnutrition and an increased in inflammatory syndrome associated with advanced heart failure.
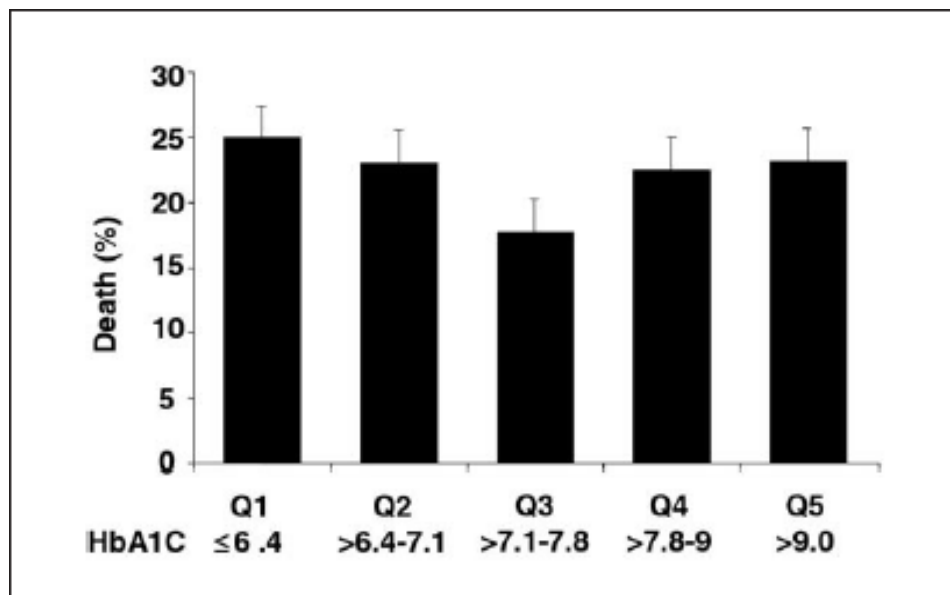
FIGURE 3: The association between mortality and HbA1C in diabetic patients with HF appears U-shaped, with the lowest risk of death in those patients with modest glucose control (7.1% < HbA1C ≤7.8%).

The graph represents the proportion of patients who died at 2-year follow-up with quintiles (Q) of glycosylated haemoglobin (HbA1c). Global chi-square p=0.001. Error bars indicate the 95% confidence intervals. Aguilar et al. J Am Coll Cardiol 2009;54:422-8

ESHAGHIAN AND COLLEAGUES—A PARADOXICAL RELATIONSHIP OF HBA1C AND OUTCOME
(7)

The relationship between HbA1c and mortality in patients with advanced HF and T2DM was evaluated by Eshaghian et al. 123 patients with T2DM and advanced HF with HbA1c measured at presentation were included in the study. Patients were then divided into two categories: HbA1c>7% (n=74) and ≤7% (n=49). More than two third of the cohort was men with the mean ejection fraction of 25% +/- 7. Of which, 60% has ischaemic cardiomyopathy with mean HbA1c of 7.9% +/- 1.8, and diabetes duration of 8.6 +/- 9 years. Both groups were matched for age, sex, New York Heart Association class; body mass index; diabetes duration; anti-diabetic medications used. Patients with HbA1c >7.0 were associated with higher ejection fraction, increased β-blocker and sulfonylurea use. Contrary to previous perception of better glycaemic control leads to a better outcome, this study found a paradoxical relationship of HbA1c and adverse outcome. Patients with low HbA1c of ≤7.0 were found to have a significantly increased all-cause mortality, compared with those with HbA1c >7.0 (35% vs. 20%, hazard ratio 2.6, 95% CI 1.3-5.2, P<0.01).
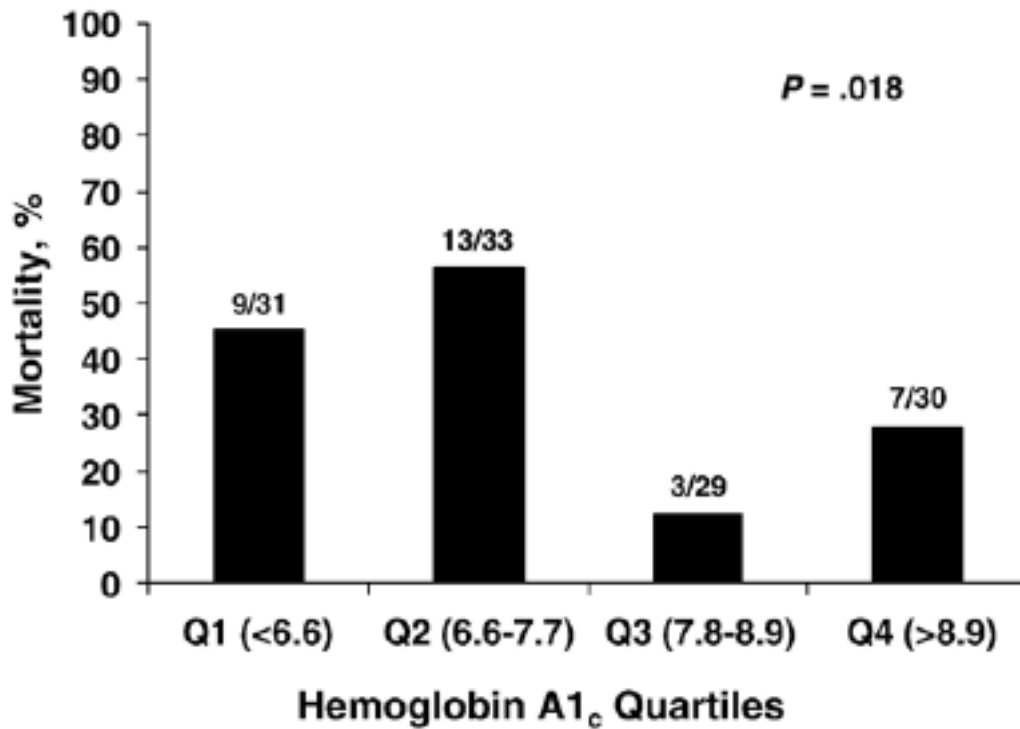
FIGURE 4: A Paradoxical Relationship of HbA1c and outcome.

Paradoxically, elevated HbA1c levels were associated with improved survival in this cohort of patients with diabetes and advanced HF. Mortality rates by Kaplan-Meier analysis for advanced systolic heart failure patients with diabetes at 2 years by HbA1c quartiles. The number of events and the number of subjects in each quartile are shown above each bar. Eshaghian et al. Am Heart J 2006;151:91

WHAT DO WE LEARN FROM CHARM? —LINEAR RELATIONSHIP BETWEEN HBA1C AND ADVERSE OUTCOME (8)

From the Candesartan in Heart failure: Assessment of Reduction in Mortality and Morbidity (CHARM) program, we learned that diabetes is a progressive risk. The CHARM study followed up 2412 patients (907 with a previous history of diabetes) with at least one HbA1c measurement for a median of 34 months. The primary outcome consisted of CV death, HF hospitalization, and total mortality was determined. Almost all the patients were followed up until they have developed outcome or until the study finished. Authors have found a linear relationship between HbA1c and risk of adverse events. After adjusted for sex and age, hazards ratios of these outcomes per 1% higher HbA1c level were 1.25 (95% confidence interval [CI], 1.20-1.31) for CV death, 1.24 (95% CI, 1.17-1.31) for HF hospitalization, and 1.22 (95% CI, 1.16-1.29) for total mortality. This relationship remained evident in patients with and without diabetes, with reduced or preserved ejection fraction and persisted after adjustment for diabetes, other risk factors, and allocation to preserved EF or low EF (Figure 5).
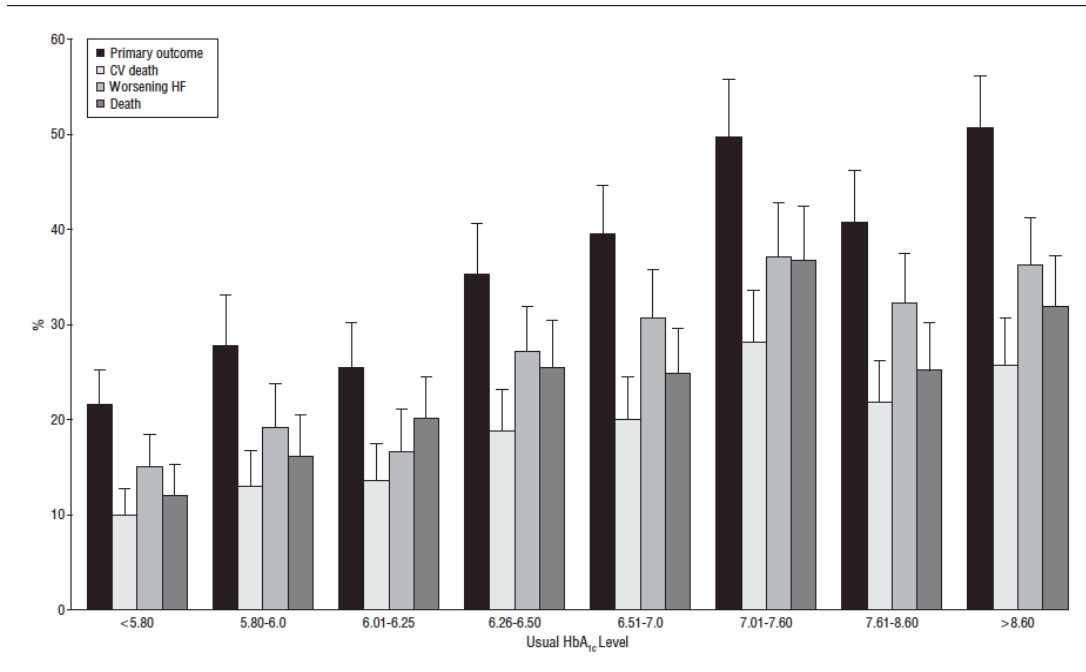
FIGURE 5: Candesartan in Heart failure: Assessment of Reduction in Mortality and Morbidity (CHARM) program.

In diabetic and non-diabetic patients with symptomatic chronic HF, HbA1c level is an independent progressive risk factor for CV death, hospitalization for HF, and total mortality. (P for trend <0.001). Error bars indicate 95% confidence intervals. Gerstein HC et al. Arch Intern Med 2008;168:1699-704

ATHEROSCLEROSIS RISK IN COMMUNITIES (ARIC) STUDY-INCREASED HBA1C ASSOCIATE WITH INCREASED RISK OF DEVELOPING HF (9)

In a prospective population-based study, Pazin-Filho et al studied the incidence of death and HF hospitalization in patients with diabetes but no evidence of HF at baseline. Each per cent increase of HbA1c was associated with 17% increased risk of developing HF in the non-coronary heart disease group. The risk was 20% in patients with coronary heart disease. HbA1c has been shown to be an independent predictor of risk of developing HF in diabetic patients with and without coronary heart disease(9).

SWEDISH NATIONAL DIABETES REGISTRY STUDY- POOR GLYCAEMIC CONTROL WITH HBA1C>7% ASSOCIATED WITH INCREASED RISK OF HOSPITALISATION OF HF

In a Swedish National Diabetes Registry study, Lind et al has identified 10,969 (13.2%) among 83,021 patients with T2DM who were hospitalized with a primary or secondary diagnosis of heart failure during a mean follow-up of 7.2 years. Male sex ($p < 0.001$), older age ($p < 0.001$) and longer diabetes duration ($p < 0.001$) correlates with increased incident HF hospitalization. After adjusting for risk factors of heart failure, the HR per each percentage unit higher HbA_{1c} for heart-failure hospitalization was 1.12 (95% CI 1.10, 1.14). By category of mean HbA_{1c}, the HR for heart failure hospitalization was: HbA_{1c} 6.0 to <7.0%, 0.91 (95% CI 0.84, 0.98); HbA_{1c} 7.0 to <8.0%, 0.99 (95% CI 0.91, 1.07); HbA_{1c} 8.0 to <9.0%, 1.10 (95% CI 1.01, 1.20); HbA_{1c} 9.0 to <10.0%, 1.27

(95% CI 1.15, 1.41); HbA$_{1c}$ ≥10.0 %, 1.71 (1.51, 1.93) (reference HbA$_{1c}$ <6%). The HR for patients with HbA$_{1c}$ 7.0 to <8.0% compared with patients with HbA$_{1c}$ 6.0 to <7.0% was 1.09 (95% CI 1.03, 1.14). They concluded that HbA$_{1c}$ >7% is associated with an increased risk of heart failure hospitalization in patients with T2DM (10).

CHAPTER SUMMARY

The bi-directional relationship between DM/IR and CHF is well established. NYHA functional class is the strongest predictor of the development of DM in CHF, whereas glycaemic control is the strongest predictor of the development of CHF in DM. The level of glycosylated haemoglobin (HbA1c) provides a measure of the glycaemic control of patients with T2DM during the previous 2–3 months (11). It is a useful prognosticator in patients with DM and CHF as demonstrated from the above studies. However, we are not certain about what is the optimal glycaemic control in patients with DM and CHF. Studies that assessed the importance of glycaemic control in diabetic patients with CHF (6-9,12) usually used a single measure of HbA1c which underestimated the importance of overall glycaemic control (13). Calculation of a mean HbA1c has been found to be a better predictor of diabetic complications (2,14,15) as it incorporates multiple measures over time (2,13). Therefore, we conducted a study to determine the relationship between *mean HbA1c* and outcome in a large cohort of patients with T2DM and incident CHF.

CHAPTER 2: GLYCAEMIC CONTROL AND OUTCOME IN PATIENTS WITH CHF

STUDY AIM AND OBJECTIVE

In a retrospective analysis, we sought to determine the relationship between mean HbA1c and outcome in a large cohort of patients with T2DM and incident CHF.

STUDY POPULATION AND DESIGN

We performed a retrospective cohort study within the population of Tayside, Scotland (population 400,000) between 1st January 1992 and 31st March 2010 exploiting the unique advanced medical informatics infrastructure available in the region. This makes use of a unique health record identifier – The Community Health Index number (CHI) which has been used for all patient healthcare activity in the region for the past 20 years. It means multiple clinical data sets can be deterministically linked at the level of the individual with high accuracy. Study subjects had both T2DM and CHF and were anonymously identified from three data resources; the Diabetes Audit and Research in Tayside Study (DARTS) (16), the Tayside echocardiographic database (>100,000) maintained by the Department of Cardiology, Ninewells Hospital and the Health Informatics dispensed prescribing database developed by the Medicines Monitoring Unit (MEMO)(17), which holds details on all dispensed prescriptions for all individuals in the region since 1993.

CHF was defined as a record of an echocardiogram with evidence of left ventricular systolic dysfunction (LVSD) and either a prescription for a loop-diuretic (provided not greater than 1 year prior to echocardiogram) or an admission to hospital with an associated heart failure diagnostic code (ICD-9 428, ICD-10 I50). The index date for development of CHF was defined as the minimum of first echocardiogram, valid prescription for a loop diuretic or admission to hospital with CHF. We previously used similar criteria to identify CHF from large datasets in Tayside, and have refined the criteria for CHF with the inclusion of echocardiographic information (18).

HBA1C MEASURES

To be included in the study, patients were required to have at least two HbA1c measures recorded between index date and end of study. HbA1c was analysed as an updated mean. The updated mean was calculated for each individual from each year of follow-up e.g. in year 1 the mean of the baseline HbA1c and all other HbA1cs measured in the first 12 months was calculated, year 2 is the mean of all measures at baseline years 1 and 2 and so forth until the end of the study period. A weighted mean HbA1c was calculated using all available HbA1c measures during the 'at risk' study time. The mean was weighted by time between measures and was then used to group patients into five categories of HbA1c (≤6%, >6-≤7%, >7-≤8%, >8-≤9% and >9%).

STATISTICAL ANALYSIS

Cox's proportional hazards models (19) were used to model time to all cause death. The entry date was index date for diagnosis with CHF. Updated mean HbA1c was analysed as a time-dependent covariate. Other covariates, averaged over the study period, which were utilized as continuous variables in the model included: age at diabetes diagnosis, age at index date, and estimated glomerular filtration rate (eGFR). History of smoking, history of ischaemic heart disease and cardiovascular medication use (aspirin, statins, thiazide diuretics, beta blockers, ACE inhibitors or ARBs, calcium channel blockers) were included as dichotomous variables. Diabetic medications (insulin therapy, oral hypoglycaemics only or no drug therapy) were considered as dichotomous time-dependent covariates. Differences in patient characteristics were determined by chi-squared test for linear trend for categorical variables and ANOVA test for linear trend for continuous variables with a two sided p-value of < 0.05 considered significant. All statistical analysis was performed using SPSS for windows (v9.2)

RESULTS

PATIENT CHARACTERISTICS

From an initial 2567 T2DM subjects in the echocardiographic database with evidence of left ventricular systolic dysfunction, 1597 (62%) had a hospitalisation for CHF and/or valid loop diuretic prescription. After exclusion for CHF incident date preceding DM diagnosis, 1100 subjects were left, of those

795 had an HbA1c measurement during their observable study period (Figure 6).
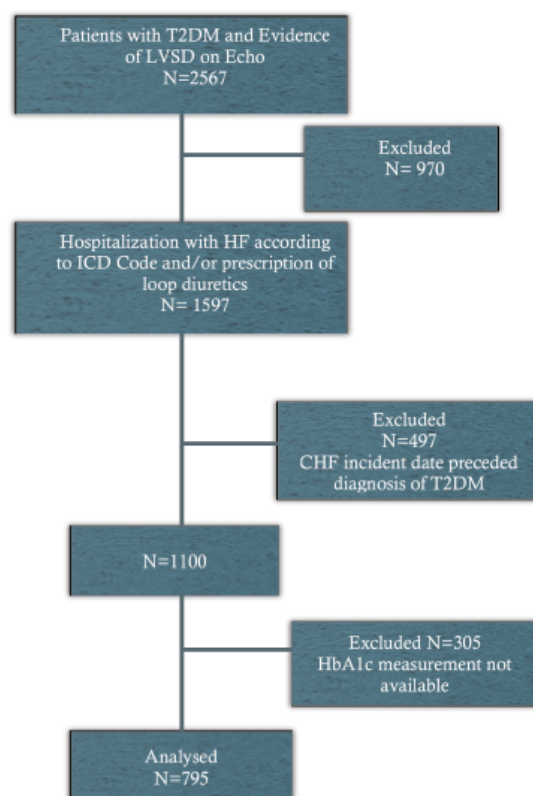
FIGURE 6 CONSORT (Consolidated Standards of reporting trial) Diagram for Glycaemic Control and outcome in patients with CHF

Characteristics of the 795 patients in the study population are provided in Table 2 split by HbA1c category. Patients in the lowest two HbA1c categories had shorter study duration and therefore fewer HbA1c measures. They were diagnosed with CHF and T2DM at an older age, and had a lower BMI and eGFR at baseline. In addition, although not statistically significant, they tended to be more likely to smoke but had fewer myocardial infarction (MI) events prior to baseline. With respect to prescribing, there were relatively more diet treated

and fewer insulin treated patients. They were also more likely to be prescribed thiazide diuretics at baseline. In addition, patients in the lowest category were prescribed less aspirin at baseline. In contrast, patients in the highest HbA1c category were diagnosed with CHF and T2DM at a younger age, had a higher BMI and eGFR, relatively more MI events at baseline, and comprised the smallest proportion of diet and largest proportion of insulin treated patients. In addition, they were more likely to be prescribed aspirin at baseline.

HBA1C AND MORTALITY

Over a median follow up of 3.8 years, there were 491(61.8%) all-cause deaths. In a Cox regression model, adjusted for all other significant predictors, with the middle HbA1c category (>7-≤8%) as the reference, we found a U shaped relationship of HbA1c and outcome with the two lowest and the highest HbA1c categories significantly associated with a higher risk of death (HR 95% CI 1.78(1.26-2.52); 1.29(1.01-1.66) and 1.38(1.03-1.84) respectively) (Figure 7).

HBA1C AND MORTALITY: DIET AND DRUG TREATED T2DM

To more carefully explore this U shaped association, we considered the HbA1c ≤7% group and made a comparison of patients split by diet and drug treatment (Table 3). The diet treated T2DM patient group were diagnosed with diabetes at an older age, had a lower study and baseline HbA1c and were prescribed less ACE inhibitors at baseline. Significantly, when comparing

baseline HbA1c and study HbA1c, there was no difference in the diet treated group ((mean ±SD) (6.01± 0.64 vs. 6.07± 0.52), p=0.29), but in the drug treated group HbA1c was significantly lower after CHF diagnosis ((7.42± 1.18) vs. (6.36± 0.47), p<0.0001) indicating that drug treatment resulted in more aggressive HbA1c lowering.

We therefore went on to split the entire study population into diet and drug treated to investigate the relationship between HbA1c and death in these groups separately. As the number of patients in each group is smaller and Figure 6 indicated a U-shaped relationship between HbA1c and death, we reduced the number of HbA1c categories to three (≤7, >7-≤9, >9). The adjusted and unadjusted Cox regression models are presented in Table 4. In the diet treated group, lower HbA1c was associated with lower risk of death. Whereas the U shaped association observed in the overall study population remained in the drug treated group.

DISCUSSION

This study had 2 main findings. Firstly, in our cohort of T2DM patients with incident CHF, we observed a U shaped relationship between mortality and glycaemic control, as assessed by a mean HbA1c. Secondly, additional analysis show that this U shape relationship is present in drug treated but not in diet treated T2DM patients. In diet treated patients, lower HbA1c was associated with lower mortality risk. These latter observations may suggest that the outcomes observed in the low HbA1c categories may be related to the response of patients to the DM drug medications.

The relationship between glycaemic control and outcome in patients with CHF and T2DM has previously been studied in at least 4 retrospective studies with different conclusions reported. The relationship between glycaemic control and outcome has been reported to be "U" shaped (6), "J" shaped (12), linear (8) and even inverse (7). In the most recent analysis, Aguilar *et al* (6) performed a retrospective analysis of 5815 veterans (94 % male) with T2DM and CHF defined by clinic coding, 45.5% of which had significantly impaired LV function. Over a 2 year follow-up they observed a U shaped relationship between HbA1c and mortality, with a "sweet spot" seen with individuals in quintile 3 (HbA1c 7.1-7.8 %). Compared to Q3, all other quintiles had significantly elevated risk of death at 2 years with those in the lowest and highest quintiles faring worst. Our data would support these findings. It should be noted that Aguilar's study like all the previous studies, only a single HbA1c was used to assess glycaemic control. However, a single HbA1c may not be reliable, especially if sampled at the time of the diagnosis of CHF when it is potentially influenced by recent alterations in therapy. Individuals may consult physicians with symptoms prior to diagnosis leading to alterations in oral hypoglycaemics or initiation of diuretic therapy that may affect the single HbA1c measurement recorded in the specialist clinic at the time of CHF diagnosis. Indeed, the practice of using baseline HbA1c in studies on diabetes complications can lead to underestimation of the importance of HbA1c as a risk factor, as only one value is used (13,15). Studies have shown that HbA1c levels have a persistent effect on complications several years after their measurement (20,21). Our data are unique as we were able to utilise all HbA1c measures recorded for each individual, enabling us to consider the

importance of longer term glycaemic control over a long period of time in a large patient cohort. In our study, we used a weighted mean to examine the impact of glycaemic control on outcome. The mean HbA1c has been shown to offer superior predictive power over time when compared to a single baseline measure, which can result in underestimations of the impact of glycaemic control (2,13,22). It should be noted that other HbA1c variables have been studied including the last HbA1c value and HbA1c variation as described by standard deviation, neither of which has been shown to be superior to the mean (14,23). Importantly, the weighted mean HbA1c and our median follow-up of 3.8 years enhances the ability of this study to accurately determine the relationship between HbA1c and mortality, as the predictive power of mean HbA1c, out with CHF, is known to increase with longer study length (20-22).

The finding of a higher mortality risk in patients in the lower HbA1c categories (HbA1c ≤6% and HBA1c >6-≤7%) deserves some consideration. In our study, patients in these low HbA1c categories had both favourable and less favourable clinical characteristics. On one hand, these patients had fewer previous MIs and had less intensive DM treatment with less use of insulin. On the other hand, these patients were older when they developed their CHF and they had a lower eGFR. Interpretation of these findings is always going to be limited by a lack of information on the underlying cause of death. However, our finding that this U shaped relationship was present in drug treated but not in diet treated T2DM patients may suggest that the outcomes observed in the low HbA1c categories may be related to the response of patients to the DM medications. This group of patients had developed their CHF later in life and

may be more vulnerable to hypoglycaemia as a result of sulphonylurea and insulin use, which can contribute to morbidity and mortality in high-risk patients with low HbA1c (24). It should be noted that the current findings are concordant with recent ACCORD(25) study, which demonstrated that very tight control of glucose in patients with diabetes may not be beneficial in patients with existing cardiovascular disease and a longer duration of diabetes.

In our study, we also observed a poor outcome in CHF patients with the highest HbA1c. In a sense, this was not unexpected. These patients had more previous MIs at baseline, had more aggressive DM therapy with the largest proportion of insulin treated patients. With respect to CHF patients, there is conflicting data on the relationship between insulin use and outcome in T2DM patients with CHF. Although T2DM on insulin had a higher risk of death in CHF trials (8) (26), the UKPDS 33 study (27) as well as a retrospective cohort study, of 16 000 Medicare diabetic beneficiaries with CHF, showed that insulin use did not predict mortality (28). Those with poorer control also tended to come from more deprived socio-economic groups which is known to be an independent risk factor for poor outcome in diabetes (29). Furthermore, our finding of a poor outcome among patients with poor glycaemic control is concordant with studies showing the wide spread and detrimental effects of hyperglycaemia including progressive atherosclerosis, elevated levels of advanced glycation end products which may lead to increased myocardial stiffness (30), diabetic nephropathy, endothelial dysfunction (31), microangiopathy (32), increased oxidative stress (33) and protein kinase C activation (34).

Obviously, the mechanisms for reduced survival associated with both very tight glycaemic as well those with poor glycaemic control in CHF must remain speculative and cannot be inferred directly from this study.

LIMITATIONS

We recognize the limitations of our study, which are inherent with any retrospective, non-randomized, observational data. However, the current study reflects the true population and a "real world scenario" and adds to previous studies by selecting a large number of patients with T2DM and CHF with a long follow-up period. In common with all observational studies, it was impossible in our study to account for all confounding influences that may have biased the observed differences between the groups considered. We have sought to minimise these as far as practicable by utilising multivariate models and incorporating data on drug prescribing, laboratory blood tests and smoking status. Additionally we utilised multiple HbA1c measures for each individual, and as these were not sampled at specified intervals this may potentially result in survival bias for those who have a greater number of measures, in turn this was minimised by utilising a mean weighted for time. Due to the incidence of recording of renal function and BMI, we utilised a mean value throughout the study period in our model. The study does have much strength including the large number of subjects, the large number of HbA1c measures available, the high event rate (62% died) and the reliable and comprehensive data, which were available with which to build the statistical model.

CONCLUSION

In patients with T2DM and CHF, our observational study shows that there is a U shaped relationship between HbA1c and mortality with the lowest mortality risk in patients with modest glycaemic control (HbA1c, >7-≤9%). This observational data adds support to the growing concern that we need to redefine the optimal HbA1c level in this high-risk group of patients with co-existing T2DM and CHF.

Table 2 Clinical characteristics by HbA1c category

	ALL	HbA1c Category (%)					P*
		≤6	>6 - ≤7	>7 - ≤8	>8 - ≤9	>9	
Total number of subjects	795	81(10.2)	232(29.2)	210(26.4)	158(19.9)	114(14.3)	
Follow-up time (years)	3.8(2.0-6.8)	2.7(1.6-6.0)	3.3(1.7-6.1)	4.6(2.4-7.4)	4.4(2.2-7.2)	3.8(2.0-6.4)	0.0006a
Time at risk (years)	2.5(1.0-4.8)	1.5(0.7-2.6)	2.1(0.7-4.2)	2.9(1.1-5.4)	2.9(1.2-5.7)	2.6(1.2-4.4)	<0.0001a
Time from baseline to study entry (years)	0.6(0.2-1.5)	0.7(0.3-1.5)	0.5(0.2-1.4)	0.5(0.2-1.5)	0.5(0.3-1.6)	0.6(0.3-1.9)	0.6253a
Dead	491(61.8)	50(61.7)	143(61.6)	125(59.5)	97(61.4)	76(66.7)	0.8057b
Males	489(61.5)	49(60.5)	136(58.6)	137(65.2)	94(59.5)	73(64.0)	0.6154b
Age at diabetes diagnosis (years)	62.7(11.4)	65.2(10.7)	67.0(10.4)	61.7(11.5)	59.5(11.6)	58.3(10.4)	<0.0001c
Social deprivation:							0.7895b
1 (most)	217(27.7)	24(30.0)	51(22.3)	62(30.0)	47(30.3)	33(29.2)	
2	168(21.4)	13(16.3)	56(24.5)	39(18.8)	35(22.6)	25(22.1)	
3	158(20.2)	21(26.3)	47(20.5)	37(17.9)	28(18.1)	25(22.1)	
4	127(16.2)	13(16.3)	37(16.2)	37(17.9)	24(15.5)	16(14.2)	
5 (least)	114(14.5)	9(11.3)	38(16.6)	32(15.5)	21(13.6)	14(12.4)	
Mean study HbA1c (%)	7.53(1.28)	5.60(0.30)	6.54(0.28)	7.48(0.28)	8.46(0.28)	9.72(0.59)	<0.0001c
Number of study HbA1c measures	6 (3-14)	3(1-6)	5(2-10)	8(3-17)	8(4-16)	8.5(3-14)	<0.0001a
Baseline Characteristics:							
Age (years)	71.8(9.6)	73.6(9.5)	74.1(9.5)	71.3(9.2)	70.4(9.2)	68.5(9.8)	<0.0001c
Mean HbA1c (%)	7.70(1.30)	6.64(1.29)	7.28(1.17)	7.81(1.19)	8.15(1.24)	8.41(1.08)	<0.0001c
Ever smoked	450(56.6)	50(61.7)	139(59.9)	124(59.1)	75(47.5)	63(54.4)	0.0913b
MI	408(51.3)	38(46.9)	113(48.7)	104(49.5)	80|(50.6)	73(64.0)	0.0626b
MAP (mmHg)	122.5(11.7)	123.8(11.1)	123.0(12.6)	122.4(11.8)	122.2(11.1)	121.1(10.9)	0.5761c
BMI(kg/m²)	29.6(5.5)	29.1(6.7)	29.0(5.3)	29.5(5.3)	29.9(5.2)	31.0(5.4)	0.0364c
eGFR(mmol/L)	61.4(19.3)	57.2(20.3)	59.8(19.7)	61.9(18.4)	62.1(19.1)	65.5(19.1)	0.0324c
Diabetes drugs:							<0.0001b
Diet	202(25.4)	41(50.6)	79(34.1)	43(20.5)	25(15.8)	14(12.3)	
Oral: No Sulphonylurea	114(14.3)	16(19.8)	39(16.8)	28(13.3)	17(10.8)	14(12.3)	
Oral: Sulphonylurea	321(40.4)	13(16.1)	92(39.7)	97(46.2)	70(44.3)	49(43.0)	
Insulin	158(19.9)	11(13.6)	22(9.5)	42(20.0)	46(29.1)	37(32.5)	
Cardiovascular drugs:							
Statins	332(41.8)	27(33.3)	99(42.7)	89(42.4)	68(43.0)	49(43.0)	0.6173b
ACE	356(44.8)	30(37.0)	105(45.3)	96(45.7)	68(43.0)	57(50.0)	0.4764b
Aspirin	428(53.8)	33(40.7)	131(56.5)	113(53.8)	79(50.0)	72(63.2)	0.0249b
Beta Blockers	254(32.0)	25(30.9)	82(35.3)	68(32.4)	41(26.0)	38(33.3)	0.4048b
Thiazide Diuretics	108(13.6)	15(18.5)	43(18.5)	27(12.9)	12(7.6)	11(9.7)	0.0115b
Rate limiting CCB	102(12.8)	11(13.6)	24(10.3)	32(15.2)	20(12.7)	15(13.2)	0.6580b
Other CCB	225(28.3)	27(33.3)	58(25.0)	61(29.1)	50(31.7)	29(25.4)	0.4561b

Data are mean (standard deviation), median (interquartile range) or n(%).a Kruskal-Wallis test. b chi-square test. c ANOVA. Bold values indicate a statistically significant test with P<0.05. Frequency missing: social deprivation 11, baseline HbA1c 71, MAP 78, BMI 39, eGFR 30.

Figure 7: Hazard ratio by different HbA1c categories
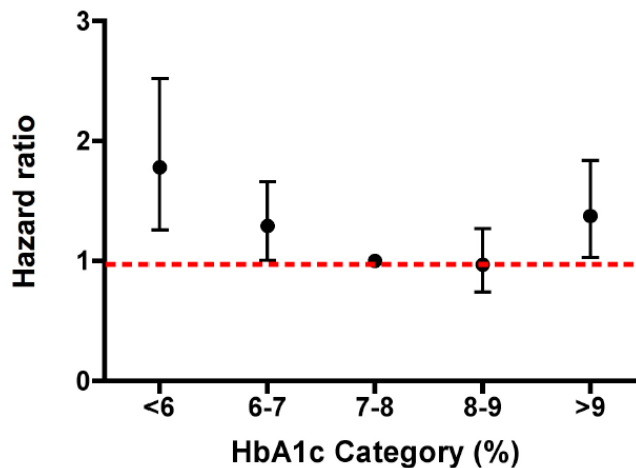
Table 3: Clinical characteristics of HbA1c split by diabetes treatment

	Diabetes treatment		
	Diet only	Drug	P*
Total number of subjects	74(23.6)	239(76.4)	
Follow-up time (years)	3.4(2.0-6.7)	3.9(1.6-5.7)	0.1758a
Time at risk (years)	2.1(0.8-4.8)	1.8(0.7-3.8)	0.1438a
Time from baseline to study entry (years)	0.5(0.2-1.5)	0.6(0.3-1.4)	0.2639a
Dead	40(54.1)	153(64.0)	0.1235b
Males	42(56.8)	143(59.8)	0.6381b
Age at diabetes diagnosis (years)	70.5(10.8)	65.3(10.0)	**0.0001c**
Social deprivation:			0.8358b
1 (most)	17(23.0)	58(24.7)	
2	18(24.3)	51(21.7)	
3	19(25.7)	49(20.9)	
4	10(13.5)	40(17.0)	
5 (least)	10(13.5)	37(15.7)	
Mean study HbA1c (%)	6.07(0.52)	6.36(0.47)	**<0.0001c**
Number of study HbA1c measures	4(2-8)	4(2-10)	0.1485a
Baseline Characteristics:			
Age (years)	75.7(9.2)	73.4(9.5)	0.0591c
Mean HbA1c (%)	6.01(0.64)	7.42(1.18)	**<0.0001c**
Ever smoked	51(68.9)	138(57.7)	0.0858b
MI	35(47.3)	116(48.5)	0.8522b
MAP (mmHg)	124.1(11.7)	122.9(12.4)	0.5241c
BMI(kg/m²)	28.1(5.9)	29.3(5.6)	0.1242c
eGFR(mmol/L)	58.7(17.3)	59.2(20.6)	0.8289c
Cardiovascular drugs:			
Statins	30(40.5)	96(40.2)	0.9544b
ACE	24(32.4)	111(46.4)	**0.0335b**
Aspirin	33(44.6)	131(54.8)	0.1241b
Beta Blockers	24(32.4)	83(34.7)	0.7160b
Thiazide Diuretics	11(14.9)	47(19.7)	0.3530b
Rate limiting CCB	11(14.9)	24(10.0)	0.2500b
Other CCB	18(24.3)	67(28.0)	0.5307b

Data are mean (standard deviation), median (interquartile range) or n(%).a Mann-Whitney test. b chi-square test. c t-test. Bold values indicate a statistically significant test with $P<0.05$. Frequency missing: social deprivation 4, baseline HbA1c 35, MAP 43, BMI 19, eGFR 14.

TABLE 4: Cox models analysing HbA1c by 3 categories

HbA1c group (%)	All (n=795)		Diet only (n=86)		Drug treated only (n=709)	
	Adjusted HR (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Unadjusted HR (95% CI)
≤7	**1.34** **(1.09-1.66)**	**1.43** **(1.18-1.73)**	**0.17** **(0.07-0.39)**	**0.28** **(0.14-0.57)**	**1.46** **(1.18-1.82)**	**1.64** **(1.33-2.03)**
>7 - ≤9	1.00	1.00	1.00	1.00	1.00	1.00
>9	**1.31** **(1.00-1.70)**	1.25 (0.96-1.62)	*	*	**1.36** **(1.04-1.77)**	1.29 (0.99-1.68)

HbA1c >7 - ≤9% is the reference category. Bold values indicate a statistically significant test with P<0.05. *There were no individuals in this group

CHAPTER 3: INSULIN RESISTANCE AND CHF

INTRODUCTION

Diabetes and chronic heart failure (CHF) often co-exist with an inter-relationship such that each condition may impact on each other in terms of causation and outcome. This bi-directional inter-relationship between CHF and diabetes also extends to insulin resistance (IR). Longitudinal epidemiological data such as the Uppsala study of 1187 middle-aged and elderly men showed that IR precedes and predicts the development of CHF, independent of established risk factors for CHF, including diabetes itself. They found a striking inverse relationship between the risk of incident CHF and insulin sensitivity. Insulin sensitivity was defined by euglycaemic insulin clamp glucose disposal rate (49). Other groups have also investigated the relationship between IR and CHF. Swan et al has previously assessed insulin sensitivity in patients with CHF using minimal modeling analysis (HOMA-IR) during a weight adjusted intravenous glucose tolerance test in which they have established the relationship between the degree of IR and severity of CHF (Figure 8) (50). Patients with CHF were associated with marked IR. Insulin sensitivity was reduced by 58% in patients with CHF compared with healthy control subjects. Moreover, the degree of IR also correlates positively with severity of heart failure characterized by low peak oxygen consumption (VO2). Peak VO2 derived from cardiac pulmonary exercise testing is a useful prognosticator to assess outcomes in CHF (51,52). Doehner et al has shown that the presence and the

severity of IR in patients with CHF were independently associated with a poorer

outcome, characterized by reduced peak VO2 and NYHA classes (53).
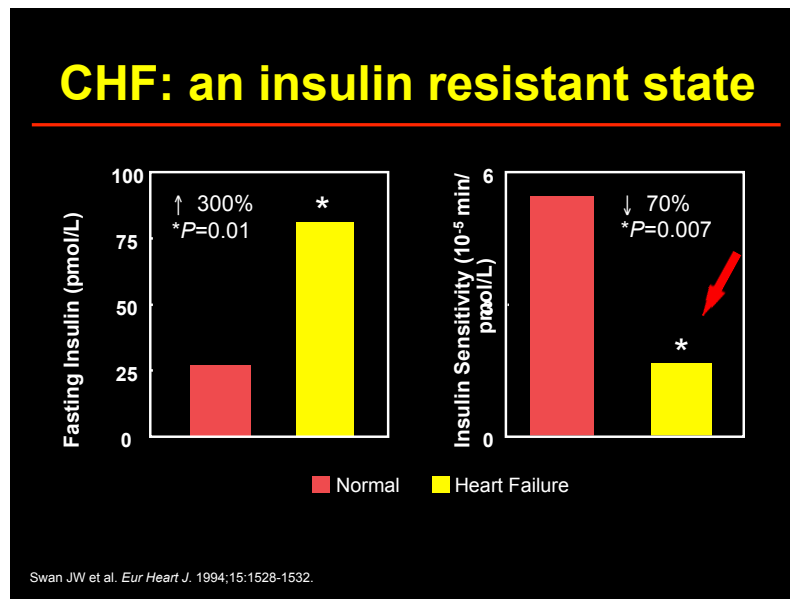
Figure 8: Heart Failure: An Insulin Resistant state

Heart Failure is associated with dysglycaemia and inversely correlates with insulin sensitivity. Swan et al. J Am Coll Cardiol 1997;30:527-32

It should be noted that all these studies have utilized the HOMA-IR

during an intravenous glucose tolerance test to determine insulin resistance/

sensitivity, which is a demanding technique. We recently evaluated the

prevalence of IR in patients with CHF using fasting insulin resistance index

(FIRI), which consists of the product of plasma insulin and glucose divided by

25. The presence of IR is defined by FIRI of ≥2.7 according to local laboratory

normal ranges (54). Among 92 non-diabetic patients with CHF, 67% of these

patients were insulin resistant. IR is highly prevalent in CHF population, and the

degree of IR also correlates with severity of CHF, characterized by higher NYHA

classes and lower peak VO2 and cardiac output (55) (Figure 9). The presence of

IR also correlates with poorer endothelial function compared to CHF patients without IR.
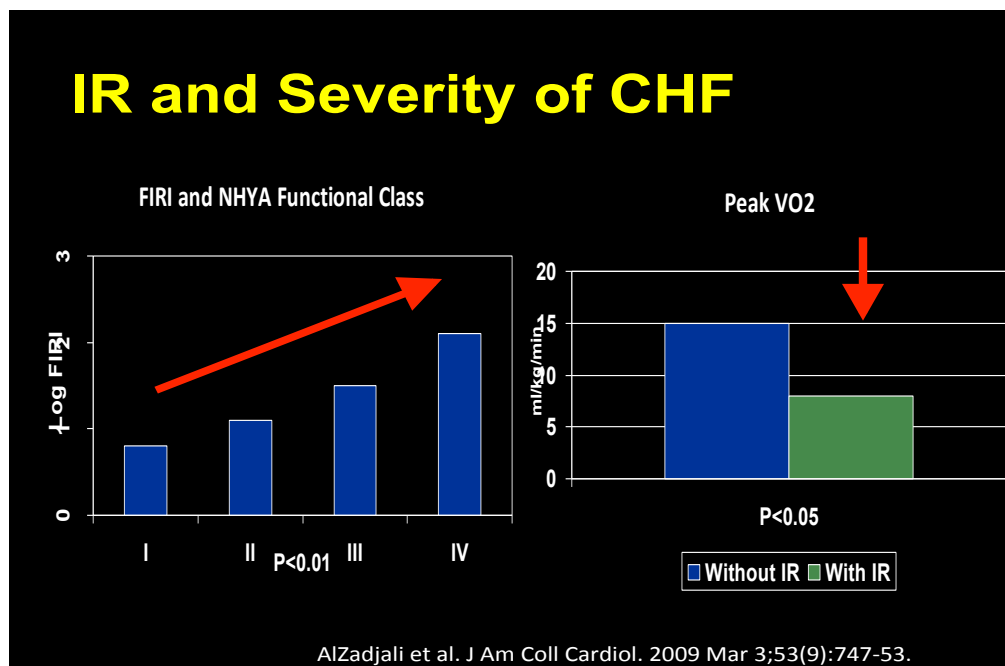
Figure 9: Relationship between Insulin Resistance and Severity of CHF

Degree of insulin resistance correlates with severity of heart failure and insulin resistance is associated with significant reduction in exercise capacity. Alzadjali et al. J Am Coll Cardiol 2009;53:747-53

More importantly, IR in CHF not only predicts disease severity but also mortality (35). In a community-based HF clinic, Goode et al assessed the prognostication of HbA1c for mortality in patients with HF in 970 non-diabetic patients who were referred to HF clinic. Patients with reduced EF of ≤ 45%, HbA1c of >6.7% were associated with an abrupt increase in mortality (n=68) compared with those with HbA1c of ≤6.7% (n = 368) (hazard ratio (HR): 2.4, p<0.001). This observation persisted after adjustment for age and comorbidity (HR 1.9, p = 0.008) with respective 1-year mortalities of 26.5% and 9.4%. Conversely, this increase in mortality was not observed in those with LVEF of

greater than 45% (HR 1.44, p = 0.36 after adjustment)(12). The exact role of IR in patients with CHF is still yet to be defined, as these observational studies do not distinguish between the cause and effect.

PATHOPHYSIOLOGY OF IR AND CHF

Does IR lead to CHF or CHF lead to IR? There are still no definite answers to these questions. IR and CHF are a pair of intricate disease with overlapping pathophysiological processes. What we do know from observational studies on certain rare genetic diseases associated with severe IR such as Alstrom syndrome, 60% of these patients progressed to severe dilated cardiomyopathy (DCM) (56). The presence of cardiomyopathy in the absence of coronary artery disease and hypertension is increasingly recognized in diabetic patients, but the exact pathophysiology is still remained to be defined. Rodent models were used to study the relationship between diabetes and cardiomyopathy as rodents are resistant to atherosclerosis; therefore, these have provided evidence on the occurrence of IR or diabetic cardiomyopathy in the absence of coronary artery disease and hypertension. Various in vivo or in vitro measurements were performed during the study of these animal models. Type 1 diabetes (either streptozotocin-induced rats or genetic non-obese diabetic mice) (57-60) rodent models have been shown to have echocardiographic evidence of systolic and diastolic dysfunction (57,61), endothelial dysfunction (58), elevated LV end-diastolic pressure, reduced LV systolic pressure, cardiac output and cardiac power (62). However, studies on Type 2 diabetic, insulin resistant, obese Zucker rats have yielded conflicting results (63,64). It is likely that these

conflicting findings arose from different models used; as Type 1 and Type 2 diabetic rodent models have different cardiac energy metabolism and neurohormonal changes, resulting in different impact on cardiac function. The severity of cardiomyopathy may vary among different models depending upon the severity and duration of alterations of plasma parameters such as insulin level, leptin, glucose, fatty acids, cytokines, tumour necrosis factor-alpha (TNF-α). Therefore, experimental data obtained using animals models of diabetes should be used in caution when extrapolating to the human diabetes. Additionally, aetiology of cardiomyopathy, neurohormonal changes and severity of these alterations may vary between animal and patients with diabetes mellitus (65).

In a chronically pacing dog model, Nikolaidis et al have successfully shown us how CHF can lead to IR (66). Thirty-four conscious, chronically instrumented dogs were studied at four stages during the evolution of dilated cardiomyopathy (DCM) induced by rapid right ventricular pacing. They showed that severe DCM is associated with the development of myocardial and systemic IR. There was impaired myocardial glucose uptake and altered myocardial insulin signaling, involving decreased Ser 473 phosphorylation of Akt-1. Myocardial insulin resistance in advanced, severe DCM was also associated with reduced myocardial adenosine triphosphate (ATP) levels. There are no clear explanations of how IR leads to worsening of CHF. Various research groups have proposed a few possible hypotheses on the overlapping pathophysiological processes in both conditions over the last decade.

(1)   SYMPATHETIC NERVOUS SYSTEM AND RENIN-ANGIOTENSIN ALDOSTERONE SYSTEM ACTIVATION

CHF invokes compensatory sympathetic nervous system (SNS) and renin-angiotensin aldosterone system activation, which leads to increase free fatty acids (FFA), thereby inhibits glucose uptake by muscle, and causes pancreatic damage. The increased plasma glucose elicits a compensatory insulin response but it is inadequate to compensate the hyperglycaemia because of the pancreatic damage mediated by cytokines such as TNF-α, angiotensin II and FFA. This leads to aberrant metabolism and IR. The increased FFA and glucose predispose to increased hepatic synthesis of triglycerides (TG) and increased angiotensin II, which in turn increases tissue TG levels and promote insulin receptor substrate-1 damage in the pancreas, thereby magnifies IR. Angiotensin II also promotes vasoconstriction, which in turn increase cardiac afterload (36). Angiotensin II activation and SNS activation are also common features of IR secondary to the compensatory hyperinsulinaemia (37,38).

(2)   INFLAMMATION

Systemic abnormalities in CHF have become a focus of cardiology research lately. IR and CHF are increasing recognized as an inflammatory state. There is mounting evidence to suggest that these inflammatory mediators can predict the development of IR or diabetes in population at risk (39-42). Opie et al and Wisniaki et al (43) have both demonstrated that in insulin-resistant CHF patients, C-reactive protein, interleukin-6, TNF-α and its soluble receptors were all significantly elevated secondary to neuro-hormonal activation. High level of circulating TNF-α has been detected in cachectic patients with severe CHF

(44,45), and it has been shown to correlate with leptin level, disease severity and poorer exercise tolerance (46). At a molecular level, TNF-α increases the Ser phosphorylation of insulin receptor substrates results in reduction of auto-phosphorylation of tyrosine and tyrosine kinase activities. The docking and interaction of IRS to its downstream effectors and kinase such as PI3-K are markedly reduced, resulting in reduced glucose transport (47). These lead to IR and reduce myocytes' ability to utilize glucose as a substrate for ATP production. These maladaptive stress responses of IR and CHF have been recognized by Lip et al as a pro-thrombotic state, and can lead to thrombogenesis and poorer clinical outcome (48). They concluded in their study that IL-6 and tissue factors (but not vascular endothelial growth factor (VEGF), plasma viscosity, von-Willebrand factor, fibrinogen or soluble P-selectin) levels were predictors of mortality and poor prognosis in patients with CHF.

(3)   ALTERED ADIPOKINES LEVELS

LEPTIN

Leptin, a product of ob-gene, has received a great deal of attention in recent research on metabolic syndrome and heart failure. Leptin is an adipose tissue specific protein with immune-modulatory properties. It plays an important role in weight regulations and energy expenditure. Leptin deficient mice have been shown to be susceptible to infections (49). Leptin release can be induced by pro-inflammatory cytokines and catecholamines (50,51). Besides IR/ diabetes (52,53) and CHF, elevated leptin levels were found in other

conditions with a high level of circulating proinflammatory cytokines and catecholamines such as obesity (54), sepsis (55,56) and chronic obstructive airway disease (57). Leptin stimulates fatty acid oxidation (58) and glucose uptake (59). It prevents lipid accumulation in non adipose tissue, which can otherwise lead to lipotoxicity (60). Lipotoxicity of pancreatic beta-cells, myocardium, and skeletal muscle leads, respectively, to type 2 diabetes, cardiomyopathy, and insulin resistance. High level of leptin has been consistently observed in patients with CHF (61,62) and correlates positively with IR (63) and levels of TNF-α (64). Doehner et al hypothesized that hyperleptinaemia may result in impaired cardiac energy metabolism. Serum level of leptin also correlates with the progressive functional impairment of advanced CHF (46). But interestingly leptin level was found to be low in patients with advanced CHF and cachexia (65,66). These findings may be the end results of excessive loss of muscle and fat mass in patients with advanced CHF.

ADIPONECTIN

Adiponectin, an adipocyte derived protein, which has anti-inflammatory, insulin sensitizing, anti-atherogenic properties (67). It also plays an important role in vascular remodeling. It has been researched extensively in various disease states lately particularly in patients with metabolic syndrome and CHF. Overweight individuals have reduced serum adiponectin levels. . Clinical studies have shown that adiponectin levels were low in patients with acute coronary syndrome (68) and T2DM (69), and were more closely related to the severity of insulin resistance and hyperinsulinaemia than to the degree of adiposity.

Paradoxically, adiponectin levels are higher in patients with CHF. It was not related to BMI in these patients (64). The levels of adiponectin also correlate positively with severity of CHF determined by NYHA functional class and serum NT-pro BNP levels. More importantly, elevated adiponectin levels were found to be a predictor of morbidity and mortality in CHF (70-72). As stated earlier, CHF is an inflammatory condition associated with elevated inflammatory markers. Of particular interest, serum adiponectin levels were negatively correlated with serum inflammatory markers such as highly sensitive C-reactive protein (hs-CRP) (73,74). Why are adiponectin levels high in CHF rather than low as in other disease states such as atherosclerosis and acute coronary syndrome? Owing to its anti-inflammatory and anti-diabetic properties, it has been speculated that the elevated adiponectin levels parallel the neurohormonal and inflammatory axes in the pathophysiology of CHF (71,75), as part of the physiological protective response to counteract inflammation and neurohormonal activation (71). Recent clinical studies have demonstrated the significance of adipocytokines modulation in HF patients (76). Van Berendoncks et al have shown that adiponectin mRNA expression was increased in CHF patients. Whereas its receptors (AdipoR1) and its downstream metabolic genes (i.e. PPAR–α and AMPK) expressions were decreased in CHF patients. More importantly, they have shown that four months of endurance resistance exercise training normalized these levels, suggesting that modulation of these adipocytokines altered glucose and lipids metabolism at the muscle level. This further consolidates our understanding of the beneficial effects of exercise training in CHF.

RESISTIN

We have now known that the adipose tissues play an essential part in the regulation of glucose and insulin metabolism through the release of adipocytokines. The adipocytokines also plays an important role in regulation of endothelial function and inflammation. Resistin has attracted a great deal of interest and attention in recent years. Resistin was originally described as an adipocyte-secreted peptide that induced insulin resistance in rodents (77). Administration of resistin to healthy rats impairs glucose tolerance and insulin action. Resistin has recently been shown to induce beta cell apoptosis in rats (78). There is increasing recognition of its role in the inflammation (79). Recent study has shown that resistin activates human endothelial cells through the up-regulation of cell adhesion molecules (80) and is a significant local and systemic regulatory cytokine involved in inflammation on vessels' walls in a rodent model (81). Serum resistin levels are increased in patients with T2DM. It is strongly associated with body mass index, and the degree of IR measured by HOMA-IR and various ED and inflammatory markers (82). More importantly, there is a strong positive correlation between resistin level and the development and degree of mircoangiopathies (i.e. retinopathy, neuropathy and nephropathy) in patients with T2DM independent of age, gender, BMI, and either the duration of T2DM (P = 0.0318) or serum creatinine (P = 0.0092) (83).

In a correlation study of the serum level of resistin and exercise capacity in patients with stable coronary artery disease, elevated serum resistin was also associated with poor exercise capacity and exercise-induced cardiac ischaemia. Adjustment for inflammatory markers attenuated these associations, suggesting

a possible role for resistin in inflammation and the pathophysiology of coronary heart disease (84). Long-term resistin over-expression was associated with a complex phenotype of oxidative stress, inflammation, fibrosis, apoptosis and myocardial remodeling and dysfunction in a rodent model (85). Therefore, with increased understanding of the pathophysiological role of resistin, it is not a surprise to see that serum resistin levels were associated with incident heart failure, even after accounting for prevalent coronary heart disease, obesity, and measures of insulin resistance and inflammation from the Framingham Offspring study (86). This study suggested the pathophysiological role of resistin in HF. Similar findings were shown from the Heart and Soul Study where patients with coronary heart disease with resistin levels in the highest quartile were at an increased risk of heart failure (hazard ratio [HR], 2.06; 95% confidence interval [CI], 1.26–3.39) and death (HR, 1.56; 95% CI, 1.11–2.18), adjusted for age, sex, and race. However, these effects were neutralised after adjusting for traditional cardiovascular risk factors such as obesity, hypertension, insulin resistance, dyslipidaemia, and renal dysfunction (87). Nonetheless, the current literatures seem to suggest that resistin plays an important role in vascular biology, inflammation and is highly related to the development of IR and the development of HF.

(4)   FORMATION OF ADVANCED GLYCOSYLATION END PRODUCTS

This process is greatly accelerated in diabetic patients. High level of advanced glycosylation end products in the myocardium leads to increased collagen cross-linking and myocardial stiffness (88). This causes further deterioration of ventricular relaxation and contraction in patients with CHF.

Chronic metformin use has been shown to prevent the above process and improve ventricular function in canine diabetic models (89).

(5)   *HYPERINSULINAEMIA*

Insulin is a catabolic hormone, and chronic hyperinsulinaemic state has been shown to increase myocardial mass and reduced cardiac output in rats (90). It can also lead to sodium retention (91) and subsequently decompensated heart failure. Besides that, hyperinsulinaemia also leads to activation of SNS (37), and increased pressor response to angiotensin II (38). Elevated catecholamine levels in CHF further antagonized insulin's actions, which promote lipolysis, results in increased free fatty acid and worsened insulin resistance (92) and cardiac energy metabolism. The results of these effects are increased cardiac hypertrophy, collagen formation, myocardial fibrosis (93), and eventually worsening of CHF.

(6)   *SUBSTRATE UTILIZATION*

In a normal, unstressed heart, energy in the form of ATP is mainly derived from free fatty acids (FFA) oxidation (94). Under pathological stress, the heart will switch from FFA oxidation to more fuel-efficient glucose metabolism (95) (amount of ATP generated per molecule of oxygen consumed). IR is associated with a high level of circulating FFA. High supply of FFA exceeds the heart's oxidative capacity, leading to accumulation of intra-myocardial triglycerides, and hence lipotoxicity that worsens CHF (95-98). High level of

FFA impairs the heart's ability to utilize glucose as a main source of ATP generation in different ways:

1. It impairs insulin mediated glucose uptake through inhibition of insulin receptor substrates and protein kinase-B.

2. PPAR-α is activated, and this leads to the promotion of genes involved in FFA oxidation and pyruvate dehydrogenase kinase-4 (PDK-4), which inhibits pyruvate dehydrogenase (PDH) and influx of pyruvate into mitochondrial.

3. High level of acetyl-CoA from β-oxidation of FFA further activates PDK-4, leading to further inhibition of PDH, hence pyruvate influx.

4. Augmented acetyl-CoA also leads to accumulation of citrate, which subsequently inhibits phosphofructose kinase-1 (PK1), a rate-limiting enzyme of glycolysis.

5. Increased FFA level also correlates with decreased myocardial phosphocreatinine-to-ATP (PCr/ATP) ratios, suggesting impaired ATP production (99). ATP production depends on the energy of the proton gradient across the mitochondrial inner membrane. High level of FFA activates the transcription factors of PPAR, leading to increased expression of mitochondrial uncoupling proteins (UCPs) expression (100). UCPs are also being up regulated in heart failure (100). UCP lowers the proton gradient by allowing protons to re-enter the mitochondrial matrix with the production of heat rather than ATP (101).

In severe CHF, myocardial IR results in reduced membrane translocation of GLUT-4 (decreased glucose uptake) and decreased phosphorylation of Akt-1 (decreased glucose metabolism) resulting in decreased ATP production. It further prevents the heart adaptive response to stress (deriving ATP from glucose metabolism rather than FFA oxidation). CHF in the setting of IR is probably the worst of all possibilities for energy metabolism. Gene expression for metabolizing FFA is down regulated due to CHF, and genes for metabolizing glucose are down regulated secondary to IR, preventing the heart from utilizing either fuel (102,103).

(7)   ENDOTHELIAL DYSFUNCTION (ED)

The endothelium represents an active and reactive single layer cells that line all the blood vessels in the body (104). A healthy endothelium should consist of a smooth surface that limits the activation of clotting cascades and pro-inflammation. Endothelial dysfunction is defined as inadequate or abnormal endothelial-mediated vasodilatation, which eventually leads to activation of coagulation and clotting cascades and inflammation, and eventually development of atherosclerosis. It was first observed in patients who underwent diagnostic coronary angiography (105). ED and IR often co-exist, and the combination of ED and IR were found among the individuals who are at higher risk of developing cardiovascular event (106). IR and ED represent the fundamental pathophysiological disturbance responsible for the clusters of metabolic and cardiovascular disease (107,108). The presence IR and ED are increasing recognised as a precursor to the development of atherosclerosis (109). ED leads to inadequate vasodilatation and/or paradoxical

vasoconstriction in coronary and peripheral arteries in response to stimuli that release nitric oxide (NO). Deficiency of endothelial-derived NO is believed to be the primary defect that links insulin resistance and endothelial dysfunction. NO deficiency can result from either decreased synthesis or accelerated degradation by high levels of reactive oxygen (ROS) and nitrogen (RNS) species, which are produced by cellular disturbances in glucose and lipid metabolism (110). ED is found in patients with insulin resistance prior to the development of diabetes, and is well described in obese patients, metabolic syndrome and in patients with gestational diabetes (111-113). ED was detected in patients with early asymptomatic HF (114) as well as symptomatic HF (115). The presence of IR was associated with ED in patients with CHF (116). Deficiency in endothelial-derived nitric oxide is believed to be the link between IR and ED (117). IR and ED have an impact on each other in terms of causation and outcome. There is a large body of evidence to show that IR is associated with ED (118,119). The exact mechanisms of how IR affects ED are not fully understood. Oxidative stress, hyperglycaemia, attenuation of insulin medicated NO release from vascular bed; dyslipidaemia and increased arginase activity have been suggested (120-122).

ED can impact on insulin action by altering its trans-capillary passage of insulin to target tissues, which results in abnormal tissues metabolisms, and accumulation of toxic metabolites, which in turns worsen IR. Vascular damage from oxidative stress and lipids deposition further induce localised inflammatory process that worsens IR and ED.

The presence of ED is also an important prognosticator in patients with established cardiovascular disease independent of other established cardiovascular risk factors (123). Therefore, improving ED and IR may represent therapeutic targets to prevent the development of atherosclerosis and cardiovascular disease.

CHAPTER SUMMARY

IR and CHF are an intricate pair of disease. There is increasing evidence to suggest that IR plays an important role in the pathophysiological processes in CHF. Activation of SNS and RAS, inflammation, altered adipocytokines levels, formation of advanced glycosylation products, changes of substrate utilization in the myocardium and endothelial dysfunction are possible explanations of how IR affecting disease process in CHF. With increased understanding of the pathophysiological role of IR in CHF, improving IR may potentially result in improvement of CHF.

CHAPTER 4: PHARMACOLOGICAL TREATMENT FOR INSULIN RESISTANCE AND CHRONIC HEART FAILURE

In this section, we will consider pharmacological tools that can be utilized to reverse IR in patients with CHF. We are mindful that lifestyle changes are important, most notably exercise which has been shown to improve IR in patients with impaired glucose tolerance in the Diabetes Prevention Program (DPP) study (124).

CHF DRUGS THAT IMPACT ON IR

A recent meta-analysis showed that drugs that inhibit the renin-angiotensin system might prevent the onset of diabetes mellitus (DM). In their meta-analysis, Andraws and Brown showed that in angiotensin converting enzyme (ACE) inhibitor trials, the odds of developing DM were reduced by 28% (OR 0.72, 95% CI 0.63 to 0.84, p<0.001), and in the angiotensin receptor blocker trials, there was a 27% reduction (OR 0.73, 95% CI 0.64 to 0.84, p<0.001) in the odds (125) compared to placebo or other antihypertensive agents such as calcium channel blockers and diuretics. With respect to beta-blockers, there may be differences between beta-blockers in their impact on insulin resistance (126). In the setting of CHF, carvedilol has shown superior effects in prevention of diabetes when compared with metoprolol in the COMET study (127).

Mineralocorticoid receptors are expressed in non-epithelial tissues, such as blood vessels, the heart and adipose tissue. Mineralocorticoid receptors

antagonist (MRA) had been shown to be beneficial in HF trials. There is increasing body of evidence to suggest the role of aldosterone in the pathophysiological process of HF, it is associated with fibrosis, worsening catecholamine process, inflammation and endothelial dysfunction (128). The Randomized Aldactone Evaluation Study (RALES) has shown mortality and morbidity benefit of adding spironolactone in addition to existing HF therapy during that time (129). Additionally, the newer MCA Eplerenone has also been shown to have striking mortality benefit in patients with LVSD following myocardial infarction (130) and also in milder HF population (131) in addition to RAS modulators (ACEi or ARB). Although both spironolactone and eplerenone are both MCAs, they have very different metabolic effects, particularly on IR or glycaemic control. In an animal model, the use of spironolactone was associated with an increased level of plasma aldosterone and impaired glucose tolerance compared to eplerenone (132). Whereas in a study examining the effects of eplerenone and spironolactone on cortisol and HbA1c levels in patients with chronic stable HF, plasma cortisol levels and HbA1c were significant higher, and adiponectin levels significantly decreased in patients taking spironolactone but remains unchanged in the eplerenone group (133). Eplerenone seems to have neutral metabolic effects on IR whereas spironolactone has been shown to upset glycaemic control in HF patients. Therefore, these metabolic effects should be considered when prescribing MCA to CHF patients with DM or IR.

DIABETIC DRUGS THAT IMPROVE IR

THIAZOLIDINEDIONE (TZDS)

TZDs (e.g. rosiglitazone and pioglitazone) belong to the high affinity ligands for the nuclear hormone receptor family member PPAR-γ (134). They modulate the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism in adipose, muscular tissue and in the liver. They improve insulin sensitivity in the liver and peripheral tissue, and reduce plasma insulin level and glycated haemoglobin. TZDs also improve lipid profiles and are associated with anti-inflammatory and anti-atherosclerotic properties (135). However, there have been reports of adverse cardiovascular outcomes associated with the use of TZDs, most notably with CHF hospitalizations. In a recent meta-analysis, Lago and colleagues reported that TZDs increased risk for development of CHF, probably as a result of fluid retention, across a wide background of cardiac risk (relative risk [RR] 1.72, 95% CI 1.21-2.42, p=0.002) (136). At this time, the Food and Drug Administration (FDA) indicates that TZDs are not recommended for use in patients with NYHA functional class III/IV CHF, and have introduced a 'black box' warning regarding the increased risk of CHF. There are additional concerns regarding the risk of myocardial infraction with TZDs especially with rosiglitazone. Recent results of different meta-analyses were inconclusive as to whether rosiglitazone caused real adverse effects of myocardial infarction (137,138). The US FDA have placed a 'black box' warning on rosiglitazone to signal potential of myocardial infarction and heart-related deaths until additional safety data is available. The UK Commission on Human

Medicines has reviewed the available data in year 2010 and has concluded that there is an increased cardiovascular risk for rosiglitazone. The Commission has therefore concluded that the benefits of rosiglitazone no longer outweigh its risks. The European Committee on Medicinal Products for Human Use has recommended the suspension of the marketing authorisations of rosiglitazone (Avandia, Avandamet) across the European Union.

METFORMIN

Another insulin sensitizing drug that may have potential use in CHF is metformin. Metformin has been on the market for almost 50 years and is widely prescribed in diabetic population. It was first described in the scientific literature in 1957 (139) and was first marketed in France in 1979, but did not receive approval by FDA for the treatment of Type 2 diabetes until 1994 in the USA . It is cheap and has potent insulin sensitizing effect (140). Metformin, according to the package insert, is contraindicated in all patients with ''heart failure requiring pharmacologic treatment'' because of increased risk of potentially lethal lactic acidosis (LA) (141). This stems from the previous reports of severe LA with phenformin, another biaguanide that was removed from the market after 306 cases of LA were reported in the 1970s. However, the available evidence of the risk of LA with metformin is somewhat lacking (142,143). Indeed, the reported incidence of LA related to metformin have been extremely low in large observational studies and metformin levels do not correlate with lactate levels in individuals who develop LA which may support the notion that metformin may be 'an innocent bystander'' in sick patients

rather than the causal agent (144,145). The overall rate of LA with metformin has been estimated at 6.3 cases per 100,000 patient-years (143).

With respect to studies reporting the use of metformin in CHF, there has been several analyses of prescribing databases. In a retrospective cohort analysis by Masoudi et al (28), 16417 Medicare beneficiaries with diabetes discharged after hospitalization with the principle discharge diagnosis of heart failure were assessed. The primary outcome of the study was time to death due to all causes. Secondary outcomes included time to readmission for all causes or for heart failure. Crude 1-year mortality rates were lower among the 2226 patients treated with TZDs (30.1%) or the 1861 treated with metformin (24.7%) compared with that among the 12069 treated with neither insulin-sensitizing drug (36%, P=<0.0001 for both comparisons)(Figure 10). The study concluded that these treatments are not associated with increased mortality and may improve outcomes in older patients with diabetes and heart failure. Admissions for all causes did not differ with either insulin sensitizer. There was a higher risk of readmission for heart failure with TZDs treatment (HR 1.06, 95% CI 1.00 to 1.09) and lower risk with metformin treatment (HR 0.92, 95% CI 0.92-0.99). Notably, there was no excess of admissions for LA in patients treated with metformin.
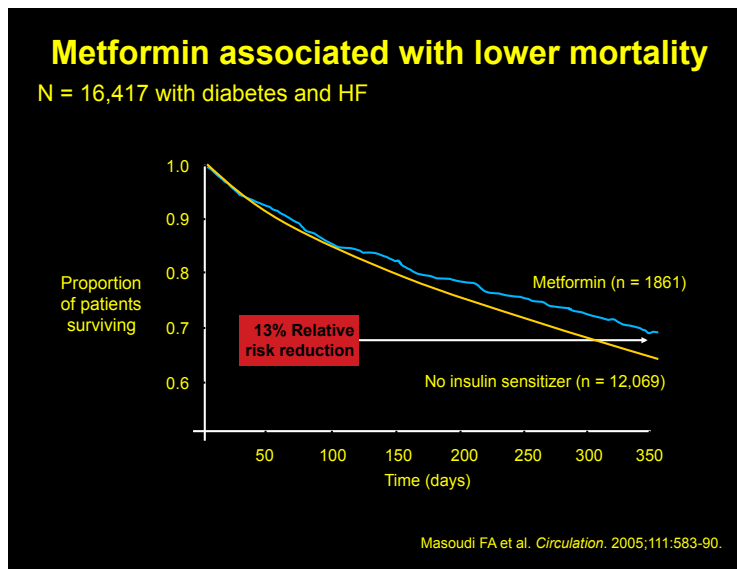
Figure 10: Observation data suggests that metformin may have mortality benefit in Type 2 Diabetes Mellitus. Masoudi et al. Circulation 2005;111:583-90

Eurich DT and colleagues had analyzed the Saskatchewan Health database to identify patients with CHF and diabetes for a retrospective analysis (146). 12272 new users of oral anti-diabetic agents were identified. The average follow-up was 2.5 years. Compared with sulfonylurea monotherapy, fewer deaths occurred in subjects receiving metformin: 404 (52%) for sulfonylurea monotherapy versus 69 (33%) for metformin monotherapy (HR 0.70 [95% CI 0.54-0.91]) and 263 (31%) for combination therapy (0.61 [CI 0.52-0.72]). Reductions in deaths or hospitalization were also noted in metformin treated groups. Therefore, metformin, alone or in combination, in subjects with heart failure and Type 2 diabetes was associated with lower morbidity and mortality compared with sulfonylurea monotherapy.

GLUCAGON LIKE PEPTIDE (GLP-1) AND DIPEPTIDYL-PEPTIDASE-4 INHIBITORS

The incretin system has received a great deal of attention in the treatment of diabetes mellitus. Glucagon like peptide (GLP-1) is a gut derived incretin hormone that has glucose-dependent insulinotropic effects. GLP-1 stimulates insulin release, suppresses glucagon secretion, inhibits gastric emptying and reduces appetite and food intake. The current approach to enhance incretin system is either to increase the incretin level (incretin mimetic, i.e. GLP-1) or to reduce the degradation of incretin (dipeptidyl-peptidase-4 inhibitors (DPP-4), i.e. sitagliptin and vildagliptin). The incretin concept was developed from an observational study in the 1960's, when enteral nutrition was shown to be a more potent insulinotropic stimulus than iso-glycaemic intravenous challenge (147). GLP-1 levels increase rapidly after eating but also inactivated swiftly by DPP-4. Activation of incretin systems lead to insulin biosynthesis, stimulation of β- cell proliferation (148), promote resistance to apoptosis and enhanced β-cell survival (149,150). Nikolaidis et al shared some insights into how GLP-1 affects cardiac function from canine models. He demonstrated that recombinant GLP-1 infusion resulted in dramatic improvement of LV and systemic haemodynamics in conscious dogs with advanced dilated cardiomyopathy induced by rapid pacing (151). This was followed by a clinical study, which showed improvement of LV ejection fraction, functional status, and reduced brain natriuretic peptide (BNP) in patients with CHF (95). The expected insulinotropic effects of GLP-1 were observed in this study. Plasma glucose and non-esterified fatty acid were significantly reduced in GLP-1 group, in association with an increase in plasma insulin levels but

measurement of insulin resistance (insulin-mediated glucose uptake) were not performed in this study. GLP-1 and DDP-4 inhibitors have not been shown to ameliorate IR directly to date. Therefore, improvement of cardiac function may not be a direct effect of GLP-1 on IR. It may be the results of improvement of endothelial function (152,153), GLP-1 variable inotropic effects (154,155) and more efficient myocardial ATP production from reduced myocardial FFA.

It should be noted that the available DPP-IV inhibitors, which are currently used in the treatment of diabetes mellitus, could increase GLP-1 levels (156). To date, it has not been shown to have any cardioprotective effects post myocardial infarction (157) although smaller animals studies have show positive effects on BNP expression (158) and improvement of cardio-renal function in a porcine models (159).

A few randomized controlled trials are currently underway to define the utility of targeting the incretin system in HF patients with DM. Incretin-based therapy may represent a novel therapeutic strategy in the treatment of HF patients with diabetes, in particular for their cardioprotective effects independent of those attributable to tight glycaemic control.

CHAPTER SUMMARY

Lifestyles changes such as diet and exercise are important in improving IR in CHF patients but difficult to achieve and prescribe. CHF trials seem to suggest that most conventional CHF medications have favourable impact on glycaemic control or prevent the development of diabetes. In terms of diabetic medications, insulin sensitizers are limited to TZDs and metformin. The use of TZDs have reduced greatly because of risk of exacerbating HF and its association with increased risk of myocardial infarction, whereas metformin is increasing recognised as a safe and beneficial drug in T2DM and CHF from large observational studies. Lastly, our increased insights and understanding of the incretins system has opened a new horizon in the potential treatment options in CHF, and outcome trials are awaiting.

CHAPTER 5: METFORMIN USE AND MORTALITY IN PATIENTS WITH CHF

INTRODUCTION

Chronic heart failure (CHF) and type 2 diabetes mellitus (T2DM) frequently coexist (160-162). In population based studies and in CHF trials, the prevalence of T2DM in patients with CHF is estimated to be between 11% and 28% and among all patients hospitalized for CHF it has been reported that 25-30% have T2DM. This combination can be lethal since diabetes has consistently been shown to be an independent predictor of increased morbidity and mortality in patients with either symptomatic heart failure or asymptomatic left ventricular dysfunction (163,164).

Unfortunately, patients with a combination of CHF and T2DM have limited drug therapy options for their diabetes. Because insulin resistance is a key pathophysiological process in these patients (165), it would seem intuitive to administer an insulin-sensitizing agent such as a thiazolidinedione or metformin. However, thiazolidinediones have the potential to exacerbate CHF in patients with poor cardiac reserve and are currently contraindicated in CHF patients with New York Heart Association (NYHA) functional class III or IV. While metformin is a widely prescribed drug in the management of T2DM, its use in patients with T2DM and CHF has previously been discouraged due to concerns over the risk of lactic acidosis originating from earlier experience with phenformin. This was withdrawn from U.S. market in the 1970s due to case

reports of fatal lactic acidosis. However, both clinical experience and the literature suggest that the risk of metformin–associated lactic acidosis is very low, and similar to that of other anti-diabetic drugs (28,166).   In fact, observational data suggest that metformin may actually be beneficial for CHF through its insulin sensitizing properties. A retrospective cohort study in a group of Medicare recipients discharged from U.S. hospitals with a principal diagnosis of CHF, suggests that metformin (and thiazolidinediones) were not associated with increased mortality and may improve outcomes in older patients with T2DM and CHF (28). It has been proposed by several groups that compensated CHF can no longer be upheld as contraindication for metformin (167-169). The beneficial effect of metformin in CHF may extend to patients with other manifestations of cardiovascular disease; an observational study in the UK demonstrated that cardiovascular risk was lower among metformin users compared to sulphonylureas users (169). Further evidence for the potential benefit of metformin in patients with both T2DM and CHF may result in expanding the therapeutic options for managing this important common and complex group of patients. Therefore, the aim of this study was to add to the growing body of evidence on the safety of metformin, and to explore benefits of its use among patients with T2DM and CHF in Tayside, Scotland.

AIMS AND OBJECTIVES

To investigate the use of metformin therapy for treating type 2 diabetes mellitus (T2DM) with Chronic Heart Failure (CHF) in a large population-based cohort study.

METHODS

DATA SOURCES

This study was carried out in the population (approximately 400,000) of Tayside in Scotland, using the Diabetes Audit and Research in Tayside Scotland (DARTS) (171) information system and the dispensed prescribing database maintained by the Health Informatics Centre (HIC) of the University of Dundee (formerly known as the MEMO database). This contains records of all dispensed community prescriptions dating back to 1993. The DARTS dataset contains detailed clinical information on every patient diagnosed with diabetes in Tayside. Clinical information is collected according to the national clinical dataset for the care of diabetic people in Scotland, and includes diabetes type, date of diagnosis, duration, therapy, HbA_{1c}, presence (and date) of microvascular and macrovascular diabetic complications and cardiovascular risk factors.

Datasets available from HIC also include hospital discharge data, biochemistry data, mortality data, socio-demographic descriptors, and other data that are linked by a unique 10 digit patient identifier, the community health index (CHI) number that is used for all health care activities in Tayside. The first six digits are the date of birth, with a figure coding sex (odd male, even female) in the remaining four distinguishing digits. This number facilitates high accuracy record linkage at the level of the individual across the region. All research data are robustly anonymised and approved by the Tayside NHS

Caldicott Guardians. The study was granted ethical approved by the Tayside Committee on Medical Research Ethics.

STUDY POPULATION

The study population was defined as residents of Tayside who were registered with their GP during the study period 1994-2003. Using the DARTS database, we identified all Tayside residents who were diagnosed with T2DM prior to December 2003. We then identified all those who were newly treated with oral hypoglycaemic agents during the study period (January 1994 to December 2003). Any patients who received oral hypoglycaemic agent prescriptions before 1994 or received insulin at any point during the study period were excluded.

We identified patients who had incident CHF during the study period and defined a date of CHF diagnosis for each patient. This was the earliest date of patients fulfilling any one of the following criteria: 1. Patients with a hospital admission ICD9/10 diagnostic code for CHF during the study period (ICD-9 428 ICD-10 50). The date of admission was defined as the date of CHF diagnosis. 2. Patients commenced on CHF medications defined as a combination of loop diuretics and ACEi. Co-prescribing had to occur within a 90-day period, and the date of the second drug was defined as the date of CHF diagnosis. 3. Patients who had at least one admission for myocardial infarction and then received loop diuretic medication. The date of diuretic medication was defined as the date of CHF diagnosis. Patients were excluded if their plasma creatinine concentration was > 200 μmol/L prior to the prescribing of a loop diuretic. This

was to exclude patients with renal hypertension without CHF who might receive loop diuretics and ACE inhibitors. We have previously used similar criteria to identify CHF from large datasets in Tayside (172).

To be eligible for the study, the date of CHF diagnosis had to occur after the date of diagnosis of T2DM. The patient also had to receive a first prescription for either metformin or sulphonylureas within 1 year after their date of CHF diagnosis. Patients were then divided into three cohorts: those who received prescriptions for metformin only (metformin monotherapy), those who received prescriptions for sulphonylureas only (sulphonylureas monotherapy) and those who received prescriptions for both (combination).

STATISTICAL ANALYSIS

All subjects were prospectively followed up from their index date (the date of CHF diagnosis) until the primary outcome, termination of HIC health care coverage, or termination of the study. The study outcomes were all-cause mortality, both at 1 year (short term), and by the end of the follow-up period (long term). All cause mortality was determined from death certification records of the General Register Office (GRO) of Scotland. We compared survival between cohorts for each outcome using Kaplan-Meier survival plots and used Cox regression analyses to estimate the relative risks of each outcome for patients in the study cohorts. For this analysis, the metformin monotherapy cohort and the combination cohort were merged into a larger cohort, with the sulphonylureas monotherapy cohort as the reference group. Survival times were censored if patients left Tayside, or at the end of the study period. The

following confounding variables were investigated: sex, age at index date, duration of diabetes at index date, creatinine (divided into quartiles in ascending orders) and HbA1c. We also accounted for whether the patient had been admitted to hospital with an ICD9/10 diagnostic code for a major cardiovascular event (myocardial infarction, coronary heart disease or stroke) prior to their diagnosis with CHF. Finally, we calculated the proportions of patients in each cohort who had received prescriptions for any of four drug types: angiotensin-converting enzyme inhibitors aspirin, diuretics or beta-adrenoceptor blocking drugs (beta blockers). Continuous covariates were categorised into quartile groups where appropriate, and all covariates were included in the final models only if they were statistically significant in univariate analyses ($p < 0.05$), to produce adjusted risk estimates for all covariates. All analyses were conducted using SPSS version 16.0 (Chicago, USA).

RESULTS

There were 1,141 patients who were diagnosed with T2DM prior to December 2003, who received oral hypoglycaemic agents during the study period (1994 to 2003) but were not on insulin. All of these patients had been admitted to hospital with a diagnostic code for CHF, although 218 patients were not eligible for the study as their hospital admissions occurred out with the study period. It was not possible to identify a date of diagnosis of CHF for 9 patients who were also excluded from the study. From the remaining 914 patients, we identified 769 whose date of CHF diagnosis occurred after date of diagnosis of T2DM.

Four hundred and ninety patients were prescribed either metformin or sulphonylureas after their date of CHF diagnosis. However, we excluded a further 59 patients whose first oral hypoglycaemic agent was prescribed more than 365 days after date of CHF diagnosis, and 9 without a valid date of diagnosis of T2DM. Of the remaining 422 patients (mean age 75.4 ± 0.56yrs), 68 were prescribed metformin only, 217 were prescribed sulphonylureas only and 137 received prescriptions for both. The characteristics of these patients are presented in Table 5. We compared patients who had received any metformin (metformin monotherapy cohort or combination cohort) with patients in the sulphonylureas monotherapy cohort. Although they were slightly younger and more likely to be female, the only statistically significant differences were that they had lower mean creatinine and a higher proportion was treated with aspirin and ACE inhibitors.

Both 1-year and long-term mortality were higher in the sulphonylureas monotherapy cohort compared with patients prescribed metformin. In the Cox regression analysis, the unadjusted hazard ratios were 0.56 (95% CI 0.38-0.84) and 0.53 (95% CI 0.33-0.67) for 1 year and long-term mortality respectively, when the metformin and combination cohorts were grouped together and compared with the sulphonylurea monotherapy cohort (Table 6). After adjusting for baseline differences between the two groups, users of metformin, alone or in combination had a 30-40% lower risk of the outcomes (Table 6 and Figure 11).

DISCUSSION

Metformin is a widely prescribed potent insulin-sensitizing drug, which is cheap, and has been on the market for almost 50 years. However, in the relatively large group of T2DM patients with concomitant CHF, it is largely contraindicated. According to the package insert, metformin is contraindicated in patients with CHF requiring pharmacological therapy due to a possible increased risk of lactic acidosis. This is due to historical experience of lactic acidosis with phenformin, despite the fact that metformin dose not predispose to this when compared with other therapies (167). Other contraindications such as old age, renal impairment and CHF are increasingly disregarded in clinical practice. The key finding of this study is that patients with T2DM and CHF who were treated with metformin alone or in combination with sulphonylureas were at significantly lower risk of all cause mortality during 1 year and long-term follow-up than those who were treated with sulfonylurea alone. This remained so even after full correction for multiple possible confounding influences. This reduces the possibility that this finding might be due to differences in baseline co-morbidities, medication or other patient characteristics that may influence channelling bias.

How does metformin mediate these beneficial cardiovascular effects? Metformin has potent insulin sensitizing effects although its precise mechanisms of action are not fully understood (140). Arguably, the insulin sensitizing properties might confer in part some of the beneficial effects. CHF is increasingly recognized as an insulin resistant state (165,173). Studies that

have demonstrated an association between CHF and insulin resistance have found that the degree of insulin resistance is independently associated with the severity and exercise intolerance in CHF in terms of exercise capacity and peak oxygen consumption (VO_2) or the 6-min walk test (116,174,175). Insulin resistance also predicted mortality in patients with CHF, independent of body composition and other established prognostic indicators (35). These findings support the notion that insulin resistance is pathophysiologically linked with CHF and is implicated in the disease progression. This is likely because insulin resistance is associated with endothelial dysfunction, inflammation, increased oxidative stress and myocardial remodelling; processes that accelerate the progression of disease in CHF. Therefore, it is likely that the observed beneficial effects of metformin might be related to its potent insulin sensitizing effects (176,177). Indeed, metformin has been shown to offer protection from cardiovascular disease in general (140,177,178). In the UK Prospective Diabetes Study (UKPDS), metformin decreased the risk of mortality and morbidity among obese patients with type 2 diabetes who had cardiac disease (179). In keeping with our finding that CHF patients in the metformin monotherapy group or in combination had better survival compared to those on sulfonylurea alone, evidence from previous studies showed clearly that its benefits outweigh its risks in patients with haemodynamically stable heart failure and adequate renal function (28,166).

It should also be noted that metformin activates 5'-AMP-activated protein kinase (AMPK), a heterotrimeric enzyme that is expressed in many tissues, including the heart and vasculature (137). In animal models, cardiac

AMPK has been shown to mediate ischaemic glucose uptake and prevent post-ischaemic cardiac dysfunction (180). Metformin may also have specific vasculo-protective effects and can improve endothelial function (181).

Our study is observational and in common with all studies of this nature, it is impossible to account for all possible confounding influences that may have biased the observed differences between the groups considered. For example, patients in the sulphonylurea monotherapy group were somewhat older and have more prevalent use of loop diuretics. This may imply that this group have greater prevalence of left ventricular dysfunction and degree of heart failure than the metformin cohort. This may well have led to non-prescription of metformin given the well-known perception of contraindication of this agent in patients with heart failure. In this context, the presence of left ventricular dysfunction could easily lead to non-prescription of metformin, and therefore, confounding by contraindication. Patients in the sulphonylurea group have higher creatinine level, which is another potential confounder. Metformin is contraindicated in patients with renal impairment and physicians would be likely to withhold metformin from patients with the worse renal function, a well recognised independent predictor of adverse outcome in patients with heart failure. Lastly, the findings of lower hazard ratios in patients with higher creatinine is counterintuitive, this may be a reflection of higher usage of diuretics and ACE inhibitors. However, this analysis is based on one creatinine measurement and therefore, evidence of higher creatinine level associated with better outcome in heart failure patients with T2DM must remain speculative and cannot be inferred directly from this study. Clearly, further randomised

placebo controlled trials in this area would be required to provide definitive evidence of the benefit of metformin in this group of patients and further define the underlying mechanisms.

CONCLUSION

This large observational data suggest that the insulin sensitizer metformin is probably more effective than sulphonylurea based monotherapy in the treatment of CHF in patients with T2DM. A clinical trial of metformin in CHF is warranted to corroborate the observational study results

CONTRIBUTIONS TO STUDY

This study was published in the American Journal of Cardiology in year 2010. PMID: 20854965. The first author of the paper is Dr JM Evans. I contributed to the conception and design of the study, analysis of data and interpretation, manuscript writing and revision

ACKNOWLEDGEMENTS

Table 5: Characteristics of patients in the study cohorts with p values for differences between the 'any metformin' cohort and the sulphonylureas monotherapy cohort.

	Sulphonylureas monotherapy	Metformin monotherapy	Metformin + Sulphonylurea Combination	Any metformin: Metformin monotherapy AND Combination	p*
Total	217	68	137	205	
% female	45.2	48.5	46.7	47.3	0.66[1]
Age in yrs (SD)	76.7 (SD 9.8)	75.5 (SD 8.9)	73.4 (SD 8.7)	74.1 (SD 8.8)	0.19[2]
Mean diabetes duration in yrs. (SD)	6.7 (SD 6.2)	6.7 (SD 6.2)	7.7 (SD 5.8)	7.4 (SD 5.9)	0.86[2]
Previous hospital admission (%)	72 (33.2%)	23 (33.8%)	37 (27.0%)	60 (29.3%)	0.39[1]
Mean last creatinine µmol/L (SD)	170.8 (SD 139.4)	135.1 (SD 71.0)	133.2 (SD 82.8)	133.8 (SD 78.0)	<0.001[2]
Mean last HbA1c (SD)	7.20 (SD 1.6)	7.35 (SD 1.5)	7.59 (SD 1.9)	7.51 (SD 1.8)	0.56[2]
% on ACE inhibitor	32.6	41.8	43.3	42.9	0.03[1]
% on aspirin	51.6	68.7	67.6	68.0	0.001[1]
% on diuretic	**21.9**	**14.9**	**17.6**	**16.7**	**0.13[1]**
% on beta-blocker	11.6	19.4	15.4	16.7	0.13[1]

Pearson Chi square[1]. Independent samples t test[2]

* p comparison is between sulphonylurea monotherapy vs 'Any metformin: Metformin monotherapy and Combination'

Table 6: Cox regression analysis showing unadjusted and adjusted odds ratios (with 95% confidence intervals) for all covariates for 1- year and long-term mortality.

	1- Year mortality		Long-term mortality	
Cohort	Unadjusted	Adjusted[1]	Unadjusted	Adjusted[1]
Sulphonylurea monotherapy	1.00	1.00	1.00	1.00
Metformin monotherapy + Combination	0.56 (0.38-0.84)	0.60 (0.37-0.97)	0.53 (0.33-0.67)	0.67 (0.51-0.88)
Sex				
Male	1.00	1.00	1.00	1.00
Female	0.82 (0.67-1.00)	0.47 (0.29-0.77)	1.16 (0.93-1.45)	0.64 (0.49-0.84)
Age				
< 60 yrs.	1.00	1.00	1.00	1.00
60-69 yrs.	5.60 (0.76-41.4)	4.62 (0.62-34.64)	1.15 (0.57-2.32)	1.11 (0.52-2.39)
70-79 yrs.	5.07 (0.70-36.9)	4.20 (0.59-34.64)	1.66 (0.85-3.28)	1.57 (0.75-3.25)
80-89 yrs.	7.06 (0.97-51.5)	7.33 (0.98-54.86)	2.82 (1.43-5.56)	2.61 (1.24-5.49)
>89 yrs.	8.57 (1.09-67.7)	12.39 (1.52-101.06)	3.24 (1.51-6.94)	3.44 (1.47-8.05)
Duration				
< 5 yrs.	1.00	-	1.00	1.00
5-9 yrs.	1.44 (0.91-2.28)	-	1.26 (0.97-1.64)	0.92 (0.67-1.26)
10-14 yrs.	1.70 (1.00-2.91)	-	1.28 (0.92-1.78)	1.13 (0.77-1.64)
>14 yrs.	1.38 (0.74-2.58)	-	1.80 (1.25-2.58)	1.77 (1.17–2.65)
Previous hospital admission				
Yes v No	1.23 (0.83-1.83)	1.08 (0.67-1.74)	1.01 (0.79-1.28)	0.97 (0.73-1.27)
Creatinine[1]				
Quartile 1	1.00	1.00	1.00	1.00
Quartile 2	0.54 (0.30-0.96)	0.46 (0.26-0.84)	0.55 (0.38-0.80)	0.52 (0.35-0.76)
Quartile 3	0.29 (0.14-0.59)	0.23 (0.11-0.47)	0.62 (0.43-0.88)	0.53 (0.36-0.77)
Quartile 4	0.67 (0.38-1.17)	0.51 (0.28-0.91)	1.09 (0.78-1.53)	0.87 (0.61-1.23)

HbA1c[2]				
< 7.5	1.00	-	1.00	-
7.5 – 9.49	1.14 (0.70-1.83)	-	1.08 (0.83-1.40)	-
>9.49	1.06 (0.48-2.34)	-	1.03 (0.67-1.57)	-
ACE				
Yes v No	0.56 (0.36-0.86)	0.57 (0.34-0.97)	0.66 (0.52-0.84)	0.76 (0.58-1.00)
Aspirin				
Yes v No	0.61 (0.41-0.89)	0.67 (0.42-1.08)	0.65 (0.52-0.82)	0.80 (0.61-1.05)
Diuretic				
Yes v No	1.14 (0.72-1.81)	-	1.09 (0.83-1.43)	-
Betablocker				
Yes v No	0.46 (0.22-0.94)	0.41 (0.16-1.04)	0.52 (0.36-0.74)	0.52 (0.34-0.80)
Available for 346[1] and 363[2] patients				

Figure 11: Kaplan-Meier plot for 1-year follow-up, comparing mortality in the sulphonylureas cohort with mortality in the any metformin cohort.
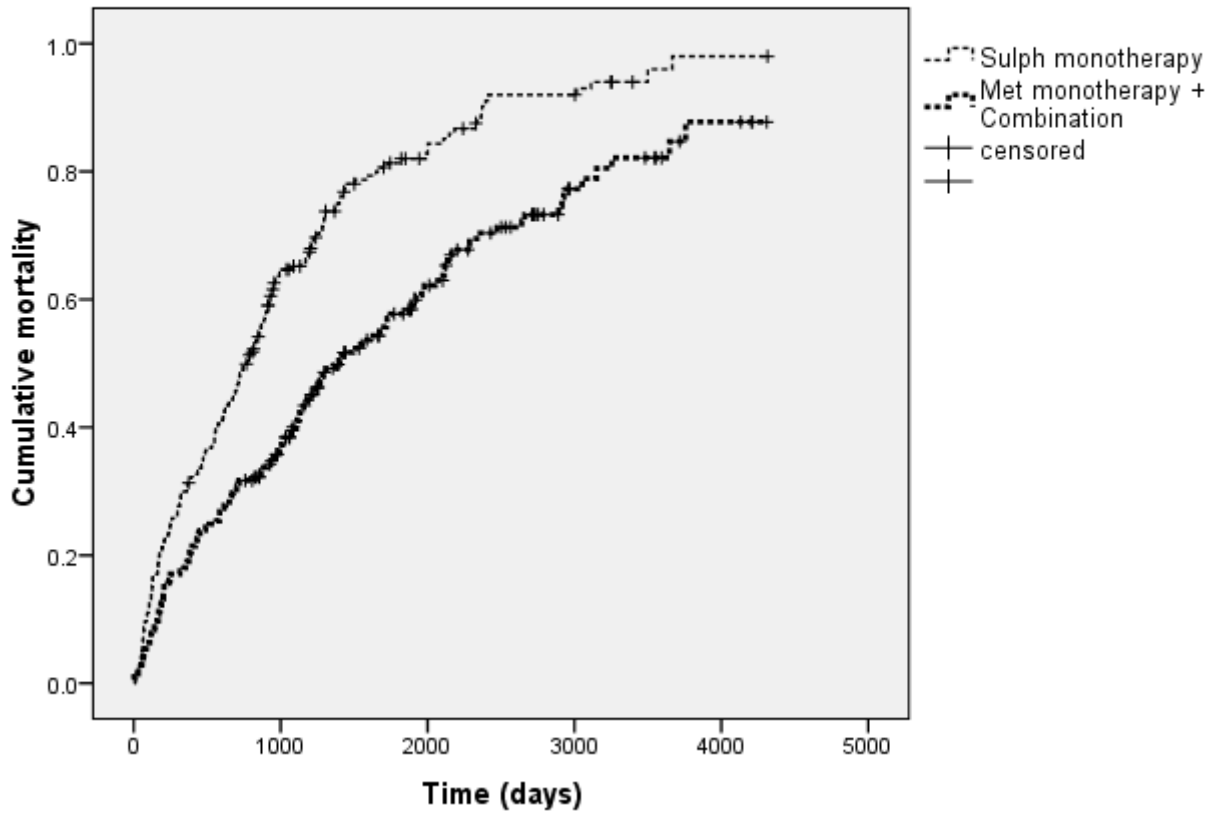
CHAPTER SUMMARY

Insulin resistance and CHF is an intricate pair that forms a vicious cycle that worsens each other at both tissue and cellular levels. Our increased understanding of the relationships between IR and CHF provides the rationale for targeting IR in the development of new CHF therapy. However, there appear to be some conundrum in the choice of available insulin sensitizers as there are safety issues of regarding TZDs and metformin. While the concerns regarding TZDs appear to be justified, there is now emerging evidence that metformin may not only be safe in CHF but may indeed be good in CHF. However, prospective trials are needed to prove this. TAYSIDE study (Me<u>T</u>formin in Insulin Resist<u>A</u>nt LV D<u>YS</u>funct<u>I</u>on, <u>D</u>ouble blind, plac<u>E</u>bo-controlled trial) will determine if reversing IR with metformin will have beneficial effects in patients with CHF.

CHAPTER 6: THE EFFECTS OF METFORMIN ON INSULIN RESISTANCE AND EXERCISE PARAMETERS IN PATIENTS WITH HEART FAILURE

INTRODUCTION

Chronic heart failure (CHF) is an insulin resistant (IR) state (165). We and others have shown that IR is highly prevalent among non-diabetic patients with CHF (116,174,182), and the degree of IR correlates with disease severity and outcome (35,116). Furthermore, it is associated with reduced exercise capacity (116,175). It is unclear whether IR is a bystander reflecting disease severity or whether it is a culprit contributing to the pathophysiology of CHF. Previous association studies did not distinguish the cause and effect. Therefore, a proof of concept study is required to test the hypothesis that IR is a culprit, and that reversing IR will lead to clinical improvement in CHF. However, the numbers of insulin sensitizers that are suitable to be tested are limited. Thiazolidinediones have the potential to exacerbate CHF in patients with reduced cardiac reserve. Although metformin is a widely prescribed drug in the management of T2DM, and its use in diabetic patients with CHF has previously been discouraged due to concerns over the risk of lactic acidosis originating from earlier experience with phenformin. However, clinical experience suggests that the risk of metformin–associated lactic acidosis is very low and similar to that of other anti-diabetic drugs (183). Indeed, there is observational data to suggest that metformin may actually be beneficial for CHF (18,184). We

have therefore conducted a proof of concept study to evaluate the impact of metformin on IR and its effects on exercise capacity in non-diabetic patients with CHF.

RESEARCH DESIGN AND METHODS

This is a randomized, placebo-controlled trial designed to evaluate the impact of metformin on IR and its effects on exercise capacity in non-diabetic patients with CHF. The primary endpoint of the trial was to determine if improvement of IR with metformin lead to improvement of peak VO_2. However, many patients with CHF are unable to perform maximal exercise, and oxygen requirements for activities of daily living rarely approach maximal levels (185). Therefore, we have included the sub-maximal derived exercise variable of the slope of the ratio of minute ventilation to carbon dioxide production (VE/VCO_2) as a secondary end-point of this study. VE/VCO_2 slope is an index of ventilatory response to exercise, unlike peak VO_2, VE/VCO_2 is not influenced by the mechanical work done during exercise testing but reflects alterations in the peripheries caused by the disease in CHF, which can in turn lead to the progression and symptomatology of CHF (186). Furthermore, we have also explored possible mechanisms of improvement of exercise capacity by measuring left ventricular ejection fraction by echocardiography, endothelial function and related biomarkers. The study was approved by the East of Scotland Research Ethics Service (07/S1401/59).

PATIENT POPULATION

Every patient provided written informed consent prior to participation in this study. Patients with CHF were recruited from out patient cardiology clinics and local echocardiography database. Patients with a history of CHF and left ventricular systolic dysfunction on echocardiography had enrolled for a fasting blood test to determine IR status by an empirical fasting insulin resistance index (FIRI), consisting of the product of plasma insulin and glucose divided by 25. CHF patients with a FIRI ≥ 2.7 were considered to have IR (116) and invited to participate in the study. Exclusion criteria included: patients with history of type 2 diabetes or fasting glucose of more than 7mmol/L; patients aged >80 years; patients with NYHA functional class IV and decompensated CHF; renal dysfunction (serum creatinine > 160 μmol/L); patients unable to exercise including patients that were excluded for reasons of safety or potential effects on exercise performance. Patients had to be on stable dose of CHF medications at least one month prior to screening. 127 patients were screened (Figure 12) and 53 patients were excluded based on exclusion criteria, and 21 were found to be non-IR. 62 patients with evidence of CHF and IR were randomised to receive metformin or placebo (2:1 ratio) with 39 patients randomised to receive metformin and 23 to receive a placebo. Treatment allocation was masked for patients and investigators until after database lock. Compliance was assessed by tablet counting.
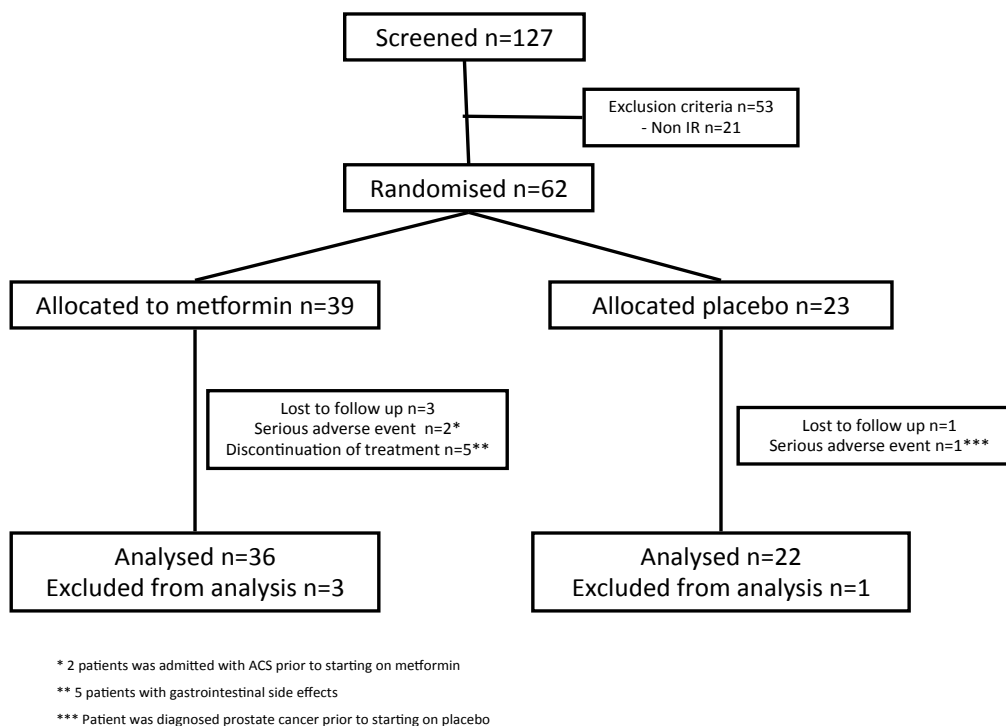
Figure 12: The CONSORT (Consolidated Standards of Reporting Trial) diagram describing outcomes of all patients within the study

STUDY PROTOCOL

The study consisted of 6 visits (Figure 13). At visit 1, patients underwent physical examination, fasting blood tests, cardiopulmonary exercise testing (CPET), two-dimensional echocardiography (2-D echo), endothelial function assessments, 6-minute un-encouraged walk test (6MWT) and Minnesota Living with Heart Failure Questionnaires (MLHF). CPET was repeated at a separate visit (Visit 2) within a week in order to achieve consistent exercise parameters with a variation of exercise duration of less than 15% prior to randomization. Following this visit, patients were randomized to receive either 4 months of metformin (1000mg b.d.) or matching placebo using a pre-established computer generated sequence from our study drug provider (Western

Infirmary, Glasgow). The dose and duration of metformin therapy was based on

a previous study in patients with impaired glucose tolerance, which showed

that this dose regimen was well tolerated and had a beneficial effect on

endothelial function (187). The metformin study drug was commenced at

500mg b.d. for two weeks and was up titrated if well tolerated based on

symptoms and measurement of plasma lactate and renal function. At

subsequent visits, patients were reassessed and doses of study drug altered

according to tolerability. All measurements of interests were repeated after 4

months of intervention.
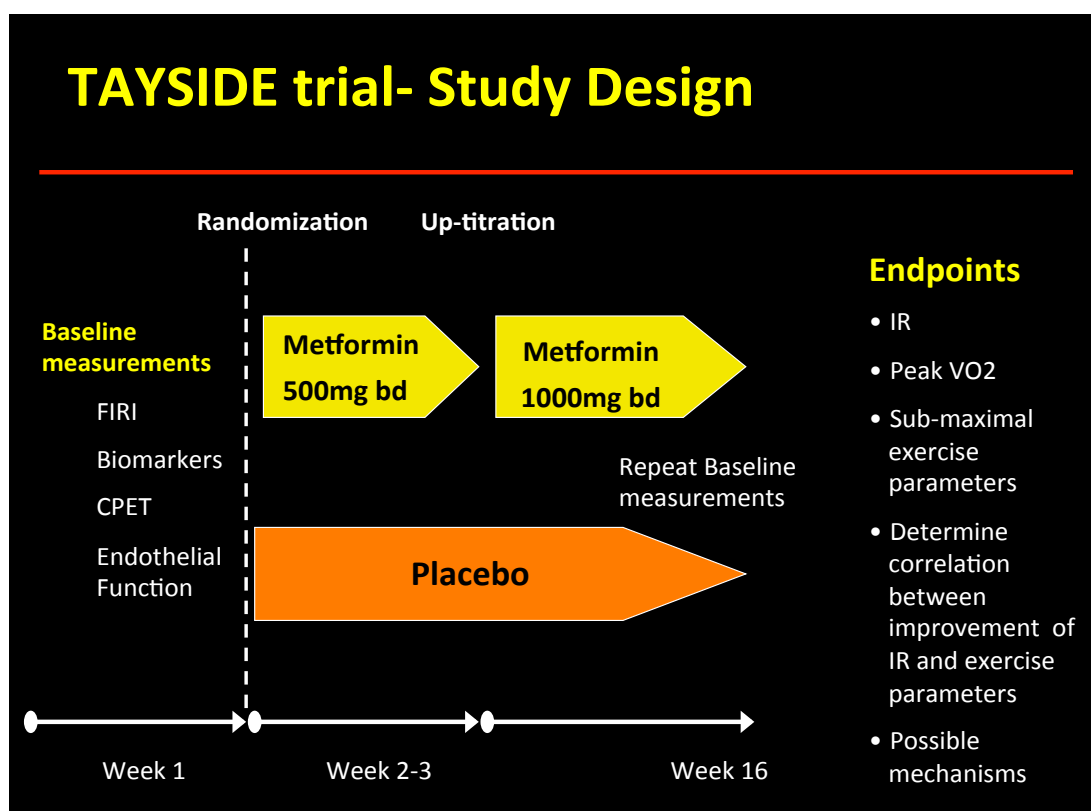
Figure 13 TAYSIDE Trial Design

CARDIOPULMONARY EXERCISE TESTING (CPET)

CPET was performed in the fasting state as previously described (188). Prior to exercise, the patient was instructed on the re-breathing technique. An incremental symptom-limited bicycle exercise testing was performed using an upright, braked cycle ergometer. After 3 minutes rest, exercise was begun at 0 Watts (W) and increased every 3 minutes by 25W until symptom-limited maximal exercise was achieved. Patients were instructed to signal approximately 1 minute before peak exercise. ECG was monitored continuously during the test. Cuff blood pressure was measured at rest and every 3 minutes. Expired gas analysis was performed continuously throughout the test with the Innocor system (Innovision A/S, Odense, Denmark). Peak VO_2 was defined as the highest value of VO_2 achieved in the final 20 seconds of exercise. VE/VCO_2 slope was calculated from the start of incremental exercise to the anaerobic threshold, by least squares linear regression. Cardiac output (CO) measurements were made at the end of the rest period and at peak exercise. VO_2 (ml/kg/min), VCO_2 (L/min), and VE (L/min) (minute ventilation) were measured on a breath-by-breath basis. CPETs were performed at visit 1 and visit 2 to achieve a variation of exercise duration of less than 15% prior to randomisation to minimise the "learning effect". If variation was more than 15%, a further CPET was repeated and the highest value of peak VO_2 was chosen to be the baseline.

ENDOTHELIAL FUNCTION

A number of non-invasive methods of assessing endothelial function are available.   We have chosen Reactive Hyperaemia Peripheral Arterial Tonometry, RH-PAT (Itamar Medical Ltd. Caesarea, Israel) (Figure 14) and Flow-Mediated Dilatation (FMD) (116,189) (Figure 15) to assess vascular function in different vascular beds.

FLOW-MEDIATED DILATATION (FMD)

Endothelial function has been shown to predict future cardiovascular events (190). Flow-mediated dilatation (FMD), available since 1992, is currently viewed as the gold standard for assessing endothelial function non- invasively (109). FMD provides useful prognostic information based on the concept that direct assessment of the function of the arterial wall has more predictive power compared to assessing traditional risk factors. Table 7 summarises the studies using FMD as a prognosticator in subjects with cardiovascular disease or high risk for developing cardiovascular disease (191). FMD has been documented to correlate with invasively assessed endothelial function in the coronary arteries. (192). Endothelial function in the brachial circulation is impaired as in the coronary circulation in the setting of traditional and novel risk factors and responds to interventions known to reduce CVD risk (190) . FMD measures change in diameter of a conduit vessel following a period of ischaemia, and the brachial artery is the most commonly studied vessel. A sphygmomanometer cuff is placed on the forearm distal to the brachial artery and inflated to supra-

systolic blood pressure for 4-5 minutes and the cuff is released. The resulting reactive hyperaemia increases shear stress leading to NO release, and therefore endothelium dependent vasodilatation (184). Endothelium independent vasodilatation is assessed by response of brachial artery to sublingual GTN. FMD is widely used and correlates well with coronary vascular endothelial function (109). However FMD has several limitations, which precluded its integration into clinical practice. It is technically difficult to perform and require extensive sonographer training, together with the expense of equipment and the requirement of labor-intensive image analysis, and lack of methodological standardization that have prompted a search for techniques inherently faster and easier to perform.

First Author	Journal	N	Male, %	Age, y	Group	Follow-Up	End Points (n)	Prognostic Impact
Meyer	*J Am Coll Cardiol*	75	89	56	CHF	1.5	Progression	FMD predicts progression disease (Independent)
Neunteufl	*Am J Cardiol*	73	52	51	Chest pain+CAG	5	CV events (23)	FMD predicts CV events (Independent)
Gokce	*J Am Coll Cardiol*	199	82	67	PAD	1.2	CV events (35)	FMD predicts CV events (Independent)
Fathi	*J Am Coll Cardiol*	444	59	59	CAD	2	CV events (119)	Low risk: FMD does not predict CV events; high risk: FMD predicts CV events (Independent)
Brevetti	*Circulation*	131	90	64	PAD	1.9	CV events (39)	FMD predicts CV events (Independent)
Karatzis	*Am J Cardiol*	98	100	63	NSTEMI	2	CV events (20)	FMD predicts CV events (Independent)
Fischer	*European Heart Journal*	67	61	82	CHF	3.9	CV events (24)	FMD predicts CV events (Independent)
Chan	*J Am Coll Cardiol*	152	56	84	CAD	2.8	CV events (22)	FMD/GTN ratio predicts CV events (Independent)
Frick	*J Am Coll Cardiol*	398	100	54	Chest pain+CAG	3.3	CV events (44)	FMD does not predict CV events
Patti	*Circulation*	136	81	63	CAD+stent	0.5	Restenosis (20)	FMD predicts restenosis
Kitta	*J Am Coll Cardiol*	141	68	66	PCI	0.5	Restenosis (46)	FMD (during follow-up) predicts restenosis

CV indicates cardiovascular; CAD, coronary artery disease; PCI, percutaneous coronary intervention; CAG, coronary angiography; CHF, congestive heart failure; NSTEMI, non-ST elevation myocardial infarction; PAD, peripheral artery disease; GTN, glyceryl trinitrate.

Table 7: FMD as a prognosticator in subjects with cardiovascular disease or at high risk for cardiovascular disease. Green D et al. Hypertension 2011;57:363-369

PULSE AMPLITUDE TONOMETRY (PAT)

Digital pulse amplitude tonometry (PAT) is a new non-invasive technique to measure endothelial function. It measures volumetric changes in the fingertip, using a probe that quantifies pulse amplitude in response to reactive hyperemia using a commercially available device (EndoPAT, Itamar Medical, Ltd). Signals in the contralateral hand not experiencing hyperemia are simultaneously recorded, controlling for systemic effects. Proprietary software provides a reactive hyperemia PAT ratio in relation to the control arm that is expressed after natural log transformation owing to skewed variable distribution. The potential advantage of this technique relates to the use of an automated, computerized analysis system that minimizes operator dependency and inter-observers variability. Small-scale preliminary studies showed that PAT hyperemic responses depend on NO and were reduced in the presence of coronary artery disease or its risk factors, suggesting that clinically important group differences can be detected using this method (193).
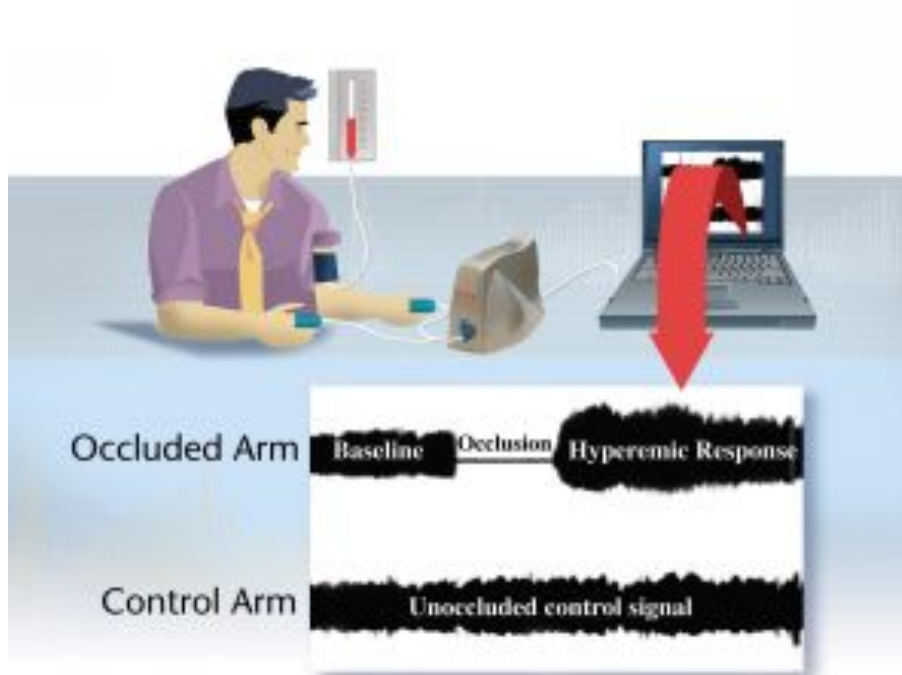
Figure 14: Reactive hyperaemic tomography (Adopted from ITamar Medical)

Figure 15: Flow mediated dilatation. (Adopted from BMPE, University of Tokyo)
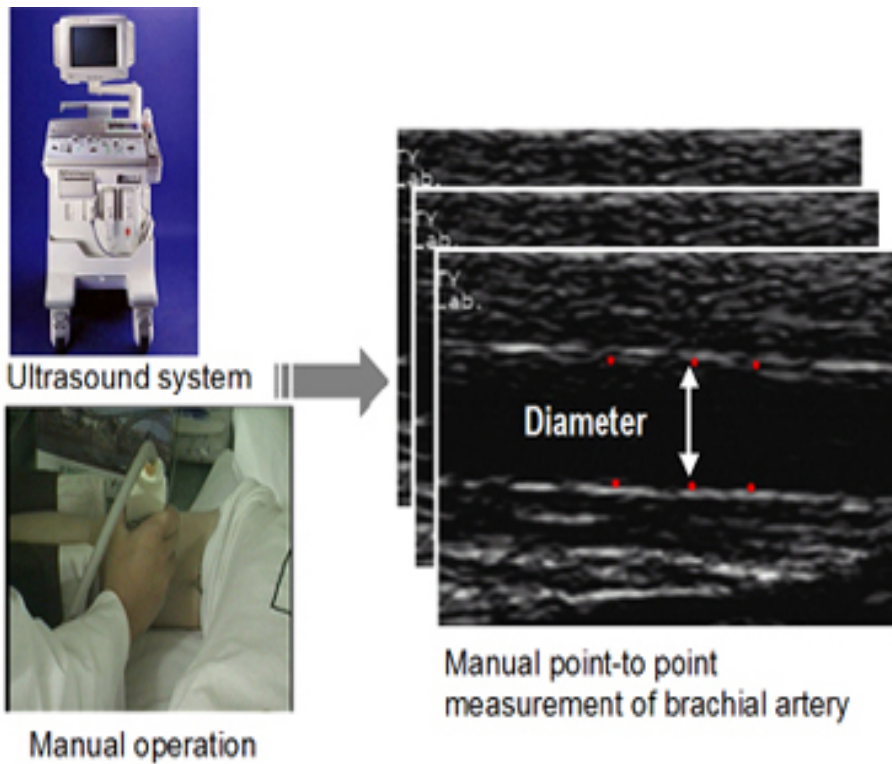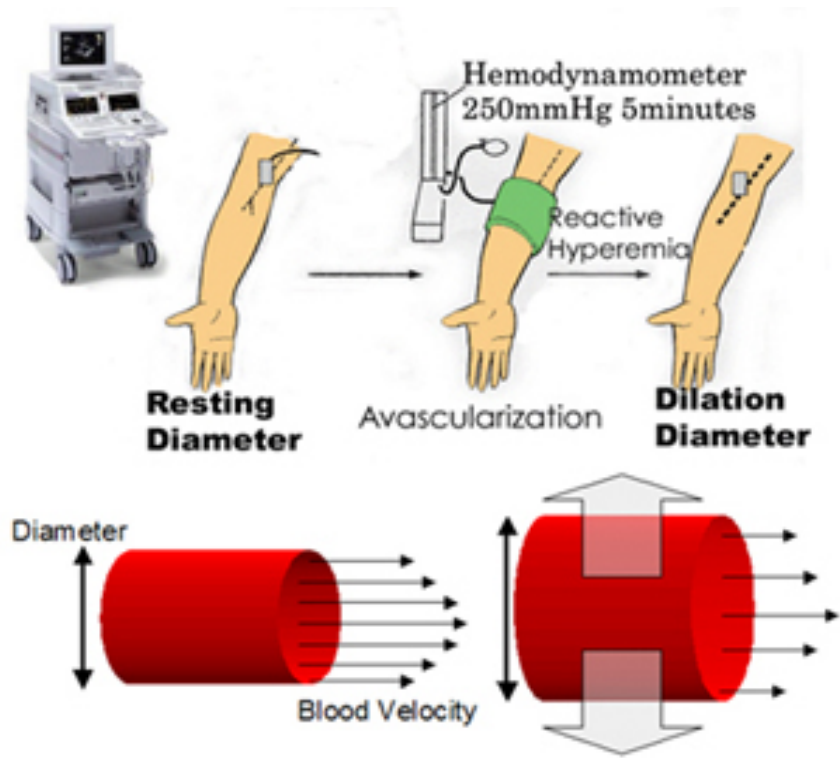
ECHOCARDIOGRAPHY

Standard 2-D echo was performed in all patients. Biplane left ventricular ejection fraction (LVEF) was calculated using the Simpson's method.

6MWT WAS PERFORMED USING A STANDARDISED APPROACH OVER A 25 METERS COURSE.

MINNESOTA LIVING WITH HEART FAILURE QUESTIONNAIRE (MLHF)

This is a 21-item questionnaire, which assesses physical activity, subjective symptoms and psychosocial issues.

BIOMARKERS

Venous blood samples were obtained following a 20 min semi-recumbent rest in the fasting state at baseline, and at end of study for measurement of full blood count, renal function, glycated haemoglobin (HbA1c), glucose, lipids, lactate, insulin (INSIK-5, DiaSorin, UK), brain natriuretic peptide (BNP), adiponectin (Quantikine, R&D System, UK), leptin (Quantikine, R&D System, UK) and resistin.

SAFETY ASSESSMENTS

Safety was assessed via monitoring for adverse events (AEs), clinical examination, standard laboratory testing, ECG recordings and regular measurements of vital signs. Lactate levels were measured 2 weeks after initiation of study treatment, and at the end of study visit.

POWER CALCULATION AND STATISTICAL METHOD

We targeted 66 subjects and the power calculations were based on our previous observational study of CHF with IR with a mean peak VO_2 of 11 ml/min/kg and standard deviation of 1.8ml/min/kg, which would provide 80% power to detect a 13.5% change in peak VO_2 in the 2 groups of patients with CHF (alpha=0.05) allowing for a 10% drop out rate. Statistical analysis was performed using SPSS for Windows version 16 (SPSS Inc., Chicago, IL). Numeric values were expressed as mean ± standard deviation. An intention to treat analysis was used. The significant of differences between the two treatment groups of changes from baseline was analyzed using independent t-tests and chi-square tests. Correlations were made using Pearson product moment correlation coefficients. P value of less than 0.05 was considered statistically significant.

RESULTS

Baseline characteristics were well matched between the 2 study groups (Table 8). Baseline measurements of interests are shown in Table 9.

Table 8: Baseline characteristics of TAYSIDE Study

	Metformin	Placebo	All Patients	P Value
	N=39	N=23	N=62	
Age	64 ± 8	68 ± 7	65 ± 8	0.063
Sex (Male/Female)	35/4	22/1	57 / 5	0.409
Systolic Blood Pressure (mmHg)	113 ± 16	114 ± 17	113 ± 16	0.761
Diastolic Blood Pressure (mmHg)	71 ± 9	73 ± 10	72 ± 9	0.563
Mean Heart Rate (bpm)	74 ± 16	71 ± 19	73 ± 16	0.597
Body Mass Index (BMI)	30 ± 5	29 ± 4	29.7 ± 4.6	0.224
LV Ejection Fraction (%)	34 ± 8	30 ± 8	33 ± 8	0.083
NYHA (I/II/III/IV)	7/29/3/0	3/17/3/0	10 / 46 / 6 / 0	0.725
Aetiology of Heart Failure				
Ischaemic	28	21	49	0.068
Non-ischaemic	11	2	13	
Past Medical History (%)				
Ischaemic Heart Disease	80	91	63	0.036
Atrial fibrillation	31	17	26	0.245
Revascularisation	33	35	34	0.944
Peripheral vascular disease	3	9	5	0.277
Hypertension	41	43	55	0.850
Chronic Obstructive Pulmonary Disease	3	13	7	0.105
Medications (%)				
Diuretics	51	52	53	0.946
Angiotension Converting Enzyme inhibitors	74	82	74	0.473
Angiotensin receptor blockers	21	14	18	0.474
Betablockers	82	91	82	0.329
Aldosterone antagonist	37	14	27	0.055
Digoxin	8	14	10	0.475
Calcium channel blocker	11	18	13	0.401
HMG Co-A reductase Inhibitors (Statin)	82	100	91	0.031
Isosorbide Mononitrate	18	36	12	0.122

Table 9: Baseline measurements of TAYSIDE Study

	Metformin	**Placebo**	**P Value**
	N=39	**N=23**	
Exercise Parameters			
▪ Peak VO$_2$ (ml/kg/min)	19.5 ±4.9	18.5 ±5.1	0.312
▪ Peak Cardiac Output (L/min)	8.3 ±2.9	7.8 ±2.6	0.631
▪ VE/VCO$_2$ Slope	32.9 ±15.9	32± 5.9	0.821
▪ Ventilatory Class I/II/III/IV	13/12/7/1	8/7/5/0	0.845
▪ Respiratory Gas Exchange Ratio (R)	0.9 ±0.1	0.8 ±0.1	0.088
▪ Total exercise duration (secs)	1049 ±207	940 ±288	0.091
▪ 6 Minute Walk Test (meters)	438 ±76	414±86	0.280
Endothelial Function			
▪ Reactive Hyperaemic Index (RHI)	1.8 ±0.3	1.9 ±0.6	0.264
▪ Flow Mediated Dilatation (FMD) (%)	7.1 ±3.7	5.4 ±3.7	0.119
Laboratory Parameters			
▪ Haemoglobin (g/dL)	14.8 ±1.4	14.5 ±1.3	0.361
▪ Creatinine (μmol/L)	87.8 ± 16.3	88.3 ± 19.5	0.912
▪ Glucose (mmol/L)	5.6 ± 0.6	5.3 ± 0.4	0.029
▪ Insulin (mU/L)	26.8 ± 14.3	23.2 ± 10.5	0.198
▪ Fasting Insulin Resistance Index (FIRI)	6.6 ± 3.9	6.5 ± 4.6	0.116
▪ Total Cholesterol (mmol/L)	4.3 ± 1.0	3.8 ± 0.6	0.023
▪ Adiponectin (mg/ml)	8.4 ± 6.7	8.7 ± 5.5	0.879
▪ Leptin (ng/ml)	16.6 ± 23.2	10.9 ± 6.4	0.265
▪ BNP (pg/ml)	131.7± 158.5	187.1 ± 251.3	0.362
▪ Resistin (ng/ml)	5.1 ± 2.3	4.0 ± 1.1	0.054

CHANGES IN WEIGHT, FIRI AND BIOMARKERS

Compared to placebo, metformin resulted in a significant reduction in weight and BMI (p< 0.001, p=0.037 respectively). Metformin significantly decreased FIRI (p<0.001) and serum HbA1c (p=0.002). Serum leptin levels were significant reduced with metformin (metformin, -4.56 ± 11.0 ng/ml vs placebo, 0.58± 3.5 ng/ml, p=0.038). There was no significant change in plasma BNP with metformin (metformin, -20.2 ± 78.7 pg/ml vs placebo, 7.5 ± 131.2 pg/ml).

MAXIMAL AND SUB-MAXIMAL EXERCISE PARAMETERS AND 6MWT

Peak exercise parameters did not differ between treatment groups (Table 10). Compared to placebo, metformin decreased the sub-maximal parameters of VE/VCO_2 slope (from 32.9±15.9 to 28.1± 8.8, p=0.034) and ventilatory class (χ-square test, p=0.008). There was no difference in 6MWT.

CORRELATIONS BETWEEN VE/VCO2 SLOPE, WEIGHT AND FIRI

Pearson correlations and linear regression model showed that weight reduction on its own was not correlated with the reduction of VE/VCO_2 slope (p=0.801). In the metformin treated group, FIRI and serum leptin levels were significantly related to the reduction of VE/VCO_2 slope (R=0.41, Difference in FIRI: Beta: -14.07, p=0.036; Difference in leptin: Beta=0.29, p=0.023).

SYMPTOMS

There was no significant change in NYHA functional class between the treatment groups (χ-square test, p=0.124). Although it was noted that 4 patients in the metformin arm reported an improvement in NYHA functional class with a drop of one NYHA functional class whereas 1 patient in the placebo arm had an increase in one NYHA functional class. MNLHF Questionnaire scores did not differ between the groups. Heart failure medications including diuretic dosage remained unchanged throughout the study.

ECHOCARDIOGRAPHY AND ENDOTHELIAL FUNCTION

There was no significant change in LVEF. Changes in RH-PAT (metformin, 0.12 ± 0.4 vs. placebo, 0.06 ± 0.70) and FMD (metformin, -0.38 ± 4.46 vs. placebo, -1.74± 2.71) were not statistically significant.

TOLERABILITY AND SAFETY OF METFORMIN

There were no SAEs in either treatment groups. AEs were more frequent with metformin treatment although majority of these AEs were transient, and were mild to moderate in severity. The main AEs were abdominal discomfort (16% metformin, 4% placebo, p= 0.18), diarrhoea (47% metformin, 13% placebo, p= 0.008), nausea (29% metformin, 0% placebo, p= 0.005), and anorexia (21% metformin, 0% placebo, p= 0.021). AEs led to premature discontinuation in five metformin treated patients. The average tolerable dose of metformin was 1675mg daily. Lactate levels did not differ between treatment groups and no lactic acidosis was reported.

Table 10: TAYSIDE study. Changes after 4 months of metformin treatment

	Metformin N=36	Placebo N=22	P value
Peak Exercise Parameters			
Peak VO$_2$ (ml/kg/min)	-0.38 ± 1.40	3.60 ± 3.90	0.08
Peak CO (L/min)	0.03 ± 3.10	-0.35 ± 2.10	0.682
Sub-maximal Exercise Parameters			
VE/VCO$_2$ Slope	-4.45 ± 10.72	-0.23 ± 3.54	0.034
Ventilatory Class I/II/III/IV	21/8/2/2	8/6/7/1	0.008
NYHA Functional Class I/II/III/IV	10/25/1/0	3/15/4/0	0.124
Heart Failure Questionnaires	1.58 ± 14.78	0.45 ± 8.57	0.746
6 Minute Walk Test (meters)	6 ± 40	6 ± 32	0.988
Biomarkers and laboratory parameters			
HbA1c (%)	-0.12 ± 0.19	0.08 ± 0.17	0.035
Glucose (mmol/L)	-0.36 ± 0.45	0.09 ± 0.71	0.005
Insulin (mU/L)	-6.60 ± 8.80	4.10 ± 13.10	0.000
FIRI (Log)	-1.44 ± 0.16	0.05 ± 0.18	0.000
Leptin (ng/ml)	-4.56 ± 11.0	0.58 ± 3.50	0.038
Adiponectin (mg/ml)	-0.44 ± 2.16	0.43 ± 2.54	0.168
Resistin (ng/ml)	0.01 ± 0.08	0.04 ± 0.06	0.094
BNP (pg/ml)	-20.2 ± 78.7	17.5 ± 131.2	0.184
Creatinine (μmol/L)	0.50 ± 12.46	1.41 ± 7.39	0.758
Lactate (mmol/L)	0.09 ± 0.63	0.00 ± 0.42	0.562
Cholesterol (mmol/L)	-0.32 ± 0.54	-0.01 ± 0.65	0.055
Triglyceride (mmol/L)	-0.17 ± 0.60	-0.04 ± 0.80	0.467
Weight (Kg)	-1.9 ± 2.3	1.1 ± 2.5	0.000
Body Mass Index	-2.03 ± 6.07	0.39 ± 0.89	0.037
Waist Hip Ratio	-0.08 ± 0.03	-0.49 ± 2.02	0.341
Ejection Fraction (%)	0.35 ± 5.50	-1.10 ± 4.20	0.356
Endothelial Function			
Reactive Hyperaemic Index (Endo-PAT)	0.12 ± 0.54	0.06 ± 0.70	0.743
Flow Mediated Dilatation (%)	-0.38 ± 4.36	-1.74 ± 2.71	0.201

DISCUSSION

This study had two main findings, Firstly, we showed that in patients identified to have insulin resistance and CHF, metformin treatment significantly reduced FIRI and that this treatment was associated with a weight loss of 1.9 kg. Secondly, although metformin had no effect on peak exercise parameters including peak VO_2, metformin treatment did result in a significant improvement in VE/VCO_2 slope, a pre-specified endpoint of this proof of concept study.

Although diabetes predispose to the development of CHF, CHF may also lead to the development of IR and diabetes. Previous clinical studies utilizing the hyperinsulinaemic-euglycaemic clamps have demonstrated fasting hyperinsulinaemia and insulin resistance in patients with both ischaemic and non-ischaemic CHF (174). IR is highly prevalent amongst patients with CHF. We and others have showed that close to two thirds of patients with CHF has IR determined by either FIRI or oral glucose tolerance test (116,182). IR is associated with decreased exercise capacity, endothelial dysfunction (116) and more importantly worse prognosis in CHF (2,3). The prognostic impact of IR is independent of other variables, including peak VO_2 and left ventricular ejection fraction, which may imply that IR maybe pathogenic rather than simply a marker for worsened CHF (175,194). These findings support the notion that IR may be pathophysiologically linked with CHF, and is implicated in the disease progression in CHF (195). This is likely because IR is associated with endothelial dysfunction, inflammation, increased oxidative stress, changes in

cardiac metabolism and myocardial remodelling, processes that accelerate the progression of disease in CHF (195).

If IR is important to the pathogenesis of CHF, it could be argued that therapies directed toward improving IR could be beneficial. To the best of our knowledge, this is the first proof of concept study to examine the impact of metformin on IR and exercise parameters in patients with CHF identified to have IR. In this study, we have chosen to use metformin although we recognize that the precise mechanisms of metformin's action are not entirely understood (176). However, there is increasing evidence to suggest that metformin may have cardio-protective effects in the setting of CHF that are not attributed to the glucose lowering effects alone. Recent experimental studies suggest ancillary potential mechanisms. These protective effects may be conferred via the 5'-AMP-activated protein kinase (AMPK) pathway, which is activated by metformin (165,196,197). Metformin has also been shown to improve endothelial function by increasing nitric oxide production (198). Clinical studies show that metformin may reduce plasma dipeptidyl peptidase-4 activity and increase circulating levels of glucagon-like peptide 1 (GLP-1), which is an incretin hormone that has protective effects on the heart and the vasculature (199,200). Finally, there are observational studies of patients with CHF and type 2 diabetes mellitus taking metformin that suggest a morbidity and mortality benefit (18,184). Although these data were encouraging, the main limitations of these observational studies were the potential for selection bias imposed by different therapies. What is needed are prospective placebo controlled studies such as our study.

In this study, we were interested in determining if reversing IR with metformin in patients with CHF would result in an improvement in exercise capacity. In this study, we did not observe an effect of metformin on peak exercise parameters including peak VO_2, the primary endpoint of our study. However, metformin treatment did result in VE/VCO_2 slope and ventilatory class. The VE/VCO_2 slope reduced from 32.9 to 28.1. The VE/VCO_2 slope had been reported to be a more accurate prognostic index for cardiac related mortality and hospitalization than peak VO_2 (201,202), and the ventilatory classification system has been proposed to guide therapy in patients with CHF (203). We acknowledge that there are several possible explanations for our findings. One possible explanation for the improvement in functional capacity might be the weight loss of 1.9 kg associated with metformin therapy. Studies of both diet and drug-induced weight loss have been shown to improve functional status in CHF patients (204). However, it should be noted that our regression model showed that weight reduction did not correlate with the reduction in VE/VCO_2 slope. Another consideration is that the insulin sensitizing properties of metformin might confer some beneficial effects on exercise capacity. Improving insulin sensitivity has been shown to improve exercise capacity. Regensteiner and colleagues have previously shown that improvement in insulin sensitivity with rosiglitazone resulted in a significant improvement in exercise capacity and peak VO_2 in diabetic individuals (205). In this regard, we found that with metformin treatment, reduction of FIRI and serum leptin level was significantly correlated with the reduction of VE/VCO_2 slope. Doehner et al had previously demonstrated that hyperleptinaemia is an independent predictor of IR in patients with CHF, and it may play an important role in

energy metabolism in these patients (63). Reduction of serum leptin levels have been previously reported following chronic metformin therapy (206), and might be due to a direct effect of metformin on leptin secretion (207). Recent studies have shown the significance of adipocytokines modulation in HF patients; and high levels of adiponectin were associated with adverse outcomes in CHF (75,76). There were no significant change of adiponectin levels in our study (p=0.168). However, we did notice that patients in the metformin treated group have decreased adiponectin level whereas adiponectin levels were higher in the placebo group. Therefore, the improvement of sub-maximal exercise capacity in our cohort may in part be due to an improvement of IR and the reduction of serum leptin level. A third consideration is metformin's ability to activate AMPK, which is expressed in various tissues including the skeletal muscle, myocardium as well as the vascular endothelium (165). Therefore, activation of AMPK could impact on central as well as peripheral haemodynamic mechanisms, which in turn leads to changes in VE/VCO_2 slope and hence, ventilatory class. Improvement of myocardial substrate utilization and glucose uptake through activation of AMPK may improve myocardial contraction and increase cardiac output (196). In animal models of heart failure, metformin has been shown to activate AMPK and improve left ventricular function and to attenuate oxidative stress-induced cardiomyocyte apoptosis, resulting in improved survival (197). However, we did not observe an effect of metformin on echo derived LVEF or CO at rest and during peak exercise. With respect to peripherally mediated mechanisms, an effect of metformin on exercising skeletal muscles could account for the improvement in VE/VCO_2 slope. Alterations in skeletal muscle energy metabolism, IR and

functional adiponectin resistance have been reported, and linked to exercise intolerance in patients with CHF (208). A study of the effect of metformin on skeletal muscle enzyme activities in our subjects would be of interest. In our original proposal, we had planned to do this but this invasive procedure was offered as an option and no patient consented to the procedure. We did not see an effect of metformin on endothelial function. Obviously any evidence that metformin improves exercise capacity through central cardiac or peripheral mechanisms in patients with CHF must remain speculative and cannot be inferred directly from this study. Clearly, further studies are required to define the mechanisms underlying these effects of metformin in CHF.

In this study, we did not record any incidence of lactic acidosis and SAEs. The most common AE was diarrhoea, which is well described with metformin use. The 2 kg reduction in weight is consistent with findings of previous studies of metformin when used in non-diabetic populations (209).

LIMITATIONS OF STUDY

In order to comply with our strict inclusion criteria, patients recruited had to be able to perform repeated CPETs. We believe that these strict inclusion criteria might have resulted in us recruiting a cohort of patients with milder CHF as our patients had a higher peak VO_2 (19 ml/kg/min) than the peak VO_2 (11 ml/kg/min) that we based our power calculations on. This might explain why we did not observe an effect of metformin on the primary endpoint of the study, peak VO_2. Even though this is a randomised controlled study, there were inevitable potential confounding factors noted although they were not

statistically significant between groups. Patients in the metformin group were somewhat younger, LVEF was higher and with more non-ischaemic aetiology of heart failure. Therefore, they may have been able to exercise more within the period of the study.

CONCLUSIONS

This proof of concept study has shown that in non-diabetic CHF patients identified to have IR, treatment with metformin significantly improved IR, VE/VCO_2 slope and resulted in significant weight loss, but did not improve peak VO_2, the primary endpoint of the study. Although the improvement of VE/VCO_2 slope was correlated with the improvement of IR, we were not able to ascertain if this was the cause of improvement of sub-maximal exercise performance owing to the complexity of action of metformin. Our findings are however hypothesis generating, and further studies are clearly required to determine the effects of metformin on exercise performance in patients with CHF.

CHAPTER 7: THE FUTURE INSULIN RESISTANCE MODULATORS: AMP-ACTIVATED PROTEIN KINASE ACTIVATORS

ABSTRACT

The 5'-AMP-activated protein kinase (AMPK) is a heterotrimeric enzyme that is expressed in many tissues including the heart and vasculature, and plays a central role in the regulation of energy homeostasis. It is activated in response to stresses that lead to an increase in the cellular AMP: ATP ratio caused either by inhibition of ATP production (i.e. anoxia, ischaemia) or by accelerating ATP consumption (i.e. muscle contraction, fasting). In the heart, AMPK activity increases during ischaemia and functions to sustain ATP, cardiac function and myocardial viability. There is increasing evidence that AMPK is implicated in the pathophysiology of cardiovascular and metabolic diseases. A principle mode of AMPK activation is phosphorylation by upstream kinases (e.g. LKB1, calcium calmodulin dependent protein kinase), which leads to direct effects on tissues and phosphorylation of various downstream kinases (i.e. eEF2 kinase, p70S6). These upstream and downstream kinases of AMPK have fundamental roles in glucose metabolism, fatty acid oxidation, protein synthesis and tumour suppression; consequently, they have been implicated in cardiac ischaemia, arrhythmias and hypertrophy. Recent mechanistic studies have shown that AMPK has an important role in the mechanism of action of metformin, thiazolidinediones and statins. Increased understanding of the beneficial effects

of AMPK activation provides the rationale for targeting AMPK in the development of new therapeutic strategies for cardio-metabolic disease.

INTRODUCTION

The prevalence of cardiometabolic diseases is reaching epidemic proportions in industrialized nations and in developing countries (210-212). Despite aggressive treatment of the individual cardiometabolic risk factors, death from cardiometabolic conditions remains unacceptably high. Therefore, there is an urgent need to identify new strategies for treating and preventing cardiometabolic diseases. In this respect, the AMP-activated protein kinase (AMPK) pathway has become the focus of a great deal of attention as a novel therapeutic target in cardiometabolic disease, because it has been demonstrated to mediate, at least in part, the effects of a number of physiological and pharmacological factors that exert beneficial effects on the vasculature and the heart. AMPK has several important metabolic effects including increasing muscle glucose uptake (213) (214), and ameliorating insulin resistance (215). It regulates cardiac muscle glucose and lipid metabolism both directly and indirectly in order to provide ATP in response to energy depletion (specifically a rise in the AMP: ATP ratio). AMPK activity can also be modulated by hormones and adipocytokines, which may have protective effects against cardiovascular disease. AMPK has also been shown to regulate transcription of genes involved in lipid and glucose metabolism (216,217). Dysregulation of this process (e.g. in obesity) can lead to the development of insulin resistance and dyslipidaemia, both of which are major risks factors for

CVD. Thus, the identification of a compound that specifically and safely activates the AMPK pathway might contribute significantly to the treatment, management and even prevention of CVD. We aim to discuss the direct and indirect role of AMPK in normal cardiac physiology and in cardiometabolic disease, and therapeutic strategies in modulating APMK activity.

STRUCTURE AND REGULATION OF AMPK

Understanding of the role of AMPK in key physiological pathways has increased several folds in recent years. Its discovery can be traced back to two independent findings reported in 1973 (218) which observed that crude preparations of acetyl-CoA carboxylase (ACC) and 3-hydroxy-3-methyl (HMG)-CoA reductase became inactivated when incubated with ATP. Both groups predicted that the effects were due to phosphorylation of the enzyme by an endogenous protein kinase that contaminated their preparations. It was subsequently shown that this protein kinase was itself activated by phosphorylation by an upstream kinase (219). In 1987, Hardie *et al* made the discovery that the inactivation of ACC and HMG-CoA reductase were both catalyzed by a single protein kinase (220). As it became clear that it was a true multi-substrate kinase, they renamed it AMP-activated protein kinase after its allosteric activator, 5'-AMP (221). Hardie et al have described AMPK as a 'fuel gauge' and 'guardian of energy status', implying the fundamental role of AMPK in energy metabolism and maintaining body energy balance. AMPK is a heterotrimetric enzyme complex which consists of α, β, and γ subunits, each of which has two or more isoforms that are encoded by distinct genes and are

differentially expressed in various tissues (222). The α subunit contains the catalytic domain, including the important regulatory Thr-172 residue, which is phosphorylated by upstream kinase. The β subunit has a glycogen-binding C-terminal domains that are sufficient on their own to form a complex with α and γ subunits. High cellular glycogen content exerts an inhibitory effect on AMPK through interaction with the β subunit in skeletal muscle, although the exact mechanism is unknown (223). The γ subunit of AMPK was first recognized by Bateman (224) and contains 4 repeats, forming two domains. Each of these domains binds one molecule of AMP or ATP ion in a mutually exclusive manner (225), consistent with the findings that high concentrations of ATP antagonize activation of AMPK by AMP.

For many years, the upstream kinases(s) that phosphorylate Thr-172 on the α subunit of AMPK remained unidentified. In recent years, it has been established that the major upstream kinase in mammalian cells is a complex of protein kinase LKB1 and two accessory subunits, STRAD and MO25 (226-228). LKB1 also acts as an upstream kinase of at least 12 other AMPK-related kinases (229,230). It has also been found to be a tumour suppressor and was identified in humans as a gene carrying an autosomal dominant mutation in Peutz-Jeghers syndrome (228,231). The STRAD subunit is essential for the ability of the LKB1 complex to phosphorylate Thr-172 on AMPK (227). Besides LKB1, STRAD and MO25, AMPK can also be activated by an LKB1-independent mechanism involving Ca^{2+}/calmodulin dependent protein kinases.

AMPK exerts its metabolic effects through its interactions with various metabolic pathways. Activation of these metabolic pathways via AMPK

activation leads to remodeling of various components of metabolic syndrome (232) (Figure 16). AMPK plays a major role in providing ATP in the midst of energy depletion via its interactions with various metabolic pathways (Figure 17). Furthermore, AMPK also has direct and indirect effects on cardiovascular system. The understanding of such effects provide the rationale of targeting AMPK as a new therapeutic modality for the treatment and prevention of CVD.
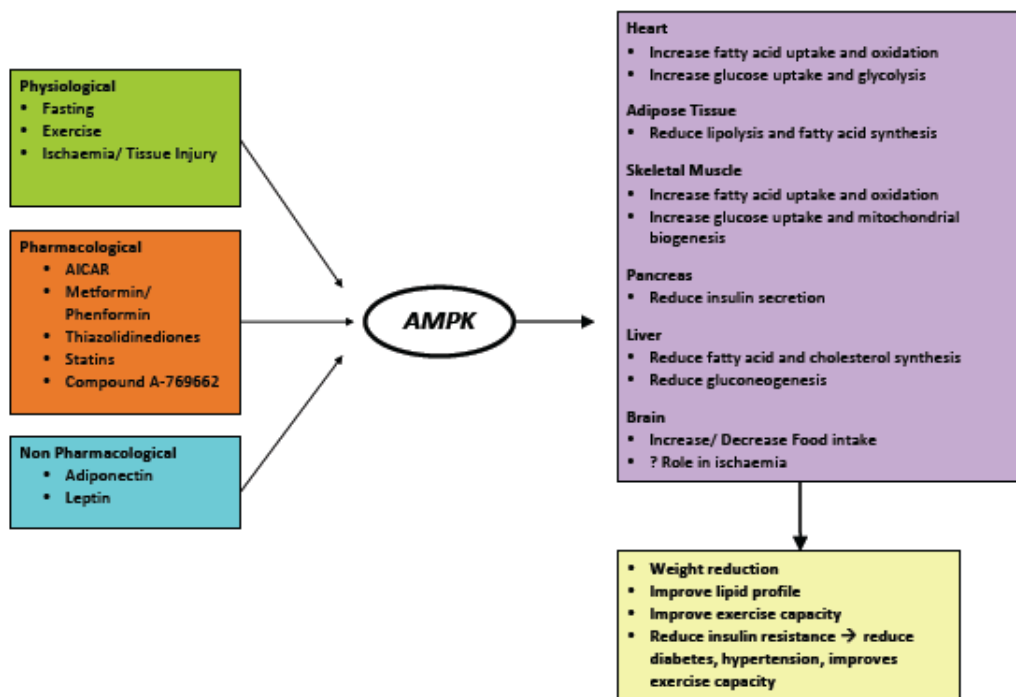
Figure 16: Activation of various metabolic pathways via AMPK activation leads to remodelling of various components of metabolic syndrome

AMPK: DIRECT EFFECTS ON CARDIOVASCULAR SYSTEM

Congestive cardiac failure, left ventricular hypertrophy, myocardial ischaemia and diabetic cardiomyopathy are all associated with disturbance of cardiac energy homeostasis. In these pathological states, AMPK activity is up regulated in response to increased AMP/ATP ratio (energy-depleted state). AMPK switches on energy generating pathways to increase cardiac myocytes fatty acid uptake (233), and increases glucose uptake by increasing translocation of GLUT-4 in an PI3-K independent manner (214) while also enhancing glycolysis via PFK-2 activation (234). At the same time, AMPK turns off protein synthesis pathways by activating eEF2 kinase, resulting in the phosphorylation and inactivation of eEF2 and by decreasing Thr-389 phosphorylation of p70 ribosomal protein S6 kinase (p70S6K), another important kinase which is involved in protein synthesis (235) via mammalian target of rapamycin (mTOR) inhibition (235,236) (See Figure 17).
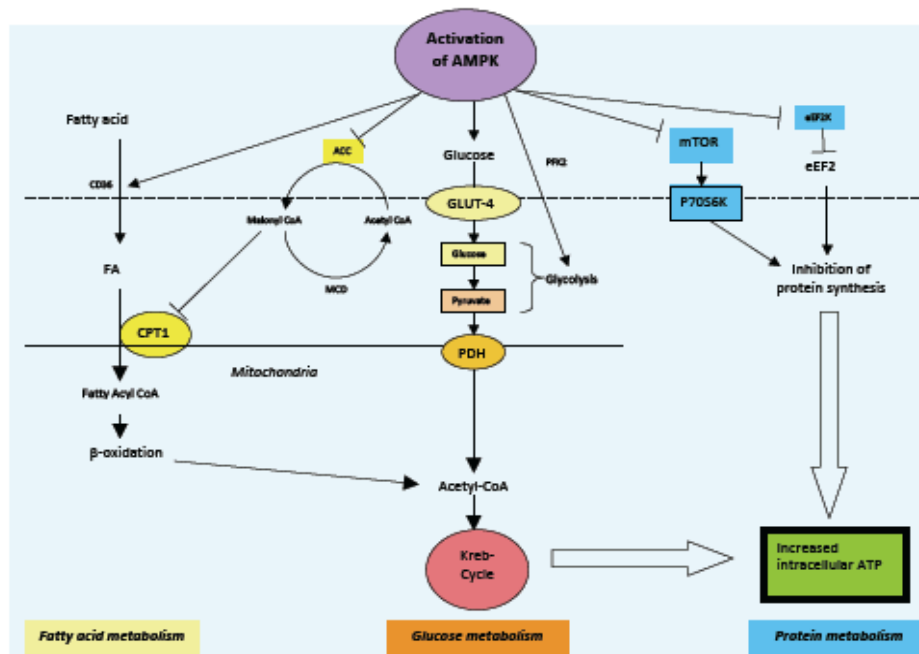
Figure 17: AMPK activation leads to activation of different metabolic pathways

AMPK plays an important role in whole body energy homeostasis. It regulates and interacts with different key metabolic pathways. Activation of AMPK secondary to change of AMP:ATP ratio or activation by upstream kinases such as CAMKK, LKB1 leads to leads to switching on energy production pathways such as glucose and lipids metabolism and turning off energy metabolic process such as protein synthesis, which is not required for immediate cell survival.

Fatty Acid metabolism. AMPK activation leads to increased translocation of CD36, a fatty acid transport protein. It increases fatty acid into cells and subsequent uptake into mitochondria for β-oxidation. Carnitine palmitoyl transferase (CPT-1) inhibits fatty acid influx and acts as a gatekeeper for mitochondrial uptake of fatty acid. Activation of AMPK leads to inhibition of acetyl CoA carboxylase (ACC2 isoform), which normally converts acetyl CoA to malonyl CoA. The inhibitory effect of malonyl CoA of CPT-1 is hence removed, leading to unopposed intake of fatty acid into mitochondria. Furthermore, phosphorylating and inactivation of the ACC1 isoform of ACC by AMPK activation reduces fatty acid synthesis and turning off expression of lipogenic genes such as fatty acid synthase.

Glucose metabolism. Activation of AMPK increases translocation and retention of glucose transporter-4 (GLUT-4) in the plasma membrane as well as increased transcription of GLUT-4 gene, leading to increased glucose uptake. It also enhances glycolysis via activation and phosphorylation of phosphofructokinase (PFK2).

Protein metabolism. p70 ribosomal protein kinase 6 (p70S6K) is a one of the key kinase involved in protein synthesis. mTOR activates p70S6K and leads to increased protein synthesis. When AMPK is activated, the activation of p70S6K is blocked as a result of inhibition of mTOR. Activation of AMPK also results in phosphorylation and inactivation of eEF2, subsequent inhibition of protein synthesis.

(A)   AMPK *AND* CARDIAC *ISCHAEMIA*

During cardiac ischaemia, the AMP/ATP ratio is increased as a result of decreased oxidative metabolism of both free fatty acids and glucose due to diminished oxygen supply in the face of increased glycolytic ATP production and glucose transport (237). Russell et al have shown that AMPK activation using 5' aminoimidazole-4-carboxamide-1-β-4-ribofuranoside (AICAR) in an *in vitro* rat model increased translocation of glucose transporters (i.e. GLUT -4) into the sarcolemma, and hence increased glucose uptake(214). Furthermore, AMPK also phosphorylates and activates phosphofructokinase (PFK-2), leading to the production of fructose 2,6-bisphosphate, a potent stimulator of glycolysis. AMPK may be necessary for adiponectin to exert its cardio-protective effect against ischaemia-perfusion injury (238). Both the $\alpha 1$ and $\alpha 2$ subunits of AMPK are activated during myocardial ischaemia, with $\alpha 2$ activated to a greater extent (180,239). Previous studies in transgenic mice have shown that decreased $\alpha 2$ activities resulted in reduced cardiac glucose uptake following ischaemia (240) and impaired recovery of left ventricular systolic function (180). Additionally, in transgenic mice expressing a kinase dead (KD) form of the enzyme, phosphocreatinine was also lower after reperfusion (180). These observations suggested that activation of AMPK following ischaemia has a cardio-protective effect, and results in lesser cardiac injury and faster recovery. Calvert et al have also shown that activation of AMPK with metformin resulted in decreased myocardial injury in both diabetic and non-diabetic mice (241). This may be a result of deriving ATP from more energy efficient glucose

metabolism from increased AMPK-mediated glucose uptake and glycolytic flux in the face of oxygen deprivation (242,243).

However, ischaemic-induced activation of AMPK may be detrimental to the ischaemic heart, as suggested by Dyck and Lopaschuk (244). During ischaemia, circulating fatty acid levels are elevated (245), which may be detrimental to the ischaemic heart (246,247). Activation of AMPK leads to increased fatty acid uptake and inhibition of malonyl-CoA, a potent endogenous inhibitor of mitochondrial fatty acid uptake. This results in accelerated mitochondrial fatty acid uptake and hence increased mitochondrial acetyl CoA production from β-oxidation. High level of acetyl CoA has an inhibitory effect on pyruvate dehydrogenase (PDH), reducing the amount of pyruvate being converted into acetyl-CoA, hence reduced glucose oxidation (the exact mechanisms remain undefined). The proposed mechanisms of these detrimental effects of high circulating fatty acids include: (1) accumulation of toxic intermediates of fatty acid oxidation such as long chain acyl-CoA thioesters and long chain acylcarnitines (246), (2) inhibition of glucose oxidation via inhibition of PDH complex by fatty-acid-derived acetyl CoA, (3) accumulation of glycolytic by-products such as protons and lactate. These valuable observations have affirmed the role of AMPK in cardiac ischaemia and implicated a potential role for therapeutic targeting in the treatment of myocardial ischaemia and infarction.

(B)   AMPK AND CARDIAC ARRHYTHMIAS

Mutations of the γ2-subunit of the AMPK have also been shown to contribute to glycogen storage disease and Wolff-Parkinson-White syndrome (248). Gollob et al identified in 2001 a mutation (Arg531Gly) in the AMP-activated protein kinase (AMPK) γ2 subunit (PRKAG2 gene) to be responsible for Wolff-Parkinson-White Syndrome and early onset of atrial fibrillation and conduction disease (248). Using a transgenic model targeting the murine gene, Davies et al demonstrated striking cardiac manifestations such as hypertrophy, impaired contractile function, electrical conduction abnormalities, and marked glycogen accumulation (249). Furthermore, Sidhu et al have identified a distinct atrial ventricular accessory pathway and prolonged QRS duration on electrocardiography in this transgenic mice model (250). However, the effects of the mutations described in this gene on the overall activity of AMPK vary in the different experimental models (251,252). It is still uncertain whether these cardiac manifestations are the result of disease-causing mutations *per se* or secondary to glycogen deposition. Murphy el al postulated that the manifestations of AMP kinase disease might be due to defects in energy utilization or in specific cellular substrates, rather than mere passive deposition of glycogen (253). Nonetheless, these data illustrate an important role for AMPK in cardiac hypertrophy and arrhythmias.

(C)   *AMPK AND CARDIAC HYPERTROPHY, CELL GROWTH AND GENE TRANSCRIPTION*

AMPK may play a further role in the regulation of normal cardiac cell growth (180,240) and energy regulation in the hypertrophied heart (254) via its effects on protein synthesis (255,256). γ2 mutations not only cause glycogen overload in the heart and the Wolff-Parkinson-White syndrome, but also hypertrophy and heart failure (248,257-259). Severity of the defect also correlates with severity of the disease. Eukaryotic elongation factor-2 (eEF2) is the main mediator of the translocation step in protein synthesis and is inhibited through phosphorylation of eEF2 kinase. p70S6 kinase regulates protein synthesis through the same pathway or via phosphorylation of ribosomal protein S6. Chan et al have shown that AMPK not only regulates eEF2 kinase, but also exerts effects on protein synthesis via the mammalian target of rapamycin (mTOR) pathway, ultimately leading to inhibition of p70S6 (256). Furthermore, Chan et al have shown that activation of AMPK using metformin and AICAR results in inhibition of protein synthesis, and is associated with prevention and regression of cardiac hypertrophy. However, studies in transgenic mice have shown that elevated AMPK activity is associated with accumulation of large amount of glycogen, leading to dramatic left ventricular hypertrophy and arrhythmias (254,260).   It remains uncertain therefore whether AMPK activation in the hypertrophied heart is beneficial (256,261) or deleterious and further studies are required.

(D)   AMPK AND VASCULAR AND ENDOTHELIAL FUNCTION

AMPK also plays an important role in the regulation of vascular function and structure. It activates endothelial nitric oxide synthase (eNOS) in endothelial cells and cardiac myocytes by phosphorylation at Ser-1177 (human sequence) (262,263). eNOS activation leads to augmentation of vascular tone, platelet aggregation, leukocyte adherence and vascular smooth muscle proliferation (264).

Using a diabetic rat model, Suzuki et al has shown that activation of AMPK using a cyclic AMP (cAMP) phosphodiesterase inhibitor, cilostazol, restores endothelial function independently of cAMP (265). Administration of cilostazol leads to phosphorylation of AMPK and subsequent phosphorylation of eNOS and increased nitric oxide (NO) production. Other AMPK activators, 5-aminoimidazole-4-carboxamide riboside (AICAR) (266), metformin (267) and rosiglitazone (268) have all been shown to increase NO production in human aortic endothelial cells via the AMPK pathway. Additionally, AMPK also appears to have a role in angiogenesis, promoting the action of the HIF-1α/ vascular endothelial growth factor VEGF pathway (269) (270), and inhibiting angiotensin II-induced smooth muscle cell proliferation (271). Furthermore, activation of AMPK activation using AICAR has been shown to inhibit palmitate-induced endothelial cells apoptosis through suppression of reactive oxygen species (272). It is clear that AMPK plays a central role in vascular biology.

AMPK: INDIRECT EFFECTS ON CARDIOVASCULAR

SYSTEM

Recent data have shown that levels of adipocytokines such as adiponectin and leptin correlate with the development of different components of metabolic syndrome (273). AMPK has been suggested to play a role in mediating the metabolic and vascular effects of the key adipocytokines (274,275).

(A)   *AMPK AND LEPTIN*

Leptin is an adipocyte-secreted hormone that plays a pivotal role in the regulation of food intake, energy expenditure, body weight, and neuroendocrine function (276). Leptin stimulates fatty acid oxidation (58), and glucose uptake (59), and prevents lipid accumulation out with adipose tissue, preventing lipotoxicity (60). Deposition of ectopic fat in pancreatic beta cells, myocardium, and skeletal muscle contributes to the pathogenesis of type 2 diabetes mellitus, cardiomyopathy, and insulin resistance respectively. Leptin is known to exert effects via the AMPK pathway, stimulating phosphorylation and activation of the α2 catalytic subunit of AMPK selectively in skeletal muscle (58). Leptin also suppresses ACC2 activity, thereby stimulating fatty acid oxidation in muscle. AMPK also inhibits lipogenesis and ectopic fat deposition in the liver (277). AMPK is also a key regulator of leptin action in the hypothalamus and a "master regulator" of food intake. Minokoshi et al have shown that inhibition of AMPK

activity by leptin specifically in the arcuate and paraventricular nuclei is essential for its anorexigenic and weight loss effects (278).

(B)   AMPK AND ADIPONECTIN

Adiponectin, an adipose-specific protein present in high concentrations in the circulation, was discovered in 1996. It possesses anti-atherogenic, insulin-sensitizing and anti-inflammatory properties. Yamauchi et al have shown that adiponectin stimulates glucose utilization and fatty acid oxidation via the AMPK pathway (274). Furthermore, adiponectin has been shown to reduce infarct size, improve left ventricular function and remodelling, and increase coronary flow during reperfusion in animal models. The underlying mechanisms are thought to be phosphorylation of eNOS, AMPK Thr 172 and Akt Ser 473 (279). Adiponectin deficient mice have been shown to have progressive cardiac remodelling in a pressure overloaded condition due to reduced AMPK signalling and worsening insulin resistance (280). Therefore, the AMPK pathway is not only critical for the metabolic- and insulin sensitizing actions of adiponectin, but also its cardio-protective effects in myocardial ischaemia and reperfusion.

AMPK ACTIVATORS: PHARMACOLOGICAL TOOLS AND THERAPEUTIC POTENTIAL

As we have seen, AMPK is a pivotal enzyme that regulates diverse signals in metabolic pathways and has direct and indirect effects on the heart and vasculature. AMPK activation has not only been shown to alleviate various components of the metabolic syndrome, but may also improve left ventricular hypertrophy and reduce cardiac injury in ischaemia. AMPK is also a key mediator of the anti-atherosclerotic and insulin-sensitizing effects of adiponectin. Therefore, it is clearly an attractive therapeutic target in cardio-metabolic disease. A number of AMPK activators are available as pharmacological tools and some are in clinical use (Table 11).

(1)   5-AMINOIMIDAZOLE-4-CARBOXAMIDE RIBOSIDE (AICAR)

AICAR is an adenosine analogue, which activates AMPK through direct binding followed by allosteric modification. It is initially taken up by adenosine transporters and subsequently phosphorylated to 5-aminoimidazole-4-carboxamide-1-ß-D-ribofuranotide (ZMP) within the cell, which mimics AMP in AMPK signalling (281). AICAR was first developed to block adenosine reuptake in the ischaemic heart, promoting stimulation of adenosine membrane receptors. In 1997, treatment with acadesine (AICAR) before and during surgery was showed to reduce early cardiac death, myocardial infarction, and

combined adverse cardiovascular outcomes (282), although the mode of action via AMPK was not fully appreciated at that time.

AICAR is now widely used in the laboratory setting, particularly in experiments relating to glucose metabolism, insulin signalling pathways and lipid metabolism. In recent years, AICAR has been shown to reverse various aspects of metabolic syndrome in animal models (232,283-285) and healthy human subjects (286)(Table 12). AICAR has also been shown to stimulates adiponectin and inhibit cytokines such as TNF-α and IL-6, which have been implicated in the development of obesity-induced insulin resistance (287-290). Unfortunately, AICAR is far from an ideal activator of the AMPK pathway in the clinical settings because of its short half-life, requirement for intravenous infusion and variable effectiveness. It also causes bradycardia and can lead to hypoglycaemia when administered intravenously. Therefore, there is great interest in developing a more potent, safer and more specific activator.

(2)   METFORMIN

Metformin has been used to treat diabetes for more than 50 years and is associated in observational studies with reduced mortality and improved outcomes in patients with chronic heart failure (28,146). It is the preferred anti-diabetic medication for obese patients with Type 2 diabetes mellitus because of its property to stabilize weight and reduce cardiovascular events when used as monotherapy (291). Recent clinical studies have shown that the effects of metformin may go beyond improving glycated haemoglobin and may include reductions in cardiovascular endpoints in Type 2 diabetes mellitus and heart

failure. This wide spectrum of cardiovascular protective effects may be attributable to its activation of AMPK and its downstream pathways.

Metformin has been shown to activate AMPK in myocytes (292-294), hepatocytes (295) and skeletal muscle cells (295). Metformin decreases hepatic glucose production and increases skeletal muscle glucose disposal. Therapeutic doses of metformin have been shown to increase AMPK $\alpha2$ activity in human skeletal muscle with an associated increase in phosphorylation of AMPK on Thr172 and decreased ACC2 activity (296). Metformin can also up-regulate eNOS and increases nitric oxide bioactivity via AMPK activation (297). Furthermore, AMPK activation by metformin enhances fatty acid oxidation, which leads to alleviation of endothelial lipotoxicity and improved endothelial function (298). Moreover, metformin has also been shown to have anti-cancer effects in recent study via its indirect AMPK activation (299). However, the precise mechanisms of how metformin activates AMPK are still poorly understood.

Even though metformin is regarded as an AMPK activator, it has not been shown to bind directly to AMPK; neither does it regulate its own phosphorylation and dephosphorylation in cell-free assays (300). One hypothesis is that it activates AMPK by inhibiting complex I of the respiratory chain, which subsequently causes a rise in the AMP: ATP ratio (301,302). In fact, inhibition of the respiratory chain in the intestinal mucosa may account for the gastrointestinal side effects of the drug and this property may account for the propensity of its predecessor biguanides phenformin to cause lactic acidosis. Metformin is transported into intestinal cells mainly by the organic cation

transporter OCT-1, but phenformin penetrates cell membranes without active transport. Recent identification of polymorphisms in genes encoding cation transporters proteins may ultimately explain differences in tolerance and response to metformin (303). Interestingly, there are also studies suggesting that AMPK can be activated by metformin without changes of AMP/ATP ratio (300,304) and metformin can also exert its beneficial metabolic effects on cardiac myocytes in an AMPK-independent manner (305).

However, we should be mindful that extra caution is required if we are to use these results to extrapolate to the effects of metformin on AMPK. Firstly, variable doses of metformin have been used in these studies. The plasma metformin concentration in clinical use is usually around 10 μM (140) whereas the doses used in vivo and in vitro experiments are consistently higher, in the range of 1-10mM (Table 13). Saeedi et al has shown that lower doses of metformin (i.e. 2mM) failed to activate AMPK and cause no changes of energetic state. On the contrary, Hardie et al have shown that lower doses of metformin can actually produce AMPK activation without significant change of cellular AMP: ATP ratio (300). Other research groups reported that AMPK activation required higher doses of metformin (i.e. 5-10mM) (292,294) (see table 13). They suggested that higher doses of metformin are required to cause changes in the energetic state and hence subsequent AMPK activation. However, these diversified results may be the result of different exposure time of metformin. For instance, Yang et al has shown that lower dose of MF (1mM) activated AMPK and increased cardiac myocytes glucose uptake after a prolonged exposure of 18 hours (293). On the other hand, Bertrand et al had shown that

short exposure (4 hours) of metformin could result in AMPK activation if much higher doses of metformin were used (5-10mM) (306). Therefore, AMPK can be activated by metformin in a time and concentration dependent manner. Clearly further studies are required to determine the time and concentration of metformin, which will result in the maximal beneficial effects of AMPK activation without intolerable side effects.

Table 11: Different "AMPK activators" and their limitations in clinical use

AMPK activators	Possible mechanisms of AMPK activation	Activations of other pathways	Limitations
AICAR	▪ Direct activation followed by allosteric modification	▪ Stimulate adiponectin release ▪ Inhibit cytokines such as TNF-α and IL-6 ▪ Please refer to table 2	▪ Short half life ▪ Variable effectiveness ▪ Only intravenous forms available ▪ May cause bradycardia and significant hypoglycaemia
Metformin (MF)	▪ Indirect activation ▪ Via alteration of AMP:ATP ratio as a results of inhibition of complex I in the respiratory chain ▪ Other unknown mechanisms ▪ Please refer to table 2	▪ Anti-cancer effects via its effects on p53 ▪ Up-regulate eNOS and increases nitric oxide bioactivity ▪ Enhances fatty acid oxidation which leads to alleviation of endothelial lipotoxicity ▪ Please refer to table 3	▪ Indirect AMPK activation ▪ Doses and duration of MF required for AMPK activation not determined ▪ Higher doses of MF results in intolerable gastrointestinal side effects
TZDs	▪ Indirect activation ▪ Via alteration of AMP:ATP ratio, possibly similar to MF ▪ Via adiponectin	▪ Anti-atherosclerotic and anti-inflammatory effects via adiponectin ▪ Effects on mitochondrial biogenesis ▪ Exerts anti-oxidative effects by inhibiting PKC via AMPK activation	▪ Indirect inhibition ▪ Risk of developing fluid retention ▪ Risk of developing cardiovascular events yet to be determined
Statins	▪ Indirect activation ▪ Does not alter AMP:ATP ratio ▪ Other unknown mechanisms	▪ HMG-CoA reductase inhibition ▪ Activation of AMPK-eNOS-ACC	▪ Doses required for AMPK activation in human still to be determined
Compound A-769662	▪ Direct activation	▪ Increased fatty acid oxidation ▪ Decreased plasma and liver triglyceride level ▪ Inhibit fatty acid synthesis ▪ Stabilize weight	▪ Poor oral bioavailability ▪ Data on long term AMPK activation are awaited

Table 12: Various Studies on AMPK activation using AICAR and their major findings

Study	Type of Subjects	Dosage	Duration	Major findings
Iglesias M A, Diabetes 2002	Insulin resistant (IR) high-fat-fed rats	Subcutaneous injection of 250mg/kg	24 hour	• Enhanced whole body, muscle and liver insulin action • Reduced hepatic glucose output
Buhl E S Diabetes 2002	Obese Zucker rats exhibiting IR, hyperlipidaemia and hypertension	Subcutaneous injection of 0.5mg/g	7 weeks	• Reduced plasma triglyceride, free fatty acids, increased HDL • Lower systolic blood pressure • Normalised oral glucose tolerance test and reduced fasting glucose and insulin • Showing tendency toward decreased intra-abdominal fat content
Bergeron R Diabetes 2001	Obese Zucker rats	Bolus 100mg/kg Constant infusion 10mg/kg/min	60 mins	• Increased glucose transport in red gastrocnemius muscle whereas insulin showed no effects • Suppression of endogenous glucose production and lipolysis
Song X. M Diabetologia 2002	Ob/Ob Mice	Subcutaneous 1mg/g of body weight	7 days	• Corrected hyperglycaemia, improved glucose tolerance, and increased GLUT4 and hexokinase II protein expression in skeletal muscle
Cuthbertson, D. J. Diabetes 2007	Healthy men	Intravenous infusion at 10mg/kg/hour	9 hours	• Increased human skeletal muscle 2-deoxyglucose uptake and whole-body glucose disposal.

Table 13: Recent studies of AMPK activation using metformin and their major findings

Study	Aims (A) and Subjects(S)	Dosage	Key findings	Clinical application
Calvert JW et al Diabetes 2008	A: Examine the cardioprotective effects of MF S: Murine models	125 mcg/kg vs saline (286 fold lower than maximum antihyperglycaemic dose)	▪ Reduction of myocardial injury in both diabetic and non diabetic mice ▪ Increased AMPK activity and eNOS phophorylation	Cardioprotective effects of MF might be secondary to eNOS activation via AMPK pathway
Solskov L et al Basic Clinical Pharmacology Toxicology 2008	A: To determine the effects of single dose MF on cardiac protection against IRI S: Wistar rats	Single dose of MF (250mg/kg) vs saline	▪ Reduction of myocardial infarction size ▪ Two fold increased in AMPK-α1 activity	MF might reduce myocardial infarction size in pre-treated subjects via AMPK activation
Saeedi R et al Am J Physiol Heart Cir Physiol 2008	A: Determine if MF has effects on metabolism of heart muscle independent of AMPK pathway S: Sprague-Dawley rats	2mM (this dosage has greatest cellular metabolic effects without an impact on cellular energy status	▪ Increased rate of glycolysis, glucose uptake and fatty acid oxidation ▪ AMPK was not activated by 2mM of MF	MF has AMPK-independent metabolic effects, possibly via protein kinase C and p38 mitogen-activated protein kinase pathways
Kovacic S et al J Biol Chem 2003	A: Akt activation induced by insulin negatively regulates AMPK activities S: Akt transgenic mice and adenovirus infected noenatal rat cardiac myocytes with mutant form of Akt1 and Akt2	5mM of MF	▪ Insulin increased Akt phosphorylation and reduced AMPK phosphorylation ▪ Administration of MF overcome Akt-dependent AMPK suppression ▪ Study suggests a cross talk between Akt and APMK pathway	AMPK can be activated by MF via insulin-independent pathway but higher doses of MF are required
Zhang L et al Am J Physiol Heart Cir Physiol 2007	A: MF activates AMPK in heart via increasing cytosolic AMP S: Sprague-Dawley rats	10mM of MF	▪ MF increases AMPK activity preceded by and correlated with increased cytosolic AMP but overall AMP/ATP remained unchanged	MF activates AMPK without altering total AMP/ATP ratio. High dosage of MF is required for AMPK activation.

(3)   THIAZOLIDINEDIONES (TZDS)

Thiazolidinediones (e.g. rosiglitazone and pioglitazone, "TZDs") are ligands for the nuclear hormone receptor family member PPAR-γ (134). Both the TZDs have been shown to activate AMPK in intact cells (307,308). TZDs can also activate AMPK by stimulating the release and expression of circulating adiponectin from adipose tissue (274,275), or indirectly by increasing cellular AMP/ATP ratio, possibly by a similar mechanism to biguanides (309). Both rosiglitazone and pioglitazone have been suggested to have additional and protective beneficial anti-atherosclerotic and anti-inflammatory effects (310). Furthermore, TZDs have also been shown to have diverse beneficial effects on endothelial function, TNF-α, nitric oxide and endothelial cell proliferations via AMPK-dependent and PPARγ-independent mechanisms (311-315). These effects may translate into improvement of clinical outcomes in patients with cardiometabolic disease. Previous studies have raised the intriguing possibilities that these effects may be mediated via AMPK activation (308,316,317). However, like metformin, we are not certain if AMPK activation is the key to these clinical beneficial effects on cardiovascular system. Furthermore, we also need to be very cautious when we try to translate these observations in animal studies to the clinical setting. The doses and type of TZDs that have been shown to activate AMPK vary among different research groups and the doses used in these animal studies may not be applicable to human subjects. Furthermore, the majority of these in vivo studies are short-term studies examining the effects of acute AMPK activation and its metabolic effects. However, the effects of long-term AMP activation by TZDs have yet to be

determined. Nonetheless, the cardiovascular protective effects of TZDs are evidenced in the recently published post hoc analysis from the PROspective pioglitAzone Clinical Trial In macrovascular Events (PROactive) [116]. It showed that patient who has chronic kidney disease who received pioglitazone are less likely to reach composite end-points of all-cause death, MI, and stroke, independent of the severity of renal impairment.

However, it should be noted that TZDs use is associated with the risk of fluid retention, which may exacerbate heart failure (177) (PROACTIVE, United State Food and Drug Administration (FDA) statement). In a recent meta-analysis, Lago and colleagues reported that TZDs increased risk for development of CHF, probably as a result of fluid retention, across a wide background of cardiac risk (relative risk [RR] 1.72, 95% CI 1.21-2.42, p=0.002) (136). There is also a concern that these agents may be associated with additional cardiovascular (MI, stroke) risk in patients with Type 2 diabetes mellitus (T2DM) with rosiglitazone. However, it should be noted that these meta-analyses which included many small trials (138), while large clinical trial data have shown no signal of these CV events (RECORD, PROACTIVE) (177,318). The European Medicines Evaluation Agency (EMEA) for Medicinal Products for Human Use has adopted a scientific opinion in January 2008, recommending the inclusion of a new warning stating that the use of rosiglitazone in patients with ischaemic heart disease and/or peripheral arterial disease is not recommended. A recent FDA review suggested that more large randomized studies with active comparators should be conducted (FDA) by the manufacturers. In 2010, EMEA has recommended the suspension of the

marketing authorisations of rosiglitazone (Avandia, Avandamet) across the European Union.

(4) STATINS

Statins are widely prescribed in patients with metabolic syndrome owing to the high incidence of hypercholesterolaemia in this group of patients. There is mounting evidence to suggest that the clinical benefits of statins are beyond its lipid lowering effects. The clinical efficacy of statins treatment in reducing cardiovascular mortality and morbidity in patients with diabetes and without diabetes are well proven in clinical trials such as the Heart Protection Study and the Collaborative Atorvastatin Diabetes Study (319-322). Besides its cholesterol lowering effect via 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibition, statins has also been shown to activate AMPK in human and bovine endothelial cells (323). Xenos et al have shown that AMPK protein levels in human endothelial cells were increased after being treated with fluvastatin for 2 days (324). Sun et al have also shown that atorvastatin and lovastatin caused rapid activation of AMPK-eNOS/ACC in mouse myocardium and endothelial cells [125]. The atorvastatin dose used in this study was 50mg/kg in mice, which is equivalent to 80mg per day in human. This study has shown that atorvastatin did not alter cellular AMP: ATP ratio, suggesting different mechanism of AMPK activation. The beneficial effects of statins on endothelial function have been suggested to be the results of its ability to up regulate eNOS (319,320,325-327), and its anti-inflammatory and anti-atherogenic effects (322,328).These observations have all suggested that AMPK activation might be the key to the pleiotropic effects of statins on cardiovascular

protection. However, further mechanistic and translational studies are required to show that AMPK activation is indeed the key these effects of statins treatment as well as the examining the doses of different statins required to activate AMPK.

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(5)   COMPOUND A-769662

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Cool et al. have identified a thienopyridone family of AMPK activators (329), compound A-769662. It stimulates AMPK directly in partially purified rat liver and inhibits fatty acid synthesis in primary rat hepatocytes. Short-term treatment of normal Sprague Dawley rats with A-769662 decreases liver malonyl CoA levels and the respiratory exchange ratio, VCO_2/VO_2, indicating an increased rate of whole-body fatty acid oxidation. In ob/ob mice, treatment with compound A-769662 has been shown to decrease plasma glucose, reducing weight gain and significantly decreasing both plasma and liver triglyceride levels. These results demonstrated that small molecule-mediated activation of AMPK in vivo is feasible, and represent therefore a promising approach for the treatment of Type 2 diabetes mellitus and the metabolic syndrome. However, the compound has poor oral bioavailability, limiting its use in clinical settings.

An alternative small molecule compound that is safe, potent, acts directly on AMPK with good oral bioavailability would be an attractive candidate to progress towards clinical development.

CONCLUSIONS

Activation of AMPK pathway may be the key in treating and preventing various cardiometabolic diseases. AMPK pathway and its association with its upstream and downstream kinases have fundamental roles in glucose metabolism, fatty acid oxidation, and protein synthesis. AMPK pathway has been the focus of many researches of late owing to his central role in modulating cardio-metabolic processes, and recent mechanistic studies have shown that AMPK has an important role in the mechanism of action of metformin, thiazolidinediones and statins. Activation of AMPK may be responsible for the insulin sensitizing and beneficial cardio-metabolic effects of these drugs. However, it is still uncertain whether direct activation of the AMPK pathway in the absence of a physiological stress will be beneficial or deleterious overall in humans. It is hoped that chronic activation of AMPK will not result in "over-compensatory" activation of other systems such as the renin-angiotensin-aldosterone system activation in heart failure. Alterations in cardiac AMPK activity are associated with a number of cardiovascular-related diseases such as pathological cardiac hypertrophy (258), myocardial ischemia (244), glycogen storage cardiomyopathy (260), and Wolff-Parkinson-White syndrome (259), suggesting a possible maladaptive role in such conditions. Andersson et al described anti-satiety effects of AMPK, which may lead to weight gain (330). Furthermore, McCullough et al also demonstrated that activated AMPK might be harmful in stroke (331). All these uncertainties will need to be clarified by further translational studies and much effort is still required to define the roles of AMPK activation in various conditions that we have already discussed.

Furthermore, it is also a great challenge for pharmaceutical companies to produce a specific AMPK activator, which has predictable effects owing to its heterotrimeric structure and its complex interactions with various upstream and down stream kinases. The other approach in which many researchers adopted was to develop a compound that targets the down stream kinases of AMPK (i.e. malonyl CoA activator, CPT-1 activator). The AMPK-malonyl CoA-CPT-1 axis might represent an interesting pathway for further research in cardiac substrate utilization and fatty acid metabolism. AMPK-adipocytokines interaction has also formed the rationale of developing of new treatment modalities for the treatment of obesity. Lastly, the AMPK-MTOR-eEF2-p70S6K axis modulations may the key to understand the pathogenesis of cardiac myocytes hypertrophy and mitochondrial biogenesis. The greater understanding of the biochemistry and physiology of AMPK and better understanding of the mechanism of actions of existing agents have nonetheless opened up a new horizon for the treatment and prevention of cardiovascular and metabolic disease.

CHAPTER 8: FINAL DISCUSSION

Diabetes Mellitus and HF commonly coexist, and each condition impact on each other in terms of causation and outcome. DM is highly prevalent amongst HF patients and vice versa. The prevalence of DM increases with severity of HF. Up to one third of patients who are hospitalised with HF are found to be diabetic.

NYHA functional class has been shown to be a predictor of risk of developing DM in HF from population based study, whereas HbA1c measurements are predictors of risk of developing HF in diabetic patients. Elevated HbA1c level is also a predictor of incident HF in diabetics as well as non-diabetics. More importantly, suboptimal glycaemic control as measured by HbA1c is associated with adverse outcome. However, there were some conflicting reports regarding the degree of glycaemic control in T2DM and CHF. In patients with T2DM and CHF, our observational study shows that there is a U shaped relationship between HbA1c and mortality with the lowest mortality risk in patients with modest glycaemic control (HbA1c, $>7 \leq 9\%$). This observational data adds support to the growing concern that we need to redefine the optimal HbA1c level in this high-risk group of patients with co-existing T2DM and CHF. These findings may be partly explained by the differences in severity of CHF, duration of diabetes, and differences in the choices of drugs used.

The bi-directional inter-relationship between CHF and diabetes also extends to insulin resistance (IR). IR precedes and also predicts the development of CHF, independent of established risk factors for CHF including diabetes itself. The degree of IR positively correlates with severity of CHF and is associated with adverse functional consequences (i.e. reduced endothelial function, exercise capacity and associated with abnormal serum biomarkers). The exact pathophysiology of IR and CHF is not fully understood. Activation of SNS, RAS, inflammation, altered adipocytokines levels, formation of advanced glycosylation products, changes in substrate utilization of the myocardium and ED are possible explanations of how IR affecting disease process in CHF. With our increased understanding of the pathophysiological role of IR in CHF, improving IR may represent a new target for treatment for patients with CHF.

So, how can we improve IR? Lifestyle changes such as diet and exercise are possible but very difficult to prescribe, and patients' adherence can be of great challenge. Therefore, pharmacological approach is needed for most patients with evidence of IR/DM and CHF. We are mindful that certain conventional HF medications only have modest beneficial effects on glycaemic control and the prevention of the development of diabetes. Therefore, more potent "insulin sensitizers" are needed to reverse IR in CHF. TZDs were initially thought to be a blockbuster drug when it was first marketed owing to its potent insulin sensitizing property and favourable impacts on cardiovascular parameters such as lipids, blood pressure, inflammatory biomarkers, endothelial function, and fibrinolytic status (332,333). However, its use in CHF was restricted because of its ability to increased fluid retention caused by

increased re-absorption in the distal nephron as well as increased vascular permeability in adipose tissue (334). Furthermore, there are additional concerns regarding the risk of myocardial infarction with TZDs especially with rosiglitazone, which has now been withdrawn from the market.

The incretin system has received a great deal of attention in the treatment of diabetes in recent years. A few randomized controlled trials are currently underway to define the utility of targeting the incretin system in HF patients with DM. Incretin-based therapy may represent a novel therapeutic strategy in the treatment of HF patients with diabetes, as it has been shown to have cardioprotective effects independent of those attributable to tight glycaemic control. Our increased insights and understanding of the incretin system has opened up new horizons in the potential treatment options in CHF, and outcome trials are awaiting.

Metformin, another insulin sensitizing medications, has been on the market for almost 50 years. The use of metformin in patients with CHF has been discouraged previously because of previous experience with phenformin back in the 1970's. The precise mechanisms of action are not fully understood. However, with increased understandings and refreshed insights from accumulating experience of metformin use, metformin has proven to be safe in patients with CHF and DM. More importantly, there are now large observational data and retrospect studies to support the notion that metformin is not only safe, but also its use was associated with a better outcome in patients with CHF and DM. Although these data were encouraging, the main limitations of these observational studies were the potential for selection bias imposed by different

therapies. What is needed is prospective placebo controlled studies to examine the impact of metformin in CHF and DM.

Therefore, in a randomized placebo controlled trial, we evaluatedthe impact of metformin on IR and its effects on exercise capacity in non-diabetic patients with CHF. The primary endpoint of the trial was to determine if improvement of IR with metformin lead to improvement of peak VO_2. However, many patients with CHF are unable to perform maximal exercise and oxygen requirements for activities of daily living rarely approach maximal levels (185). Therefore, we have included the sub-maximal derived exercise variable of the slope of the ratio of minute ventilation to carbon dioxide production (VE/VCO_2) as a secondary end-point of this study. VE/VCO_2 slope is an index of ventilatory response to exercise. Unlike peak VO_2, VE/VCO_2 is not influenced by the mechanical work done during exercise testing but reflects alterations in the peripheries caused by the disease in CHF, which can in turn lead to the progression and symptomatology of CHF (186). Furthermore, we have also explored possible mechanisms of improvement of exercise capacity by measuring left ventricular ejection fraction by echocardiography, endothelial function and related biomarkers.

Our study had two main findings. Firstly, we showed that in CHF patients with insulin resistance, metformin treatment significantly reduced FIRI and this treatment was associated with a weight loss of 1.9 kg. Secondly, although metformin had no effect on peak exercise parameters such as peak VO_2, metformin treatment did result in a significant improvement in VE/VCO_2 slope, a pre-specified endpoint of this proof of concept study. There are several

possible explanations of improved VE/VCO$_2$ slope. Firstly, the improvement in functional capacity might be related to the weight loss of 1.9 kg associated with metformin therapy. However, in our regression model, we showed that weight reduction alone did not correlate with the reduction in VE/VCO$_2$ slope. Secondly, the insulin sensitizing properties of metformin might confer some beneficial effects on exercise capacity. Improving insulin sensitivity has been shown to improve exercise capacity. In this regard, we found that with metformin treatment, reduction of FIRI and serum leptin level was significantly correlated with the reduction of VE/VCO$_2$ slope. Doehner et al had previously demonstrated that hyperleptinaemia is an independent predictor of IR in patients with CHF, and it may play an important role in energy metabolism in these patients (63). Reduction of serum leptin has been previously reported following chronic metformin therapy (206) and might be due to a direct effect of metformin on leptin secretion (207). Recent studies have shown the significance of adipocytokines modulation in HF patients and high levels of adiponectin were associated with adverse outcomes in CHF (75,76). There was no significant change in adiponectin levels in our study (p=0.168). However, we did notice that patients in the metformin treated group have decreased adiponectin level, whereas adiponectin levels were higher in the placebo group. Therefore, the improvement of sub-maximal exercise capacity in our cohort may in part be due to an improvement of IR and the reduction of serum leptin level. A third consideration was metformin's ability to activate AMPK, which is expressed in various tissues including the skeletal muscle, myocardium and vascular endothelium (165). Therefore, activation of AMPK could impact on central as well as peripheral haemodynamic mechanisms which in turn leads to

changes in VE/VCO$_2$ slope and hence, ventilatory class. Improvement of myocardial substrate utilization and glucose uptake through activation of AMPK may improve myocardial contraction and increase cardiac output (196). In animal models of heart failure, metformin has been shown to activate AMPK and improve left ventricular function, and attenuate oxidative stress-induced cardiomyocyte apoptosis, resulting in improved survival (197). However, we did not observe any significant effect of metformin on echo-derived LVEF or CO at rest and during peak exercise. With respect to peripherally mediated mechanisms, effects of metformin on exercising skeletal muscles could account for improvement in VE/VCO$_2$ slope. Alterations in skeletal muscle energy metabolism, IR and functional adiponectin resistance have been reported and linked to exercise intolerance in patients with CHF (208). A study of the effects of metformin on skeletal muscle enzyme activities in our subjects would be of interest. In our original proposal, we had planned to do this, but this invasive procedure was offered as an option and no patient consented to the procedure. We did not see any significant effect of metformin on endothelial function. Obviously any evidence that metformin improves exercise capacity through central cardiac or peripheral mechanisms in patients with CHF must remain speculative, and cannot be inferred directly from this study. Clearly, further studies are required to define the mechanisms underlying these effects of metformin in CHF.

AMPK pathway has been the focus of recent research, owing to his central role in modulating cardio-metabolic processes. Recent mechanistic studies have shown that AMPK has an important role in the mechanism of

action of metformin, thiazolidinediones and statins. Activation of AMPK may be responsible for the insulin sensitizing and beneficial cardio-metabolic effects of these drugs. For example, in animal models of heart failure, metformin has been shown to activate AMPK, improve left ventricular function and to attenuate oxidative stress-induced cardiomyocyte apoptosis, resulting in improved survival (197). Increased understanding of the beneficial effects of AMPK activation provides the rationale for targeting AMPK in the development of new therapeutic strategies to improve IR, cardio-metabolic disease and heart failure.

PUBLICATIONS AND PRESENTATIONS

PAPERS

1. Aaron K Wong, Allan D Struthers, Anna-Maria Choy, Chim C Lang. Insulin sensitization therapy and the heart: focus on metformin and thiazolidinediones. Heart Fail Clin. 2012 Oct;8(4):539-50. doi: 10.1016/j.hfc.2012.06.002. Epub 2012 Aug 9.

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PRESENTATIONS

1. Title: Metformin in Insulin Resistance LV systolic dysfunction, A Double-blind, placebo controlled trial. (Short-listed for Philip Poole-Wilson Young Investigator award in Clinical Research) in European Society of Cardiology Heart Failure Congress, Berlin May 2010

2. Title: Reversing Insulin Resistance: A New Target for Treatment in Chronic Heart Failure. Annual Meeting of the Association of Physician of Great Britain and Ireland, Dundee April 2010

3. TAYSIDE trial. Scottish Society of Physicians 52nd Annual Meeting, Edinburgh 25th September 2010 (Awarded Fitzgerald Peel Prize)

4. Title: Prevalence of Insulin Resistance and CHF. Scottish Cardiac Society Annual Conference, Glasgow September 2008

POSTERS

1. Moderated poster presentation in European Society of Cardiology Congress, Stockholm, August 2010. *Title: Glycaemic control and the development of heart failure and its importance in diabetic patients with established heart failure.*

2. European Society of Cardiology Congress, Stockholm, August 2010. *Title: Metformin in Insulin Resistance LV systolic dysfunction, A Double-blind, placebo controlled trial.*

3. Moderated poster presentation in British Cardiac Society Annual Scientific Conference, Manchester June 2010. *Title: Metformin in Insulin Resistance LV systolic dysfunction, A Double-blind, placebo controlled trial.*

4. Annual Meeting of the Association of Physician of Great Britain and Ireland, Dundee April 2010. Title: *Metformin in Insulin Resistance LV systolic dysfunction, A Double blind, placebo controlled trial.*

5. Moderated poster presentation in British Cardiac Society Annual Scientific Conference, Manchester June 2008. Title: *Insulin resistance is highly prevalent and is associated with reduced exercise tolerance in non-diabetic patients with heart failure*

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